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APPLICATIONS OF METABOLOMICS AND METABOLIC PROFILING TO STUDY PLANT INTERACTIONS WITH PLANTS, MICROBES AND GRAZING HERBIVORES

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Abstract: Plant secondary products are the drivers of many plant interactions, both above and below ground, with other plants, herbivores and soil-borne macro- and microbiota. During plant growth and cellular ontogeny, secondary plant products are biosynthesized and often accumulate in a highly regulated manner in specialized tissues and organelles. Metabolites released by plants and their residues play important roles in rhizosphere signalling, plant defence and responses to abiotic stresses. Plants employ a variety of sequestration and transport mechanisms to store and release bioactive metabolites and export them selectively into the surrounding environment. Transport systems in plant roots and shoots are generally closely linked to soil environmental conditions and plant health. Release of key metabolites often involves enzymatic activity leading to tissue degradation, but is also achieved by active transport, simple diffusion, extrusion or active exudation. In actively growing roots, specialized cells generate and release large quantities of allelochemicals in response to stressors in local environments.

Our research has focused on the production, localization and release of plant-produced metabolites by combining fluorescence and confocal microscopic observations with metabolic profiling. We have employed both targeted and non-targeted metabolomics to identify novel metabolites and pathways in response to plant-plant and plant-microbe (resulting in symbiosis, allelopathy and/or phytotoxicity) and plant-grazing herbivore interactions (resulting in photocytotoxicity/poisoning). By identification of bioactive metabolites of interest, the regulation of biosynthetic pathways in plants can be studied, as well as their microbial or chemical transformation products. Here we present examples of metabolites playing key roles in crop interference with weeds, invasion success of exotic weeds, transformation processes leading to bioherbicide formation, livestock poisoning, reproductive failure and photosensitization. Opportunities for bioprospecting of novel metabolites as lead molecules for biologically-based crop protection are also discussed.

Key words: Metabolomics, secondary plant products, photosensitization, phytotoxicity, allelopathy, plant defense

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CABBAGE PLANTS AND CABBAGE PESTS: PLANT PROTECTION AFTER 100 MILLION YEARS OF COEVOLUTION

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Abstract: The taste and smell of cabbages arises from mustard oil glycosides or glucosinolates, compounds that have long been assumed to act as plant defenses against herbivores. However, how and why glucosinolates act in defense is still uncertain. We have been studying the glucosinolates of the Brassicaceae to learn more about their mode of action in defense and how some herbivores have managed to circumvent them.

The textbooks tell us that glucosinolate toxicity arises from their hydrolysis to reactive hydrolysis products, such as isothiocyanates, which then readily bind to proteins. We found, however, that for a generalist-feeding caterpillar glucosinolate toxicity is due rather to reaction with glutathione that depletes the supply of glutathione and its precursor cysteine and eventually leads to protein breakdown.

Certain herbivores feed on glucosinolate-rich plants without apparent negative effects, and are known to metabolize glucosinolates in various ways. But, it is not easy to prove that these reactions actually serve in detoxification. By employing isotopic tracers and silencing herbivore genes, we demonstrated that desulfation of glucosinolates by diamondback moth larvae is an effective detoxification mechanism that improves herbivore fitness when feeding on plants with glucosinolates. Nevertheless, we were also able to detect costs of detoxification because larvae showed increased fitness when fed on non-glucosinolate diets where desulfation did not occur.

Once herbivores have developed the ability to detoxify glucosinolates, plants may be selected for counteradaptations. For example, the deployment of mixtures of glucosinolates instead of single compounds and the attraction of herbivore enemies that prey on or parasitize herbivores may help plants protect themselves against herbivores resistant to individual glucosinolates. The end result of such selection on both plants and herbivores is likely the coevolutionary persistence of both cabbages and their herbivores that we observe today along with the multiple chemical and biochemical features mediating their interactions. More knowledge of these features may be useful in reducing insect damage on cabbages and other Brassicaceae crops.

Key words: glucosinolate, Brassicaceae, plant defense, herbivore detoxification

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CURRENT AND FUTURE PROSPECTS IN HERBICIDE DISCOVERY

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Abstract: Herbicides have been the most successful tools to manage weeds. There was a steady discovery of approximately one unique mechanisms of action (MOA) every two years from the early 1950s to the mid-1980s. While this situation changed dramatically after the introduction of glyphosate-resistant crops, evolution of resistance to glyphosate has renewed the agrichemical industry interest in new chemistry interacting with novel target sites (Dayan 2019). An exciting recent development has been the discovery that cinmethylin inhibits fatty acid thioesterases (FAT), a key enzyme in fatty acid metabolism (Campe et al 2018). The biosynthesis of plastoquinone was already known to be sensitive to herbicides that inhibit p-hydroxyphenylpyruvate dioxygenase (HPPD). However, two new herbicidal target sites have been identified on this pathway. The herbicide discovery group of Bayer CropScience, in collaboration with Targenomix, recently reported that, in addition of mild inhibition of protoporphyrinogen oxidase (PPO), aclonifen has a novel MOA targeting chloroplastic solanyl diphosphate synthases (SPS) (Kahlau et al. 2020). Mitsui Chemical Agro Inc. discovered that the bleaching effect of cyclopyrimorate is due to inhibition of homogentisate solanesyltransferase (HST) (Shino et al. 2018). Cyclopyrimorate is in fact a proherbicide that needs to be bioactivated into its desmorpholinocarbonyl cyclopyrimorate (DMC) herbicidal metabolite to inhibit HST. FMC Agricultural Solutions recently announced a new herbicide chemical class (aryl pyrrolidinone anilide) that includes tetflupyrolimet targets dihydroorotate dehydrogenase (DHODH), a key enzyme in pyrimidine nucleotide biosynthesis. Plant DHODHs have different substrate specificity and inhibition from the animal form of this enzyme. Commercialization of this tetflupyrolimet is projected to be in 2024.

This review will cover other topics such as a new insight on the MOA of glufosinate that accounts for its synergy with PPO inhibitors, and promising new chemistry such as BASF's new isoxazolopyridines with selectivity on monocotyledonous crops while providing excellent post-emergence control of dicot weeds and good activity on some grasses, Sinochem's novel uracil herbicides containing an isoxazolopyrimidine ring for broad weed control in cereals, and Corteva's work on benzoxaboroles. Innovative approaches to discovery of novel herbiicdes and their target sites will also be discussed.

Key words: Amino acid biosynthesis; Lipid biosynthesis; Plastoquinone biosynthesis; Pyrimidine biosynthesis

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PLANT-MICROBE INTERACTION AND CLIMATE CHANGE

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Abstract: Soil holds a great part of the world's biodiversity, dominated by microorganisms - bacteria and fungi - in terms of numbers and biomass. Besides these organisms, soils generally contain a variety of animals, nematodes, micro-arthropods, and earthworms. In addition, a large number of macrofauna species are living in the uppermost soil layers, the soil surface and the litter layer. Soil biota is involved in the global cycling of organic matter, energy, and nutrients.

Human activities and their effects on the climate and environment cause loss in biodiversity and endanger animal and plant life on Earth. Losses of species, communities and habitats are comparatively well researched, but microorganisms are generally not discussed in the context of climate change. Considering the global climate change scenarios, a major obstacle to global food security is sustained loss of crops due to climatic stresses (particularly drought and salinity) (Ma et al., 2020). In this talk we will discuss the effects of climate change on soil microorganisms and plant-microbe interactions, and explain the underlying mechanisms used in plant growth promoting microorganisms to assist crops to cope with drought and salinity stresses (Ma et al. 2016, 2017, 2019, 2020).

Key words: Climate change, Crops, Soil microorganisms

References: please follow the examples below

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GRAPEVINE FACING A FUTURE CLIMATE: RESPONSE OF TEMPRANILLO CLONES TO CLIMATE CHANGE SCENARIOS

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Abstract: Increases in the atmospheric temperature and carbon dioxide (CO_2) levels, and a reduction in water availability are predicted in the coming decades (IPCC 2013). These conditions will likely affect grapevine (*Vitis vinifera* L.) physiology, through alterations in leaf carbon metabolism, fruit ripening rates and berry composition. The use of the intra-varietal diversity has been proposed as a strategy for keeping regional varieties cultivation and wine typicity under future growing conditions. We will present a research aimed to evaluate the response of Tempranillo clones, differing in the length of reproductive cycle, to the interaction among climatic factors, focusing on plant growth and fruit composition. Experiments with fruit-bearing cuttings were performed in temperature gradient greenhouses and growth chamber greenhouses to simulate different climate change scenarios: high temperature (+4 °C), elevated CO_2 (700 ppm) and water deficit, both in combination or independently. The results showed higher vegetative growth but reduction in yield due to high temperatures. Elevated CO_2 increased photosynthesis, and thus vegetative growth, although signs of a photosynthetic acclimation process (i.e. reduced C/N) were observed especially when combined with high temperature. Drought overshadowed the positive effects of temperature and CO_2 on photosynthesis and vegetative growth. Regarding fruit composition, elevated temperature, both individually and combined with high CO_2, hastened sugar accumulation and advanced maturity, these effects being mitigated by water deficit. Malic acid degradation was enhanced by high temperature, especially when combined with elevated CO_2 and drought. Elevated CO_2 reduced the effect of temperature decoupling the anthocyanin:sugar accumulation; however, the combination of elevated temperature, high CO_2 and water deficit led to the imbalance between these two components. Climate change-related factors modified anthocyanin profile towards more stable forms. The clones studied showed differences in their phenology, growth and grape composition. The impact of the environmental

conditions was dependent on the clone, thus indicating certain intra-varietal variability in the response of Tempranillo, which not always was solely explained by differences in the ripening dynamics. The findings obtained highlight the importance of multi-stress approaches to assess the potential effects of predicted changes of climate on grapevine physiology, and provides insights into the response of clones as the basis for the adaptation of cultivars in their traditional growing regions.

Key words: drought, elevated CO_2 and temperature, grape quality, growth, phenology

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VEGETATION RESPONSES TO SIMULATED CLIMATE CHANGE IN SEMIARID SHRUBLAND COMMUNITIES

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Abstract: In the Mediterranean region, temperatures will rise by 2-5ºC in the next few decades, accompanied by a lower amount and frequency of precipitations. These projected changes will increase aridity and are expected to have a strong negative impact on plant ecophysiology. We have conducted a 7-10 year manipulative experiment in two semiarid shrublands in Southeastern Spain in which we simulated the warmer and drier climate conditions forecasted for the Mediterranean Region. We evaluated the long-term impacts of warming (W), rainfall reduction (RR) and their combination (W+RR) on the ecophysiological performance of these phylogenetically and functionally diverse native shrub species. Warming (W and W+RR treatments) consistently decreased the ecophysiological performance of native shrubs strongly decreasing their net photosynthesis rate and increasing plant water loss (stomatal conductance) and thus reducing the shrubs' water use efficiency. Leaf nutrient (N, P, K, Fe, Zn, Cu) concentrations and pool sizes in foliage were decreased by warming across species, indicating reduced plant nutrient uptake and status. Warming also led to large reductions in the photosynthetic use efficiencies of nitrogen, phosphorus and potassium (PNUE, PPUE and PKUE, respectively) across species and sites. Structural equation modelling showed that reductions in PNUE, PPUE and PKUE were mostly the consequence of large reductions in net photosynthesis rates (almost halved in warmed shrubs), accompanied by more modest reductions in leaf nutrient concentrations. We also observed a negative relationship between time-integrated water use efficiency ($\delta^{13}C$) and PNUE, PPUE and PKUE but the slopes of these relationships were always lower for plants growing under warming conditions. This decoupling of the adaptive trade-off between WUE and NUE in warmed plants could be indicative of a maladaptive physiological behaviour leading to an increasing inability of the plants to cope with warmer conditions. Projected climate warming will thus push semiarid shrubland vegetation beyond its optimum temperature

threshold for photosynthesis, which will become increasingly decoupled from transpiration and nutrient use efficiency, thereby degrading the ecosystem's capacity to act as a carbon sink.

Key words: Climate change; ecophysiology; Mediterranean ecosystems; warming

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CHLOROPLAST PROTEOMICS REVEALED MOLECULAR CLUES SUPPORTING TRANSGENERATIONAL STRESS MEMORY IN GYMNOSPERMS

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Abstract: Plants do have stress memory and chloroplast signaling has been revealed as crucial element to acquire this memory (Van Buer et al., 2019). Nevertheless, little is known about the dynamics of chloroplast proteome triggered by stressful situations and its role mediating memory effects. Thus, two different provenances of *Pinus radiata* seedlings exposed to UV-B radiation were used to carry out a transgenerational cross-stress memory study: seeds whose parents were grown in field under controlled (Provenance E) or natural environmental ("mild stress" events) (Provenance T) conditions. Photosynthetic activity was measured employing a pulse-amplitude modulation fluorimeter and several metabolism biomarkers were analyzed by colorimetric method (López-Hidalgo et al., 2021). Chloroplast proteome fraction was isolated and purified using a discontinuous Percoll-Sucrose gradient (Lamelas et al., 2020) and proteins were analyzed by nUPLC-Orbitrap/MS (Orbitrap Fusion). Proteome Discoverer software, six independent plant-specific subcellular location tools, pRocessomics and agricolae packages (RStudio) were employed to analyze data. Although both provenances seem to achieve "acclimation" at the end of the stress, univariate and multivariate analyses, as well as Venn diagrams, showed clear differences at both physiological and proteomic levels along stress. Results suggested a faster, stronger, and more coordinated, uniform, and specialized response in provenance T than E, as well as a reduction in biological variability among samples. At short-term, redox homeostasis was the main differential pathway: provenance E showed enhanced malondialdehyde levels just as a reduction of its protein abundance, while provenance T "a priori" exhibited less cellular damage and a progressive increase in proteins linked to this category. At long-term, secondary metabolism (protective role) and proteome remodeling were revealed as key pathways in provenance T response, which was better since the beginning of the stress. According to the results, this study sheds light on the dynamics of chloroplast proteome under UV-B stress and results support transgenerational cross-stress memory hypothesis, where cellular homeostasis, protein remodeling, and secondary metabolism seem to be essential to notice and face a new stress event.

Key words: chloroplast signaling, transgenerational memory, subcellular proteomics, pine

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HOW TO SIMULTANEOUSLY INCREASE FRUIT QUALITY AND STRESS TOLERANCE BY DELVING INTO SUGAR METABOLISM IN A TOMATO RIL POLULATION

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Abstract: Current predictions for climate change indicate an increase in temperatures and a significant reduction in the quality of irrigation waters that, together with the growing world population, make it urgent the need to seek alternatives in the generation of crops with greater tolerance to different abiotic stresses. To study the effect of stress due to salinity and heat, acting together, on the assimilation of sugars, a study of two lines of a population of RILs (*Recombinant inbred lines*), obtained by crossing a wild line (*S. pimpinellifollium L.*) and a commercial line (*S. lycopersicum L.*) was carried out. An initial population of 84 different RILs was subjected to combined salinity and heat stresses to select a sensitive and a tolerant line. This study was carried out on tomato fruits of the RIL-18 (sensitive) and the RIL-76 (tolerant) tomato lines, in two different ripening stages, mature green and red ripe. These plants were grown under control conditions (25ºC and 0mM NaOH) and simulated field conditions (35ºC and 60mM NaOH). Under these conditions, the expression and activity of the main genes and enzymes involved in the synthesis, degradation and accumulation of sugars were determined. The final concentration of the main metabolites of this pathway was also measured. The tolerant line showed highly significant differences in red ripe fruits in comparison to the sensitive one under the simulated field conditions, with an overexpression of the genes *SlFBP*, *SlSPS*, *SlSUS3*, and *SlNi*. These expression patterns correlated with a higher activity of the enzymes *FBP*, *SPS*, *SUS3*, *AI*, and *G6PDH*, which resulted in the accumulation of fructose, glucose and UDP-glucose. Also, overexpression of the sugar transporters *SlGLCT*, *SlINT4*, *SlSUT4*, *SlTMT1*, and *SlVGT1* was identified. These results will allow establishing the tomato fruit quality bases, as well as its resistance to abiotic stress caused by climate change constraints. In this way, this study empathized the importance of studying abiotic stress in combination and how recombinant material with different degrees of tolerance can be highly important for the improvement of fruit quality in horticultural plants under a scenario of climate change.

Key words: abiotic stress, simulated field conditions, tomato, heat, salinity, fruit quality

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POLLINATOR-ASSISTED PLANT PHENOTYPING UNDER CLIMATE CHANGE SELECTION PRESSURE

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Abstract: Abiotic stresses as drought, salinization, flooding and rising temperatures aggravated by the climate change are threatening food production for the current growing population. Such environmental pressure mainly affects crops during the flowering stage and also decline ecosystem services, as pollinating insects, essential for the production of many crop species (Soroye et al., 2020). Thus, phenotyping and selection of flower traits is key for securing food production through increasing plant resilience and ensuring animal pollination (Borghi et al., 2019). Improving crop resistance and resource use efficiency is the main objective of breeding programs, whose main bottleneck is the precise selection of most suitable genotypes. Modern phenotypic platforms aim to improve plant productivity by analysing chlorophyll status (proxy of the 'sources') and estimate growth (proxy of the 'sinks') using non-invasive monitoring of plant growth. Imaging analysis acquires these proxy measurements, generating a large amount of data difficult to process and complex because of the architecture, size and arrangement of the plants. Those platforms rarely analyse floral traits, probably due to the difficulties to procure accurate and high spatio-temporal resolution. We hypothesise that flowers integrate 'source-sink' relationships and determines energy and nutrient availability for pollinators via nectar/pollen chemical composition. Our objectives are (i) to check whether pollinator preference reflects the degree of optimization of the source-sink relationships and if that preference can serve as a tool to identify the most resilient individuals under an environmental selection pressure by analysing the genotype x pollinator x environment (GxPxE) interaction; and (ii) to develop an automated geo-positioning system to quantify GxP at large phenotyping scale. For that purpose, we scored pollination visits to the flowers and correlated them with production and adaptive parameters of the visited plants, under an environmental pressure (salinity). Interestingly, yield and biomass parameters, chlorophyll content and fruit quality correlated positively with the pollinator preference. Moreover, we were able to quantify number and duration of visits under controlled conditions. This approach based on natural ecosystemic interactions could change the current paradigm of plant phenotyping and opens new perspectives for plant breeding in crop species under a climate change scenario.

Key words: abiotic stresses, climate change, flower traits, food security, plant x pollinator interaction.

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USE OF LEGUMINOUS COVER CROPS AS A SUSTAINABLE STRATEGY ON RAINFED OLIVE ORCHARDS: EFFECTS ON PHYSIOLOGY, YIELD AND FRUIT COMPOSITION

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Abstract: Olive tree is one of the most important crops in the Mediterranean basin with a huge social and economic impact. Considering the predicted scenarios of climate change, there is a growing need for the adaptation of agronomic practices, in order to mitigate the negative effects of those changes on plant physiology, yield, and olive fruit and oil quality. However, in Portuguese rainfed orchards the most frequently used soil management practice is the conventional tillage, which significantly contributes to the decline of soil health and fertility, with serious consequences to plants and environment. In this way, cover cropping has emerged as a sustainable practice of soil management, able to provide numerous ecosystem services, which contribute to crop productivity and quality.
The objective of this study was to compare the effects of a cover crop of self-reseeding annual legumes of short growing cycle (LC) with the conventional tillage (T) on physiology, yield and fruit composition of twenty-seven-year-old olive trees (*Olea europaea* L. cv. Cobrançosa) grown under rainfed conditions. The experiment was carried out during 2018 and 2019 in Trás-os-Montes region, northeast of Portugal.
The physiological performance was significantly improved on LC plants, considering the rise of net photosynthesis, transpiration rate and stomatal conductance. Moreover, LC trees registered consistently higher yields, accompanied by a significant increase on fruit weight, length and maturation index. Regarding fruit biochemistry, results were more evident on the first year, showing a decrease of total phenols, ortho-diphenols and total antioxidant activity of LC fruits, probably associated with their higher ripening state. The fruit fatty acid profile,

obtained on a Trace GC-FID (gas chromatograph with flame ionized detector), showed that in 2018 LC fruits presented higher concentration of oleic acid (C18:1) and a lower content of palmitic (C16:0) and linoleic acids (C18:2). These results give evidence that leguminous cover crops provided several benefits comparing with the conventional tillage technique. Therefore, this is a very promising strategy of sustainable soil management to implement in olive orchards under drought conditions.

Key words: Climate change, Conventional tillage, Leguminous cover crops, Olive tree, Sustainable soil management

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Poster Communications



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ABA-DEFICIENT MUTANTS '*flc*' SHOWED A SPECIFIC DISRUPTED METABOLISM UNDER ABIOTIC STRESS COMBINATION COMPARED TO SINGLE STRESS APPLICATION

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Abstract: Abiotic stress is known for reducing growth and yield worldwide. Current climate change predictions point to an increase in environmental temperature that will act synergistically with an increase in the salinity of the irrigation water, while this water will become more scarce for agriculture. Consequently, in the last decades, different strategies to induce tolerance to abiotic stress applied in combination have been developed. ABA is a well-known phytohormone with many physiological functions in plants, being especially important as a signaling molecule in the plant stress response. With the objective of shed some light on the specific ABA interaction mechanisms with other important stress-response molecules under abiotic stress combination, such as ROS and NO, *Solanum Lycopersicum* L. (cv. Micro-Tom) ABA deficient plants (flacca, *flc*), and wild type (Wt) were subjected to single stress (75 mM NaCl or 35ºC) and their combination (75 mM NaCl or 35ºC). Stomatal responses to stress were highly inhibited in *flc* plants, showing opened stomata under any stress applied and the concomitant reduction in the CO_2 assimilation rate, being heat treatment the most dramatic treatment. The ABA deficiency in *flc* was highly correlated with an increase in JA and its precursor, which was more evident under the combination of salinity+heat. These mutants also exhibited a significant accumulation in Zn, Cu and Mn under stress conditions respect to Wt. The expression of the ABA synthesis pathway-related transcript indicated an overexpression of SAM and ABA2 in *flc* under salinity+heat. ABA deficiency in *flc* was correlated with a higher and accumulation in ROS and NO as compared to Wt under the combination of salinity and heat due to a strong upregulation of *Cu/ZnSOD* and GSNOR genes as compared to Wt under these conditions. Our results showed that salinity+ heat combination induced a specifics stress response in Wt plants, but more importantly, that this specificity is truncated in ABA-deficient mutants, showing the importance of this hormone in the stress combination-related signaling mechanisms.

Key words: ABA-deficient mutant, *flc*, photosynthesis, ionomics, transcriptomics, ROS, hormone homeostasis, ABA synthesis pathway.

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ADAPTION TO CLIMATE CHANGE AND BREEDING POTENTIAL OF SUMMER CROPS IN A TEMPERATE HUMID AREA

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Abstract: Climate change threatens agricultural production worldwide, including the temperate humid Northwestern Spain where water availability is not a major limiting factor nowadays. Introducing sustainable crops with better adaption to forthcoming stress conditions and breeding for future climatic conditions can make more resilient agricultural systems, ensuring high productivities and environmental sustainability. This study assesses the potential adaption of summer crops to present and future climatic conditions with the aim to make available for farmers a wider range of summer crops. Also, we aim to identify the physiological characteristics required for breeding each crop for drought conditions. In a greenhouse experiment, we compared an autochthonous, well-adapted legume crop (common bean) and two allochthonous legume crops (chickpea and pigeonpea) under control and drought conditions. Allochthonous legumes showed lower photosynthetic performance under optimal conditions, but better water use efficiency (WUE) under drought stress. In addition, we evaluated the photosynthetic performance of four autochthonous crops (maize, pepper, tomato and common bean), and allochthonous chickpea and pigeonpea to assess their adaption and breeding potential. CO_2 assimilation was highest for maize and lowest for chickpea; transpiration was lowest for maize and chickpea and highest for pigeonpea and tomato; finally, WUE was highest for maize and lowest for pigeonpea. Maize had significant variability for all traits, but its C4 photosynthesis limits its breeding potential; chickpea had not significant variability for any trait; pigeonpea had the largest variability and breeding potential for photosynthetic traits.

Key words: Climate change, Crop breeding, Photosynthesis, Authochthonous crops, Allochthonous crops.

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ADVANCES IN THE VALORIZATION OF CAULIFLOWER BY-PRODUCTS: POSITIVE EFFECTS OF FOLIAR PUTRESCINE APPLICATION, AMMONIUM EXPOSURE AND HEAT STRESS

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Abstract: Climate change is the greatest environmental threat facing humanity (Zúñiga-González *et al*., 2020), and heat stress can alter the normal growth and development of plants Ahmar *et al*., 2019). Polyamines, a family of plant growth regulators, have been used to improve the tolerance of plant under different abiotic stresses (Gupta *et al*., 2013; Collado-González *et al*., 2021). It has been observed that nitrogen fertilization that provides ammonium (NH_4^+), aside from promoting plant growth, can also reduce the negative effects of heat stress (Piñero *et al*., 2019).

Due to the health-promoting effects of cauliflower (*Brassica oleracea* L. var. Botrytis), its production and consumption has dramatically improved in the last few years. Unfortunately, negative effects for the environment are produced due to the very high generation of by-products during the processing of this crucifer. Therefore, in this work, we have tried to enhance the quality of cauliflower *cv*. Moonshine by-products in order to promote the use of cauliflower waste for nutraceutical purposes and to increase the sustainability of this crop. As a result, a synergistic effect between polyamines and the nutritional treatment for coping with heat stress was observed. In fact, heat stress combined with a 50:50 NO_3^-/NH_4^+ ratio and the foliar application of 2.5 mM putrescine decreased peroxidation (35.2 % and 14.6 % in young and outer leaves (YL and OL), respectively) and significantly increased the photosynthetic rate (88.6 %), and the concentrations of chlorophyll (25.2% in YL and 54.5% in OL), and total proteins (28 % in YL and 8.6 % in OL), with an important change observed in the nutrient status.

From the results found, it can be concluded from a physiological and agronomic point of view, the foliar application of putrescine and the 50:50 NO_3^-/NH_4^+ treatment managed to alleviate the negative effects of the high-temperature abiotic stress suffered. On the other hand, the increase in pigments and proteins, and the decrease in lipid peroxidation obtained in the cauliflower by-product suggests that these by-products could be used for health or nutraceutical purposes. This highlights a possible advance in the valorization of the cauliflower by-products.

Key words: Cauliflower waste, Heat stress, Nitrogen forms, Plant nutrition, polyamines

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EXPLOITING TOMATO GENOTYPES TO STUDY HEAT STRESS TOLERANCE

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Abstract:
Increased temperatures caused by climate change are a significant threat to agriculture and food security. Current models predict an increase in the global average temperature between 2–5 °C over the next hundred years. Extreme weather events (including heat waves) will have devastating consequences for crop productivity (Bita and Gerats., 2013). To overcome this challenge, special attention is being paid to the genetic potential that wild and traditional plants constitute, since they represent a genetic resistance reservoir not yet explored. Therefore, the main objective of this work is the characterization of traditional tomato varieties responses to heat stress. For this purpose, four traditional *S. lycopersicum* genotypes, originated from Mediterranean area: TH-30 (Greece*); A*DX (Spain); ISR-10 (Israel); MO-10 (France) and two commercial genotypes: *Ailsa Craig (*Ailsa*)* and *Moneymaker* (MM), were used. To achieve heat stress, the temperature of the culture chamber was increased to 42ºC during 6 hours and the recovery capacity was subsequently evaluated after two hours under normal temperature conditions. In order to check how heat shock stress could affect to different tomato genotypes, leaf damage, relative water content (RCW) and photosynthetic parameters were evaluated. Based on the results obtained, it has been characterized the tomato resistance against heat stress, demonstrating that MO-10 and MM are the most tolerant genotypes and ADX and TH-30 the most susceptible. For example, MO-10 plants displayed after 6h of heat shock (6hpHS), less than 60% of leaflets damaged and displayed a slight reduction on RCW. Likewise, it has been observed how these plants displayed higher levels of photosynthesis, transpiration and stomatal conductance compared with the other genotypes. In contrast, TH-30 plants are highly affected by heat stress, showing the 100% of its leaflets damaged at 6hpHS and a strongly reduction on RCW at this time. These plants showed an accused reduction of photosynthesis, transpiration, and stomatal conductance at 6hpHS. These results demonstrated that genetic variability exists in tomato germplasm for heat tolerance. This finding implies a possibility to identify high temperature tolerant genotypes and the use of this traits could be exploited to generate tomato cultivars better adapted to uncertain climatic scenarios.

Key words: heat stress, traditional tomato varieties, climate change.

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IDENTIFICATION AND CHARACTERIZATION OF *DAM* GENES MUTATIONS ASSOCIATED WITH LOW-CHILLING AND EARLY BLOOMING IN SWEET CHERRY

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Abstract:

Dormancy release and bloom time on sweet cherry (*Prunus avium* L.) cultivars depend on the environment and the genotype. The knowledge of these traits is essential for cultivar adaptation to different growing areas, and to ensure fruit set in the current climate change scenario. In this work, a major sweet cherry bloom time QTL (*qP-BT1.1*^{m}) spanning 327 Kbps at bottom of chromosome 1 (Calle et al. 2020) was scanned for candidate genes using the 'Regina' sweet cherry reference genome. Six *MADS-box* genes, orthologs to *DAM* (*Dormancy associated MADS-box*) genes of other *Prunus* species, were identified as candidate genes for bloom time regulation (*PavDAM*s). To investigate intra-specific variation of *PavDAMs,* 13 sweet cherry genome sequence reads (Ono et al. 2018) from cultivars with contrasting chilling requirements and bloom times were then mapped to the sweet cherry reference genome. A high level of protein sequence conservation was observed (98.8-100%). Additionally, a higher variability and several structural mutations were identified in the low-chilling and extra-early blooming *cv.* 'Cristobalina'. Specifically, a large deletion (694 bp) upstream of *PavDAM1,* and various INDELs and SNPs in contiguous *PavDAM4* and *-5* UTRs were identified in this cultivar. *PavDAM1* upstream deletion include several cis-acting motifs, potentially involved in *PavDAM* expression. Also, the structure of a non-coding gene expressed in late-blooming cultivars seems truncated in 'Cristobalina'. Additionally, *PavDAM4* and *-5* UTRs mutations revealed different splicing variants in *PavDAM5*. The results indicate that the regulation of *PavDAM*s expression and post-transcriptional regulation in 'Cristobalina' may be altered due to structural mutations in regulatory regions. Previous transcriptomic studies showed differential expression of *PavDAM* genes during dormancy in this cultivar. Together, the results indicate that

‘Cristobalina’ shows significant amino acid and structural mutations in *PavDAM*s, which correlate with low-chilling and early bloom phenotype, but the direct implication of these mutations remains to be determined. The presence of these mutations and their association with low-chilling and early-blooming was confirmed in a segregating population and in a cultivar collection, and markers were designed for selection breeding.

Key words: *Prunus avium*, dormancy, low-chilling, bloom time, *DAM*, mutation, expression.

Acknowledgments: This work has been funded by ‘Ministerio de Ciencia e Innovacion’ ‘Agencia Estatal de Investigación (AEI)’ (project PID2019-103985RR-I00); ‘Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria’ (INIA) (projects RTA2015-00027-00-00 and RFP2015-00015-00-00); and FEDER funds. A. Calle was funded by ‘Gobierno de Aragón’.

References: please follow the examples below

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PLANT PHENOTYPING FOR MONITORING BROCCOLI PLANTS INFECTED BY *XANTHOMONAS CAMPESTRIS* PV. *CAMPESTRIS* UNDER DIFFERENT CLIMATE CONDITIONS

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Abstract: *Xanthomonas campestris* pv. *campestris* (Xcc) is a pathogen bacterium causing black rot, a worldwide devastating disease. The virulence of pathogens could be altered by climate change since climate conditions may affect plant and pathogen physiology and/or their geographical distribution, among other factors (Juroszek and von Tiedemann, 2015). Plant phenotyping by imaging sensors is a valuable tool for the diagnosis and detection of plant diseases even in the absence of symptoms (Barón et al., 2016). In this work, broccoli plants were infected with race 1 or 4 of Xcc and compared to mock-infected control plants. Their response was monitored in terms of leaf temperature (transpiration) with a thermal camera, and of secondary metabolites accumulation with a multicolour fluorescence imaging system. Alterations in pigment composition and plant fitness were also examined by the analysis of vegetation indexes using a hyperspectral reflectance camera. The reaction of control and infected plants at current climate conditions (CCC) has been compared to the response under two Representative Concentration Pathways (RCP) in years 2081-2100, according to the Assessment Report of Intergovernmental Panel on Climate Change (IPCC 2014. AR5: Climate Change 2014. Synthesis Report): an intermediate (RCP4.5), and an extreme scenario (RCP8.5). Several imaging parameters have been related to symptoms coverage in every climate scenario.

Key words: Hyperspectral reflectance imaging, Multicolour fluorescence imaging, Thermography.

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QUINOA IS SUITABLE TO BE GROWN IN ALAVA'S PLAIN AREA (NORTH OF SPAIN)

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Abstract: To keep pace with increasing world population, food production must be increased despite the limited availability of arable land and water by adopting different sustainable management approaches. One of them includes the diversification of cropping systems, with the inclusion of drought tolerant crops, to ensure food security under future climatic change conditions. Quinoa is a suitable candidate because it can be grown under harsh environmental conditions. The objective of this work was to know if quinoa could be grown in Alava's plain area (North of Spain), where generally a summer drought occurs. A field experiment was settled, with plots of 12 m^2 arranged in a split plot design, with four replicates. The whole-plot factor was the irrigation level: rainfall (187 mm) *vs.* rainfall (187 mm) plus supplementation of irrigation (265 mm), and the split-plot factor was the cultivar (Vikinga, Titicaca, Puno and Pin). Plots consisted of 4 rows with a row spacing of 30 cm. The plots were established on May 15, 2019, and harvested on September 6, 2019. Under irrigated conditions, the four cultivars presented a similar production, although Vikinga showed lower height and developed faster than the other three cultivars. Regarding quality parameters of the grains, no differences between cultivars were detected except for Puno which exhibited higher protein levels and lower antioxidant capacity, compared to the other cultivars. When the non-irrigated conditions were analyzed, neither final grain production nor protein content, phenol content, or antioxidant capacity were affected in any of the cultivars. However, the weight of 1000 grains was affected by non-irrigated conditions, and reductions in all cultivars were detected, except for Pin. So, we conclude that quinoa can be grown in the Alava's plain area and the summer drought does not affect grain production or quality, although the grains tend to be smaller. Despite the possibility of including quinoa to diversify cropping systems in Alava, its profitability is not yet proven, as farmers are having many problems selling the grain, and crop technology needs to be improved.

Key words: Alava's plain area, Drought, Quinoa

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REDUCING EXTREME WEATHER IMPACTS IN GREENHOUSE HORTICULTURE: THE EFFECT OF PASSIVE CLIMATE CONTROL SYSTEMS ON CATION CONCENTRATION AND AMINO ACIDS PROFILE OF PEPPER FRUITS

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Abstract: In the Mediterranean Region, most of the greenhouses are low-tech, and many of these greenhouses do not have effective climate-control systems (Tuzel et al., 2017) to deal with climate change impacts. Greenhouse climate control in Northern Europe often requires high energy inputs, resulting in a high carbon footprint of the product. However, in Mediterranean climate conditions, a decreased environmental impact, along with water and nutrient reuse can be established through the use of new techniques for controlling non-optimal climate conditions. In this way, we can minimize reductions in the potential yield, and fruit quality. The objective of this work was to evaluate the effect of the use of a passive heating and cooling system on the quality of pepper fruits. Thus, a sweet pepper crop was grown from August 7th, 2019, to March 8th, 2020. The assay was developed in two greenhouses located in the IFAPA "La Mojonera" (Almería, Spain). In one of the greenhouses (P) a passive hybrid air conditioning system was installed; the other greenhouse was considered as the control (C). For the warm period, the passive hybrid system had textile screens made of a hydrophilic material with a high water-retention capacity. It was hung parallel to the lines of cultivation, allowing for the evaporation of water. During this period, a 100% polyester mobile screen was also activated for shading. On the other hand, for the cold months, the system had a series of flexible polyethylene sleeves filled with water, which accumulate heat during the day to release it at night. In addition, the activation of the mobile thermal screen before sunset limited the loss of heat stored in the greenhouse to the outside

during the night. At the end of the experiment, the content of cations and amino acids were measured in harvest fruits.
The results showed that the fruits grown under passive system conditions had a lower concentration in some of the amino acids measured, such as Glu, Thr, and Met, while others, such as Ser, Arg, and Ala were not affected. On the other hand, the concentrations of Fe and Zn were reduced (24% and 11%, respectively), although, the concentrations of P and K increased (9% in both cases). Therefore, these passive systems could produce an increase in production, with a differential quality with respect to the control fruits.

Keywords: Greenhouses, passive heating and cooling system, pepper fruit, amino acids, cation.

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ROOT TEMPERATURE IS KEY FOR PLANT DEVELOPMENT UNDER HIGH TEMPERATURE

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Abstract: Climate change threads plant development and growth, affecting severely crop yield. Roots are organs protected from extreme temperature (temp) fluxes due to the soil buffering capacity, which generates a temp-gradient in the root zone (TGRZ). However, in many *in vitro* or greenhouses studies analyzing the heat stress, effect the entire plant (roots and shoot) are challenged to a homogeneous high temp. We hypothesized that the TGRZ might be critical to understand the complex interactions between the root-nutrients-microbiota during heat adaptation. We developed a novel device that generates a temp-gradient in the root zone and analyzed morphological and transcriptional changes in response to high temp. Arabidopsis plants were grown under homogenous high temp (HHT) of 32ºC, high temp in the shoot and TGRZ in roots (32-TGRZ) or under homogeneous standard temp (HST) of 22ºC. HHT plants showed a severe root growth arrest while the hypocotyl length and petiole angle increased. Interestingly, 32-TGRZ plants showed longer main root and more laterals roots than HHT, but similar hypocotyl length and petiole angle. Comparative transcriptomic analyses between HHT and 32-TGRZ revealed expression level changes in a large number of genes in roots, while in shoots (grown at similar temp), changes were smaller, but significant, suggesting a root-to-shoot communication. Finally, using this device and pots containing natural soil, we found that tomato plants showed phenotypic, ionome and root-associated bacterial microbiome composition differences. Our data demonstrate that cultivating roots in adequate temperature is essential for proper plant development and responses to high atmospheric temperatures.

Keywords: root temperature, climate change, heat stress, mineral nutrition, microbiome

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THE USE OF *VICIA SATIVA* GENETIC RESOURCES AS A DROUGHT RESILIENCE STRATEGY

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Drought causes large losses in the crop production, including the legume common vetch (*Vicia sativa* L.). Its effect will presumably be enhanced under the current climate change scenario. Vetch is a legume of high protein content and its nitrogen fixation ability makes its cultivation suitable in sustainable agriculture systems. Genebank collections are essential to provide genetic variability for breeding to safeguard future crop productivity.

Transcriptomic analysis has allowed the characterization of drought response and drought adaptation networks underlying molecular mechanisms in common vetch. Presence of polymorphic variants (SNPs and SSRs) in differential expressed genes of drought-sensitive and tolerant genotypes has enabled the design of molecular markers that will be used in trait prediction association analysis (De la Rosa *et al.*, 2020).

The screening of *V. sativa* germplasm collection using markers related to crop drought resilience and the use ecogeographical data as a complementary approach has allowed the identification of potential drought-tolerant candidates to be screened. To validate the efficiency of these complementary approaches, we are performing the field evaluation of selected accessions as drought-tolerant candidates by analysis of drought-associated parameters, as stomatal conductance, epicuticular wax content (EWC), Normalized Difference Vegetation Index (NDVI), and thermographic measures. Yield and grain production data will be measured.

The data suggest that these complementary strategies, including genomic tools, would be useful to select good candidates for traits related to crop drought resilience, adaptability or yield by screening of germplasm collections and would be therefore suitable in future breeding strategies.

Key words: drought tolerance, plant genetic resources, common vetch, *Vicia sativa*

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UNRAVELLING THE MOLECULAR MECHANISMS UNDERLYING CHANGES IN THE NUTRITIONAL PROPERTIES OF QUINOA SEEDS UNDER DIFFERENT ENVIRONMENTAL CONDITIONS

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Abstract: *Chenopodium quinoa* Willd, commonly known as quinoa, belongs to the *Amaranthaceae* family native to the Andean region. It is characterized by its high biodiversity, for being a very nutritious food and by its outstanding resistance to abiotic stress conditions. The remarkable nutritional traits of quinoa seeds rely on the high protein content and protein quality, which is higher than that of cereal crops, the balance of amino acids, minerals, and the presence of polyphenols which are linked to a large antioxidant capacity. These features have resulted in the expansion of its cultivation worldwide as its consumption has risen globally. Besides, quinoa can tolerate drought, high soil salinity, frost, and low temperatures, making it an ideal crop to be exploited and introduced in marginal environments. Thus, several studies in quinoa have taken place aiming at studying its adaptability to different environments but still, very little is known about how the environmental factors influence on the nutritional parameters and the exact mechanisms controlling these responses. In order to shed light on this, our works have centered on investigating the physiological, biochemical and genetic pathways responsible for the changes observed in the nutritional properties of seeds that may depend on the environmental conditions and on the genetic background. We present here the main recent studies we have performed on quinoa under Mediterranean field environments and under controlled greenhouse conditions. We show that environmental conditions play a key role determining the nutritional and physiological characteristics of quinoa seeds, which affects overall seed quality. Furthermore, we identify which nutritional properties are more susceptible of fluctuating when changing the environmental conditions. Our results highlight the importance of selecting a specific cultivar(s) for a particular location due to the distinctive response linked to the climatological conditions.

Key words: Quinoa; Nutritional traits; Seeds; Environmental adaptability; Environment x Genotype.
Acknowledgments: This work was supported by the Ministerio de Ciencia e Innovación (MICINN, Spain) (PID2019-105748RA-I00), the Comunidad de Madrid (CM)-Universidad Autónoma de Madrid (UAM) (SI1/PJI/2019-00124), the CYTED (ValSe-Food 119RT0567), the FPI UAM Fellowship Programme 2019 (to S. G. R.), the CM research assistant fellowship (to I. M. G.) and the Ramón y Cajal Programme 2019 (to M. R.).



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UNTANGLING THE BIOLOGY OF THE FEEDING SITES INDUCED BY ROOT-KNOT NEMATODES

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Abstract:

Plant-parasitic nematodes cause serious economic losses in agriculture (Singh et al., 2015). Among them, the obligate endo-parasitic root-knot nematodes (RKN; *Meloidogyne* spp.) are one of the major contributors. The larvae, J2, penetrates the host-roots in the elongation zone and moves through the cortex towards the meristematic zone entering the vascular cylinder, where they induce a new organ assisted by secreted effectors (a gall including several giant cells; GCs) that nourish them till life cycle completion. The GCs are multinucleated and are formed by mitosis with incomplete cytokinesis from vascular cells (Kyndt et al., 2013; Escobar et al., 2015).

Our group firstly combined cell biology and molecular biology techniques to study the transcriptomes of GCs after isolation by laser microdissection in Arabidopsis and tomato (Barcala et al., 2010; Portillo et al., 2013). Those transcriptomes of early developing GCs and galls revealed a massive gene repression conserved in both plant species only at early stages of development. Accordingly, we confirmed the role of several induced microRNAs that act by repressing plant genes encoding transcription factors, i.e., miR390/ARF3, miR159/MYB33 and miR172/TOE1 at different stages of the RKNs (Cabrera et al., 2016; Medina et al., 2017, Díaz-Manzano et al., 2018; respectively) in galls/GCs.

However, other putative mechanism, as transcriptional gene silencing (TGS) that acts by directing repressive epigenetic modifications, such us DNA methylation and histone modifications, to homologous regions of the genome (Ruiz-Ferrer & Voinnet, 2009) were explored. Sequencing of small RNAs (sRNAs) revealed the accumulation of 24-nucleotide (nt) siRNAs at early (3 days post infection; dpi; Cabrera et al., 2016), matching repetitive

regions of the genome (repeat associated small interfering RNAs; rasiRNAs; Cabrera et al., 2016; Ruiz-Ferrer et al., 2018) as a gall hallmark in Arabidopsis, and it correlates to extensive DNA hypermethylation in several regions of the genome at early stages of gall formation that is dynamically regulated.

Furthermore, the transcriptional patterns and regulatory pathways of GCs and galls revealed high parallels to developmental processes of new organogenesis and/or root regeneration, such as lateral root formation, callus formation and also of root meristem regeneration (Olmo et al., 2020).

Key words: root-knot nematodes, gene silencing, new-organogenesis, galls, giant cells.
Acknowledgments: This study was supported by the Spanish Government (PID2019-105924RB-I00) and by the Castilla-La Mancha Government (SBPLY/17/180501/000287) to CE

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VERSATILITY OF BACILLUS EXTRACELLULAR MATRIX IN INTERSPECIES INTERACTION AND COMMUNICATION WITH PLANTS

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Abstract: Microbes are able to live as individual or collectively in communities generally known as biofilms. To do so, cells genetically coordinate to generate different subpopulations, and to produce a megastructure called extracellular matrix (ECM). The ECM is complex in composition, but mostly contains proteins, exopolysaccharides or eDNA. Functions of bacterial ECM includes control of signalling, protection to cells from external aggressions, or mediating the interaction with hosts. *Bacillus subtilis* is a soil dwelling bacterium able to sporulate and to form biofilms. We do know much of the genetic pathways and regulation of biofilm formation *in vitro*, a knowledge granted to the investigation of the revelance in real scenarios. Several studies have demonstrated the beneficial contribution of diverse *Bacillus* strains to the health of crops; thus, it is a suitable interaction to explore fundamental questions of bacterial cell development and ecology. In our laboratory we are interested in the study of the composition and assembly of bacterial ECM, and how each of the structural components contributes to the ecology of *Bacillus* on plants. The ECM components define the mechanical, chemical and physical features of the ECM, and we have demonstrated how they complementary mediate the interaction with the environment. Exopolysaccharides, an amyloid protein or certain secondary metabolites acts synergistically in: i) providing protection against aggressors, ii) permitting the persistence of cells over phylloplane, or iii) mediating the communication with the plants.

Key words: *Bacillus*, biofilms, plant-microbe interactions, mutualism

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REACTIVE OXYGEN SPECIES IN NODULES OF LEGHEMOGLOBIN-DEFICIENT MUTANTS OF THE MODEL LEGUME *Lotus japonicus*

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Hemoglobins are ubiquitous in all organisms, where they perform multiple functions, including the transport, delivery, and scavenging of O_2 and nitric oxide (NO)[1]. Legumes contain symbiotic hemoglobins or leghemoglobins (Lbs) and, like other plants, nonsymbiotic hemoglobins or phytoglobins (Glbs)[2]. Lbs occur only in nodules, whereas Glbs are present in virtually all plant tissues and can be categorized, in turn, into three classes according to their phylogeny and biochemical properties. In legume nodules, Lbs provide O_2 to the N_2-fixing bacteroids, thereby maintaining an optimal O_2 environment for efficient N_2 fixation. The genome of the model legume *Lotus japonicus* encodes three Lbs and six Glbs (two class 1, one putative class 2, one unknown globin, and two class 3)[2].
In this work, we have characterized single (*lb3*), double (*lb13* and *lb23*), and triple (*lb123*) knockout mutants of *L. japonicus* defective in the *Lb* genes, which were generated by CRISPR/Cas9 technology[3]. First, we phenotyped in detail nodulated plants after four weeks of inoculation with *Mesorhizobium loti* MAFF303099. Mutant plants showed a delay in growth that was directly related to the number of Lbs lacking in the nodules. Expression (mRNA and protein) of Lbs and Glbs was examined in the mutant nodules. Compared with the wild-type, the expression of Lb1 and Lb2 was greater in the *lb23* and *lb13* mutants, respectively, indicating that these nodules tend to compensate the total amount of Lbs for maximizing N_2 fixation. Expression of *Glb1-1* and *Glb2-2* decreased, whereas that of *Glb3-1* was enhanced in the *lb123* nodules. Next, we determined the production of reactive oxygen species (ROS) and the activities of superoxide dismutase (SOD) isoforms and catalase in nodules. Superoxide radical and H_2O_2 production was localized based on their reactions with nitroblue tetrazolium and diaminobenzidine, respectively. The *lb123* nodules showed a higher production of ROS, a decrease of catalase (activity) and FeSOD (mRNA and activity), an increase of CuZnSOD (mRNA and activity), and no significant changes in MnSOD (mRNA and activity). Our results indicate that the lack of the three Lbs alters ROS homeostasis, probably due to a loss of O_2 buffering capacity within the infected zone of nodules.

Key words: (Leg)hemoglobin, Legume nodules, Reactive oxygen species, Superoxide dismutases

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VIRULENCE-RELATED METABOLISM MAY BE ACTIVATED IN *BOTRYTIS CINEREA* MOSTLY IN THE INTERACTION WITH TOLERANT GREEN GRAPES THAT REMAIN LARGELY UNAFFECTED

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Botrytis cinerea is responsible for the gray mold disease, severely affecting Vitis vinifera grapevine and many other economically important crops. However, mechanisms involved in this fruit-pathogen interaction remain largely unknown. The combined analysis of the transcriptome, hormonome and metabolome of green fruits infected with *B. cinerea* from susceptible and tolerant genotypes was never performed in any fleshy fruit, mostly because green fruits are widely reported to be resistant to this fungus. However, the proximity with the Atlantic and/ or the presence of highly virulent fungal strains may lead to the frequent observation of green grapes from very susceptible varieties naturally in-field infected with *B. cinerea* in Lisbon area.

In this work, peppercorn-sized fruits were infected in the field or mock-treated at EL29, and samples were collected at green (EL32) stage from a susceptible (Trincadeira) and a tolerant (Syrah) variety. The transcriptome, hormonome and metabolome of these fruits were analysed using RNAseq, HPLC-MS/MS, GC-EI/TOF-MS and GC-EI/QUAD-MS

platforms. Data suggested that Syrah exhibited basal defense relying on specific signaling pathways (enrichment in genes coding for protein kinases, transcription factors, and involved in Ca^{2+} signaling), hormonal regulation (salicylic acid, auxins, jasmonates, and ethylene), and phenylpropanoid metabolism. In addition, putative defensive metabolites such as ursolic acid, *trans*-4-hydroxy cinnamic acid, and epigallocatechin were more present in Syrah than Trincadeira before infection. On the other hand, Trincadeira underwent a broad metabolism reprogramming upon infection but was unable to contain disease progression and exhibited heavy symptoms of infection. RNA-seq analysis of the fungus *in planta* revealed an opposite scenario with *Botrytis cinerea* presenting higher and to some extent specific transcriptional reprogramming in the tolerant cultivar. Among the *B. cinerea* virulence- and growth-related were noticed genes encoding CAZymes, MFS transporters, involved in germination, conidia regulators, and oxidative stress. Many genes coding for transcription factors were expressed only in Syrah and might be novel elements of pathogenesis that deserve functional characterization. The results suggested a more active virulence state in Syrah even without visible disease symptoms probably as a strategy to manage a successful infection.

Together, this study brings novel insights related to *B. cinerea* early infection strategies and green berry responses involved in tolerance/susceptibility against necrotrophic fungi.

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WHAT IS THE CONTRIBUTION OF THE POLYAMINE PUTRESCINE TO PLANT DEFENSE?

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Abstract: Polyamines, and putrescine in particular, often accumulate during biotic stress. However, the contribution of polyamines to defense and their molecular mechanisms remain largely unknown. Using a comprehensive RNA-sequencing analysis, we show that early transcriptional responses triggered by each of the most abundant polyamines (putrescine, spermidine, spermine, thermospermine, and cadaverine) exhibit specific quantitative differences, suggesting that polyamines (rather than downstream metabolites) can elicit defense responses. Signaling by putrescine, which accumulates in response to bacteria that trigger effector-triggered immunity (ETI) and systemic acquired resistance (SAR), is largely dependent on the accumulation of hydrogen peroxide and is partly dependent on salicylic acid (SA), the expression of ENHANCED DISEASE SUSCEPTIBILITY (EDS1) and NONEXPRESSOR of PR GENES1 (NPR1). Putrescine elicits local SA accumulation as well as local and systemic transcriptional reprogramming that overlaps with SAR. Loss-of-function mutations in arginine decarboxylase 2 (ADC2) -which is required for putrescine synthesis-, and copper amine oxidase (CuAO) -which is involved in putrescine oxidation- compromise basal defenses, as well as putrescine and pathogen-triggered systemic resistance. These findings confirm that putrescine elicits ROS-dependent SA pathways in the activation of plant defenses.

Key words: Salicylic acid, putrescine, systemic acquired resistance, Arabidopsis, defense

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A NEW *IN VITRO* MODEL FOR TESTING THE EFFECTS OF THE FO12 STRAIN OF *Fusarium oxysporum* ON IRON DEFICIENCY RESPONSES IN *Arabidopsis thaliana*

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Abstract: To solve the problem of low solubility of iron (Fe) in alkaline soils, dicot plants induce physiological and morphological responses in their roots, where some hormones and signaling substances, such as ethylene and nitric oxide, have been involved. Some key genes related to these responses are *FRO2* (Ferric Reductase-Oxidase), encoding a ferric reductase that reduces Fe^{3+} to Fe^{2+}; *IRT1* (Iron Regulated Transporter), encoding an iron transporter that transports Fe^{2+} into the root cells; and *HA1* (H^+-ATPase) encoding a H^+-ATPase that releases protons to the rhizosphere. In addition, it is known that the Induced Systemic Resistance (ISR), triggered by some beneficial rhizosphere microorganisms, is also regulated by similar hormones and signaling substances, in such a way that these microorganisms also promote the induction of the Fe-related genes described above (Romera et al. 2019).
In this work we adapted an inoculation method for the treatment of *Arabidopsis thaliana* Col-0 plants grown in agar with the non pathogenic strain of *Fusarium oxisporum* Fo12 (Masachis *et al*., 2016; Zamioudis *et al.,* 2015). The image analysis of *A. thaliana* roots manifested that the inoculation with the Fo12 strain promotes the formation of radical hairs, and alters the root architecture (higher number of lateral roots and shorter primary root). Furthermore, the fungus induces growth promotion of the plants, a higher ferric reductase activity both upon Fe deficiency and Fe sufficiency conditions, and the induction of key

genes involved in Fe deficiency responses.

Key words: Fe deficiency, *Arabidopsis thaliana*, *Fusarium oxysporum*, ISR, *in vitro.*

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A NEW PYRIDINE DERIVATIVE HAS ANTIFUNGAL ACTIVITY AGAINST *Botrytis cinerea* AND POSITIVELY REGULATES PLANT DEFENSE

Néstor Carrillo-Barral[1,3], Raquel Núñez[1,3], Breogán Villar[1], Marta Lois[1], Ismael Marcos[2,3], Alberto Fernández[2,3], Jesús José Fernández[2,3], José Díaz[1,3]

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Abstract: Chemical control is essential for integrated pest and disease management of many crops. However, the repeated application of the same product against a pathogen usually leads to the emergence of resistant strains. Therefore it is necessary to develop new compounds that are effective at low concentrations. In this study, several hydrazone derivatives of pyridine were evaluated as potential fungicides. As a first step, we assayed the effect of a moderate concentration (100 µM) of the different compounds on the pathogens *Botrytis cinerea* (a true fungi) and *Phytophthora capsici* (a fungal-like organism: an oomycete). Compounds L1 and L2 were chosen in this first round for further determination of their ED50s because both showed a strong antibiotic activity against *B. cinerea* and L1 also inhibited significantly *P. capsici*. Eventually, ED50 of L1 against *P. capsici* was higher than 50 µM and L2 did not inhibit the oomycete at all. However, ED50 for both compounds was below 15 µM when assayed in *B. cinerea*. These results suggest that both compounds have a high specificity for true fungi and would be promising compounds to be used as fungicides in agriculture. The next step was to test compounds L1 and L2 *in planta*, using French bean (*Phaseolus vulgaris*) as host plant. Surprisingly, only L1 protected the plant against *B. cinerea*, both locally and systemically, despite of having a higher ED50 than L2. Searching for the reason of this result, we checked the effect of L1 and L2 on several plant defense components. According to the results, the overall impact on plant defense of L2 leads to a more susceptible plant, whereas L1 leads to a more resistant plant. The main conclusion of this research is that it should be compulsory to test the effect in plant defense of any compound proposed as a candidate to be included in pesticide formulation.

Key words: *Botrytis*, fungicide, *Phytophthora*, plant defense, pyridine derivatives.

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APOPLASTIC PROTEINS, AN ALTERNATIVE FOR PLANT DISEASE CONTROL

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Abstract: Plants synthesize antimicrobial compounds in order to protect themselves from enormous number of potential enemies in natural habitats including herbivores and pathogenic microorganisms. Despite the current preoccupation with synthetic chemistry as a vehicle to discover and manufacture pesticides, the search of novel safe alternatives against pathogen microorganisms is still enormous because of accessibility and affordability. The aim of the present work was to evaluate the antibacterial activity of an apoplastic protein (AP) extracted from tomato plants treated with the inductor, 1 Methyl tryptophan (1-MT), against *Pseudomonas syringae* pv. tomato DC3000 (Camañees et al. 2015, Scalschi et al. 2018, Llorens et al. 2020). According to results, AP represents a prospective safe alternative for combating antimicrobial resistance in this plant pathogen *P. syringae*. Results evidenced a linear decrease of bacterial growth rate with the increase in protein concentration. Moreover, tomato plants were treated with AP and further infected with *P. syringae*. The plants treated with the AP showed significantly lower symptoms of bacterial infection as well as lower levels of colony forming units per leaf compared with non-treated control plants. In these works, it was observed that the presence of the protein alters the balance of the hormones involved in plant defense pathways in treated plants compare to controls. Therefore, AP could be used as natural alternative preventives to control plant diseases and preserve avoiding health hazards of chemically antimicrobial applications.

Key words: Apoplastic proteins, disease control, Pseudomonas, tomato

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Arabidopsis plants lacking plastid phosphoglucose isomerase respond to microbial volatiles through GLUCOSE-6-P/PHOSPHATE TRANSLOCATOR2 action

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Abstract: Recent studies have shown that PGI1 is an important determinant of photosynthesis, growth and starch production, likely as a consequence of its involvement in the synthesis of plastidial isoprenoid compounds such as hormones and photosynthetic pigments (Bahaji et al. 2015; Bahaji et al. 2018). We have shown that volatile compounds (VCs) emitted by microbes promote growth, photosynthesis, and accumulation of cytokinins (CK) and starch (Sánchez-Lopez et al. 2016a). This response was PGI1-independent, as fungal VCs exposed PGI1 null *pgi1-2* plants grew faster and accumulated exceedingly higher levels of starch and CKs than plants not exposed to VCs (Sánchez-López et al. 2016b). The most up-regulated gene in leaves of fungal VCs exposed plants was At1g61800, encoding the plastidial GPT2 glucose-6-phosphate (G6P)/phosphate translocator. Whether the response of *pgi1-2* to fungal VCs could be due to the GPT2-mediated transport of cytosolic G6P into the chloroplast was investigated by characterizing *pgi1-2/gpt2-2* PGI1- and GPT2-null double mutants cultured in the presence or absence of VCs emitted by the fungal phytopathogen *Alternaria alternata*. We found that photosynthesis, active CKs content, growth and leaf starch content in *pgi1-2/gpt2-2* plants exposed to fungal VCs were lower than in VCs exposed WT, *gpt2-2* and *pgi1-2* plants. Proteomic analyses revealed that fungal VCs strongly up-regulate the expression of proteins involved in photosynthesis in WT, *gpt2-2* and *pgi1-2* plants, but in much lower extent in *pgi1-2/gpt2-2* plants. The overall

data show that the combined action of PGI1 and GTP2 is an important determinant of the plant´s response to microbial VCs. The possible involvement of these functions in the production of growth- and foliar metabolism-regulating isoprenoid hormones in heterotrophic organs is discussed.

Key words: starch, growth, volatile compounds.

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ASSAYING BIOCHAR AS A POTENTIAL SUSTAINABLE ALTERNATIVE FOR THE CONTROL OF PLANT ENDOPARASITIC NEMATODES

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Plant-parasitic nematodes represent a major threat to agricultural manufacture and the economy worldwide (Nicol et al., 2011). Within them, the root-knot nematodes (RKN), *Meloidogyne* spp., are sedentary obligate parasites that feed on their host plants to complete their life cycles (Escobar et al., 2015). The use of biochar to control plant pathogens is a non-chemical control strategy that could be valuable since chemical nematicides are progressively banned. Biochar is a product derived from the decomposition of organic material in an oxygen-deficient environment at high temperatures, with the potential of improving plant growth and defence against pathogens (Poveda et al., 2021). We pyrolyzed residues of the wine industry (grape skins) at 350 and 700 °C, obtaining two different biochars (B350 and B700, respectively). Elemental characterization through ICP-MS revealed higher concentrations of C and plant macro (Mg, K, Ca, P, Fe) and micronutrients (Cu, Ni, Mn) in both biochars, compared to the raw material. Water extracts obtained from heat and maize straw biochar have been shown to be beneficial for plant growth (Lou et al., 2016); therefore, we checked whether water extracts from both biochars could have a plant-growth effect and/or a reduced nematode infection that could be considered an added value. *In vitro* growth assays with tomato seeds and water extracts from both biochars showed a reduction of root length, revealing some phytotoxicity that disappeared at higher dilutions of the extracts (1/10 for B350 and 1/100 for B700). *In vitro* infection assays with *Meloidogyne javanica* in tomato seedlings growing in the non-phytotoxic dilutions of the biochar water extracts, showed no significant differences in the number of galls per plant. None of the extracts showed relevant direct nematicidal activity against the infective J2 larvae (10%). Therefore, we assayed biochar directly in soil. Preliminary results with low concentrations of biochar showed increased growth and relative water content of tomato plants. Further research will involve a deep analysis of its potential for nematode control in soil.

Key words: biochar, plant-parasitic nematode, nematode control, plant growth

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BENEFICIAL ROLE OF EXTREMOPHILE MICROORGANISM EXUDATES IN SEEDLINGS OF TOMATOES AND CORN

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Abstract

Microorganisms that live in regions of difficult survival for other species are known as extremophiles; for this reason, their secondary metabolites have begun to be used and studied and their possible role in the promotion and adaptation of plant growth. The present work evaluated the potential role in the growth promotion of saladette tomato plants (*Solanum lycopersicum* L.) and corn (*Zea mays* L) of the exudates of bacteria and Trichoderma fungus isolated from extreme environments of Veracruz, Mexico. The microorganisms were grown in a liquid medium (medium PDA and LB) under constant stirring at room temperature when 10^7 cfu / ml were obtained; they were filtered with a 45 µm filter under vacuum to eliminate the structures of the microorganisms. The experiments were carried out with tomato and corn seedlings with the first true leaves; they were inoculated with 3 ml of solution per treatment. A randomized complete block treatment design was used that included independent microorganisms: bacteria (Bac33, Bac34, Bac41, Bac45), Trichoderma (TC3, TC5, TC7), absolute control (T), and dual inoculation in the corn bioassay. For the tomato bioassay, treatments with commercial microorganisms (TC-co and Bac-co) were added. For the comparison of means, the Newman-Keuls test was 95% confident. The inoculum with the best result for corn for the variables evaluated height, stem diameter, and the number of leaves was Bac41-TC5 compared to the control (p =0.004). For the tomato trial, the best treatment in height and diameter of the stem was the simple inoculation of Bac 45 compared to the control and commercial microorganisms (p =0.001). These results imply that single or dual exudate inoculation plays a significant role in the plants' growth and fitness under study. To know better microorganism behavior, production trials under field conditions are recommended.

Keywords: Extremophilic microorganisms, Bacteria, Secondary metabolites, Trichoderma.

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BIOFORTIFICATION OF PRUNUS ROOTSTOCKS THROUGH SYMBIOSIS WITH CHILEAN NATIVE AND WIDESPREAD ARBUSCULAR MYCORRHIZA UNDER DIFFERENT WATER REGIMES

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Abstract: Biofortification has been described as a potential method to enhance plantlet fitness during plant production process. In the present work, symbiosis with arbuscular mycorrhiza fungi (AMF) and deficit irrigation have been used as mechanisms to increase the fitness of *Prunus* rootstocks 'Adara' and 'Maxma14'. Experimental design has been performed as three levels of mycorrhizal symbiosis and two levels of water regime, for each rootstock: non symbiosis (M0), symbiosis with *Clareidoglomus clareidum*, obtained from chilean soils (M1) and symbiosis with *Rhizophagus intraradices*, as a widespread mycorrhizal fungus (M2), under well-watered (WW) and water-deficit conditions (WD). Stem elongation rate was measured as an integrate parameter of plant development under those experimental conditions. After two months, predawn and midday water potential, mycorrhizal colonization, root hydraulic conductivity (Lp), plant biomass and water use efficiency of whole plant (WUEp) were measured. Both rootstocks stablished mycorrhizal symbiosis with M1 and M2, while M0 did not showed colonization by AMF. Soil water content (SWC) was 95% in WW plants during all the experimental period, and the deficit irrigation decreased progressively the SWC of WD ones. SWC of M1 and M2 of both rootstocks decreased faster than M0, at the same time that increased stem elongation. At the end of the experiment, both AMF symbiosis showed longer stems, increasing these differences with M0 under WD conditions. Both, M1 and M2 symbiosis showed higher Lp compared with M0 and negative values of water potential at midday when plants were under

WD conditions. ‘Adara’ and ‘Maxma14’ in symbiosis with M1 and M2 showed significantly higher biomass accumulation, and despite of WD conditions enhance WUEp in all mycorrhizal conditions, M1 and M2 showed higher values than M0. On one hand, the effect of mycorrhizal symbiosis on water relations contributed to produce taller rootstocks. On the other, WD contributed to root development and increased water use efficiency without biomass losses. All in all, mycorrhizal symbiosis and WD performed a good tool as biofortification for *Prunus* rootstocks, ‘Adara’ and ‘Maxma14’.

Key words: Biofortification, AMF symbiosis, *Prunu*s rootstock, WUEp

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BLACK ROT DISEASE DECREASES YOUNG BRASSICA OLERACEA PLANTS' BIOMASS BUT HAS NO EFFECT IN ADULT

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Abstract: Black rot disease, caused by the bacterium *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson (*Xcc*), causes important yield losses in *Brassica oleracea* L. crops worldwide. In temperate areas, yield losses are mostly due to the discarding of those plants showing chlorotic and necrotic lesions, since they may be unmarketable. However, the biomass loss caused by the diversion of resources from the primary to the secondary defense metabolism could also affect the final crop yield. In this work, we have focused on studying the impact of *Xcc* race 1 invasion on the biomass production of young and adult *B. oleracea* plants. The results have shown that *Xcc* infection reduces biomass and photosynthesis in the aerial parts of seedlings and modifies their water percentage in a time-dependent manner. When adult plants were inoculated in the field, no effect was detected on the leaves or the biomass of marketable products. This was probably due to a better immune response when compared to seedlings. Since the first developmental stages of *B. oleracea* crops are especially vulnerable to *Xcc*, plant disease control should be increased in order to avoid yield losses of marketable products at the adult stage.

Keywords: yield loss; plant-pathogen interaction; *Brassica*; *Xanthomonas campestris* pv. *campestris*

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BULK-SEGREGANT RNA-SEQ TO UNCOVER PATHWAYS INVOLVED IN MAIZE RESISTANCE TO KERNEL CONTAMINATION WITH FUMONISINS

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Abstract: *Fusarium verticillioides* has been known as a causing agent of maize ear rot for long time, but the discovery of fumonisins, mycotoxins produced by *F. verticillioides*, increased concerns on kernel infection by *F. verticillioides*. In the current study, a bulk-segregant RNA-seq approach has been used to identify differentially expressed genes (DEG) between bulks of resistant and susceptible inbreds to Fusarium ear rot (FER) and kernel fumonisin contamination in order to uncover the pathways involved in resistance.
All inbreds were originated from the same F_2 population. Plants were selfcrossed and 15 days later the main ear of the plant was inoculated. RNA-seq analysis was done with RNA extracted from kernels collected 10 days after inoculation using three biological replicates for each bulk. The six bulk samples were sequenced at Novogene and a pseudo alignment approach was used to speed up the alignment procedure. Genes with FDR adjusted p-values < 0.05 were treated as DEG.
Three-hundred sixty-four DEG were identified when comparing transcript counts of resistant *versus* susceptible bulks. Infected kernels of the resistant inbreds clearly differentiated from those from susceptible inbreds attending to the expression of genes involved in hormone biosynthesis and signaling pathways. In general, genes involved in strigolactone, brassinosteroid and ethylene signaling were upregulated in resistant inbreds; meanwhile, genes involved in phytosulfokine, jasmonic acid and salicylic acid signaling pathways were downregulated. The expression differences for genes involved in hormone signaling were accompanied by expression changes in genes implicated in the cell cycle, metabolic processes of RNA, proteasome-ubiquitination system, post-translational modifications of proteins, respiration, and primary and secondary metabolism. A growth-defense tradeoff is proposed since sink metabolic processes such as fatty acid and starch biosynthesis were downregulated as well as phytosulfokine signaling, well known as regulator of cell proliferation and growth, and many other genes involved in cell division;

meanwhile genes involved in secondary metabolism were upregulated, especially those involved in cell wall biosynthesis and modification and flavonoid biosynthesis that could play important roles in resistance (Bashline *et al.*, 2014; Perrot-Rechenmann, 2010; Bernardi *et al.*, 2018; Venturini *et al.*, 2016; Yao *et al.*, 2020).

Key words: *Fusarium verticillioides*, Maize, Fumonisins, Resistance, Differentially expressed genes (DEG)

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DAMAGE - ASSOCIATED MOLECULAR PATTERNS AS GREEN VACCINES: EXTRACELLULAR DNA INDUCES PLANT RESISTANCE AGAINST PATHOGENS AND PESTS

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Abstract

Damage-associated molecular patterns (DAMPs) are endogenous signals released from attacked host cells to the extracellular space leading to activation of general immune responses against present challenges (Gust, Pruitt, & Nurnberger, 2017; Heil & Land, 2014). The role of extracellular DNA (eDNA) acting as a DAMP signal in mammals and plants has gained much attention within the last years (Duran-Flores & Heil, 2016; Hemmi et al., 2000). However, the pathway of eDNA perception and its role in enhancing resistance in plants is still unknown. In this study, we extended the knowledge of eDNA as a DAMP signal in plants. Our results showed that eDNA treatments upregulate the mitogen-activated protein kinases marker genes (*MPK3* and *MPK6*), ROS signaling gene (*OXI1*), and the calcium signaling gene (*CML37*). Additionally, we observed an induction in H_2O_2 production and with no associated changes in callose accumulation following eDNA treatments. This suggested that signaling defenses but not direct defense responses are induced after eDNA applications. Using different eDNA sources from Arabidopsis ecotypes and phylogenetically distant plant species, a direct correlation between the phylogenetic closeness and the level of relevant eDNA-Induced Resistance markers (eDNA-IR) was observed. Moreover, we reported broad-range plant protection against pathogens and pests measuring damage parameters after challenged eDNA-treated plants by detrimental pathogens with different lifestyles *Hyaloperonospora arabidopsidis*, *Botrytis cinerea*, and *Pseudomonas syringae* and insect pests *Myzus persicae* and *Frankliniella occidentalis*. Our study provides strong evidence for the role of eDNA as a DAMP signal. However, more studies are required to get a better understanding of the mechanisms behind eDNA-IR.

Key words: Damaged-associated molecular patterns, Extracellular DNA, Induced Resistance

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DO PUTRESCINE BIOSYNTHESIS PATHWAYS PLAY A ROLE IN TOMATO PLANT DEVELOPMENT UNDER NH_4^+ NUTRITION?

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Abstract: Plants can uptake nitrogen mainly as nitrate (NO_3^-) and ammonium (NH_4^+) forms. NO_3^-based nutrition causes environmental problems due to its emission to the atmosphere or by leaching into groundwater (Xu *et al*., 2016). Moreover, high concentrations of NH_4^+ lead to the *ammonium syndrome*, which is characterized by different symptoms such as biomass suppression, rhizosphere acidification or changes in root system architecture (Britto and Kronzucker, 2002; Liu and von Wirén, 2017; Xuan *et al*., 2017; González-Hernández *et al*., 2020). However, NH_4^+ nutrition seems to be a greater alternative for N supply to crops due to its high adsorption into the soil exchange complex, the low energy required for its assimilation into amino acids, as well as the induction of basal responses that allows plants to activate systemic acquired acclimation to cope stresses (Miller & Cramer, 2004; Fernández-Crespo *et al*., 2014; González-Hernández *et al*., 2019). Two of these amino acids are arginine (Arg) and ornithine (Orn), which are involved in putrescine biosynthesis through the arginine decarboxylase (ADC) and ornithine decarboxylase (ODC) enzymes, respectively. The role of polyamines in plant development has been widely studied, but the study of the relevance of each biosynthetic pathway in plant development under NH_4^+ nutrition is still lacking. Therefore, in the present work, we proposed to study the involvement of both biosynthetic pathways in tomato shoot and root development when grown under this N source. To accomplish this objective, we have obtained two knock-down independent tomato lines in *ADC* or *ODC* genes (*SiADC- 1*, *SiADC- 2*, *SiODC-1*, *SiODC-2*), which displayed a reduced ADC or ODC expression at basal level compared to the control. The results showed that *SiADC*-1 and *SiADC-2* plants displayed an increase in root and shoot length, while *SiODC-1* and *SiODC-2* plants showed a slightly reduction in lateral root number and density. Altogether suggests the involvement of these pathways in different plant development processes.

Key words: *Solanum lycopersicum***,** ammonium nutrition, root architecture, polyamines.

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EFFECT OF A BIOSTIMULANT TREATMENT ON THE GERMINATION POTENTIAL OF CAMELINA SATIVA SEEDS

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Abstract: Seed enhancement technologies have the potential to improve germination and seedling growth under environmental stress, as they improve the germination rate and uniformity (Campobenedetto et al., 2020; Qiu et al., 2020). Therefore, this technology could improve the cultivation of camelina, to position it as an alternative and profitable crop in rotation with cereals or legumes in the Community of Madrid. The main objective is to obtain by-products and biomass (mainly oil for biofuels or animal feed), as well as a cover crop to reduce soil erosion in semi-arid areas as the center of Spain (Valentin et al., 2004; Lu, 2008; Iskandarov et al., 2014; Lohaus et al., 2020; Kabelka et al., 2021). We have investigate the effects of FINDER® from TRICHODEX S.A (Seville, Spain), a biostimulant based on *Sinorhizobium arboris* and *Azospirillum brasilense* formulated to increase nitrogen fixation, on the seed Germination Potential [GP] of eight Varieties [V] of camelina (*Camelina sativa* (L.) Crantz). We used non-treated seeds [C] and two biostimulant doses: [D_1]) 10mL/kg_{seed} and [D_2]) 100mL/kg_{seed}. As camelina is an autumn-winter crop, we first incubated the seeds in Petri dishes in a refrigerated chamber (4°C) for 4 days, followed by 5 days at 20°C with no light; 50 seeds in each Petri dish and four repetitions per treatment and variety (96 samples). We considered as germinated those seeds with cotyledon and radicle. The D_1 dose increased the PG up to a 3.82% in V11, 6.98% in V13, 5.62% in V3, 1.13% in V4, 3.46% in V6, 3.01% in V8, and 12.44% in V9. While, the D_2 batch increased the GP up to a 13.58% in V11, 17.31% in V13, 11.25% in V3, 15.15% in V4, 4.39% in V6, 11.93% in V8, 19.90% in V9 and 1.57% in V10. The assessment of the variability of the PG by camelina varieties showed statistical significance on V4 and V8 (p_{value}<0.05) and in V11 (p_{value}<0.10). Therefore, the use of FINDER® biostimulant has a remarkable effect on the increase of the PG of these varieties. Future works aim to test the biostimulant on field, to assess the effects on biomass production and seed quality.

Key words: camelina, biostimulants, germination potential

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EPIGENETIC REGULATORY MECHANISMS DURING THE NEW ORGANOGENESIS PROCESSES LEADING NEMATODE FEEDING-SITE FORMATION

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Abstract: Plant parasistic nematodes (PPNs) affect all mayor crops causing losses that has been estimated as a projected yield loss of 12.3% worldwide (Singh et al., 2015), therefore, they constitute a serious problem in agriculture. Two of the most important groups are the sedentary endoparasites, root-knot (RKNs; *Meloidogyne* spp.) and cyst nematodes (CNs; *Heterodera* and *Globodera* spp). RKNs form new organs in the root, called galls, mainly by the recruitment of developmental pathways of new organogenesis (Olmo et al., 2020). Inside the galls, they induce the formation of giant cells (GCs), specialized nourishing cells that feed the obligate parasite nematode until life cycle completion (Escobar et al., 2015). CNs nematodes also form specialized feeding structures, called syncytia from the fusion of adjacent cells (Piya et al., 2019). The formation of nematode feeding sites is driven partly by phytohormones, mainly auxin and cytokinin, also implicated in a large number of plant development processes (Escobar et al., 2015; Olmo et al., 2020). Moreover, the role of several miRNAs, e.g. *miRNA172* and *miR390*, during gall formation has been recently addressed and interestingly, they are also implicated in different aspects of plant development (Díaz-Manzano et al., 2018; Cabrera et al., 2016). Thus, miRNA regulation and developmental processes of new organogenesis leading to GCs formation are somehow connected. The presence of AuxRE elements in the *miRNA172* and *miR390* promoter regions and their modulation by auxin signaling, highlights the key role of auxins during GCs and gall development (Cabrera et al., 2016; Dastidar et al., 2019; Marin, E., 2010). In this respect, we are focused on the dissection of the *miR390* promoter, and the identification of putative AuxRE elements with regulatory roles during RKNs and CNs infection. In addition, recent approaches performed in our laboratory, by using confocal microscopy, marker Arabidopsis lines, and a battery of mutants, showed the expression and function of several marker lines and genes of the root meristem, of lateral root formation, callus formation and root regeneration (i.e ARF5, GATA23, AHP6, SCZ, among others; Olmo et al., 2020) in galls and syncytium.

Key words: Auxins, lateral root, galls, syncytium
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IDENTIFICATION OF SOURCES OF RESISTANCE AND CHARACTERIZATION OF RESISTANCE MECHANISMS IN A GERMPLASM COLLECTION OF MEDITERRANEAN WHITE AND RED OAT AGAINST *Puccinia coronata f.sp. avenae*

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Abstract: Crown rust caused by *Puccinia coronata f.sp. avenae* is one of the most important diseases of oat crop and causes high yield and grain quality losses (Simons, 1985). This disease is particularly important in the Mediterranean basin (Hemmami, 2006) where populations are more virulent than in the centre and north of Europe (Sánchez-Martin et al., 2012). In order to identify alternative sources of resistance, a collection of 709 oat accessions, constituted mainly by red and white Mediterranean oat landraces (*Avena byzantina* and *Avena sativa*, respectively), from 24 different countries, was screened against a virulent isolate of *Puccinia coronata f.sp avenae* collected in oat fields in Córdoba, Spain. Accessions were macroscopically assessed for infection frequency and other disease parameters by image analysis, with the Robust, User-Friendly Script Tool (RUST) software (Gallego-Sánchez et al., 2020). The most interesting accessions were selected for further microscopical analysis to determine the cellular responses underlying the resistance response observed. At microscopical level, a range of defense responses has been observed, acting alone or in combination, such as a reduced fungal growth before mesophyll penetration, penetration resistance by cell wall strengthening and/ or papilla deposition, and hypersensitive response leading to cell death, which impeded fungal development at different stages. The novel sources of resistance will be useful for introducing durable resistance in oat cultivars.

Key words: hypersensitive response, image analysis, oat, penetration resistance, rust.

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IMPACT OF THE COMBINED ARSENIC AND FLOODING STRESS ON PEANUT NODULES

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Abstract: Arsenic (As) is a harmful metalloid affecting living organisms, being groundwater the major source of contamination. The use of this water for crop irrigation, or the direct absorption of As-containing water from high phreatic layers by roots, could increase metalloid accumulation in plant edible parts. Argentina is one of the top peanut exporting countries. This legume engages in a symbiotic interaction with rhizobia, where a nitrogen fixing nodule is developed in the roots. In Córdoba province, 90% of its groundwater is affected by high As levels of geochemical origin. This issue is aggravated by the occurrence of flooding episodes. Thus, the aim of this work was to elucidate the effects of As, flooding (F) and the combined treatment (As/F) on peanut nodules. Peanut pre-germinated seeds were transferred to pots with a mixture of perlite:sand (1:2) and irrigated with Hoagland solution in the presence or absence of 3 µM As. Seven days post-emergency plants were inoculated with *Bradyrhizobium* sp. SEMIA6144 mycrosimbiont. After 30 days of growth, plants were randomly distributed in flooding and non-flooding conditions, with or without As addition for 8 days. Results showed that nodule number and nitrogen content decreased in all treatments, being the nodules of plants exposed to As/F conditions the most affected. The applied stress modified the morphology of the bacteroids characterized by an increase of the spaces of the peribacteroid membrane. Nodule As content of plants exposed to As was lower than that obtained under combined stress and the activity of arsenate reductase enzyme showed a significant increase in As/F treatment compared to As. Regarding the oxidative status, all treatments increased the production of reactive oxygen species. Jasmonic acid increased under F treatment but was reduced under As. The levels of salicylic acid decreased in all treatments compared to control. No treatment effects on methyl jasmonate concentrations were observed, while the content of 1-aminocyclopropane 1-carboxylic acid was significantly increased under F treatment. All these findings allow us to conclude that peanut exposition to an environmentally relevant As concentration, to flooding conditions, or to combined As/F treatments negatively impacts

the morpho-physiological and biochemical response of peanut nodules.

Key words: Arsenic, *Bradyrhizobium* sp.-peanut interaction, oxidative status.

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IN VIVO METABOLIC REGULATION OF ALTERNATIVE OXIDASE UNDER NUTRIENT DEFICIENCY—INTERACTION WITH ARBUSCULAR MYCORRHIZAL FUNGI AND RHIZOBIUM BACTERIA

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Abstract: The interaction of the alternative oxidase (AOX) pathway with nutrient metabolism is important for understanding how respiration modulates ATP synthesis and carbon economy in plants under nutrient deficiency. Although AOX activity reduces the energy yield of respiration, this enzymatic activity is upregulated under stress conditions to maintain the functioning of primary metabolism. The in vivo metabolic regulation of AOX activity by phosphorus (P) and nitrogen (N) and during plant symbioses with Arbuscular mycorrhizal fungi (AMF) and Rhizobium bacteria is still not fully understood. We highlight several findings and open questions concerning the in vivo regulation of AOX activity and its impact on plant metabolism during P deficiency and symbiosis with AMF. We also highlight the need for the identification of which metabolic regulatory factors of AOX activity are related to N availability and nitrogen-fixing legume-rhizobia symbiosis in order to improve our understanding of N assimilation and biological nitrogen fixation.

Key words: alternative oxidase; arbuscular mycorrhizal fungi; nitrogen and phosphorus nutrition; Rhizobium; plant primary metabolism

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INHIBITION OF *COLLETOTRICHUM GLOEOSPORIOIDES* USING EXTRACTS OF MEXICAN MISTLETOE (*PSITTACANTHUS CALYCULATUS*)

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Abstract: Mistletoe is a hemiparasitic plant that grows and develops on several species of fruit and forest trees in central and southern Mexico [1]. In the last decade, the number of infected areas has increased, becoming a potential forest health problem since it parasitizes more than 150 plant species [2]. Due to its chemical components, this species of mistletoe could be an important source of compounds that confer antimicrobial activity which has been tested on microorganisms pathogenic to humans. [3,4] However there are no reports on its use for the phytopathogens inhibition such as *C. gloeosporioides*, that is the main causal agent of the anthracnose in avocado fruits [5]. Therefore, our aim was to test the in vitro antifungal capacity of different mistletoe extracts for the *C. gloeosporioides* inhibition. For this, we obtained extracts by the mistletoe leaves and flowers. Firstly, our plant materials was dried (50 °C, up to constant humidity) and was ground in an industrial blender (80 mesh). A solid/liquid extraction was carried out using acetone After it, we removed the organic phase, using a rotary evaporator at 65°C for 4h. Subsequently, we mix PDA medium and the extract at different concentrations (0.1, 0.5, 1.0 % (v/v)) and we placed the inoculum in the center of the petri dish. The control was petri dishes with PDA medium and with the phytopathogen without extract. All treatments and control were incubated at 28±2 °C in darkness for 7 days. Our evaluations were inhibition percentage of mycelial growth in a petri dish (%IMG), final sporulation (FS) and, germination (G). Our results show that the leaves extracts had the highest inhibition which presents 73 %IMG at concentrations of 1.0 and 0.5% (v/v). Besides, we detected a reduction in sporulation and germination. These results are evidence of the antifungal capacity of mistletoe extracts and their potential use for the inhibition of important phytopathogens.

Key words: phytopathogen, anthracnose, inhibition, plant extracts.

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MYCORRHIZAL FUNGI VOLATILES AFFECT PLANT DEVELOPMENT THROUGH DIFFERENT HORMONAL PATHWAYS

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Abstract: Beneficial mycorrhizal associations with roots are critical for plant development, as they facilitate the acquisition of nutrients and water, specially under environmental constrains (Calvo-Polanco *et al*. 2019). The signals exchanged among the fungi and their host include hormones and volatile organic compounds (VOCs) (Ditengou, *et al.* 2015). In particular, the production of sesquiterpenes by the ectomycorrhizal fungus *Laccaria bicolor* was related with the proliferation of lateral roots and the conformation of root architecture (Ditengou, *et al.* 2015). Within our current research, we have studied the main plant hormonal pathways affected by the VOCs of two different ectomycorrhizal fungi, *Laccaria bicolor* and *Hebeloma crustuliniforme* (typically found in symbiosis with tree species) and the mycorrhizal fungus *Piriformosfora indica* (inducing stress tolerance in plants), using an *Arabidopsis* mutant collection impaired in different hormonal pathways (i.e. abscisic acid, ABA; auxin; gibberellins; citokinins; ethylene; jasmonic acid and nitric oxide). The plants were subjected to the VOCs of the different fungi for two weeks in order to detect their effect during seed germination and early plant development. The results indicated that all the fungi modified seed germination of the ABA mutants under study (*abi1-1, abi3-1, abi4-1*, *abi5-1* and *hab1-1; abi1-1*), while seedling growth was particularly affected in the mutants related with auxin (*shy2-24, shy2-31, aux1-7, axr1-3* and *axr6-3*), cytokinins (*cre1*, *cre1; ahk3, arr1-3, arr1-4)* and ethylene (*ein2-5*) pathways. Reporter *Arabidopsis* lines of the auxin pathway fused to ß-GUS (*SHY2:GUS, DR5:GUS, SKP2B:GUS*) were used to quantify the effects of fungal VOCs, using GUS histological and western blot assays. The western blot assays indicated a significant increase in the accumulation of the proteins analysed, especially in the aerial parts of SHY2 and SKP2B. Also, the histological analysis showed a higher amount of secondary root primordia in the presence of fungal VOCs, mainly in the case of SKP2B:GUS. All these results may explain the promoting growth effects of the ectomycorrhizal and mycorrhizal fungi under study.

Key words: Mycorrhizal associations, Arabidopsis, Volatile organic compounds (VOCs), Hormonal pathways

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NO SIGNALLING DURING THE *Botrytis cinerea* – HOST PLANT INTERACTION: A TWO-SIDED STORY.

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It is widely known that nitric oxide (NO) is involved in a great variety of plant responses, including abiotic and biotic stresses (reviewed in Manrique *et al.* 2020; Martínez-Medina *et al.*, 2019 and Sanz *et al.*, 2015). Basal defences and the hypersensitive response rely on NO (Mur *et al*., 2013) and during plant immune responses against fungi NO leads to a substantial reprogramming of gene expression (Mur *et al.*, 2017). This free radical also plays a major role in the signalling pathways of phytopathogenic fungi, and it is hypothesized that the necrotroph *Botrytis cinerea* takes advantage of NO production to promote plant colonization (Turrión-Gómez *et al*., 2010; Turrión-Gómez and Benito, 2011). Despite the importance of NO as a signalling molecule, research about the presence and function of NO in plant-fungal interactions is limited, and this limitation widens as we enter further into the influence of NO in the production of secondary metabolites. An approach by Kliebestein *et al.* (2005) has already demonstrated that glucosinolates (GSLs), which are sulfur-containing defense metabolites produced in the Brassicales, influence Arabidopsis/Botrytis interactions, but if NO has some influence in this kind of defence response involving GSLs and their degradation products remains to be elucidated. Here in this work, using available NO homeostasis mutants both of *A. thaliana* and *B. cinerea*, we aim to study further the involvement of NO during the interaction of this fungus and the host plant and how NO produced by both the plant and *B. cinerea* affects the production of key metabolic elements, such as glucosinolates and flavonols, some of them already linked to NO homeostasis (Sanz *et al.* 2014). Our results indicate that the phenolic and GSLs profile varies between mutants with impaired in NO homeostasis and this variation could be further influenced by *Botrytis cinerea* infection. We also show that NO deficient and overproducing Arabidopsis mutants may be affected in the degradation of GLS hydrolysis products such as nitriles, as a result of an impairment of nitrilase gene expression. Taken together these results provide new insights in the involvement of NO in the metabolism of GSLs during plant-pathogen interactions.

Key words: Nitric oxide, *Arabidopsis thaliana*, *Botrytis cinerea*, Glucosinolates

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PLANT GROWTH PROMOTING ABILITY OF A FUNGAL ENDOPHYTE FROM TRADITIONAL TOMATO

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Abstract:
Beneficial microorganism is one of the most promising resources in agriculture to reduce dependence of chemical inputs and confront the consequences of climate change. In this context, fungal endophytes has been shown to improve performance of crops and their resistance against environmental stress factors (Hossain, Sultana and Islam, 2017; Rho *et al.*, 2018), yet studies about these microorganism and their role are still focused on only a few known endophytes. We isolated a novel fungal endophyte from traditional tomato plants (*S. lycopersicum*), named e27. After some test assays, we performed experiments in growth chamber and greenhouse to assess the growth traits of three traditional tomato varieties (ADX, TH-30 and MO-10) when inoculated with the endophyte. For the experiments, tomato seeds were inoculated with fungal suspension for 6 hours, and then germinated and grown in growth chamber under controlled conditions (16h photoperiod, 26ºC:17ºC day:night, 80% humidity). For greenhouse experiments, plants grew in growth chamber for one week before moving to greenhouse, where they were transplanted to appropriate containers, and reinoculated by applying 1mL fungal suspension to root. In sum, all plants were cultivated for 3 to 5 weeks until 7th leaves stage before recollection. Then, growth parameters were measured for fresh and dry tissue. Results of growth chamber experiments were greater than greenhouse ones, yet both showed the same trend. Improvement of growth was observed in different degrees, but was especially significant for TH-30. For this variety, a representative experiment in greenhouse determined that stem length was improved up to 17%, basal stem diameter increased around 10% and root length was boosted more than 20%. Weight for stem and root also increased at least 20% in both fresh and dry tissue. Chlorophyll content was also slightly increased. In contrast, flowering stage remained unaltered. Thus, these results provide new insights for the ability of e27 to improve growth for its host, which effects are seemingly enhanced for less resistant varieties like TH-30.

Key words: plant-microorganism interactions, *S. lycopersicum*, fungal endophytes, growth promotion

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POTENTIAL OF *GLOMUS IRANICUM* VAR. *TENUIHYPHARUM* TO ENHANCE FIELD ESTABLISHMENT OF YOUNG NECTARINE TREES

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Abstract: Applications of *Glomus iranicum* var. *tenuihypharum* (a commercial type of arbuscular mycorrhizal fungi) are a vegetable production practice capable of improving crop yield and sustainability of agricultural ecosystems (Nicolás et al., 2015; Alarcón et al., 2020). Although recent studies conducted under field conditions have shown promising results (Basiru et al., 2021), no studies in nectarine trees have been addressed. In this work, the studied mycorrhiza was introduced through drip irrigation to inoculate young early-maturing 'Flariba' nectarine trees cultivated in south-eastern Spain to assess their effects on plant water status, and vegetative and reproductive growth compared with Control trees (untreated). Root dynamics was measured using minirhizotrons during a complete growing season, and revealed that mycorrhiza greatly increased by 51% the root growth compared with Control trees, while no changes in plant water status and canopy development were noted. The productive response improved in inoculated trees, as demonstrated by a significantly higher yield, fruit size, number of fruits per tree and crop load efficiency values than Control trees. Since the same amount of irrigation was applied in both treatments, the irrigation water use efficiency increased by 19.5% in mycorrhizal trees as compared with Control ones. The colonization of roots resulted in further soil exploration by new roots, due to the production of extra-matrix mycelia that connected to the tree root system, leading to an increase in nutrient and water uptake as indicated the higher values registered of crop and water use productivity (Butcher et al., 2009; Diagne et al., 2021). Furthermore, the minirhizotrons technique used in the study was a useful tool in studying young "Flariba" nectarine root dynamics, similar to that observed in adult peach trees by Abrisqueta et al. (2017). The findings of this study suggest that a straightforward mycorrhiza inoculation can be considered a good cultural practice in semi-arid agro-systems to firmly establish efficient young nectarine tree plantations and their adaptation to field conditions.

Key words: minirhizotrons, fruit yield, arbuscular mycorrhizal fungi, plant water status, root dynamics

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PP2A5: TWO DOMAINS, ONE FUNCTION?

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Plants have developed an enormous battery of direct and indirect defenses to face enemies like pests or pathogens. One of the most important plant foes is the phytophagous spider mite *Tetranychus urticae* Koch (Tetranychidae). This pest can infest more than 1100 plant species thanks to its stylet, with which they suck vegetal cell content, resulting in a reduction in crop yield (Santamaría M.E. *et al*; 2017). Once *T. urticae* attack plants, signal transduction occurs to activate genes with a defensive role (Santamaria M.E. *et al*; 2018). One of these genes, *PP2-A5*, was identified in our group. It encodes a protein related to resistance in *Arabidopsis thaliana* plants when mites infest it. This protein has 411 amino acids and owns two domains: a Toll-Interleukin Receptor (TIR) in the N-terminal part of the protein and a PP2 or lectin domain in the C-terminal region. Usually, TIR domains are implicated in the transduction of signals while lectins bind specific carbohydrates and are toxic to insects, bacteria, and fungi (Santamaría M.E. *et al*; 2019). Due to this special protein structure, we engaged our work on the role of each domain separately. Subcellular location assays showed that the individual domains are placed at most cell compartments. However, through Y2H (yeast two-hybrid) studies we have not noticed any interaction between our TIR-TIR; lectin-lectin, and TIR-lectin domains. The possibility of the formation of heterodimers with other TIR domains is being tested. Besides, several nucleotide mutations in the TIR domain have been performed to analyze the importance of amino acids putatively involved in its potential function, and both domains are being expressed as recombinant proteins in bacteria or yeast to determine their molecular functions. Finally, Arabidopsis plants overexpressing each domain independently have been done to perform in vivo mite assays and determine their role in the defensive response alongside the mite perturbation. Furthermore, *Solanum tuberosum* (potato) plants have been transformed with the full *PP2-A5* gene to investigate the defensive role of the PP2-A5 protein in a crop.

Key words: *Arabidopsis thaliana*, PP2-A5, PP2/Lectin, *Solanum tuberosum*, *Tetranychus urticae* Koch, Toll-Interleukin Receptor (TIR).

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RELATIONSHIP AMONG STALK CORN BORER RESISTANCE, AGRONOMIC TRAITS, AND CELL WALL HYDROXYCINNAMATES IN A SET OF RECOMBINANT INBRED LINES FROM A MAIZE MAGIC POPULATION.

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Abstract: Corn borers are the most important pest affecting maize causing high yield losses. Against the attack of this pest, cell wall bound hydroxycinnamates have been described as structural barrier and defence mechanism. On the other hand, resistance to corn borer attack may compromise plant fitness being detrimental for some important agronomic traits as yield. In this study, agronomic characterization and cell wall bound hydroxycinnamates quantification by High Performance Liquid Chromatography (HPLC) was performed in a subset of Recombinant Inbred Lines from a MAGIC population classified for resistance to corn borer attack. The goal of this research is to deepen into the knowledge of: 1) the relationship between resistance to corn borer and the main agronomic traits and 2) the role of cell wall bound hydroxycinnamates as defence mechanism against corn borer. Our results indicated that resistant lines showed precocity, low grain moisture at harvest, and reduced plant height. Thus, selecting for tunnel length may be detrimental for yield. In this way, in this MAGIC population, we suggest a breeding strategy directly targeting grain yield to tolerate corn borer attack. As well, resistant lines showed greater concentration of p-coumaric acid, being the only hydroxyccinnamate that would take part in resistance in this vegetal material.

Key words: *corn borer resistance, yield, cell wall hydroxycinnamates, RILs, agronomic perfomance,*

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ROOT COLONIZATION OF LEAFY *Brassica* CROPS BY *Trichoderma hamatum* SYSTEMICALLY MODIFIES GLUCOSINOLATE PROFILES AND ANTIOXIDANT CONTENT

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Abstract:
Trichoderma includes several species widely studied and used as beneficial microorganisms in agriculture, primarily as biological control agents, due to different mechanisms of action, such as mycoparasitism, antibiosis, space and nutrients competition, modulation of plants local and systemic resistance, mediated tolerance to abiotic stresses and plant growth promotion. In a previous study, it was reported that *T. hamatum* did not produce a significant increase in the biomass produced by kale plants. However, it involved the activation of a systemic resistance that caused plants to be significantly less affected by the phytopathogenic bacterium *X. campestris*. In this work, we have reported how root inoculation with *T. hamatum* supposes a significant increase in the foliar biomass produced by kale, cabbage and turnip greens. Moreover, the application of *T. hamatum* in cabbage and turnip greens supposed a significant increase in the antioxidant capacity and in the content of total GSLs in its leaves. This is due to an increase in the content of aliphatic (glucoiberin and sinigrin) and indole GSLs (glucobrassicin) in cabbage, and in indole aliphatic GSLs (gluconapin) in turnip greens, representing an important result for the use of Brassica leafy crops as nutraceutical foods. The increase in plant growth and GSL content in *Brassica* crops simultaneously by *Trichoderma* inoculations has not been reported previously.

Key words: *Trichoderma*; kale; cabbage; turnip greens; leafy turnip; aliphatic glucosinolates.

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RUST: A ROBUST, USER-FRIENDLY SCRIPT TOOL FOR RAPID ASSESSMENT OF RUST DISEASE

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Abstract: During the last years phenotyping has become the main bottleneck in plant breeding and fundamental plant science. This is particularly true for the assessment of plant diseases that, in addition to the time consuming evaluations, often deal with the subjectivity of visual assessments (Sánchez-Martín et al., 2012). In this work, we have developed an open script for semi-automated evaluation of rust disease in leaves. RUST function under the free image software Fiji (developed from ImageJ), which is a well-recognized software among scientific community. The script allows evaluating rust disease in leaves by a color transformation tool and provides three different modes ranging in automation. The script opens sequentially the images after recording infection frequency (pustules per area), average size of the pustules and percentage of the leaf covered by disease permitting high-throughput analysis. In addition, it is possible to manage in a similar automated way various leaf segments scanned on the same picture, selecting consecutively the desired segments. The script has been validated on nearly 900 samples from 80 oat genotypes ranging from resistant to susceptible and from very light to very heavily infected showing a high accuracy with a concordance correlation coefficient of 0.99. Low variation coefficients were estimated when repeating the measurement of the same samples demonstrating the high repeatability of the analysis. The script has also optional steps for calibration and training that allow accuracy even in low resolution images. This will allow evaluating more efficiently hundreds of leaves facilitating the screening of novel sources of resistance to this important cereal disease.

Key words: hypersensitive response, image analysis, oat, penetration resistance, rust.

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SALICYLIC ACID KEY ROLE ON POSSIBLE BEHAVIOR OF BENEFICIAL FUNGI AS PLANT PATHOGENS: USING *MARCHANTIA POLYMORPHA* AS MODEL PLANT IN EVOLUTIONARY STUDIES

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Abstract:
Marchantia polymorpha is a non-vascular plant, within the liverwort group, whose use as a model plant in physiological and evolutionary processes is increasing in recent years. With regard to plant-microorganism interactions, very few studies have been carried out with *M. polymorpha*, being a field of study with great potential for understanding the evolutionary plant-pathogen relationships. It is widely known as the symbiotic relationship with arbuscular mycorrhizal fungi (AMFs) provides the plant with benefits not only nutritional but also resistance against pathogens and pests. Although it is known how AMFs colonize the tissues of the genus *Marchantia*, the possible beneficial or harmful effect of this interaction has not been studied. Through the use of various species of AMFs we have discovered how the species *Rhizophagus fasciculatus* behaves as a plant pathogen of *M. polymorpha* tissues, possibly due to the inability of the liver to defend itself by the salicylic acid (SA) pathway against AMF. In turn, within the *Trichoderma* filament fungal genus there are numerous species widely studied and used as biocontrol agents in agriculture due to mechanisms of action such as mycoparasitism, antibiosis or the induction of plant resistance. The study of the *Trichoderma-M. polymorpha* interaction, under different growth conditions and under different forms of interaction, has allowed us to verify how in the evolutionary step from mycoparasitic fungus to a plant-symbiont, *Trichoderma* had an intermediate stage in which it behaved as a plant pathogen, playing a key role SA-mediated plant defences.

Key words: *Marchantia polymorpha*; evolutionary molecular plant-microbe interactions; salicylic acid; arbuscular mycorrhizal fungi; *Trichoderma*.

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SEARCHING FOR COMPOUNDS IN OLIVE MILL WASTEWATER USEFUL IN AGRICULTURE: INDUCTION OF RESISTANCE OF PEPPER TO PLANT PATHOGENS

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Abstract: Soilborne plant diseases as Verticillium wilt (Deketelaere, 2017) and Phytophthora root rot (Granke, 2012) cause great losses in pepper (*Capsicum annuum* L.) production.
Induced resistance to plant pathogens is a promising tool that can play a substantial role in sustainable plant disease management. Moreover, the "Farm to fork" strategy that promotes the European Union includes recycling of residues from the food industry, and this is the case of olive mill wastewater (OMW) (Cayuela, 2008). In the present work, we initially studied several fractions derived from OMW to explore their potential as resistance inducers. Plant symptoms were assessed daily according to biometric parameters and a severity scale (*ad hoc* for each disease), and the area under the progression disease curve (AUPDC) was calculated. One of the fractions was able to reduce disease severity of Verticillium wilt and Phytophthora root rot in pepper plants growing in a phytotron, and to induce plant defences, both biochemical and structural, including gene expression (qPCR), PR proteins and lignin. Our next step was to assay X10, one of the main compounds present in such an OMW fraction, in order to check if it was responsible of such inducing resistance activity. Our last results show that the compound was able to reduce plant disease severity of Phytophthora root rot, not only in phytotron assays, but also in greenhouse conditions. In the greenhouse experiments, we also harvested the plant fruits to evaluate differences between the treatments. Yield was determined recording the number and weight of commercial fruits produced per plant, and fruit quality was determined measuring capsaicinoid levels, ºBrix, pH and some biometric parameters that are indicated in the rules of the Protected Designation of Origin "Pemento de Herbón". Moreover, RNA-seq results show that several genes involved in plant defence as lignin formation, cell wall structural proteins and cell wall polysaccharide modification, show expression changes in the plant as a response to X10.

Key words: plant disease, olive mill wastewater, resistance inducers.

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SEVERAL YEAST SPECIES INDUCE IRON DEFICIENCY RESPONSES IN DICOT PLANTS

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Abstract: Iron (Fe) is very abundant in most soils but presents low availability for plants. In order to alleviate this situation, plants develop different Fe deficiency responses, mainly in their roots, to facilitate Fe mobilization and acquisition. Even so, Fe chelates are widely used in modern agriculture, despite they are expensive and can cause environmental problems. One of the most sustainable and promising alternatives to the use of these agrochemicals is a better management of rhizosphere beneficial microbes (Romera et al. 2019; www.uco.es/fethyleno/rhizosferrum/). The objective of this research has been to evaluate the ability of *Debaryomyces hansenii*, *Saccharomyces cerevisiae* and *Hansenula polymorpha* yeasts to induce Fe deficiency responses in dicot plants. To date, there are no studies on the roles played by yeasts on the Fe nutrition of plants. Experiments were carried out with cucumber and tomato plants grown in a hydroponic growth system. The effects of these yeast species on three of the most important Fe deficiency responses, acidification of the rhizosphere, ferric reductase activity, and proliferation of subapical root hairs, was evaluated. The results obtained show the inductive character of the three yeast species on the three Fe deficiency responses evaluated in both cucumber and tomato plants. These results open the way to a promising research line involving these microorganisms in achieving a more sustainable and environmentally friendly agriculture.

Key words: *Debaryomyces hansenii*, *Saccharomyces cerevisiae*, *Hansenula polymorpha*, iron, yeast

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SOIL APPLICATION OF FILTRATES FROM FUNGAL CULTURES ENHANCES YIELD OF PEPPER *(CAPSICUM ANNUUM* L.) PLANTS

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Abstract: Microbes synthesize a multitude of substances including carbohydrates, proteins, lipids, amino acids, hormones, volatiles, etc., which regulate plant growth and morphogenesis. Irrigation with culture filtrates (CFs) of some plant beneficial microorganisms promotes plant growth and enhances yield. However, nothing is known about the response of plants to soil application of CFs of microbial phytopathogens. In this work we explored the response of two pepper *(Capsicum annuum* L.) cultivars *(i.e.* Sweet Italian and Piquillo) and soil microbiota to CFs of the beneficial fungus *(i.e. Trichoderma harzianum)* and two fungal phytopathogens *(i.e. Alternaria alternata* and *Penicilliun aurantiogriseum).* We found that soil application of CFs of the three fungal species similarly enhanced root growth (40-70% increase under greenhouse conditions) and commercial fruit yield (50-112% and 20-28% increase under greenhouse and open field conditions, respectively). High-throughput amplicon sequencing of bacterial 16S and fungal ITS rRNA genes of the soil microbiota revealed that the CF treatments led to strong enrichment of the populations of the same beneficial bacterial and fungal species. Our findings show that the application of microbial CFs to the soil represents a potentially very useful strategy for increasing the yield of horticultural crops in a sustainable and environmentally friendly way.

Key words: Yield, culture filtrate, plant pathogens, biostimulants

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SURPLUS ZINC AMELIORATES THE RESPONSE OF TOBACCO PLANTS TO *XYLELLA FASTIDIOSA* INFECTION

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Abstract: *Xylella fastidiosa, (Xf),* is a xylem-confined bacteria and a quarantine pathogen organism in the EU that causes important diseases in vineyards and almond tree orchards in Mallorca (Balearic Islands) (Gomila et al., 2019). Zinc is a key micronutrient that participates in the plant's protection against pathogens (Cabot et al., 2019). The virulence of *Xf* is affected, among other factors, by plant ionic composition. Zinc concentration has been related to planktonic growth and *Xf* biofilm production (Andersen et al., 2007; Navarrete et al., 2015). Here, the role of surplus Zn in the plant response to *Xf* has been studied in *N. tabacum* var. Petit Havana. Plants were grown in 50% Hoagland nutrient solution modified with 2 (control) or 12 µM (surplus Zn) Zn. In 2-month-old plants, the lowest non-senescent leaf in half of the plants for each Zn treatment were inoculated with *Xf* subp. *fastidiosa*. Forty days after inoculation, visual symptoms, plant biomass, and water content were recorded. Bacterial population in plant extracts was quantified by qPCR. Wilting symptoms were shown in the lower leaves of inoculated plants in both Zn treatments. Moreover, *Xf* accelerated flowering more markedly in control than in surplus Zn plants. In both Zn treatments, leaf fresh and dry biomass decreased in infected plants, with the exception of the youngest leaves in surplus Zn, which showed no differences with respect to control. *Xf* decreased water content in the leaves closest to the inoculation site, which showed the highest *Xf* colonization in both Zn treatments. Bacterial colonization decreased in the upper leaves, with surplus Zn plants showing the lowest values. *Xf* decreased total plant biomass in control plants, while no differences were found in the surplus Zn treatment. In surplus Zn, *Xf*-inoculated plants showed a significant increase in root growth with respect to control. Our results suggest that the ameliorating effect of surplus Zn in the response of tobacco plants to *Xf* could be related to promotion of the development of a larger root system, which

could contribute to maintain plant growth, rather than surplus Zn having an inhibitory effect on bacterial colonization or improving leaf water status.

Key words: *Nicotiana tabacum*, Surplus zinc, *Xylella fastidiosa*.

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THE *at*ALKBH9B m^{6}A-DEMETHYLASE: MAPPING ITS RNA-BINDING DOMAIN AND ITS REGION OF INTERACTION WITH THE COAT PROTEIN OF ALFALFA MOSAIC VIRUS.

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Abstract: The N6-methyladenosine (m^{6}A) modification is a dynamically regulated modification on cellular RNAs that affects many cellular processes and pathways (Thiel, 2019). This reversible methylation has been also identified on transcripts encoded by a wide range of mammal's viruses playing a significant role in modulating viral replication (Manners et al., 2019). In contrast to mammals, very few studies on the function of m^{6}A modification have been reported in plants-virus interaction (Arribas-Hernández and Brodersen, 2020). *Arabidopsis thaliana* protein *at*ALKBH9B was the first m^{6}A-demethylase described in plants. This protein was found to accumulate in siRNA bodies and P-bodies and interact with the coat protein (CP) of Alfalfa mosaic virus (AMV) having a profound impact in the viral infection process (Martínez-Perez et al., 2017). To dissect the functional activity of *at*ALKBH9B in AMV infection, we performed a protein-mapping analysis to identify putative domains required to regulate this process. In this context, the mutational analysis of the protein revealed that the residues between 427-467 positions are critical for binding *in vitro* to the AMV RNA. Also, we determined that *at*ALKBH9B amino acid sequence presents intrinsically disordered regions (IDRs) located both, at the N-terminal part delimiting the internal AlkB-*like* domain and at the C-terminal part. Moreover, the protein presents an RNA binding domain containing a RGxxxRGG motif and overlapping with the C-terminal IDR. The IDR could play a critical role in the protein-RNA interface inducing the formation of a flexible structure and permitting additional contacts trough the RG, RGG and YG residues located in this C-terminal region. Moreover, we observed that *at*ALKBH9B deletions either of the N-terminal 20 residues or the C-terminal last 40 amino acids impede its accumulation in siRNA bodies. Interestingly, these regions are predicted to form IDRs as well which are frequently found in proteins that undergo liquid–liquid phase separation, a process that likely contributes to the formation and stability of RNA granules (Alberti et al., 2019). Finally, we determined that residues located between 387-427 are critical for the interaction with the AMV CP, an interaction that may be critical for the modulation of the viral infection process (Martínez-Pérez et al., 2017).

Key words: N6-methyladenosine, RNA-binding proteins, plant viruses, epitranscriptomics, ALKBH.

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THE ZN-CD HYPERACCUMULATOR *NOCCAEA BRACHYPETALA* HABOURS A SPECIFIC ROOT MICROBIOME

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Abstract: Similarly to the hyperaccumulator model plant *Noccaea caerulescens*, *N. brachypetala* is able to hyperaccumulate metals even growing on non-metalliferous soils. Our aim was to evaluate whether this ability is associated to the microbiome companion. For this purpose, we performed high-throughput sequencing of the bacterial and fungal communities in the rhizosphere soil and rhizoplane fractions. The extracted rhizobiome of *N. brachypetala* was compared with those from other non-hyperaccumulator Brassicaceae species from the same locations. Our results revealed that the microbiome richness and relative abundance of *N. brachypetala* tended to increase compared to the non-hyperaccumulator species. The rhizoplane fraction contained the specific and rare taxa associated with each species confirming the key role of root compartment to describe the community composition. The fungi relative abundance in *N. brachypetala* was similar to the other plant taxa but some specific taxa were notably reduced. Related to the bacterial domain, we observed an enrichment of previously described metal-tolerant bacteria and bacteria involved in nitrogen cycling in the hyperaccumulator rhizoplane. The bacteria involved in the nitrogen cycle could contribute indirectly to the hyperaccumulator phenotype by improving soil quality and fertility. Our results suggest a potential selection of beneficial taxa on the root-fractions of *N. brachypetala* that would favor the phenotype of hyperaccumulation and/or improve plant nutrition. The detected satellite groups associated to the root niche of this hyperaccumulator plant could be of interest for improving biological strategies for heavy metal remediation.

Key words: high-throughput sequencing, non-metalliferous soil, root-associated microbia

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TO DEFEND OR TO GROW: THE DILEMMA ARABIDOPSIS FACES UPON SPIDER MITE INFESTATION

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Abstract: Plants and phytophagous arthropods have co-evolved for millions of years. During this long coexistence, plants have developed defense mechanisms including constitutive and inducible defenses (Santamaria *et al*., 2020). In an effort to survive upon herbivore attack, plants reallocate resources towards defense, which usually ends up with a penalty in plant growth, development or reproduction directly linked to crop losses (Huot *et al*., 2004). Achieving the balance to maximize crop yield requires a fine tune regulation specific for each host-arthropod combination. Our results show that herbivore infestation reduces growth, photosynthesis and reproduction, being the effect dependent on several variables surrounding plant-pest interaction such as feeding guild, plant host, ontogenetic stage of the plant, magnitude and durability of the infestation. We unveil the importance of the parameters measured to determine the cost, revealing growth and photosynthesis more differences among subgroups than reproduction parameters (Garcia *et al*., 2021). To understand the mechanisms behind the defense-growth trade-off stablished upon plant pest interaction we selected *Arabidopsis thaliana* and *Tetranychus urticae* model species. This work evaluates plant fitness parameters under spider mite infestation along plant life cycle. Differences in leaf area, leaf number, petiole length, trichome density, dry weight and water content are found between control and infested plants. The integration of fitness phenotypes with transcriptomic, hormone and metabolomic results at different time points enables us to decipher the most important events in the defense-growth balance and its development in time, allowing also the determination of the key points and regulators directing these responses. Our results show infestion leads to induction of genes related to defense responses, including genes involved in glucosinolate metabolism, response to pathogens, salicylic acid, jasmonic acid and ethylene. However, genes related to growth, cell expansion, leaf development or response to auxins, gibberellins or brassinoesteroids are repressed during the infestation. This knowledge will be crucial in designing specific pest

management strategies focused on the production of more resistant plants. It will also allow an improvement of the existing production systems by minimizing plant fitness costs, which will be very important in the current context of climate change, increasing food demand and production costs.

Key words: *Arabidopsis thaliana*, defense-growth trade-off, fitness, *Tetranychus urticae*

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Tomato spotted wilt virus INCIDENCE IN *Capsicum annuum* IN THE NORTHWEST OF SPAIN

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Abstract: Tomato spotted wilt virus (TSWV) reduced the pepper production by 10% in average in 2018. However, some farmers have lost their entire pepper harvest that year. In the region of Cambados, the pepper-producing region most affected by TSWV, had losses due to this disease of around 30,000 kilos of pepper. The TSWV uses the trip from the genera *Frankliniella* as vectors, the farmers have used biological control to reduce the presence of the trip in the greenhouses with moderate success. High-throughput sequencing was used to study the genome of the virus and the response of the different genotypes of pepper to the virus. Up to 12 variations of the TSWV strain could be identify. 4 of the variants were the most frequently found. Phylogenetic analysis of the 4 TSWV variants showed similarity with strains from Italy and Turkey indicating a putative new introduction of the virus from plant material originating in these regions. Pepper production in the northwest of Spain uses traditionally planted pepper cultivars, seeds pass on over time and selected by each individual farmer. This generates a genetic pool in the pepper cultivars of the region. This genetic heterogenicity causes the plant response to be different among plants on different greenhouses. It has been observed that the incidence or number of plants affected in nearby greenhouses was different. These greenhouses used the same control strategies and agricultural methodologies. However, the pepper varieties were not the same as each farmer grow and selected their own seeds over time. High-throughput sequencing showed genetic divergence of the different pepper varieties present in the greenhouses. The sequencing data also showed that the plant response to the virus was different among these pepper varieties. Endonucleases and Argonaute related genes were higher express on pepper plants present in greenhouses with lower incidence. The genetic pool traditionally present in the region could be used as a tool to reduce the incidence of the virus using genetic markers to identify the genetically resistant pepper varieties.

Key words: TSWV, virus, pepper.

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TWO GENES, ONE HORMONAL PATHWAY, A MILLION MITES

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Abstract: In response to biotic stresses, plants activate specific processes of defence against either pathogens and/or herbivores. To survive, plants depend on a fast and specific defence response. One of the most devastating pests is *Tetranychus urticae*, a cosmopolitan species that causes substantial crop yield losses. With an extensive host plant range and an extreme record of pesticide resistance (Bensoussan et al., 2016). To fight against Two-Spotted Spider Mite (TSSM) attack, plant resistance comprises two main events: initially, the plant must detect the presence of the mite and then, formulate an appropriate immune response. For this purpose, specific transcriptional reprogramming events occur, which rely on the induction of disease resistance genes, also known as R genes (Santamaria et al., 2020a).
LRR domain proteins belong to R genes and transcriptomic analysis suggests that play a role in plant immune response against TSSM. Most LRR proteins have multiple functional domains however, an LRR subfamily presents a unique LRR domain (Ravindran et al., 2020). Nevertheless, the role of these single LRR-domain proteins in Arabidopsis remains still largely unknown. We here report the characterization of two differentially induced genes involved in the Jasmonic Acid (JA) defence route during TSSM feeding (Santamaria et al., 2020b): a single LRR domain protein (LRR1), and the JOX2, an enzyme that converts the active form of JA into inactive 12OH-JA (Smirnova et al., 2017). Characterization assays implying both organisms, have been carried out using T-DNA insertion lines of *A. thaliana*, and have contributed to demonstrate the repercussion of the lack of such genes. Besides, mite feeding on mutant plants causes the deregulation of *LRR1*, *JOXs* and other key JA route genes accentuating the importance of hormonal crosstalk in plant defence. This also has an important effect on mite fecundity and survival capacity.
The knowledge of these plant events and the genes involved in such processes will contribute to the development of strategies, which will help overcome certain agricultural threats posed by pests.

Key words: *Tetranychus urticae*, Jasmonic Acid, Hormonal crosstalk.

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DEALING WITH TOO CLOSE NEIGHBORS: AVOIDANCE OR TOLERANCE

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Abstract: Presence of competing neighboring vegetation is signaled in nature as changes in the ratio of red to far-red light (R:FR), which are perceived by the phytochrome photoreceptors. Plants have evolved two basic and divergent strategies to acclimate to vegetation proximity: avoidance and tolerance. Seedlings of the shade-avoider *Arabidopsis thaliana* respond by elongating their hypocotyls, in addition to other growth and development responses collectively known as the shade avoidance syndrome (SAS). By contrast, seedlings of the shade-tolerant *Cardamine hirsuta*, an *A. thaliana* relative, lack the promotion of hypocotyl elongation in response to shade and have developed a variety of traits to acclimate to low light conditions. This divergent hypocotyl trait makes this pair of species ideal for comparative studies. Indeed, as a way to understand the basis for shade tolerance, we have been carrying out comparative genetic and molecular analyses between *A. thaliana* and *C. hirsuta*. Using a range of forward and reverse genetics approaches, we reported that the lack of elongation in response to shade exposure in *C. hirsuta* is caused by the acquisition of mechanisms of suppression of this trait that involve the action of components already known to repress the shade-induced hypocotyl elongation in *A. thaliana*, such as phytochrome A (phyA), HFR1 and HY5. Our latest results will be presented.

Key words: Phytochromes, photoreceptors, shade avoidance, shade tolerance.

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ROLE OF *BRANCHED1B* IN POTATO CARBON ALLOCATION AND TUBERIZATION

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Abstract: The control of carbon allocation, storage and usage is critical for plant growth and development and is exploited for both crop food production and CO2 capture. Potato tubers are natural carbon reserves in the form of starch that have evolved to allow propagation and survival over winter. They form from stolons, below ground, where they are protected from adverse environmental conditions and animal foraging. We show that potato *BRANCHED1b* (*BRC1b*) acts as a tuberisation repressor in aerial axillary buds, which prevents buds from competing in sink strength with stolons. *StBRC1b* loss of function leads to ectopic production of aerial tubers and reduced underground tuberisation. *StBRC1b* promotes dormancy, ABA responses and reduced number of plasmodesmata in buds. This limits sucrose accumulation and access of the tuberigen factor SP6A to axillary buds. Moreover, the StBRC1b protein directly interacts with SP6A and blocks its tuber-forming activity in aerial nodes. Altogether these actions help promote tuberisation underground.

Key words: Solanum tuberosum, tuberisation, bud dormancy, TCP genes, BRANCHED1, sink strength.

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MTSUPERMAN CONTROLS THE NUMBER OF FLOWERS AND INFLORESCENCE ARCHITECTURE IN *MEDICAGO TRUNCATULA*.

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Abstract: Legumes have unique features, such as compound inflorescences and a complex floral ontogeny. Thus, the study of regulatory genes in these species during inflorescence and floral development is essential to understand their role in the evolutionary origin of developmental novelties. The *SUPERMAN* (*SUP*) gene encodes a C_2H_2 zinc-finger transcriptional repressor that regulates the floral organ number in the third and fourth floral whorls of Arabidopsis thaliana. In this work, we present the functional characterization of the *Medicago truncatula SUPERMAN* (*MtSUP*) gene based on gene expression analysis, complementation and overexpression assays, and reverse genetic approaches. Our findings provide evidence that *MtSUP* is the orthologous gene of *SUP* in *M. truncatula*. We have unveiled novel functions for a *SUP*-like gene in eudicots. *MtSUP* controls not only the number of floral organs in the inner two whorls, but also in the second whorl of the flower. Furthermore, *MtSUP* regulates the activity of the secondary inflorescence meristem, thus controlling the number of flowers produced. Our work provides insight into the regulatory network behind the compound inflorescence and flower development in this angiosperm family.

Key words: *Medicago truncatula, MtSUPERMAN,* flower development. Compound inflorescence development.

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NEW INSIGHTS INTO THE ROLE OF CELL WALL MODIFICATIONS INDUCED BY A-XYLOSIDASE1: THE IMPACT IN SEED AND FRUIT SIZE

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Abstract:
Cell wall modifications are of pivotal importance during plant development. Among cell wall components, xyloglucans are the most abundant hemicellulose in primary cell walls, and can connect the cellulose microfibril surface to affect cell wall mechanical properties. Changes in xyloglucan structure are known as the major factor regulating cell growth. Therefore, the degradation of xyloglucan is an important modification that alters the cell wall. The α -XYLOSIDASE1 (*XYL1*) gene encodes the only α-xylosidase acting on xyloglucans in *Arabidopsis thaliana*. Here, we show that mutation of *α-xyl1* strongly influences seed size, seed germination, and fruit elongation. We found that the expression of *XYL1* is directly regulated in developing seeds and fruit by the MADS-box transcription factor SEEDSTICK (STK). We demonstrate that *XYL1* complements the *stk* smaller seed

phenotype. Furthermore, using Atomic Force Microscopy (AFM) combined with cell wall composition analysis, we discuss the role of XYL1 activity and xyloglucan deposition in controlling tissue stiffness and growth. Overall we provide new insights into the current models for plant organ growth concerning the role of cell wall modulation in shaping organs.

Key words: Seed development, Fruit development, Cell wall, Transcriptional regulation, Xyloglucan, Cell growth, Atomic Force Microscopy.

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RECONSTRUCTION OF LATERAL ROOT FORMATION THROUGH SINGLE-CELL RNA-SEQ REVEALS ORDER OF TISSUE INITIATION

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Abstract: Post-embryonic organogenesis is critical for plant development.[1] Underground, lateral roots (LR) form the bulk of mature root systems, yet the ontogeny of the LR primordium is not clear.[2,3] Here, we use single-cell RNA-seq through the first four stages of LR formation in Arabidopsis.[4,5] Our analysis leads to a model in which a single group of precursor cells, with different cell identity from their pericycle origins, rapidly reprograms and splits into a mixed ground tissue/stem cell niche fate and a vascular precursor fate. The ground tissue and stem cell niche fates soon separate and a subset of more specialized vascular cells form sucrose transporting phloem cells that appear to connect to the primary root. We did not detect cells resembling root cap or epidermis, suggesting that outer tissues may form later preceding LR emergence. At this stage, some remaining initial precursor cells form the primordium flanks while the rest create a reservoir of pluripotent cells able to later replace the LR if damaged. Laser ablation of the main LR primordium regions shows that identity of remaining cells is reset to the initial precursor cell identity restarting the sequence of tissue initiation. Our analysis reveals an ontological hierarchy for LR formation with an early and sequential split of main root tissues and stem cells.

Key words: Arabidopsis, lateral root, organogenesis, single- cell RNAseq, stem cells.

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STOP THAT TOMATO PLANT! SEARCHING FOR MOBILE SIGNALS AND OTHER FACTORS THAT CONTROL GLOBAL PROLIFERATIVE ARREST.

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Abstract: Lifespan of monocarpic plants is determined by the coordinated arrest of all active meristems. This phenomenon is known as "Global Proliferative Arrest" (GPA). GPA, which is linked to unknown signals from fruits/seed, has been described for many species. In parallel, genetic factors related to age-pathways also appear to play a role in GPA. Some of these factors have been identified in *Arabidopsis thaliana* (Balanzà et al., 2018): *APETALA2-LIKE* (*AP2/AP2-like*) genes promote the activity of the stem cells of the shoot apical meristem, maintaining the expression of *WUSCHEL*. In turn, *FRUITFULL* (*FUL*), together with the microRNA-172 (miR172), repress the expression of *AP2/AP2*-like genes, which results in GPA. In addition, recent studies show that auxin exported from fruits triggers proliferative arrest, hinting to this hormone as the putative seed-derived signal or a component of a more complex message (Ware et al., 2020).
We hypothesize that the factors that control GPA should be conserved. Studies in tomato described GPA and linked it to the fruit/seed formation (Murneek et al., 1926), but almost no studies have been done to identify the factors that regulate GPA in tomato. We aim to fill this gap by re-evaluating the role of fruit/seed production, attempting to identify the fruit/seed-derived signals and by assessing the functional conservation of the FUL-AP2-WUS genetic pathway in tomato GPA. Our findings show that complete flower removal keeps the meristems active for a longer time. Moreover, once tomato plants reach GPA, meristems can be reactivated by removing the fruits. To identify the signals that trigger GPA in tomato, we developed an efficient method to collect fruit exudates. In these exudates, we identified phytohormones and mobile miRNAs that could be involved in meristematic arrest. In addition, we developed molecular tools to modulate the expression of the sly-miR172 and *FUL* homologues in tomato. This approach will help us assess the conservation degree of the age-dependent pathway between *Arabidopsis* and tomato, paving the way to generate biotechnological tools to obtain plants with a longer life cycle and thus increased crop yield.

Key words: Global Proliferative Arrest, fruit exudates, phytohormones, mobile miRNas

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A NOVEL REGULATOR INVOLVED IN ALLEVIATION OF DOG1-REGULATED DORMANCY

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Abstract: Seed dormancy is a trait that governs the temporal distribution of germination and bestows an evolutionary advantage. Physiological dormancy, the most common type, depends on hormonal regulation, mainly exerted by abscisic acid (ABA) and gibberellins (GAs) (Carrera-Castaño et al., 2020). Besides the hormonal control of dormancy, other pathways have been linked to this process, like those involving DOG1 (Nonogaki, 2019). By screening a seed library (Weiste et al., 2007; an extended version was used), we have identified Arabidopsis lines constitutively overexpressing a transcription factor, hereafter FDG (Feedback on Dormancy and Germination), with improved germination under conditions known to decrease GA biosynthesis. Conversely, loss-of-function mutants present a germination delay in non-after-ripened (AR) seeds, suggesting a role as a negative regulator of dormancy. However, *FDG* expression is higher in dormant seeds compared to AR and imbibed seeds and it is enhanced and repressed in *DOG1* gain and loss-of-function mutants, respectively. Moreover, *FDG* loss-of-function mutants show enhanced expression of *DOG1* and downregulation of genes promoting germination such as *EXPA2* (Sánchez-Montesino et al., 2019). Additional genetic and molecular evidence have revealed that *DOG1* is epistatic to *FDG*, the latter one binding to *DOG1* and *EXPA2* promoters, a function counteracted by DELLA proteins.

Altogether, our results suggest that FDG could be regulating dormancy levels through a feedback mechanism. The activity of this novel regulator seems to be linked to that of key negative regulators of seed germination with the goal of maintaining an appropriate balance between dormancy and germination.

Key words: Dormancy, Germination, DOG1, EXPA2, transcription factor.

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A THERMOSWITCH MODULATING STOMATAL DEVELOPMENT UNDER SUPRA-OPTIMAL TEMPERATURE

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Stomata are epidermal bi-celled valves that control leaf gas exchange and contribute to a correct organ refrigeration via evapotranspiration. In Arabidopsis, the stomata development is controlled by several regulators of a gene network modulated by environmental cues such supra-optimal temperature, which represses stomatal formation (Lau *et al.*, 2018). Among the key drivers of stomatal development is *SPEECHLESS* (*SPCH*), which encodes a bHLH-type transcription factor essential for the first asymmetric cell division of protodermal cells that initiates a stomatal lineage. Hypomorphic *SPCH* alleles decrease, but not abolish, stomata initiation, producing viable plants with very few stomata, amenable for physiological, developmental and molecular analysis. Several genes have been involved in plant anatomical thermomorphogenesis responses, albeit the information about how plant acclimation counteracts heat-induced repression of stomatal generation is scarce.

To study the molecular basis of stomatal development in response to supra-optimal temperatures, we exploited the weak mutant *spch-5*: characterized by extremely low number of stomata but normal growth, and by its sensitivity to alterations in the gene circuits involved in asymmetric cell divisions that initiate stomatal lineages (de Marcos *et al.*, 2017). Our *spch-5* mutant displays a stomatal density reduction in response to supra-optimal growing conditions similar to the wild type. We will present confocal image analysis of *SPCH* promoter activity using GFP reporter lines with different genetic backgrounds.

Key words: Stomata, Plant development, Supra-optimal temperature.

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ARABIDOPSIS ABCE2 LINKS RIBOSOME RECYCLING AND AUXIN HOMEOSTASIS

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Most ATP-Binding Cassette (ABC) proteins function as membrane transporters. However, the most evolutionarily conserved group within this family, the ABCE subfamily, comprises soluble proteins that are present in all eukaryotes and archaea. Structural and functional analyses of ABCE proteins from several species have shown that ABCEs recycle cytoplasmic ribosomes. To examine Arabidopsis ABCE2 function, we conducted a co-immunoprecipitation assay of an ABCE2:YFP fusion protein, and identified 20 putative interactors, 13 of which participate in translation or its regulation, suggesting a conserved role for Arabidopsis ABCE2 in ribosome recycling.

We isolated the *apiculata7-1* (*api7-1*) mutant, which carries a recessive and hypomorphic allele of the *ABCE2* gene, and has small, dentate and pointed rosette leaves, with aberrant venation patterns. To uncover the biological processes deregulated in *api7-1*, we performed an RNA-seq analysis. Consistent with the presence of two iron-sulphur (FeS) clusters in ABCE2, we observed increased responses to iron and sulphur deficiencies in *api7-1*, possibly triggered to produce more ABCE2 proteins to compensate their partial depletion. In agreement with a role for auxin biosynthesis, transport and signalling in leaf venation pattern formation, our RNA-seq results pointed to increased auxin biosynthesis in *api7-1* leaves. In addition, expression of the auxin efflux carrier PIN-FORMED1 (PIN1), and auxin perception were reduced and increased in *api7-1* roots, respectively. Taken together, our results suggest that perturbed auxin homeostasis in *api7-1* contributes to its abnormal leaf venation patterning.

Arabidopsis encodes two ABCE proteins, ABCE1 and ABCE2, that share 81% identity. We found that *ABCE1* and *ABCE2* expression levels were low and high in all studied organs, respectively. A promoter exchange assay between these two paralogs showed that $ABCE2_{pro}$*:ABCE1*, but not $ABCE1_{pro}$*:ABCE2*, restores the wild-type phenotype in *api7-1* plants, indicating functional redundancy between ABCE1 and ABCE2. A phylogenetic analysis of plant *ABCE* genes showed that a gene duplication event occurred before the divergence of Brassicaceae species, and that both copies were conserved. Our results suggest that the *ABCE1* genes evolved more rapidly than their *ABCE2* paralogs, while the latter conserved the ancestral function.

Key words: ABCE proteins, translation, ribosome recycling, leaf venation, auxin homeostasis

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ARABIDOPSIS THALIANA MUTANTS DEVOID OF CYTOSOLIC GLUTMINE SYNTHETASE 1;3 (GLN1;3) IS COMPENSATED BY CHLOROPLASTIDIAL GLUTAMINE SYNTHETASE (GLN2) IN LEAF TISSUE

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Abstract:
In *A. thaliana* GS is encoded by 6 isogenes family, five of which with cytosolic (GS1) location and a plastid one (GS2), all involved in a wide variety of physiological processes throughout plant life cycle. Chloroplast located Glutamine Synthetase (GS2) plays a major role in the reassimilation of ammonium in plants, and has been previously shown that its function can be partially replaced by the cytosolic *Gln1;3* and *Gln1;2*, both under photorespiratory and non-permitting photorespiratory conditions [1]. Possibly suggesting beyond the redundancy capability, a specific compensatory mechanism.
To go further into this compensatory mechanism present in *A. thaliana* GS gene family, mutant plants devoid of several GS1 isoenzymes were analysed by RT-PCR, in order to evaluate the expression of the GS2. It was shown that *gln1;1* mutants kept the Gln2 expression while *gln1.2* and *gln1;4* single mutants had the *Gln2* expression compromised, but the *gln1;3* mutant plants showed GS2 is upregulated. The results determine that GS2 has also the capability to compensate the cytosolic GS1;3 absence. This compensation mechanism was confirmed by assessing GS activity in *gln1;3* leaves and further confirmed by detection of GLN2 recurring to Western blot.
The results presented in this work endorse a role of GS2 in the assimilation of cytosolic ammonium, further supporting the existence of a compensatory mechanism between cytosolic and chloroplastic GS forms.

Key words: *Arabidopsis thaliana* mutant, cytosolic glutamine synthetase, chloroplastidial glutamine

synthetase, nitrogen assimilation.

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EFFECT OF MELATONIN ON THE VARIATION OF PHENOLIC COMPONENTS DURING POST-HARVEST RIPENING IN GOLDEN APPLE

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Abstract: Apple is a high nutritional value fruit rich in antioxidants including flavonoids, anthocyanins and polyphenols. The content and composition of polyphenols is of great interest in apples since they contribute to the sensory quality and increase its healthy properties. Melatonin is an indolamine widely distributed in plants. Among other functions, it acts as a powerful antioxidant able to scavenge free radicals and reactive oxygen species. Therefore, melatonin is considered a protective molecule in processes of high metabolic activity and production of free radicals, such as the ripening of fruits and seeds. In addition, melatonin interferes with the secondary metabolism of plants by affecting the content of phenolic compounds during ripening. Our study investigated the effect of a melatonin treatment for 35 days on the evolution of phenolic compounds and the polyphenolic profile in Golden apple during the postharvest ripening period. The study was performed on skin and pulp using HPLC-DAD. The results showed total amounts of phenols, flavonoids and anthocyanins in the skin higher than in the pulp. In both tissues, the content of total phenols decreased in the first 7 days of the postharvest period, while flavonoids and anthocyanins increased gradually until day 35. Regarding the polyphenolic profile, we have quantified up to ten different compounds in the skin and five in the pulp. High levels of chlorogenic acid, phloricin and epicatechin were measured in the skin and pulp of the freshly harvested apple, while procyanidin B2 levels in the skin increase after the start of ripening (days 7 to 30). Melatonin treatment increased the content of phenols and total flavonoids in the skin and pulp between days 7 and 30 post-harvest, these effects being noticeable in the pulp for anthocyanins. Regarding the polyphenolic profile melatonin had a general stimulating role, increasing the levels of epicatechin, procyanidin B2, o-coumaric acid and trans-cinnamic. These data suggest that melatonin has a stimulatory effect on the contents of phenols, flavonols, flavanols, and anthocyanins. Therefore, melatonin could contribute to increase the antioxidant capacity of the apple during the post-harvest period, opposing the deterioration of fruit properties and improving its final quality.

Key words: melatonin, phenolic compounds, antioxidants, ripening, apple

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ENHANCER OF DEAL1 (*EODL*), A SYNERGISTIC INTERACTOR OF *VASCULATURE COMPLEXITY AND CONNECTIVITY* (*VCC*), REGULATES LEAF SHAPE IN ARABIDOPSIS

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Loss of function alleles of Arabidopsis *VASCULATURE COMPLEXITY AND CONNECTIVITY* (*VCC*; also called *DESIGUAL1* [*DEAL1*]), perturb rosette leaf bilateral symmetry: the leaf margin of the *vcc* mutants shows randomly distributed lobes and sinuses, which makes the leaf bilaterally asymmetric VCC might play a role in the integration of auxin and cytokinin responses during the early stages of leaf development for the coordination of growth in the medio-lateral axes to maintain bilateral symmetry (Wilson-Sánchez *et al.*, 2018).

In the *vcc-2* mutant, leaf asymmetry is more pronounced in adult than in juvenile leaves, and in the basal than in the apical lamina region. Our outcrosses of the *vcc-2* mutant to a number of wild-type Arabidopsis accessions revealed the unexpected existence of *ENHANCER OF DEAL* (*EODL*); this modifier gene substantially increases expressivity and penetrance of the mutant phenotype of *vcc-2*. Stems of the *vcc-2 eodl* putative double mutants also show altered phyllotaxy. Segregation of these phenotypes in the progeny of our outcrosses suggested that the accessions crossed share a spontaneous, dominant *eodl* allele. The mapping-by-sequencing and genome-wide association study approaches that we took led us to delimit a region in chromosome three, candidate to harbor *EODL*. Identification of *EODL* is of utmost interest for the understanding of the molecular mechanisms that establish leaf bilateral symmetry and stem phyllotaxy, and will provide further insight into the implication of auxins and cytokinins, and their interplay, in the establishment of plant leaf bilateral symmetry.

Key words: Arabidopsis, leaf margin, auxin, VCC, symmetry

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EVALUATION OF AUXIN-MEDIATED REGULATORY MECHANISMS INVOLVED IN SCION-ROOTSTOCK INTERACTIONS IN *Prunus dulcis* Mill. MICROGRAFTS

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Almond demand and production has increased in recent years due to its high nutritional value, benefits for human health and a strong tendency of consumers towards plant-based products. In Portugal, almond is traditionally cultured in the regions of Algarve, Trás-os-Montes and Alto Douro. Recently new orchards have emerged in other regions such as Beira Interior and Alentejo. Cultivated almond varieties are usually grafted to increase production and improve fruit quality. Grafting allows a cross-talk between the scion and rootstock, which involves not only nutrients but also signal molecules (e.g., hormones, proteins, small RNAs), thus affecting both stem and root growth, as well as agronomic performance. During grafting, the plant vasculature is severely damaged. However, once the scion is in contact with the rootstock, a complex process involving cell division and differentiation of vascular tissues establishes communication between the two entities. Since auxin transport (polar and vascular) is also interrupted during grafting, the auxin IAA (indole-3-acetic acid) and the expression of auxin-responsive genes are crucial to control the differentiation of the new vascular tissues. However, grafting is not always successful, and the mechanisms controlling scion-rootstock interactions remain largely unknown. To better understand grafting in P. dulcis physiological and histological aspects and auxin-responsive genes expression regulatory network were analysed in micrografts. To achieve this goal, protocols for in vitro establishment, shoot proliferation, rooting and micrografting were developed and optimized for scions from commercial and Portuguese traditional varieties, and for selected rootstocks. The results obtained showed that the best shoot proliferation was achieved on MS medium supplemented with 3% (w/v) sucrose, 0.7% (w/v) agar and 4.4 μM BAP (6-benzilaminopurine). Micrografts were established following a combinatory experimental design using scions from commercial and traditional varieties and rootstocks from seedlings of bitter almond and commercial rootstocks previously established and micropropagated. Sampling was made from the initial explants (t0) and the established micrografts at 2 time points, 1 week (t1) and 3 weeks (t2) during graft recovery, and samples treated for immunohistochemistry or fast-frozen for RNA extraction and IAA quantification. Auxin detection and quantification data will be correlated with the expression of auxin-related genes (AXR1, TIR1, IAA26 and ALF4), previously referred in literature for their role in micrografting success (Melnyk et al., 2015).

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EXPRESSION PROFILE OF WALL ASSOCIATED KINASE RECEPTORS IN COMMON BEAN

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Abstract: Wall Associated Kinase receptors (WAKs) are transmembrane proteins that function as sensors of the cell wall (CW) integrity through pectin state. When long pectin chains are detected by WAKs during development, cell expansion is promoted. However, during infection, pathogens produce pectin methyl-esterases and polygalacturonases, which degrade pectins into oligomers. As a consequence, growth is disrupted, and defense is activated [1]. In common bean, 6 different WAKs have been identified *in silico*, from which PvWAK1 could participate in perceiving the bean pathogen *Pseudomonas syringae* pv. *phaseolicola* (Pph) at early stages of infection, showing that bean could perceive the pathogen through a protein involved in "pectin state sensing" [2].
To further characterize the common bean WAKs function, a study of their expression profile was carried out in different tissues, such as meristems or leaves, at different development stages, from embryo to V3— when first true leaves were developed. For this purpose, RNA for each tissue and growth phase was isolated, retrotranscribed and then analyzed by q-RT-PCR.
As a result, *PvWAK2* showed the highest expression level in developing tissues, such as meristems and embryos, while it tended to decrease during the development, indicating a putative role in elongation. Interestingly, *PvWAK1*, previously described as involved in Pph infection, was not detected in meristems and embryos, but it was the most expressed in leaves. Considering all these results, this study sets the new steps into further research in the field of plant receptor genes, and their role in defense and growth processes, important in the tradeoff balancing yield and defense [3].

Key words: wall-associated kinases, common bean, growth, defense, Pseudomonas, cell-wall, pectin.

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Abstract: Glutamine synthetase (GS) is a crucial enzyme in plant metabolism, catalysing the assimilation of inorganic nitrogen into glutamine. It is considered a key enzyme in plant growth and has received special attention to improve nitrogen use efficiency and crop yield. In plants GS occurs as a collection of isoenzymes, with cytosolic (GS1) and plastid (GS2) location, involved in a wide variety of physiological processes throughout plant life cycle.
In A. thaliana GS1 is encoded by 5 isogenes and this work is devoted to the two most expressed genes in seeds, *Gln1;3* and *Gln1;5*. Transgenic marker line, expressing reporter-fusion proteins, revealing the expression pattern of these genes during seed development and germination. RT-qPCR expression studies in both single mutants revealed a compensatory mechanism, denoting an enzyme family redundancy. The single mutants showed only minor phenotype alterations and the GS activity in mature seeds, is only altered in the *gln1;3* mutants.

The double mutant *gln1;3/gln1;5*, still under scrutiny, shows that the absence of both genes is irreplaceable in the early germination and plant growth, leading to a delay in germination and plant development, having its seeds the GS activity compromised.
Glutamine synthetase seed related data is still scarce, particularly on *Gln1;5* and *Gln1;3* genes. Therefore, this research project helps to understand the GS importance during seed development, contributing for a future improvement of nitrogen use efficiency and seed quality.

Key words: *arabidopsis thaliana,* glutamine synthetase, nitrogen metabolism, seeds

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HIERARCHICAL CLUSTERING OF DNA HISTOGRAMS IMPROVES THE ANALYSIS OF CELL CYCLE IN MAIZE ROOT

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Abstract: Root architecture is the result of primary root elongation which provides new segments along its main axis where lateral roots are developed. Root elongation requires cell divisions in the meristematic zone and cell elongation in the elongation zone. The boundary between dividing and elongating cells is called the transition zone. In the meristem zone, initial cells are continuously dividing, but on the basal side of the meristem cells exit the meristem through the transition zone and enter in the elongation zone, where they stop division and rapidly elongate. Throughout this journey cells are accompanied by changes in cell cycle progression. Temperature controls root elongation: a decrease from 30°C (optimal temperature) to 20°C causes a 50% reduction. Maize primary root can be divided in several zones according with the processes that take place in each: meristem comprises from root cap junction to 1,5 mm from the root apex; transition zone from 1.5 to 3 mm, elongation zone from 3 to 8 mm; and finally, far away to 8 mm, cell length does no increased in the differentiate zone. Changes in cell cycle have been previously analysed at both temperatures along the entire primary root.
In this communication, we present a new hierarchical clustering analysis of DNA histograms obtained by flow cytometry. This approach improves the discrimination between similar samples. Interestingly, although the meristem and transition zones depict completely different cell cycle parameters, temperature does not significantly affect the distribution of cells among the different phases of the cell cycle. The analysis separated elongation and differentiation zones of roots grown at 30 and 20C; zones away 3 mm from the root apex are grouped by temperature rather distance to the meristem. Contrastingly, cells in meristem and transition zone, located at 0-3 mm away from the root apex, were clustered by distance.

Key words: root architecture, cell cycle, hierarchical clustering, flow cytometry
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HOW ARE DGK1 AND DGK2 INVOLVED IN MEMBRANE CONTACT SITES?

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Abstract: Eukaryotic cells have regions of interaction between two organelles where some proteins, which act as tether, bring both membranes closer (10-30 nm) without fusion, named membrane contact sites (MCS). Two organelles that can form MCS are endoplasmic reticulum (ER) and plasma membrane (PM). ER-PM CS play important metabolic functions such as communication between both membranes, lipid homeostasis and Ca^{2+} influx. Our group has identified that AtDGK1 and AtDGK2 (Diacylglycerol kinase 1, AT5G07920 and Diacylglycerol kinase 2, At5g63770) form a complex with a well-known protein located at ER-PM CS, Synaptotagmin1 (SYT1, At2g20990).
Upon perception of stress, phospholipase C (PLC) is activated at the plasma membrane to hydrolyse PIP(4,5)P2 or PI4P in order to generate DAG and inositol phosphates. Diacylglycerol (DAG) is phosphorylated by diacylglycerol kinases (DGKs) to produce phosphatidic acid (PA). DAG and PA are important cell signalling molecules. There are seven DGKs encoded in Arabidopsis thaliana genome, but only DGK1 and DGK2 have a transmembrane domain that anchors them to the endoplasmic reticulum, the rest are cytoplasmic. DGK1 and DGK2 appear to play a role in stress response as both are induced by exposure to low temperatures and wounding. Also, we found that *dgk2* knockout mutant produces lower resistance to freezing. Using confocal microscopy, we have analysed the subcellular localization of these two proteins and investigated their interaction with SYT1 and between them using FRET and co-immunoprecipitation studies. Additionally, we report that the mutation of DGK1 is lethal in homozygosity. Our studies suggest that DGK1 and DGK2 act in concert with SYT1 to regulate the production of PA at ER-PM CS and highlight the importance of these proteins for the correct response to stress tolerance.

Key words: Membrane Contact Sites, Synaptotagmins, Stress.

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IDENTIFICATION OF THE BradiGIGA: A NEW MUTANT LINE WITH ALTERED LIFE CYCLE AND BIOMASS PRODUCTION

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Abstract: Cereals are the most important staple food crop of the world for humans and feeding of domestic animals, but their harvesting has to be doubled in order to feed the ~9 billion worldwide population expected in 2050. A key objective in this direction is the development of new crop varieties with higher energy efficiency, biomass production and optimal grain yield. *Brachypodium distachyon* is a model species for the genetics and molecular genomics of cereals, due in part to its small, sequenced genome (~355 Mbp), short life cycle, self-fertility, diploidy and its close phylogenetic relationship with important crop plants of the tribe Triticeae within the Poaceae family, such as wheat and barley (*International Brachypodium Initiative*, 2010; González-Calle et al., 2015).

In this work, the T-DNA insertion mutant *BradiGIGA* is phenotypically and genetically characterized (Bragg et al., 2012). *BradiGIGA* plants show important modifications in plant development and biomass production compared to wild type individuals (*wt*: *Bd-21*). These plants grow up to ~1m of height (*wt*: 25 cm), live for more than 120 days (*wt*: 60 days), and produce a higher number of stems, spikes, and seeds than the *wt*. *BradiGIGA* seedlings show ABA hyposensitivity and are more tolerant to salt stress than those of the *wt*. A combined strategy of genomic DNA digestion and inverse PCR allowed the cloning of a single T-DNA insertion in the genome of the *BradiGIGA* mutant. Expression analysis by quantitative PCR showed important changes in the expression of the gene affected by this insertion in support of its participation in plant biomass production.

Key words: *Brachypodium distachyon,* Plant biomass, Plant growth, Salt stress

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MAPPING-BY-SEQUENCING OF NOVEL *DENTICULATA* GENES AND THEIR GENETIC INTERACTIONS WITH *ASYMMETRIC LEAVES 1* AND *2*

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In Arabidopsis, the *ASYMMETRIC LEAVES 1* (*AS1*) and *AS2* genes encode transcription factors involved in leaf dorsoventral axis specification, and synergistically interact with some genes encoding proteins involved in translation (Pinon *et al*., 2008; Yao *et al*., 2008). The *denticulata* (*den*) mutants exhibit small, serrated and pointed leaves, traits that make them candidates for carrying mutations in genes that encode proteins involved in translation (Berná *et al*., 1999; Horiguchi *et al*., 2011). We used next generation sequencing of F_2 mapping populations and the Easymap v.1 software (Lup *et al*., 2021) to identify the *den1*, *den4*, *den7*, and *den11* mutations. None of the candidate mutations found for the *den1* mutant seems to be related to translation. Conversely, the best candidates to be the *DEN4*, *DEN7* and *DEN11* genes that we found encode a putative translation initiation factor, the ribosomal protein RPL34B, and a ribosomal protein of the S8e family, respectively. To confirm that the loss of function of these genes causes the phenotypes under study, we constructed transgenes carrying their wild-type alleles, which had been transferred into *den* mutant plants. We are taking a similar approach to identify the causative mutations of the phenotypes of the *den9*, *den14*, *den15* and *den16* mutants.

One of the mutations that we used to obtain *as1 den* double mutants is *as1-14*, which we obtained by fast neutron bombardment of wild-type Landsberg *erecta* (L*er*) seeds and whose molecular nature had not been previously determined. PCR amplifications using nested primer pairs and Sanger sequencing of the amplification products allowed us to demonstrate that the *as1-14* mutation is a 5.880-bp deletion that spans part of the *AS1* gene and the whole *ACTIN1* (*ACT1*) and *SIAMESE-RELATED12* (*SMR12*) adjacent genes. We constructed transgenes harboring wild-type alleles of each of these genes, and obtained insertional mutants from public collections as well, in order to confirm that the mutant phenotype of *as1-14*—and its genetic interactions—are only due to the loss of function of *AS1*.

Key words: DEN, AS1, leaf dorsoventrality, mutation mapping.

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MUTE-dependent molecular transitions in stomatal development

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Abstract: Stomatal development in *Arabidopsis thaliana* begins with the asymmetric division of a protodermal cell, which produces the first recognizable cell-type of a stomatal lineage, the meristemoid. After a variable number of asymmetric cell divisions, the meristemoid loses its self-renewing character and differentiates into a Guard Mother Cell (GMC). This GMC irreversibly commits to a symmetric division followed by a differentiation process that generates the two guard cells (GCs) that constitute the stoma and terminate the lineage.
To date, *MUTE* is the only identified gene essential for the meristemoid-to-GMC transition. *MUTE* encodes a basic helix–loop–helix (b-HLH) transcription factor, specifically and transiently expressed in late meristemoids, and thence it might be involved in the cell differentiation processes that terminate asymmetric division of meristemoids and commit them to form a stomata.

To better understand the molecular mechanisms underlying MUTE action in this cell-fate transition, we generated several MUTE versions, carrying changes in the b-HLH domain predicted to compromise the DNA-binding capacity of the MUTE protein. These mimic partial loss-of-function alleles identified for *SPEECHLESS* (de Marcos et al., 2017), the closest paralog of MUTE, which decrease stomatal production while rendering viable and fertile plants. We will present the epidermal and molecular phenotypes of *mute-3* total loss-of-function lines complemented with each of three MUTE versions. Our results show that the b-HLH MUTE mutants retain partial MUTE functions, allowing for the completion of stomata differentiation and rendering viable and fertile plants that allow further inspection of the transcriptional consequences of partial loss of function of the MUTE protein.

Key words: Key word 1, Key word 2, Key word 3 (3 to 5 key words)

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OPTIMUM GROWTH AND QUALITY OF THE EDIBLE ICE PLANT UNDER SALINE CONTITIONS

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Abstract:
Ice plant, *Mesembryanthemum crystallinum* L., is an halophyte plant well known for its antioxidant activity (Hanen et al., 2009) and being a highly functional food (Agarie et al., 2009). In addition, it is capable of increasing the content of health-promoting compounds when it is subjected to certain stresses such as salinity (Agarie et al., 2009). This is particularly important nowadays, since soil salinity is a major global issue (Shahid et al., 2018). Therefore, the objective of this work was to determine the best growth conditions to achieve both an optimal production of bioactive metabolites and high crop yield. Therefore, seedlings of *M. crystallinum* were grown in a greenhouse under semi-controlled conditions. Four saline treatments were compared: 0, 100, 200 and 300 mM NaCl. The results indicated that a moderate salinity treatment (100 mM NaCl) induced a slight increase in shoot DW and an enhancement of leaf area. However, with higher salinity levels there was a significant decrease in shoot biomass without significant reductions in leaf area comparing with the control treatment. It was also observed that the concentration of starch and total proteins decreased as the concentration of salt increased. However, the content of TSS was reduced in treatments of 100 and 300 mM NaCl and proline increased in conditions over 100 mM NaCl. On the other hand, plants grown with 300 mM of NaCl had the greatest values of leaf glutathione, ascorbic acid and vitamin C, while the opposite occurred with dehydroascorbic acid and carotenoids. Moreover, antioxidant enzymes activity and total phenolics rose together with the salinity severity. According to these results we evidence that ice plant specially accumulate health-promoting compounds under salinity conditions of 300 mM NaCl. In contrast, this salinity conditions were significantly detrimental to plant growth. However, a moderate concentration of salinity (100 mM NaCl) not only improved the concentration of bioactive and antioxidant compounds, but also preserved the crop yield. This could be particularly interesting to promote the cultivation of this plant of high nutritional value in moderate salinity environments.

Key words: *Mesembryanthemum crystallinum*, salt stress, antioxidant properties, yield.

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POLYAMINE CONTENT AND GENE EXPRESSION DURING THE DIFFERENTIATION OF GRAPEVINE (*VITIS VINIFERA* L., CV. MENCÍA) SOMATIC EMBRYOS

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Abstract: Somatic embryogenesis is a technology currently available for a number of grapevine (*Vitis vinifera* L.) cultivars, with many applications including the isolation of natural somatic mutants or functional genomics through genetic transformation or mutagenesis. Despite this, one of its main limitations is the abnormal and/or incomplete development and maturation of somatic embryos that occurs during their culture in differentiation medium, observed as processes of precocious germination that reduce the rates of normal plant conversion. This was successfully avoided in our lab by culturing grapevine cv. Mencía embryogenic aggregates over a semipermeable membrane extended on top of the differentiation medium. The results suggested that some degree of water deficit and the involvement of abscisic acid (ABA) metabolism contribute to the correct maturation of the somatic embryos.

The existence of a co-regulation between the metabolisms of ABA and polyamines have been proposed, mainly in several stress conditions. The goal of this work was to study the involvement of polyamines in the differentiation of the aggregates and the maturation of the derived somatic embryos. We present the endogenous levels of polyamines and the expression pattern of several genes involved in their metabolism during the differentiation of grapevine cv. Mencía somatic embryos in DM1 medium with or without a semipermeable membrane.

The endogenous polyamine content in the somatic embryo aggregates increased with time of culture in differentiation medium, due to increase of the free polyamine fraction, mainly of putrescine and being higher in the aggregates cultured over the semipermeable

membrane. The free putrescine level showed a significant peak in the second week of culture of the aggregates over the semipermeable membrane, coinciding with a high relative expression of the *VvADC* putrescine synthesis gene. Another significant peak of putrescine levels was observed after 5 weeks of culture in these conditions. As *VvADC* was not expressed by that time, the results suggest that the maintenance of free putrescine levels can be supported by the decrease of conjugated putrescine and to spermidine degradation via PAO (polyamine oxidase) gene expression. With the information obtained, we discuss the relationship between the metabolisms of ABA and polyamines in this experimental system.

Key words: Grapevine, Somatic embryogenesis, Polyamines, Somatic embryo maturation, Semipermeable membrane

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POSTEMBRYONIC MORPHOLOGICAL PHENOTYPES CAUSED BY NOVEL VIABLE ALLELES OF ARABIDOPSIS *KEULE*

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Plant cell cytokinesis starts at the centre of the cell division plane with the fusion of vesicles that transport cell wall material, generating the cell plate, which expands radially until its fusion with the parental cell wall at the preprophase band. Such vesicle fusion process occurs in three steps, in which two membranes are first anchored to each other by a loose interaction (tethering), then get closer due to tighter interactions (docking), and are finally fused (fusion). The docking step is carried out by SNARE trans-complexes, assisted by Sec1/Munc18 (SM) proteins through their interaction with the Qa-SNARE component of the complexes. In plant cytokinesis, fusion of membrane vesicles requires the SNARE protein KNOLLE and the SM protein KEULE (Assaad *et al.*, 1999; 2001).

In a genetic screen for mutations induced by ethyl methanesulfonate causing leaf abnormalities in Arabidopsis, we isolated the *serrata4-1* (*sea4-1*) and *sea4-2* mutants, and these mutants have serrated rosette leaves (Berná *et al.*, 1999). Through mapping-by-sequencing (Lup *et al.*, 2021), we found that *sea4-1* and *sea4-2* are novel recessive, hypomorphic alleles of the *KEULE* gene, which is already known for its essential role in plant embryonic development. The *sea4-1* allele carries a point mutation at the splice donor site of its 9th intron; this intron is not removed in most cases, which in turn renders a mixture of truncated and wild-type protein isoforms. The *sea4-2* mutation is predicted to cause a Ser57→Leu substitution in the SNARE interaction domain.

Although all previously studied alleles of *KEULE* are seedling lethal, homozygous *sea4-1* and *sea4-2* plants are viable and fertile. These mutant plants have smaller rosettes and fewer leaves at bolting time than their wild type. The leaves of *sea4-1* and *sea4-2* are reduced in size and undulated, have increased venation pattern complexity, develop necrotic patches, and undergo premature senescence. The *sea4* mutants allow us to examine the roles of KEULE in postembryonic development, with a focus on rosette leaf whole organ and margin patterning.

Key words: KEULE, SNARE, SM proteins, leaf margin patterning

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RECEPTOR LIKE PROTEIN KINASE 2 CONSTRAINS PROXIMAL SECONDARY VEIN BRANCHING IN THE *ARABIDOPSIS* EMBRYO

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Abstract: Over time, plants have evolved flexible self-organizing patterning mechanisms to adapt tissue functionality to continuous organ growth. An example of this process is the multicellular organization of vascular cells into conductive channels in foliar organs of *Arabidopsis thaliana* (L.) Heynh such as cotyledons. The establishment of a closed vascular network is achieved through the coordinated specification of newly recruited procambial cells and their proliferation and elongation. An important component of this process is secondary vein branching; a mechanism employed to extend vascular tissues throughout cotyledons' surface. Here, we show that distal veins of embryonic cotyledons arise from the bifurcation of cell files contained in the midvein. Instead, proximal veins emerge from the division of pro-vascular cells, a process constrained by RECEPTOR LIKE PROTEIN KINASE 2 (RPK2) and independent of auxin transport. Using genetic and live imaging analyses, we show that RPK2 function is antagonized by COTYLEDON VASCULAR PATTERN 2 and CVP2 LIKE1. Our work supports a model in which RPK2 expression at cotyledon margins prevents branching of secondary proximal veins to adapt vein complexity to organ growth.

Key words: Receptor-Like Kinase, CLE peptides, Secondary Vein Branching, Cotyledon, Auxin

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REVERSIBLE S-NITROSATION OF BZIP67 REGULATES FATTY ACID COMPOSITION DURING EMBRYO DEVELOPMENT

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Abstract: Nitric oxide (NO) is an essential gasotransmitter able to regulate a plethora of developmental processes and stress conditions (Sanz et al., 2015; Albertos et al., 2016, 2021; Sánchez-Vicente et al., 2019). The NO-related modulation is tailored by specific posttranslational modifications that affect protein physiological characteristics (i.e. stability or activity). *S*-nitrosation of cysteine (Cys) residues is a key modification, described to be reversible by an enzymatic process called denitrosylation (Kneeshaw et al., 2014).

Here, we focus on the mechanisms by which NO controls the correct early plant development, including embryo maturation, seed dormancy and germination. Our previous results determined the prominent role of NO promoting seed germination through the *S*-nitrosation of the abscisic acid (ABA) master regulator ABI5, that leads to its degradation in a proteasome dependent manner (Albertos et al., 2015).

Our research deciphers an important redox network, which impacts on fatty acid composition during seed development, modulating the stability of the basic/leucine zipper transcription factor bZIP67. The main function of this TF was previously described during seed lipid storage regulation (Mendes et al., 2013).

The results presented here highlight the biological significance of SNO-bZIP67, which reversibility depends on the redoxin PRXIIE, finally controlling the correct seed fatty acid accumulation. In addition, this TF is susceptible to be modified by nitro-fatty acids, described as important signaling biomolecules that connect NO and fatty acid metabolism (Mata-Pérez et al., 2016). We analysed deeply the crosstalk between NO and fatty acids by

using molecular and pharmacological approaches, pointing to key mechanisms for bZIPs reversible regulation and improving the knowledge on NO molecular targets.
Besides, it has been recently published that bZIP67 accumulation is influenced by cold conditions (Bryant et al., 2019). By using transgenic lines overexpressing bZIP67 and bZIP67w/oCys, we highlight the relevance of Cys in bZIP67 accumulation during plant acclimation to different temperatures.

Key words: nitric oxide, bZIPs, fatty acids, redoxins, embryo

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ROLE OF MELATONIN IN POST-HARVEST RIPENING IN APPLES. INTERACTION WITH ETHYLENE SYNTHESIS

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Abstract: Fruit ripening is a complex process that involves a series of physiological, biochemical and organoleptic changes, which determine the characteristics of the ripe fruit. This process is regulated at both the gene and hormonal level, and ethylene has been shown to play a critical role in ripening of climacteric fruits. These fruits are able to ripe after they have been removed from the tree, and the onset of this process is advanced by exogenous application of ethylene. Melatonin is an indolamine widely presents in plants, acting as a powerful antioxidant. In addition, a role of melatonin in regulating growth and development processes has been proposed in several fruits. Apples are among the most cultivated climateric fruits, and the presence of melatonin has been consistently reported. However, few studies have focused on the role that melatonin might have in the ripening process in apples. This study aimed to investigate the effect of treatment with melatonin during 35 days on the post-harvest maturation of apples of the Golden variety. The production of ethylene and the differential expression of genes coding for key enzymes of ethylene synthesis, such as *Md*ACS1 and *Md*ACO1, were determined both in the skin and in the pulp of apples. Our results showed that melatonin stimulated and advanced ethylene production, therefore altering the temporality of the climacteric peak of the hormone. Likewise, melatonin caused a differential increase in the gene expression of *Md*ACS1 and *Md*ACO1 in the skin of the fruit during the climacteric ethylene peak (days 15 to 25 post-harvest). The effects of melatonin in the pulp were less consistent, stimulating the expression of *Md*ACS1 (day 25) without clear effects on the other genes studied. These results suggest that melatonin plays a role in regulating the ripening process in the Golden apple, mainly through its interaction with the synthesis of ethylene. Thus, melatonin enhances the expression of key genes of the ethylene biosynthesis pathway, promoting and advancing the production of this hormone, with a subsequent stimulatory effect on apple ripening.

Key words: ethylene, melatonin, ripening, apple

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SlTDP1 IS REQUIRED TO SPECIFY TAPETUM IDENTITY AND FOR THE REGULATION OF REDOX HOMEOSTASIS IN TOMATO ANTHERS

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Abstract:
Tomato is an important crop and a model species for fleshy fruit plants. Fruit set starts after pollination and fertilization, which makes pollen development a key step in fruit formation. Increasing unfavorable environmental conditions reduce pollen viability and consequently fruit and seed production. Thus, studying the genes controlling pollen development can give insight into this process and provide biotechnological tools to increase crop production. We used information generated in *Arabidopsis* (Yang et al., 2003), to identify the tomato *TAPETUM DETERMINANT 1* (*Solanum lycopesicum TPD1, SlTPD1*) homolog, which encodes a small protein ligand necessary for the correct development of the tapetum. Using *in situ* hybridization, we showed that *SlTPD1* transcript was present in the L2 cells of anther primordia and expression continues until later stages in the tapetum and pollen grains. Then, several mutant lines were produced using the CRISPR/Cas9 technology in the commercial tomato cultivar Moneymaker. Histological sections of mutant anthers revealed the absence of tapetum and an over-proliferation of microsporocyte cells that, at late stages, degenerated and collapsed inside the anther locules. In consequence, mutant plant were male sterile although, occasionally, they produced small, seedless fruits. RNA-Seq analysis conducted in wild-type and mutant anthers from an early stage, revealed differential expression of several reactive oxygen species (ROS)-related genes. Interestingly, the expression of several of these genes was significantly downregulated in *Sl-tpd1* mutants. Furthermore, the formation of ROS, determined by DAB staining, is lower in the mutant than in wild-type anthers. We conclude that *SlTPD1* is required for tapetum differentiation and for the regulation of redox homeostasis during anther development in tomato.

Key words: pollen, development, tapetum, redox, tomato (3 to 5 key words)

Acknowledgments: This work was funded by research grant RTI2018-094280-B-100 from Spanish Ministry of Science, Innovation and Universities.

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THE GAMETOPHYTE OF FERNS HOLDS A WIDE PROTEOMIC ARSENAL TO FACE ENVIRONMENTAL CHALLENGES

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Abstract: Ferns represent an unexplore clade of plants, hidding valuable information to deepen on plant development. In particular, the gametophyte of ferns are free-living organisms, which can be cultured *in vitro* easily and being a good experimental system to carry out deeper analyses (Grossmann et al. 2017, Rivera et al. 2018). In this work, a proteomic approach was done using gametophyte of two relative ferns: the apogamous species *Dryopteris affinis* ssp. *affinis* and its sexual relative *D. oreades.* For this purpose, apogamous and female gametophytes derived from spore, were cultured on MS medium (Murashige and Skoog 1962), and then sampled for protein extraction The proteomic profiles revealed a total of 879 proteins (Wyder et al. 2020) and, after annotation, 418 proteins were found to be in common, which are deemed here. Most of the proteins are located in the cytoplasm, chloroplasts and nucleus; they are engaged in enzymatic, binding and structure activities in the cell, and in the body in primary metabolism activities, response to stimuli and transcription. Gametophytes of *D. affinis* and *D. oreades* may have biotechnological applications in agriculture through transgenesis to improve their resistance to high temperatures, lack of water, increased carbon dioxide and ultraviolet B radiation or bacterial infections.

Key words: fern, gametophyte, proteomic

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THE ROLE OF DEVIL PEPTIDES IN CONTROLLING CELLULAR PROLIFERATION AND PLANT MORPHOGENESIS

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Abstract: *DEVIL (DVL)* genes have been identified in Arabidopsis and other plant species. They encode small size peptides of unknown function, belonging to a gene family of 24 members in Arabidopsis, and are associated to the plasma membrane. *DVL* genes were characterized by their overexpression phenotypes, which dramatically affect fruit morphology (Wen *et al.* 2004; Narita *et a*l. 2004; Guo *et al.* 2015). However, the loss-of-function mutants do not have any obvious morphological phenotype, so the biological function of these peptides is essentially unknown.

In the lab we have carried out different studies to characterize how DVL peptides interact with the plasma membrane, how are they expressed and accumulate, to identify other proteins that interact physically with these peptides, and to study whether the combination of multiple loss-of-function mutants affects development. Our preliminary results suggest that DVL peptides appear to be mobile signals not integrated in plasma membrane, that participate in the control of cell cycle and cell division processes. We are currently extending our work in these directions, generating combinations of multiple T-DNA mutants and mutants generated by CRISPR/Cas9 technology, in different *DVL* genes (*dvl1dvl4rtfl9dvl8dvl11rtfl11dvl19*). These combinations do not show obvious loss of function phenotypes, suggesting a high functional redundancy among members of *DVL* family (Wen *et al*. 2004). Therefore, we have also generated mutants for multiple target genes in only one construction by CRISPR/Cas9 (Wang *et al.* 2015) to obtain higher order combinations of *DVL* genes mutants and observe any possible morphological phenotype.

Due to the lack of a visible phenotype, we performed a transcriptomic analysis of a quintuple *DVL* mutant. The RNA-seq results showed pollen tube development and growth-related genes significantly altered.

In addition, to confirm and better understand the differences in spatial distribution of mRNA and peptides for DVL1 and DVL8, we have generated pDVLx::DVLx:GUS and

promoter:reporter lines to try to determine whether this differential distribution is due to peptide active transport. We are also starting to functionally validate the possible protein interactions uncovered in a yeast-2-hybrid screening. These and other preliminary results will be presented, along with possible future directions.

Key words: *Arabidopsis thaliana*, *DEVIL* genes**,** Small peptides.

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UNCOVERING THE DEGREE OF CONSERVATION OF THE GPA PROCESS BETWEEN ARABIDOPSIS THALIANA AND PISUM SATIVUM.

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A big proportion of the economically important crops belong to the group of monocarpic plants. In these, after the production of a certain number of fruits, the activity of the Shoot Apical Meristem (SAM) and of the meristems of the secondary axes stop in a coordinated way, leading to the cessation of flower production. This phenomenon is called Global Proliferative Arrest (GPA) and it has been interpreted as a mechanism to optimize the channeling of available resources towards seed production. Also, it has been described in numerous species, so it can be assumed that it is a general phenomenon in monocarpic plants (Hensel et al., 1994, Lockhart et al., 1961, Murneek et al., 1926 and Nooden et al., 1990).
Studies in the model species *Arabidopsis thaliana* have allowed the identification of factors that affect GPA timing. One of them is the production of fruits and seeds: sterile mutants produce more flowers than fertile ones (Nooden et al., 1990 and Landrein et al., 2018) and pruning fruits can delay GPA or induce reactivation (current work in the laboratory, Ware et al.,2020). In addition, we have recently proposed a genetic pathway that controls GPA: FRUITFULL (FUL) and miR172 regulate the levels of APETALA2 (AP2) and AP2-LIKE genes in the SAM, which finally controls SAM arrest (Balanzà et al., 2018). Recent studies working with the legume species *Pisum sativum* (pea) indicate that the mechanisms that control GPA could be very similar to those of *Arabidopsis thaliana* (Martínez-Fernández et al., 2017), and we are currently working to better characterize the differences and similarities that exist in this process between both species. For that aim, we have carried out different types of physiological studies to assess the impact of fruit and seed production in pea varieties and investigated the transcriptomic response of the pea inflorescence meristems in different stages, parallel to those already characterized in Arabidopsis.

Key words: *Arabidopsis thaliana*, GPA, *Pisum sativum*, Seed Production, Aging

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UNEXPANDED COTYLEDONS (UCO) IS A NOVEL C2H2 ZINC-FINGER TRANSCRIPTION FACTOR WITH A ROLE IN THE REGULATION OF COTYLEDON EXPANSION IN THE CONVERGENCE OF LIGHT AND RETROGRADE SIGNALING

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Abstract: Plant development is influenced by multiple internal and external factors. Among these, light is of utmost importance due to its function as source of energy and also as an information signal. After germinating in the dark, dark-grown seedlings undergo deetiolation upon the first light exposure, switching from skotomorphogenic to photomorphogenic development. This deetiolation switch is characterized by the inhibition of hypocotyl growth and separation and expansion of the cotyledons, together with chloroplast development to support autotrophic growth. Whereas hypocotyl growth is a well-studied system, the regulatory mechanism of cotyledon expansion is still elusive. Massive reprogramming of the transcriptome during deetiolation is regulated by the Phytochrome Interacting Factors (PIFs), which accumulate in the nucleus in the dark and are degraded in light by active phytochromes. Here, we describe an uncharacterized PIF-regulated C2H2 zinc-finger transcription factor that we have named UNEXPANDED COTYLEDONS (UCO), which specifically controls cotyledon expansion during deetiolation. Interestingly, UCO is targeted not only by PIFs but also by GENOMES UNCOUPLED1 (GUN1)-mediated chloroplast-to-nucleus retrograde signal (RS). RS is triggered by high light, and our previous studies have shown that RS can suppress deetiolation to prevent photodamage. Our results suggest that UCO functions in the convergence of light and RS pathways to regulate cotyledon expansion and optimize deetiolation under changing light environments.

Key words: Light signaling, cotyledon development, deetiolation, retrograde signaling, *Arabidopsis thaliana*

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COORDINATION OF FLORAL SCENT EMISSION

Marcos Egea-Cortines*[1], Julia Weiss[1] , Marta I. Terry[1], Claudio Brandoli[1], Fernando Perez-Sanz[2], Victoria Ruiz-Hernández[3], Diego Águila[1], and Raquel Alcantud[1]

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Abstract:
The chemical signature or scent is the interface between plants and most living organisms, causing deterrence or attraction. This opposite process is the result a mixture of metabolites recognized by friends and foes. Flowers produce a large amount of volatile organic compounds (VOCs), emitted to the atmosphere. Three different overlapping aspects define the scent of a flower, which can be considered its signature. First is the amount of volatiles produced, second is the actual composition of volatiles. And third is the quantitative and qualitative variation occurring as a result of changes during the day in light and temperature, or interaction with pests and pollinators. We have taken a genetic approach to identify the genes coordinating scent emission. Two groups of genes appear upstream of the cascade leading to scent emission. First floral organ identity genes are required to establish a shortlist of VOCs that comprise the standard scent. Mutations in two organ identity genes *deficiens* and *compacta*, cause drastic changes in the composition of scent in snapdragon. A second layer of genes are those involved in circadian regulation. Knockdown of *AmLHY* in snapdragon or *PhCHANEL* in Petunia cause significant changes in the overall scent complexity. Finally knockdown of the *PhGIGANTEA1* and *PhGIGANTEA2* in petunia have an overall effect on scent quantities that are significantly reduced. Exogenous temperatures also play a key role in scent quantity, composition and complexity. Using Narcissus and snapdragon we found increased complexity with increased temperature. Our results indicate that the genetic capacity to produced VOCs is not reflected in scent profiles. In fact, bouquets are formed by a small subset of VOCs that are selected by the combination of floral organ identity and clock genes.

Key words: floral scent, circadian clock, organ identity, gcProfileMaker

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SALINITY TOLERANCE IN MUTANTS OF MACROPHYLLA OBTAINED IN THE IMIDA CITRUS BREEDING PROGRAM

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Abstract: Salinity is one of the biggest challenges that need to be faced in crop production. Citrus is highly sensitive to salt stress and obtaining rootstocks with improved resistance to salinity is key for the citrus growing industry. Citrus rootstock breeding programs carried out by sexual hybridization are hampered by citrus reproductive biology (apomixis) and the high heterozygosity of the citrus genomes. This, combined with the fact that salinity seems to be regulated by many mechanisms, make any enhancement of salinity tolerance in citrus rootstock a very difficult task, so that alternative ways need to be found. One such way is gamma radiation, which has already been used to improve salinity tolerance in other species although to our knowledge, no new citrus rootstock has ever been obtained through gamma radiation. In the present study, five mutants, selected *in vitro* for their ability to cope with salt stress, were obtained through the exposure of citrus rootstock cultivar *C. macrophylla* (MA) seeds to gamma radiation. The mutants and MA were subjected to salinity in *in vitro* experiments, and their performance (growth, visual leaf damage) and their chlorophyll, proline, starch, soluble sugars and ion contents were recorded and verify *ex vitro*, in order to determine their degree of salinity tolerance at a given salinity level and the underlying mechanisms that might be operating to regulate this trait. In the saline conditions assayed, all the mutants showed better growing and less leaf damage and chlorophyll degradation than *Citrus macrophylla*, *in vitro* and *ex vitro*. Our data suggest that this improved resistance to salinity was based on their capacity to accumulate less Na or Cl^-, depending on the mutant. Furthermore, NO_3^- accumulation was also higher in mutant plants than in *Citrus macrophylla* in the saline conditions, which conferred an additional advantage against stress. Moreover, the present study demonstrates that mutation breeding by gamma radiation and the *in vitro* culture are an excellent tool to enhance salinity resistance in citrus.

Key words: Gamma rays, Plant stress, Mutagenesis, *in vitro* culture, rootstocks

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EFFECT OF THREE NATURAL COMPOUNDS AGAINST PROBLEMATIC WEEDS IN MEDITERRANEAN FIELD CONDITIONS.

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Abstract: In recent years, the use of biotechnological tools for pest control has increased due to the adoption of a more restrictive legislation in the European Union to reduce dependency on the use of pesticides (Directive2009/128/EC), which promoted the use of integrated pest management (IPM) (Dayan and Duque, 2014). One of the main crop problems nowadays is the competence caused by weeds for water and nutrients (Abbas *et al.*, 2018). Weed management, which was mainly accomplished with the use of herbicides has become more complicated due to the development of herbicide-resistant weed biotypes because of the abusive use of synthetic herbicides (Verdeguer *et al.*, 2020). Weeds are responsible for great losses in crop yields, more than 50% in some crops if left uncontrolled (Heap, 2014). Herbicides have been used as the main method for weed control since their development after the Second World War (Vats, 2015). It is necessary to find alternatives, based on environmentally friendly compounds, to synthetic herbicides that can be incorporated in an Integrated Weed Management Strategy, to produce fruits in a more sustainable way (Vasilakoglou *et al.*, 2013).

The main international fruit and vegetable markets require residue limits sometimes even lower than the limits required by the authorities. In this work, three natural compounds and formulations: pelargonic acid (PA), carvacrol (CV) and cinnamic aldehyde (CA) were evaluated, under field conditions in a post-emergence trial, against problematic and resistant weeds in a Mediterranean citrus orchard to determine their phytotoxic potential. The trial was carried out in a commercial citrus plot operated by the company Peiró Camaró, in a total area of two hectares located in Gandía (Spain). The results obtained by the natural herbicides developed by the company Seipasa in collaboration with the Polytechnic University of Valencia were similar to the results obtained by the synthetic herbicides. In particular, the mixture of the 3 natural compounds (PA + CV + CA) was more effective than the mixtures

of two of them, and its herbicidal activity (efficacy or plant death) was similar than the efficacy achieved by the synthetic formulations. The importance of the application method (type, volume, and pressure), the optimal application timing for the different species (BBCH) and the knowledge of the agronomic features of the plots were an essential factor for the final performance of the natural products on weed control.

Key words: Weeds, Abiotic stress, Natural herbicides; Secondary metabolites; Post-emergence; phytotoxicity

Acknowledgments: This research was carried out in collaboration with the commercial citrus producer Peiró Camaró, located in Gandía, Alicante (Spain), who provided us citrus orchards for the research and development of sustainable methods for weed management and helped us to carry out the treatments.

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PLANT RNA VIRUSES: A POWERFUL TOOL FOR CRISPR-CAS MEDIATED GENOME EDITING

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Abstract:
Systems based on the clustered, regularly interspaced, short palindromic repeat (CRISPR) and CRISPR associated proteins (Cas) have revolutionized genome editing in many organisms, including plants[1]. Most CRISPR-Cas strategies in plants rely on genetic transformation using *Agrobacterium tumefaciens* to supply the gene editing components, such as Cas nucleases and the synthetic guide RNAs (sgRNA). Plant virus-derived vectors offer an alternative for the fast and efficient delivery of CRISPR-Cas components into adult plants, due to their capacity to replicate and move systemically, a strategy known as virus-induced genome editing (VIGE)[2]. In this work, we first engineered a vector derived from *Potato virus X* (PVX; genus *Potexvirus*) to deliver multiple sgRNAs in a *Nicotiana benthamiana* transformed line constitutively expressing *Streptococcus pyogenes* Cas9[3]. Host endogenous genes were efficiently targeted, producing nearly 80% indels in adult tissue. Interestingly, we demonstrate that PVX allows the expression of unspaced sgRNA arrays, achieving highly efficient multiplex editing in a few days. This novel PVX vector allows easy, fast and efficient expression of sgRNAs arrays for multiplex CRISPR-Cas heritable genome editing. Secondly, we performed successful DNA-free genome editing in *N. benthamiana* by combining PVX with a compatible RNA virus vector, *Tobacco etch virus* (TEV; genus *Potyvirus*)[4]. In addition to PVX-assisted sgRNA delivery, TEV vector was employed to transiently express Cas12a nuclease. This novel two-virus-vector system improves the toolbox for transformation-free VIGE in plants and will be useful for functional gene analysis and precision breeding across diverse plant species, particularly in *Solanaceae* crops.

Key words: CRISPR-Cas, virus-induced genome editing, multiplexing, DNA-free editing, solanaceous plants

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SMALL MOLECULES TO ENHANCE *IN VITRO* CELL REPROGRAMMING AND EMBRYOGENESIS INITIATION IN CROP AND FOREST PLANTS

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Plant in vitro regeneration systems, like somatic embryogenesis, are essential players in breeding, since they permit to propagate elite genotypes, to produce double-haploids, and to convert gene editing or transformation events into plants. However, in many crop and forest species somatic embryogenesis is highly inefficient, either from microspores or other somatic cells (Testillano 2019). Recent advances in chemically-controlled reprogramming of mammalian cells have demonstrated the enormous potential of application of synthetic small molecules to regulate cellular reprogramming. We have initiated a pioneer line of research, in collaboration with experts in chemical biology to to screen novel small molecules from chemical libraries of the pharmaceutical field, to improve in vitro plant cell reprogramming and regeneration. Here, we report a new strategy using synthetic small molecule inhibitors of mammalian glycogen synthase kinase 3β (GSK-3β), never used in plants (Patent PCT/EP2020/083316). These inhibitors led to significant increase of embryogenesis initiation rates and embryo production in three different systems and species, microspore embryogenesis of Brassica napus and Hordeum vulgare, and somatic embryogenesis of Quercus suber. Further assays revealed that the compound inhibited GSK-3 activity in plant cell cultures, like it does in mamalian cells. The small molecule inhibitors increased expression of embryogenesis-genes FUS3, LEC2 and AGL15, and activated the brassinosteroid (BR) signalling pathway, probably by targeting the plant GSK-3 kinase BIN2 (Berenguer et al. 2021). These findings open the way for new strategies using GSK-3β inhibitors that could be extended to other species for improvement of in vitro embryogenesis and plant regeneration.

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CHANGES IN CELL WALL PHENOLICS AND BAHD GENE EXPRESSION THROUGHOUT EARLY MAIZE DEVELOPMENT

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Abstract: Maize lignocellulosic material accounts for a huge amount of biomass useful for forage and/or bioethanol feedstock. In the last years, several works have revealed the relationship between a high level of cell wall phenolics (ferulic acid and *p*-coumaric acid) and a reduced saccharification potential of lignocellulose (Bartley *et al*. 2013; de Souza *et al*. 2018). BAHD genes codify for enzymes that acylate cell wall polysaccharides with ferulic or *p*-coumaric acids (D´Auria *et al*. 2006). For this reason, the identification and functional characterization of the BAHD family in maize may result interesting for biotechnological purposes.

In this work we aimed to study the gene expression profile of 13 previously identified maize BAHD genes (Martínez-Rubio, 2018) and their correlation with cell wall feruloylation/*p*-coumaroylation in the B73 maize inbred. For this purpose, maize plants were grown under controlled conditions. Roots, stalks and leaves at the V1, V3 and V5 growth phases were collected. The expression profile of BAHD genes for each organ and growth phase was analyzed by q-RT-PCR. In addition, cell walls were isolated, saponified and analyzed for esterified *p*-coumarate and ferulate by HPLC-PDA.

BAHD1, *BAHD3* and *BAHD13* were the most expressed BAHD genes in maize organs. BAHD gene expression profile was similar in roots and stalks. In general, a pattern of gene repression as organs aged was found except in leaves, in which no clear trends were observed. The developmental pattern of feruloylation/*p*-coumaroylation indicated that cell wall phenolics increased along time, particularly in the transition between V3 and V5 growth phases. The results achieved provide information about organ-dependent changes in cell wall phenolics and BAHD gene expression all throughout early development of maize.

Key words: maize, lignocellulose, ferulic acid, *p*-coumaric acid, BAHD genes.

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DEVELOPMENT OF A GENE EDITING TRASFORMATION PROTOCOL IN EUROPEAN CHESTNUT

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Abstract: European chestnut or sweet chestnut (*Castanea sativa* Mill.) is a worldwide economic important tree nut species with a relevant role in Spain and Italy (Torello Marinoni *et al.,* 2020). This species is susceptible to two major diseases, ink disease and chestnut blight, caused by *Phytophthora spp.* and *Cryphonectria parasitica* respectively (Conedera *et al.,* 2004; Müllerová *et al.,* 2015). Since tolerant hybrids obtained through traditional breeding were less competitive, biotechnological methods may represent an interesting alternative to generate resistant/tolerant chestnut plants (Limera *et al.,* 2017). Nowadays CRISPR/Cas9 technology has become one of the most practical tool for genome engineering thanks to high efficiency, simplicity and flexibility (Ran *et al.,* 2013). However, this technology cannot be applicable without an efficient and reproducible *in vitro* regeneration protocol (Corredoira *et al.,*2019). Currently, bibliographic evidence of the CRISPR/Cas9 application in tree species is limited due to multiple factors including the low processing efficiency, the difficulty of *in vitro* regeneration and the slow growth compared to the herbaceous ones. The aim of the work is to set up a CRISPR/Cas9 transformation protocol in *Castanea sativa*. The target gene was *phytoene desaturase* (*pds*), involved in the chlorophyll biosynthesis, whose mutation causes the albino phenotype (Qin *et al.,* 2007). Globular and early torpedo-stage somatic embryos of *C. sativa* were used as starting materials for plant genetic transformation. The CRISPR/Cas9 construct targets two gRNAs designed on the 4th and 8th exons of the *C. sativa pds* sequence was transferred to *Agrobacterium tumefaciens* strain EHA105. After 5 days of co-cultivation and 8 weeks of subculture on medium with kanamycin, 4 kan-resistant embryogenetic lines were detected. Molecular analysis of *pds* region revealed the successful gene editing process. Somatic embryos were subsequently placed in maturation media and addressed to the germination process in order to produce albino plants. This work opens the way to the application of gene editing in European chestnut targeting genes

related to pathogen resistance/susceptibility.

Key words: *Castanea sativa*, CRISPR/CAS9, genome editing, somatic embryos

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EFFECT OF IRON DEFICIENCY ON THE GROWTH OF *IN VITRO* CULTURED *BRYOPHYLLUM* SPP. MEDICINAL PLANTS

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Iron is an essential micronutrient for plant growth and development acting as cofactor in numerous redox processes and involved in lipid metabolism and photosynthesis [1]. Therefore, the presence of iron in either excessive or limiting amounts has been shown to cause a negative impact in plant growth and development [2], thus requiring the design of specific media formulations to meet their nutritional requirements. In this study, three different species belonging to *Bryophyllum* subgenus (genus *Kalanchoe*) were subjected to *in vitro* culture as described elsewhere [3,4]: *B. daigremontianum* Raym.-Hamet et Perr. (BD), *B. tubiflorum* Harv. (BT) and *B.* × *houghtonii* D.B. Ward (BH), in order to evaluate the effect of iron deficiency on plant growth. To this purpose, an experimental design including five culture media was carried out: the full-strength Murashige and Skoog medium (MS) [5] was used as control, together with four formulations with decreasing iron concentrations, including half-strength, quarter-strength and eighth-strength of iron (½Fe, ¼Fe and ⅛Fe, respectively) and iron-free MS medium (0Fe). Culture media were supplemented with 3% sucrose and solidified with 0.8% agar at pH=5.8. Plantlets were grown under a photoperiod of 16 h light and 8 h dark at 25 ± 1 °C for 12 weeks. After this period, plant growth-related parameters (shoot length), longest root length, leaf number, aerial parts fresh weight and root fresh weight) and health were determined, as well as the reproductive capacity of plants (plantlet number). The results obtained showed that BH and BT species reached higher growth and reproduction rates, without presenting morpho-physiological disorders such as chlorosis, when grown on MS medium. On the contrary, BD achieved the best growth on ½Fe and ¼Fe, while the highest reproductive rate on ⅛Fe. These results reveal that even closely related genotypes may have different mineral requirements, requiring in some cases the modification of the most common used universal formulations, such as MS.

Keywords: *Kalanchoe*, plant *in vitro* culture, mineral nutrition

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EX SITU CONSERVATION OF SWEET AND SOUR CHERRY GERMPLASM FROM COVA DA BEIRA REGION

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Abstract:

The *ex situ* conservation of traditionally cultivated varieties, underutilized fruit tree species, and their wild relatives represents a major challenge with relevant environmental and economic impacts. Displaying characteristics of adaptation or resilience to diverse climate change scenarios such as tolerance to abiotic and biotic stresses, these species and/or varieties are a source of genetic diversity crucial to the implementation of breeding programs.

Sweet cherry (*Prunus avium* L.) and sour cherry (*Prunus cerasus* L.) are prunoids of the Rosaceae family, very important for the Portuguese fruit sector. During the last decade, the sweet cherry market kept growing with an estimated production of 3 tons per hectare (ha). In Fundão (Cova da Beira), the cultured area of sweet cherry has reached 2.500 ha and a revenue of about 20 million euros per year. Sour cherry production is subsidiary in scale, being more used as a border culture. The introduction of new improved cultivars led to a gradual abandonment of traditional varieties including valuable germplasm that is at risk of being lost, thus reducing the genetic pool that may be used to face the biotic and abiotic stresses expected to arise due to the foreseen climatic changes. *In vitro* tissue culture has a strong potential for plant conservation and has been applied to the conservation of several fruit species, sweet and sour cherry included.

The aim of this work was the *ex situ* conservation, through *in vitro* culture techniques, of sweet and sour cherry germplasm previously identified and georeferenced by local farmers of Cova da Beira. For this purpose, two types of explants were used: i) shoots collected in winter and forced to sprout from branch segments under laboratory- controlled conditions, and ii) young growing shoots sprouted in spring. For *in vitro* establishment, a MS basal medium, supplemented with 0.88 μM 6-benzyladenine (BA), was used. Currently, nine cultivars of sweet cherry and five of sour cherry have been successfully established and effective protocols for proliferation and rooting under different combinations of plant growth regulators are being implemented. This work will be followed by the genetic characterization of the cultivars and phenotyping under stress conditions.

Keywords: In vitro culture, *Prunus avium*, *Prunus cerasus*, Shoots, Underutilized varieties

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GENETIC TRANSFORMATION OF *QUERCUS ILEX* SOMATIC EMBRYOS WITH A GENE ENCODING A *Gnk2* PROTEIN

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Holm oak is a key tree species in the Mediterranean ecosystems. In the last few decades, their populations have been increasingly threatened by holm oak decline syndrome, a disease caused by the combined action of different pathogens species of genus *Phytophthora* and abiotic stresses. Genetic transformation with antifungal genes could be an alternative to conventional breeding efforts. In recent years, Serrazina et al. (2015) have identified in chestnut candidate genes associated with the plant defense differentially expressed after infection with *P. cinnamomi*. One of these candidate genes is Ginkbilobin2 homologous domain gene (*Gnk2*) which was found in seeds of the gymnosperm *Ginkgo biloba* and it is known to possess antifungal properties. The aim of the present study was to produce holm oak somatic embryos that overexpress the *Gnk2* gene. Proembryogenic masses isolated from 4 holm oak somatic embryogenic lines (Q8, E2, Q10 and E00) were used as target explants. These explants were co-cultured for 5 days with *Agrobacterium* strain EHA105pGnk2 and then cultured on selective medium containing kanamycin and carbenicillin. After 14 weeks on selective medium, the transformation efficiency (TE) was determined on the basis of the fluorescence of surviving explants. TE was low (Q8 (2.5%), E2 (2.5%), Q10 (0.0%) and E00 (0.0%)), but 5 transgenic somatic embryo lines were obtained. The presence of *Gnk2* gene on transgenic embryos was verified by PCR and the number of transgene copies was determined by digital PCR. Transgenic plants were obtained from all transgenic lines after cold storage of the somatic embryos for 2 months and subsequent transfer to germination medium. In a preliminary in vitro tolerance assays we observed that transgenic plants survival more days than wild type.

Key words: Ginkbilobin2 gene, Holm oak, Oak decline, Somatic embryos

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HERBICIDAL ACTIVITY OF MENTHA X PIPERITA L. AGAINST LOLIUM RIGIDUM GAUDIN.

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Abstract:
In the latest years, the research regarding essential oil (EO) application in pest management has increased greatly, due to the changes in the regulation of the pesticides market in the European Union (EU), through the Directive 2009/128/EC. These changes are focused in achieving the sustainable use of pesticides, and implementing, as mandatory in the EU, the principles of integrated pest management (IPM), which gives priority to the use of non-synthetic pesticides for weed control (Verdeguer, 2020). Bioherbicides could be an alternative to synthetic herbicides because they are more sustainable and environmentally friendly methods for weed control (Jouini, 2021). Some EOs and the compounds that their contain, secondary metabolites, mainly terpenoids, have demonstrated to possess phytotoxic activity (Werrie *et al.*, 2020). Many studies support the herbicidal potential of *M. piperita* against different weeds, like *Echinochloa colona* (L.)*, Portulaca oleracea* L. (Mahdavikia and Saharkhiz, 2015), *Erigeron bonariensis* L (Verdeguer *et al*., 2020) *Avena fatua* L. *A. retroflexus* L. and *Echinochloa crus galli* (L.) Beauv (Jouini *et al*., 2020)
The objective of this work was to study the herbicidal activity of *Mentha* x *piperita* against *Lolium rigidum* Gaudin., a weed species infesting winter crops, which is the number one in the list of herbicide resistant weeds because it has evolved resistance to 13 herbicide modes of action. Many secondary metabolites and their synergy have been tested to control this weed. EO components such as carvone, carvacrol, thymol, *trans*-anethol and linalool had the ability to inhibit germination and root growth of *L. rigidum* (Vasilakoglou, 2013).
The experiment was carried out in pots in greenhouse conditions. Three different concentrations of *M. piperita* EO were tested: 12, 16 and 20 µl/ml, emulsified with Fitoil at 0.05%. The treatments were applied by irrigation and spraying, ten pots were used for each treatment and each mode of application. Images of the plants were recorded 1, 3, 7, 15 and 30 days after treatment application. The images were processed with the software Digimizer, determining the efficacy (value 0 when the plant was alive and 100 when the plant was dead) and the damage level (using a damage scale from 0, no damage, to 3, dead plant). Growth parameters were registered at the end of the experiment (length of aerial parts and roots, fresh and dry weight). Only the highest dose of *M. piperita* EO was effective to control *L. rigidum*, with an efficacy of 50 equally when applied by irrigation and spraying.

Key words: Herbicidal activity, Mentha x piperita, Essential oil, Natural bioherbicides, post-emergency.

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HERBICIDAL ACTIVITY OF THYMOL AGAINST *PAPAVER RHOEAS* L.

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Abstract:
Thymol is a natural phenolic monoterpene widely produced by different species belonging to the Lamiaceae family (Araniti et al., 2020). It can be found in the essential oil (EO) extracted from *Thymus vulgaris* and *Origanum vulgare* (Grul'ová et al., 2020). Thymol is a versatile chemical with a wide range of practical applications in drug, food and cosmetic industry due to its potential antimicrobial, antioxidant, anticarcinogenesis and anti-inflammatory activities (Araniti et al., 2020; Escobar et al., 2020). Many studies have verified the phytotoxic potential of thymol (Kordali et al., 2008; Araniti et al., 2020; Grul'ová et al., 2020). In this work, the herbicidal activity of thymol was tested against *Papaver rhoeas* L., a problematic weed, especially in winter cereals in Mediterranean crops, which has developed herbicide-resistant biotypes (Rey-Caballero et al., 2017).
The experiment was carried out in pots under greenhouse conditions. Five different concentrations of thymol (IC80 for *Arabidopsis thaliana* multiplied by 16, 32,64,128 and 256) were tested as a post emergence application by both irrigation and spraying. Images of the plants were recorded 1, 3, 7, 15 and 30 days after treatments application, and growth parameters were registered at the end of the experiment (length of aerial parts and roots, fresh and dry weight). Thymol showed higher efficacy against *P. rhoeas* when it was applied through spraying than irrigation, and the efficacy increased with increased concentration of the treatment. The highest concentration of thymol managed to kill all the treated plants, both in irrigation and spraying. Thymol has exhibited great potential as a natural herbicide, however further studies need to be carried out to demonstrate its herbicidal potential in field conditions and also to test its selectivity.

Key words: weed control, natural herbicides, phytotoxicity, bioherbicides

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ISOLATION AND TRANSFECTION OF STRAWBERRY PROTOPLASTS FOR GENE EDITING

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Abstract: Strawberry is the most economically important soft fruit. The improvement of the organoleptic qualities of ripe fruit and the postharvest shelf life are main objectives of strawberry breeding programs. Fruit softening is mainly due to the disassembly of cell walls and the dissolution of middle lamella. In strawberry, functional analyses of genes encoding polygalacturonases (PGs) indicate that these enzymes play a key role in fruit softening, i.e. the antisense downregulation of PG genes *FaPG1* or *FaPG2* increased fruit firmness and postharvest shelf life (Paniagua et al., 2020). These results suggest that PG encoding genes are excellent targets for gene editing to improve strawberry fruit quality. Transfection of protoplasts with CRISPR/Cas9 ribonucleoprotein complexes is currently being explored in many species to produce DNA-free edited plants. In this research, a protocol for strawberry protoplasts transfection has been optimized with the final goal of producing non-transgenic strawberry plants with the *Fa*PG1 gene edited. Protoplasts were isolated from 9 weeks old *in vitro* grown plants of *Fragaria* x *ananassa*, cv. 'Chandler', micropropagated in Murashige and Skoog (MS) medium supplemented with 2 mg/L of BA. Protoplast extraction and purification was performed as described by Barceló et al. (2019). Using this protocol, a yield of 1 x 10^5 protoplast/g fresh tissue was obtained and nearly 50-70% of them were viable. Protoplasts were transfected with the plasmid pHBT-sGFP(S65T)-NOS using a PEG-mediated transformation system, as reported by Yoo et al. (2007). To improve the efficiency of protoplast transfection, different variables were evaluated: PEG concentration, time of incubation on PEG and DNA concentration. At 48 h after transfection, the highest percentage of protoplasts showing GFP expression, 18%, was obtained with 15 minutes incubation in 20% of PEG and 5 μg of DNA. This optimized protocol is currently being used to transfect strawberry protoplasts with CRISPR/Cas9 ribonucleoproteins to edit *FaPG1* gene.

Key words: *Fragaria* x *ananassa*, strawberry, protoplasts, transfection.

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PRESENCE OF MELATONIN IN NUTS. EFFECTS OF CULTIVAR, MATURATION AND PROCESSING

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Abstract: Nuts are important ingredients of the healthy diet and provides nutritional value and bioactive components that act as antioxidants in the body. Many studies are trying to characterize the functional bioactive compounds that are present in vegetables and derived foodstuffs, in particular those increasing antioxidant activity. Melatonin is one of these molecules that is widespread in plants and its relevance in foodstuffs is increasing. In this study, an optimized solid-phase extraction method combined with liquid chromatography techniques were used to identify and quantify melatonin that is present in walnuts and then in a number of commercial nuts. The influence of the maturation stage and the commercial processing (roasted) in nuts were also investigated. Melatonin was detected in four walnut cultivars with levels similar to those previously reported by others. Moreover, melatonin content in walnut seeds decreased sharply during ripening from ripe green stage to mature dehydrated fruit and then increased after post-harvesting when fruit leads to edible. We also found high melatonin concentrations in the green pericarp, which could be indicative that not only the seeds, but also the additional cover of the fruit (skin, epicarp and mesocarp) are melatonin-rich tissues. These data suggest that melatonin both in the green pericarp and in the kernel itself may be a relevant antioxidant constituent during walnut ripening. In addition, this molecule could have a role in preservation of the seed during the postharvest period. Melatonin was also measured in a number of other commercial nuts (almonds, peanuts, chestnuts, pistachios, etc.) with contents varying conspicuously and that in general were lower than in walnuts. In most of these nuts the presence of melatonin was negatively affected by processing (roasting) as compared to the raw form, with exception of roasted peanuts in which melatonin content increased. Therefore, this study reveals new data on the presence of melatonin in walnut seeds in natural format and its evolution with maturation, as well as in other commercial nuts. We also highlight the importance that processing that is applied to improve nuts attractiveness for consumers has on melatonin and other antioxidants that are natural constituents in these foodstuffs.

Key words: melatonin, walnuts, commercial nuts, ripening, processing

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REGENERATION AND GENETIC TRANSFORMATION OF DIFFERENT VARIETIES OF *CUCURBITA PEPO*

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Abstract. The use of biotechnological tools in crops allows a better knowledge of their genomes, permits the study of gene functions and the selection of genetic markers to be used in plant breeding techniques, opening the possibility of obtain new varieties through the use of plant transformation. Our research group has performed numerous studies related to the improvement of zucchini fruit quality during postharvest, searching for cultivars tolerant to low temperature conservation. In a recent transcriptomic study (Carvajal et al., 2018), a list of genes that showed high expression in a cold tolerant cultivar have been selected, and studied as possible candidates for resistance to this stress. However, the involvement of these genes in the process is difficult to demonstrate, since no reliable transformation protocol has been established for *Cucurbita pepo* and in general for the Cucurbitaceae family, as these plants seem to be recalcitrant for transformation. This study presents the results of the regeneration and genetic transformation of 2 lines of *Cucurbita pepo* Cavili and Muc16. To establish optimal regeneration conditions, first we have worked on the selection of the suitable explants and on the optimization of culture media conditions for *C. pepo* plant regeneration. Culture media selected for regeneration achieved up to 40% shoot induction and almost 90% root induction from cotyledon explants containing a hypocotyl fraction. In addition, the efficiency of genetic transformation has been evaluated by studying the infective capacity of several *Agrobacterium tumefaciens* strains harboring as a control vector the plasmid pGFPGUSplus. Among the different strains studied, AGL0 was the one that provided the best results, 16% of the explants contained GUS + cells. The regeneration of the plantlets is on process.

Key words: Zucchini fruit, Cold resistance, Plant regeneration, *Agrobacterium* transformation

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BIDIRECTIONAL LINK BETWEEN VITAMIN E AND OXYLIPINS IN PLANTS: A KEY TO BETTER UNDERSTANDING PLANT TRADEOFFS?

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Abstract: Tradeoffs between growth, reproduction, and defence have been well documented in plants. Despite oxidative stress is one of the physiological mechanisms that underlie trade-offs at the cellular and organ levels, the diversity of plant life forms and the complexity of scaling up limit, however, our knowledge of oxidative stress as a universal mediator of life-history trade-offs at the organism level [1]. During the last years, we have joined efforts from plant molecular, physiological, and ecological experimental approaches to provide novel insights into this topic. Using different approaches, including not only studies in the model plant *Arabidopsis thaliana* [2,3], but also research performed on Mediterranean shrubs, such as *Cistus albidus*, and high-mountain plants, such as *Saxifraga longifolia*, growing in their natural habitat [4,5], we have found evidence of a bidirectional link between jasmonates (JAs) and tocopherols (vitamin E) in plants. Most intriguingly, some evidence obtained in *A. thaliana* mutants and in several other plant species growing in their natural habitat has shown that vitamin E not only acts as an antioxidant in chloroplasts, but that it may also function as a sentinel for stress sensing and signalling, so that depending on the environmental stress conditions an increase in tocopherols results in retrograde signalling from the chloroplast to the nucleus by decreasing oxylipin (JAs) accumulation [6]. This reduction in JAs contents not only play a role in cell signalling, but it may also have important implications in plant tradeoffs, so that an increase in photoprotection (as exerted by enhanced tocopherol contents) may lead to a reduction in biotic defences (through reduced JAs levels). It is concluded that vitamin E not only acts as an antioxidant in plants, but it may also serve a role in cellular signalling by influencing JAs accumulation, so that antioxidants and phytohormones crosstalk in plant responses to environmental stresses.

Key words: Antioxidants, Chloroplasts, Jasmonates, Retrograde signalling, Tocopherols

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HEMOGLOBINS AND REDOX POST-TRANSLATIONAL MODIFICATIONS OF PROTEINS IN STRESSED PLANTS

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Nonsymbiotic hemoglobins or phytoglobins (Glbs) are widespread in plant tissues. There are three classes of Glbs based on phylogeny and biochemical properties, but their functions are poorly known with the exception of class 1 Glbs. Overexpression of this class of proteins enhances the plant's survival under hypoxic conditions. This is thought to be due to the involvement of Glbs in the 'hemoglobin-nitric oxide (NO) cycle'. Thus, hypoxic mitochondria are able to replace O_2 by nitrite as an electron acceptor, generating NO, a key signaling molecule in plants. NO is then oxidized to nitrate in the cytoplasm by the NO dioxygenase activity of ferrous Glbs. The reaction yields ferric Glb, which is reduced back to ferrous Glb by putative reductases so that the cycle can continue. The 'hemoglobin-NO' cycle would be responsible for maintaining ATP production during hypoxia. Another relevant feature of Glbs is that they can act as nitrite reductases by reducing nitrite to NO under (nearly) anaerobic conditions. We and others have shown that both reactions, NO dioxygenase and nitrite reductase, are common to class 1 Glbs and have proposed that these proteins are involved in the homeostasis of NO. In addition to NO binding to the heme iron (metal-nitrosylation), Glbs may be a target of post-translational modifications (PTMs). Some of them are dependent on the redox state, such as formation of a disulfide bridge between cysteine residues (intramolecular or intermolecular) or formation of a -SNO bond on a cysteine residue (*S*-nitrosylation). We have concluded from *in vitro* studies that the cysteines of Glbs are prone to be *S*-nitrosylated and that some Glbs may form dimers, but the *in vivo* significance of these findings is still unknown. An interesting hypothesis is that the proteins may transport NO on the cysteine residues and/or are able to change their subcellular location (nucleus, cytoplasm, plastids) in response to the redox and/or monomer-dimer states. Experiments will be conducted to tackle this hypothesis by expressing Glbs in protoplasts and exposing them to known inducers of redox PTMs, such as H_2O_2 and NO donors.

Key words: Hypoxia, Plant hemoglobins, Post-translational modifications

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CONNECTING THE DOTS BETWEEN ROOT, XYLEM AND STOMATA

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Abstract: Stomata are present on all land plants and are key features for vascular plant water content regulation on Earth. Their primary function, i.e., stomatal closure to control water loss under soil and atmospheric drought, is thought to prevent cavitation in the vascular system (Brodribb et al. 2017). However, stomata are found to close much before the xylem cavitates – i.e., the leaf water potential at which stomata close by 50% (ψ_gs50) is much less negative than the water potential at which the xylem loses 50% of its conductivity (ψ_x50) (Martin-St Paul et al. 2017). The mechanism that would allow stomata to close promptly to a decrease in transpiration in relation to a change in leaf water potential before the decrease in hydraulic conductance is still elusive. Our hypothesis is that the loss of root-soil hydraulic conductivity, more than xylem vulnerability to embolisms, is the primary constraint on transpiration during drought (Rodriguez-Dominguez and Brodribb 2020). Thus, stomata would close when the water potential around the roots drops more rapidly than the increase in transpiration. We investigated whether this loss of root-soil hydraulic conductivity, probably caused due to root shrinkage and the formation of air-filled gaps, and/or damage to fine roots, appeared to be an important constraint on transpiration during drought. We conducted physiological and imaging experiments on maize plants undergoing moderate drought. We performed high-resolution imaging (micro-CT) of leaves and the root-soil interface and measured in parallel the soil and plant water potentials. Transpiration, stomatal conductance, root hydraulic conductance and soil and plant water potential were also measured during soil drying in a similar set of plants. The formation of air-filled gaps along individual maize roots was visualized and quantified, finding an agreement between the soil water potential

at which roots shrank and root hydraulic conductance decreased, and the soil water potential at which stomata closed. These results proved the hypothesis that the loss of contact between roots and soil, and probably other root cortex modifications, triggered stomatal closure and transpiration reduction.

Key words: soil-root interface, maize, root-soil hydraulic conductance, stomatal closure

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HIGH PHENOTYPIC PLASTICITY OF THE HOLM OAK ROOT SYSTEM AS A STRATEGY TO SURVIVE DECLINE IN LOW-RESOURCE SOILS

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Abstract:

The structure and health of the tree root system are key to maintain tree and ecosystem functioning and stability and may be greatly affected by climate change-related impacts (Brunner et al. 2015). Roots play a prominent role in tree resource uptake, soil water content and nutrient cycling while contributing to soil structure and its stability (Freschet and Roumet 2017). In the Mediterranean basin, precipitation and temperature shifts related to climate change, along with increasing rates of pathogen outbreaks, have triggered worrisome

decline rates of holm oak species (*Quercus ilex* L.), which are revealed as a gradual process of defoliation that ultimately result in mortality. While tree responses at the crown level have been widely documented, the role and responses of the root systems during the process of decline are less understood. This work aimed to study root system architectural traits along with soil nutrients and root sugar interactions during the holm oak declining process. For this purpose, we characterised root functional traits in three holm oak health statuses (i.e., healthy, susceptible and declining) with focus on morphology and architecture (i.e., fine root percentage and fine root bifurcation ratio), chemistry (carbon and nitrogen contents) and physiology (non-structural carbohydrates). We further associated plant functional traits with soil chemical properties (i.e., mineral nitrogen, potassium, phosphate, pH, as well as soil stoichiometry). Results indicated that the health status of the tree determined the fine root bifurcation ratio response to soil nutrients and root soluble sugar availability. Higher root phenotypic plasticity to changes in soil nutrient availability and root soluble sugar was observed in declining trees compared to healthy and susceptible trees. These findings suggest that soil nutrients determine the architecture of the root system and this response may be triggered by a signalling cascade controlled by soluble sugar availability and the tree health status. This plasticity of the root architecture may represent a strategy used by the holm oak to better cope with adverse environmental conditions determined by climate change.

Key words: holm oak, decline, nutrient scarcity, soluble sugars, root architecture.

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INTEGRATED ANALYSIS OF TOMATO (*SOLANUM LYCOPERSICUM* L.) RESPONSES TO COMBINED NITROGEN AND WATER DEFICIT

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Abstract: Water and nitrogen (N) scarcity are two of the most relevant constraints for tomato production, thus understanding their combined effects is important to improve water and N use efficiency. This study aimed to unravel the responses of young tomato plants to N, water or the combination of both stresses. For that, three-week-old Micro-Tom seedlings were divided into four groups: control (CTR; 100%N+100%W), N deficit (N; 50%N+100%W), water deficit (W; 100%N+50%W) or combined stress (N+W; 50%N+50%W). The experiment ended when W and N+W plants reached 50% of the substrate water capacity (16 days after). Afterwards, growth parameters, physiological responses [e.g. water use efficiency (WUE), stomatal responsiveness, proline], nutritional status [total N, C/N ratio and NO_3^- levels, nitrate reductase (NR) and glutamine synthetase (GS) activity] and transcript accumulation of C and N metabolism-related genes were evaluated. Compared to CTR plants, 50% water deficit improved WUE, stomatal responsiveness and N concentration (including NO_3^-), being this effect most probably linked to increased proline and soluble protein levels, along with an upregulation of *NR* and *GS* genes in roots. Results also suggested that the impact of N starvation on morpho-physiological parameters were slightly more severe than those of W deficit, impairing

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morphological endpoints, chlorophyll content, and proline levels. N deficiency also led to a higher C/N ratio, GS and NR activity and increased expression levels of *rcbS* and *rcbL* in shoots, pointing towards a N remobilization response, which could have contributed for plants coping to mild N deficit. For most parameters, the combination of stresses resulted in similar responses to those of N-stressed plants. However, transpiration rate, NR activity and NO_3^- content, as well as *GS1* transcripts accumulation in roots were more comparable to those of water-stressed plants. Interestingly, proline showed a quite distinct response under the combined treatment, being higher than N-stressed plants, but lower than those exposed to water stress. Overall, data suggest that young tomato plants are able to employ efficient strategies to cope with N and water deficiency, being N starvation the predominant factor behind plant responses when applied together with water deficit.

Key words: Combined abiotic stresses, Gene expression, Micro-Tom cv, Nutritional status, Water use efficiency

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TOLERANCE TO DESICCATION AND HIGH TEMPERATURES OF *FUCUS GUIRYI* (PHAEOPHYCEAE) AT ITS SOUTHERN LIMIT OF DISTRIBUTION (CANARY ISLANDS)

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Abstract: Macroalgae of the order Fucales are ecosystem engineers that contribute to the balance of marine communities. The distribution patterns of these macroalgae are determined by the interaction of biotic and abiotic factors, influenced by tidal levels. During low tide, they are subjected to severe abiotic factors such as desiccation and fluctuating temperatures. In the Canary Islands, *Fucus guiryi* is the only one of the genus that can grow at such southern latitude, so studying its physiology could help predicting the behavior of other northern species in the future climate change scenario. We aimed to analyze the effect of high temperatures and desiccation on two morphotypes of *F. guiryi* (small and large) from two climatically contrasted populations of Tenerife, Canary Islands (Punta Prieta in the south and Punta del Hidalgo in the north). We analyzed the relative water content to assess the desiccation and the maximum photochemical efficiency of PSII (Fv/Fm) as an indicator of physiological state of individuals. Additionally, we compared vegetative vs reproductive structures. Thalli of *F. guiryi* recovered >90% of initial Fv/Fm upon rehydration, after achieving relative water contents <10% during desiccation. Branches were also remarkably tolerant to high temperatures up to 30°C. Significant differences were found between populations: branches and receptacles from Punta del Hidalgo were shorter, reached lower Fv/Fm values upon desiccation, and higher Fv/Fm values upon first hours of rehydration but were more susceptible to high temperatures of 32°C, than Punta Prieta samples. When reproductive branches were subjected to desiccation only vegetative part but not the receptacles dehydrated. Accordingly, Fv/Fm decreased upon desiccation in the vegetative part only, and the whole branch reached control Fv/Fm values after rehydration. We conclude that the studied individuals of *F. guiryi* are tolerant to desiccation while the receptacles, are resistant. Both structures maintain their integrity after a 24h desiccation-treatment. Our results also indicate a considerable tolerance of *F. guiryi* to short heat shocks of up to 30°C. Nevertheless, this work reveals a high heterogeneity in its tolerance to both abiotic stressors depending on population and thallus morphotype, the smaller the more vulnerable.

Key words: abiotic stresses, brown algae, climate change, chlorophyll a fluorescence, warming.

Poster Communications



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A NEW METHOD FOR ASSESSING DROUGHT STRESS IN LENTIL (*Lens Culinaris* Medik.) BY SCANNING ELECTRON MICROSCOPY

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Abstract: Lentil (*Lens culinaris* Medik.) ranks the fifth legume by global production, being also one of the most affordable and widely used sources of complex carbohydrates, protein, vitamins and minerals, especially in third world countries. Drought stress is one of the most devastating abiotic stresses in terms of crop yield nowadays. Their frequency and intensity are thought to increase in the coming decades due to the forecasted climate change, which, coupled with a predicted population growth, will endanger global food security. Although lentils are moderately drought tolerant, large differences among genotypes in response to water stress have been found. Screening and selection of tolerant genotypes at early developmental stages allows speeding up breeding programs. Relative water content (RWC), the amount of moisture in a plant sample relative to its fully hydrated state, is widely used as a marker for drought stress. Measuring RWC in the early stages of lentil seedling development is challenging due to the small leaf size. Thus, developing other more effective and accurate methods to foresee the performance of different genotypes under stress conditions becomes essential. One of the first responses of plants to drought stress is stomatal closure. By means of this mechanism, water loss by transpiration is reduced. Nevertheless, it limits CO_2 uptake as the photosynthetic rate also decreases. Here, we propose a new method, based in scanning electron microscopy, to assess the level of physiological stresss in seedlings subjected to drought. Leaf samples of four lentil genotypes were taken 2, 4 and 6 days after the beginning of the water-stress treatment. Samples were fixed, dehydrated and covered with gold for image taking. The images from stressed and control samples were analyzed to collect data on stomatal aperture, density and distribution. Statistical analysis was carried out to establish the relationship of these parameters to RWC value, for each genotype. The results were able to corroborate not only the link between water stress and stomatal size but to also classify genotypes according to their tolerance to this stress. This method is easily extensible to other plant species.

Key words: water deficit, RWC, SEM, stomatal closure, drought stress.

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A VARIATION IN VACUOLAR CALCIUM TRANSPORTER CAX1 GENERATED BY TILLING PROVIDES HIGHER TOLERANCE TO SALINITY IN *BRASSICA RAPA* PLANTS

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Salinity in soils and irrigation waters is an important environmental factor conditioning crop performance in many world regions. One of the most common salts causing salinity is NaCl and Na^+ accumulation is the main factor contributing to plant toxicity. Thus, accumulation of this ion affects negatively to photosynthesis process and causes oxidative stress in plants. However, calcium (Ca^{2+}) can counteract these adverse effects due to it has a crucial role in plant stress responses through cellular Ca^{2+} signaling fluxes. Hence, the modification of Ca^{2+} cation exchangers (CAX) transporters could be a potential strategy to increase plant tolerance to salinity. Using Targeting Induced Local Lesions in Genomes (TILLING), researchers generated single amino acidic variations in *Brassica rapa* CAX1a transporter. This study aimed to evaluate the effect of one of these variations (*BraA.cax1a-4*) on salt tolerance of *B. rapa* plants. For this evaluation, *BraA.cax1a-4* and the parental line (R-o-18) were grown under control and salinity conditions (150 mM NaCl), and parameters related to biomass, Ca, Na, and K accumulations, photosynthesis performance, and oxidative stress indicators were measured. The results showed that *BraA.cax1a-4* mutation provided higher shoot biomass which suggests a better NaCl tolerance. Furthermore, *BraA.cax1a-4* showed higher Ca and K contents and a lower Na/K. Likewise, these plants presented lower reactive oxygen species (ROS) and malondialdehyde (MDA) accumulations and enhanced superoxide dismutase (SOD) and catalase (CAT) activities. Besides, the modification of CAX1 resulted in an enhanced photosynthetic performance manifested by a higher net photosynthesis rate, low water use efficiency (WUE), and higher Fv/Fm values. Therefore, this study points out CAX1 as a potential target for crop breeding and identifies *BraA.cax1a-4* as a useful genotype that could be used to enhance the salt tolerance of crops.

Key words: *Brassica*, Calcium, Oxidative stress, Photosynthesis, Salinity

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ABSCISIC AND SALICYLIC ACID REGULATE TOMATO EARLY MOLECULAR RESPONSES TO COMBINED WATER STRESS AND TETRANYCHUS EVANSI ATTACK

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Abstract: Interaction between plants and their environment is changing as a consequence of the climate change and global warming, increasing the performance and dispersal of some pest species which become invasive species. *Tetranychus evansi* also known as the tomato red spider mite, is an invasive species which has been reported to increase its performance when feeding in tomato under water deficit conditions (Ximénez-Embún et al, 2018). In order to clarify the underlying molecular events involved, we examined early plant molecular changes occurring on cultivar Moneymaker (MM) during *T. evansi* infestation alone or in combination with moderate drought stress. Hormonal profiling of MM plants showed an increase in abscisic acid (ABA) levels in drought-stressed plants while salicylic acid (SA) levels were higher in drought-stressed plants infested with *T. evansi*, indicating that SA is involved in the regulation of plant responses to this stress combination. Changes in the expression of ABA-dependent *DREB2*, *NCED1*, and *RAB18* genes confirmed the presence of drought-dependent molecular responses in tomato plants and indicated that these responses could be modulated by the tomato red spider mite. Tomato metabolic profiling identified 42 differentially altered compounds produced by *T. evansi* attack, moderate drought stress, and/or their combination, reinforcing the idea of putative manipulation of tomato plant responses by tomato red spider mite. Altogether, these results indicate that the tomato red spider mite acts modulating plant responses to moderate drought stress by interfering with the ABA and SA hormonal responses, providing new insights into the early events occurring on plant biotic and abiotic stress interaction.

Key words: plant-herbivore interaction; drought stress; spider mites; tomato; combined stresses

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ALTERNATIVE SPLICING AND NATURAL VARIANCE IN *ARABIDOPSIS THALIANA* ABSCISIC ACID SENSITIVITY

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Alternative splicing is a posttranscriptional process playing an important role in plant responses to environmental challenges (Filichkin et al. 2015, Laloum et al. 2017), but the genes and specific events involved remain mainly unknown. Many fully-sequenced accessions of the model plant *Arabidopsis thaliana* are known to differ in both sensitivity to environmental stress (Bouchabke et al., 2008) and in their alternative splicing profiles (Gan et al. 2011, Wang et al. 2018). Aiming at the identification of transcriptional and splicing targets with functional significance in abiotic stress tolerance, we studied the response of different *A. thaliana* natural variants to abscisic acid (ABA), a major stress response phytohormone. In a preliminary phenotypic screen of 24 Arabidopsis accessions, we identified Kn-0 (Lithuania) as markedly hyposensitive to ABA when compared to the reference Col-0 genotype. This differential ABA response was further explored at several developmental stages and molecularly confirmed by expression analysis of classical ABA marker genes, which showed reduced ABA induction in Kn-0, correlating with the observed physiological phenotype. For an in-depth understanding of the different Kn-0 and Col-0 ABA responses, we conducted an RNA-sequencing experiment to compare the transcriptome of the two genotypes subjected to a transient ABA or mock treatment. We will present the first results from this large-scale analysis aimed at identifying the early response genes and alternative splicing events triggering differential physiological responses to ABA upon long-term exposure to the stress hormone.

Alternative splicing, abiotic stress, abscisic acid (ABA), *Arabidopsis thaliana*

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AMMONIUM STRESS DISRUPTS THE PUTATIVE UREA CYCLE AND THE POLYAMINE METABOLISM IN *MEDICAGO TRUNCATULA* PLANTS

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Abstract: Abiotic stresses are the major cause of losses in crop productivity worldwide. From an environmental point of view, the augmented use of fertilizers by industrial farming results in an over accumulation of nutrients in the soil, which originates lixiviation and, concomitantly, eutrophication. Ammonium (NH_4^+) nutrition represents an alternative, which binds tighter to the soil particles, and then reduces these environmental issues associated with agriculture. However, NH_4^+ as sole nitrogen source induces higher intracellular NH_4^+ content, which leads to physiological and morphological disorders, impairing the optimal plant growth and development.

Polyamines (PAs), as putrescine, are involved in responses to different environmental stresses including NH_4^+, although the underlying mechanisms are not clarified yet. We have studied the effect of different N sources and doses on a putative 'urea cycle' and PA metabolism in *Medicago truncatula* plants grown under axenic conditions to avoid bacterial interference in the signaling mechanisms within. The content of 'urea cycle'-related amino acids, polyamines and γ-aminobutyric acid (GABA) were measured in shoots and roots of *M. truncatula* seedlings. Furthermore, copper amine oxidases (CuAOs) and polyamine oxidases (PAOs) activity in PAs metabolism were studied at enzymatic, phylogenetic and transcriptional level; as well as the hydrogen peroxide (H_2O_2) content.

In general terms, we observed a marked block in the function of the 'urea cycle' under NH_4^+ nutrition, which denotes the relevance of this pathway in plants. Several low C/N metabolites such as glutamine, arginine, and putrescine importantly accumulated under high NH_4^+ nutrition. Thus, these metabolites may act as a sink of NH_4^+ to avoid the detrimental levels of intracellular NH_4^+. At the same time, stress signal molecules as H_2O_2 and GABA changed their levels. The excess of NH_4^+ may act as a feedback inhibitor of CuAOs as NH_4^+ is a by-product in the oxidation of putrescine. Moreover, the

compartmentalization of PA catabolism appears critical, as intracellular CuAO activity may well be the main disrupted by NH_4^+. In contrast, both apoplastic CuAO and PAO activities may be involved in a stress-signaling crosstalk comprising PAs, H_2O_2, nitric oxide and abscisic acid, which in last term may modulate *M. truncatula* root architecture and lignin deposition.

Key words: Amine oxidases, ammonium stress, *Medicago truncatula*, polyamines, urea cycle

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ASSESMENT OF PHOTOCHEMICAL PERFORMANCE OF A DECLINING ENDEMIC SCHRUB IN DIFFERENT LOCATIONS AT TEIDE NATIONAL PARK

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Abstract:
Spartocytisus supranubius (Teide broom) is a leguminous shrub endemic to Tenerife and La Palma. Extending from 2000 m a.s.l to 3100 m a.s.l., it represents the main keystone species of the Canarian high mountain ecosystem, which is characterised by a semiarid climate, with extended drought periods and rising temperatures consequence of climate warming. This species has several morphological adaptations to these conditions, such as deep roots, small deciduous leaves and multibranched photosynthetic stems (Olano et al., 2017). Despite this, the death of adult individuals is becoming increasingly frequent, and the populations are suffering a progressive decline (Martín-Esquivel et al., 2021). In addition, the presence of introduced herbivores (mainly rabbits) are altering the soil conditions and further compromising the regeneration of this highly palatable legume (Cubas et al., 2018,2019). The National Park (NP) has designed fenced enclosures to prevent the access of herbivores to protect and study the development of broom populations without the rabbit's influence.
In this study, we, therefore, aimed to evaluate the physiological condition of broom plants both inside and outside enclosures, as well as their evolution across seasons. Specifically, we studied photochemistry, through maximal photochemical efficiency of PSII (Fv/Fm), absorptivity, the content of photosynthetic pigments -including Car/Chl ratios-, and Stem Mass Area (SMA). Samplings were conducted in July 2018 (summer), June 2019 (spring), and October 2019 (autumn) in Fasnia, Chiqueros, and Llano de Maja, three sites located at the coolest and wettest area of the NP, the NE sector.
Our preliminary results show differences between the studied locations, Maja being the one with an overall better ecophysiological state. On the other hand, Chiqueros showed the

highest dynamism between plants inside and outside enclosures, Fv/Fm being generally higher and Car/Chl lower outside the fence. Throughout seasons, the brooms showed the highest Car/Chl in Autumn, likely indicating higher a photoprotective demand.
This work evidences the importance of studying how protection measures and location can affect the physiological fitness of this declining endemic species.

Key words: photochemistry, pigment content, plant conservation.

Acknowledgments: This research was part of the project "Fijación Biológica de nitrógeno en el Parque Nacional del Teide: simbiosis *Spartocytisus supranubius*-Rizobios (2017REC13)" funded by Fundación CajaCanarias-Caixa. LPS was awarded with a FPU fellowship from the Ministerio de Ciencia, Innovación y Universidades.

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ATG8H ROLE IN CADMIUM-DEPENDENT PEXOPAHGY INDUCTION

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Autophagy is a self-degradation mechanism involved in removing damaged or obsolete cellular constituents including organelles under starvation and stress conditions. While the role of autophagy in metal starvation has been thoroughly studied (1), its role in heavy metal toxicity has been little explored. Recently, we observed that, in Arabidopsis plants treated with 100 μM $CdCl_2$ for short periods (30 min-24 h), selective peroxisome autophagy (pexophagy) occurs, with autophagy marker *ATG8h*'s transcript levels being slightly up-regulated. To determine whether ATG8h plays any specific role in Cd-induced autophagy and whether it specifically targets peroxisomes for autophagy, we characterized Arabidopsis T-DNA mutants lacking ATG8h (*atg8h*) in response to Cd.

Arabidopsis *atg8h* grown on vertical plates showed a smaller Cd-dependent reduction in root growth than WT (Col 0). Moreover, *atg8h* plants grown hydroponically showed a delayed Cd effect, while similar results were obtained in both WT and *atg8h* plants after 3d of Cd treatment. Therefore, chlorophyll and carotenoid content was higher in *atg8h* than in Col 0 plants. Lipid peroxidation and H_2O_2 content, which showed no differences in leaves between either line, increased considerably in *atg8* in roots after 3 d of treatment. This could be explained by changes observed in catalase and peroxidase activity, particularly in *atg8h* roots. However, no statistically significant changes were observed in Cd uptake or translocation in either line. In *atg8h,* electron microscopic ultrastructural analysis of leaves showed larger peroxisome areas and numbers under control conditions, and peroxisomes in a crescent formation surrounding the vacuole after 15 h of Cd treatment. This suggests that ATG8h is involved in pexophagy and that the absence of ATG8h induces alternative pathways to regulate peroxisomal protein content. However, Cd does not appear to induce bulk autophagy after 3 d of treatment, with longer treatments being required to analyse Cd-induced autophagy.

Key words: autophagy, cadmium, oxidative stress, peroxisome, pexophagy

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AUTOPHAGY IS INVOLVED IN THE VIABILITY OF OVER-EXPRESSING THIOREDOXIN *O*1 TOBACCO BY-2 CELLS UNDER OXIDATIVE CONDITIONS

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Abstract: Autophagy is an essential process for degradation of non-useful cellular components and organelles which are lysed for their recycling or elimination, being the mechanisms involved in its regulation less known in plants than in animal systems. Redox regulation of autophagy components is emerging as a key mechanism, with thioredoxins (TRXs) proposed as candidates to regulate this process. In this work we deepen into some previously described results showing that overexpressing PsTRX*o*1 tobacco BY-2 cells presented higher viability than non-overexpressing cells under H_2O_2 treatment via antioxidants and delayed cell death (Ortiz-Espín et al., 2015). In this situation we now hypothesize an additional functional role of autophagy and TRX*o*1 in the oxidative stress response that collaborate to increase cell viability. We analyse ATG4 and ATG8 gene expression, protein levels as well as ATG4 enzymatic activity in parallel to cell viability, autolysosomes visualization and immunolocalization studies of ATG8 by fluorescence microscopy using known autophagy inhibitors. The results indicate that overexpression of PsTRX*o*1 could be favouring the establishment of an autophagic process in the response of TBY-2 cells to H_2O_2 participating in the observed increased cell survival. Also the *in vitro* interaction of autophagy related protein ATG4 and TRX*o*1 is shown by dot-blot trap analysis and the redox regulation of the ATG4 activity by the TRX system (NADPH/thioredoxin reductase/thioredoxin *o*1) is demonstrated, extending the role of TRXs in mediating the redox regulation of autophagy.

Key words: autophagy, hydrogen peroxide, TBY-2 cells, thioredoxin o1, viability

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CARBON ($\delta^{13}C$) AND NITROGEN ($\delta^{15}N$) STABLE ISOTOPE COMPOSITION PROVIDE NEW INSIGHTS INTO PHENOTYPIC PLASTICITY IN INVASIVE *Carpobrotus edulis* UNDER DIFFERENT HABITATS

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Abstract: The South African clonal plant *Carpobrotus edulis* is one of the most problematic invasive species worldwide and represents a severe threat to biodiversity in coastal areas (Campoy *et al*., 2019). In the northwestern Iberian Peninsula, it invades different coastal habitats, including cliffs, dunes, mixed forests and disturbed areas (Campoy *et al.*, 2018).
Here, we aimed to know the plastic response of *C. edulis* assessing ecophysiological differences between plants growing in these four different habitats. We selected three locations per studied habitat, distributed between O Grove (Spain) and Afife (Portugal). In each location, we selected five individuals of *C. edulis* and measured morphological traits and carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope ratios.
Our results indicated that shoot dry weight was higher on cliffs and lower in mixed forests. Contrary to this, succulence was higher in mixed forests and lower in cliffs. This suggests that plants growing on cliffs present protective structures for avoiding losing water due to high radiation exposure and substrate shortages typical of these habitats. Leaf carbon content and C:N ratio were higher on cliffs, whereas leaf nitrogen content was significantly lower than in other habitats. Moreover, leaf nitrogen content was higher in mixed forest, which could be related to a higher nitrogen concentration in the soil. Differences in $\delta^{15}N$ showed the use of different N sources in the different habitats. Values are higher in the disturbed areas with greater biological activity. Despite the different soil water retention capacities, there are no major differences in the $\delta^{13}C$ of the leaves, indicating a possible better water use efficiency on cliff and dune habitats.
In conclusion, *C. edulis* showed ecophysiological plasticity in the different coastal habitats colonized.

Key words: Alien invasive plants, *Carpobrotus edulis*, intrinsic water use efficiency, ^{15}N, ^{13}C

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CARBON METABOLISM OF WILD BARDENAS REALES *Medicago truncatula* UNDER WATER DEFICIT CONDITIONS.

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Abstract: In cultivated plants subjected to artificial selection, genetic diversity is lower than in wild species. Regarding drought tolerance, legume crops natural from semiarid areas own a genetic variability that must be explored to improve drought tolerance of cultivated genotypes.

This study was aimed at exploring the root carbon metabolism of a wild *Medicago truncatula* (*Mt.BR*) from the semiarid area of Bardenas Reales (Spain) and the cultivated *M. truncatula* cv. Jemalong A17 (*Mt*) line under water deficit conditions. Thus, the two lines were exposed to progressive water deficit levels under controlled conditions.

In both species, the carbon metabolism of the taproots (tapRs) and fibrous roots (fibRs) exhibited a different response to water deficit related to the different functioning of both parts of the root system. Sucrose and sucrose synthase activity were fundamentally located in the tapRs, which might be related to their carbon partitioning role. Conversely, alkaline invertase activity and hexose pool were mainly accumulated in the fibRs, in charge of water and nutrient absorption in the plant root system. At the shoot level, the wild Bardenas Reales ecotype exhibited a more efficient gas exchange regulation, which may contribute to the higher starch content presented by the tapRs compared to the *Mt.* Besides, the *Mt.BR* tapRs presented a higher accumulation of sucrose associated with a higher sucrose synthase activity, which may reinforce the carbon supply from tapRs to fibRs and improve the soil exploration of the root system for water absorption. Drought-tolerance traits showed in the wild *M. truncatula* from Bardenas Reales may be interesting to improve the performance of other cultivated legumes as *M. truncatula* and *M. sativa* under the future climate change scenarios.

Keywords: drought, fibrous roots, taproots, root functioning, carbon metabolism allocation.

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CITRUS IRRIGATION WITH DESALINATED SEAWATER UNDER A CLIMATE CHANGE SCENARIO

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Abstract: In arid and semiarid regions, the current lack of natural water resources is driving the use of alternative sources for crop irrigation, such as desalinated seawater (DSW). However, the use of DSW could affect crop productivity due to its chemical composition (predominance of phytotoxic ions: Na^+, Cl^- and B^{3+}). Citrus species are classified as salt and boron-sensitive; however, the rootstock plays a fundamental role in the tree's tolerance of abiotic stresses. To study the behaviour of citrus plants irrigated with DSW, one-year-old Verna lemon trees grafted on two rootstocks with differing salinity and boron tolerance (*Citrus macrophylla*, CM, and sour orange, SO) were used. The experiment was carried out at high temperature (35/27ºC, 14/10h) and the plants were irrigated with three types of water: DSW, DLB (desalinated with low B, obtained by reducing the B content of DSW) and Control (distilled water). Each type was supplemented with Hoagland nutrients. The plants were watered three times per week and harvested seven months after the treatments started. Under the high-temperature conditions, the use of DSW decreased the shoot growth of the citrus plants due to a nutritional imbalance produced by the high Na^+, Cl^- and B^{3+} levels in DSW. Plants irrigated with DSW had increased root and leaf concentrations of these phytotoxic elements, exceeding the toxic thresholds of 0.25% Na^+ and 0.6% Cl^- in photosynthetically active leaves. DLB-irrigated plants, but mainly those irrigated with DSW, also accumulated B above the toxic threshold. However, the behaviour of the rootstocks differed: CM accumulated more Na^+ and Cl^- but less B^{3+} than SO, whereas the injury to cell membranes was greater in SO plants. The greater reduction of shoot growth in CM plants, compared to SO plants, was probably due to the phytotoxic effects of Na^+ and Cl^- accumulation. Irrigation with DSW also produced foliar nutritional imbalances, reducing significantly the K^+/Na^+, Ca^{2+}/Na^+, Mg^{2+}/Na^+, NO_3^-/Cl^- and HPO_4^{2-}/Cl^- ratios, that affected plant growth. As citrus rootstocks differ in their tolerance of phytotoxic ions, the severity of negative effects on DSW-irrigated citrus trees under climate change will depend on the adequacy of the plant material selected.

Key words: Phytotoxicity, rootstocks, sodium, chloride, boron

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CITRUS ROOTSTOCKS MODIFY SCION ANTIOXIDANT SYSTEM UNDER DROUGHT AND HEAT STRESS COMBINATION

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Abstract: Climate change is altering weather conditions such as mean temperatures and precipitation patterns. Rising temperatures, especially in certain regions, accelerates soil water depletion and increases drought risk, which affects agriculture yield. Previously, our research demonstrated that the citrus rootstock Carrizo citrange (Citrus sinensis × Poncirus trifoliata) is more tolerant than Cleopatra mandarin (C. reshni) to drought and heat stress combination, in part, due to a higher activation of the antioxidant system that alleviated damage produced by oxidative stress. Here, by using reciprocal grafts of both genotypes, we studied the importance of the rootstock in scion performance and antioxidant response under this stress combination. Carrizo rootstock, under stress combination, positively influenced Cleopatra scion by reducing H2O2 accumulation, increasing superoxide dismutase (SOD) and ascorbate peroxidase (APX) enzymatic activities and inducing SOD1, APX2 and catalase (CAT) protein accumulations. On the contrary, Cleopatra rootstock induced decreases in SOD and CAT activities and CAT protein content on Carrizo scion. Taken together, our findings indicate that the activation of the antioxidant system under stress combination is a transmissible trait from the rootstock to the scion and they highlight the importance of the rootstock selection to improve crop performance and maintain citrus yield under the current scenario of climate change.

Key words: Abiotic stress, Climate change, Grafting

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COMPARATIVE EVOLUTIONARY APPROACH OF MITE-INDUCED RIN4-LIKE/NOI FAMILY PROTEINS

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Abstract: The spider mite *Tetranychus urticae* is an economically important agricultural pest, which feeds on more than 1,100 plant species and has an extensive record of pesticide resistance. Temperature rising and dryer environments as a result of climate change are providing new comfortable niches to this polyphagous species.

Plants have evolved a sophisticated innate immune system to recognize invading organisms and induce host defenses to achieve resistance. A component of this system in *Arabidopsis thaliana* is RIN4, an intrinsically disordered protein anchored to the plasma membrane. In an RNAseq experiment performed in our laboratory, 4 out of the 15 RIN4-like paralogous genes of *A. thaliana* were significantly over-expressed after *T. urticae* infestation. Two of them (NOI3 and NOI5) have one NOI (NO_3-induced) domain, which plays an important role in effector recognition and R protein activation. The other two are longer (NOI10 and NOI11) and, as RIN4, have two NOI domains linked by an intrinsically disordered region. This region is thought to function as a protein hub promoting protein interactions and is also a post-translational modification target of multiple bacterial effectors.

Comparative genomic analyses could give valuable insights into the evolution of these intrinsically disordered family of proteins involved in plant defense and help to understand plant adaptation to the invading organisms. In consequence, we performed a genomic comparative analysis of RIN4-like sequences in different species and showed that this family of proteins has an evolutionary origin as far as moss. All species analyzed have the two types of RIN4-like sequences (one or two NOI domains), although shorter ones have higher evolutionary family proliferation and may be acting as regulators of the longer ones. We also investigated the relationship between amino acid evolutionary rates and predicted sites of post-translational modifications. Intrinsically disordered regions show an expected low conservation degree and a higher amino acid evolutionary rate than NOI domains. Besides, we also found structural differences in the NOI domains of NOI10 and NOI11, compared to RIN4.

Key words: Tetranychus urticae, RIN4-like/NOI, defense, evolution.

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Comparative transcriptomics reveals changes in salicylate and jasmonate signaling pathways in leaves of carbonate-tolerant *Arabidopsis thaliana* under bicarbonate exposure.

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Abstract: High bicarbonate concentrations of calcareous soils with high pH can affect crop performance due to different constraints. Among these, Fe deficiency has mostly been studied. The ability to mobilize sparingly soluble Fe is a key factor for tolerance. Here, a comparative transcriptomic analysis was performed with two naturally selected *Arabidopsis thaliana* demes, the carbonate-tolerant $A1_{(c+)}$ and the sensitive $T6_{(c-)}$. Analyses of plants exposed to either pH stress alone (pH 5.9 vs. pH 8.3) or to alkalinity caused by 10 mM $NaHCO_3$ (pH 8.3) confirmed better growth and nutrient homeostasis of $A1_{(c+)}$ under alkaline conditions. RNA-sequencing (RNA-seq) revealed that bicarbonate quickly (3 h) induced Fe deficiency-related genes in $T6_{(c-)}$ leaves. Contrastingly, in $A1_{(c+)}$, initial changes concerned receptor-like proteins (RLP), jasmonate (JA) and salicylate (SA) pathways, methionine-derived glucosinolates (GS), sulfur starvation, starch degradation, and cell cycle. Our results suggest that leaves of carbonate-tolerant plants do not sense iron deficiency as fast as sensitive ones. This is in line with a more efficient Fe translocation to aerial parts. In $A1_{(c+)}$ leaves, the activation of other genes related to stress perception, signal transduction, GS, sulfur acquisition, and cell cycle precedes the induction of iron homeostasis mechanisms yielding an efficient response to bicarbonate stress.

Key words: Alkaline stress; *Arabidopsis thaliana*; Glucosinolate; Salicylate; Transcriptomic

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CONTRASTING FUNCTIONAL STRATEGIES FOLLOWING SEVERE DROUGHT IN TWO MEDITERRANEAN OAKS WITH DIFFERENT LEAF HABIT

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Abstract: Two types of oaks (*Quercus* L.; Fagaceae) with different palaeogeographic origin, different types of leaves, and different contrasting strategies to cope with water limitations, coexist in Mediterranean-type climates. Both *Q. faginea* and *Q. ilex* subsp. *rotundifolia* are considered as representatives of these two types of oaks that coexist in many areas of the western Mediterranean basin. However, this coexistence is a complex issue that has been the subject of debate in several articles (Nardini et al.1999, Montserrat-Martí et al. 2009, Peguero-Pina et al. 2015) and more research is needed to understand it. We induced severe water stress to both species, as occurs during summer in Mediterranean-type climates, to study the ability of plants to supply water to transpiring leaves that can be severely limited. Despite this common trend, *Q. faginea* experienced an early decrease in shoot hydraulic conductance (K_{shoot}) in response to drought, with a less

negative PLC50 than estimated for *Q. ilex* subsp. *rotundifolia*. In contrast to K_{shoot}, both species showed a similar evolution of hydraulic conductivity in the stems (K_{stem}) with increasing levels of drought stress. That is, the PLC50 in the shoots was lower than in the stems of *Q. faginea*, which is consistent with the hypothesis of "vulnerability segmentation" (Hochberg et al. 2017). According to the results obtained, the stems of *Q. faginea* were protected from an extensive xylem embolism and thus were able to develop new leaves after the dry period. This capacity is crucial for the survival and growth of this species, in addition to partially compensating for the loss of competitiveness with the coexisting Mediterranean evergreen oaks with longer leaf life. This was the case with *Q. ilex* subsp. *rotundifolia*, which showed a conservative foliar strategy, characterized by a high resistance to cavitation induced by drought and a maintenance of its functional leaves even in conditions of intense drought.

Key words: hydraulic traits, water stress, Mediterranean-type climates, vulnerability segmentation.

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CONTRASTING ROLES OF ETHYLENE IN SIGNALING OF DIFFERENT ABIOTIC STRESSES IN RICE PLANTS

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Abstract: The gaseous plant hormone ethylene is a main regulator of plant stress. In rice and other semi-aquatic plants, this hormone plays a central role in the signaling pathway of stress by submergence, in crosstalk with ABA and GAs (Rzewuski & Sauter, 2008). However, knowledge on its role in the responses to other stresses is still limited. Many reports indicate ethylene increases under stress conditions different to hypoxia, but also decreases occur, particularly in semi-aquatic plants (Carrió-Seguí et al., 2015). Also, increased ethylene synthesis may induce both growth inhibition and stimulation (Pierik et al., 2006). We investigated the effect of different stresses on plant growth, metabolic responses, and ethylene production in rice seedlings of the lowland ecotype of *Oryza sativa* L., var. *japonica,* cv. Bahia, subjected to different type, duration, and intensity stress levels. Submergence significantly promoted ethylene production while stresses induced by salinity and the heavy metal Cd caused a reduction in hormone release, together with a decrease in plant growth. Alleviation of Cd-induced stress resulted in a concomitant increase of ethylene production, carbon assimilation and plant growth. Seedlings grown in the presence of ABA and treated with Cd showed a further reduction of ethylene release, while GA_3 counteracted partially the effect of the metal. Compared to *Arabidopsis*, rice possesses conserved and diverged mechanisms for ethylene signaling, and alternative pathways have been described (Yang et al., 2015; Binder, 2020) but also, different responses may also be due to different stress signaling pathways upstream ethylene synthesis.

Key words: Abiotic stress, ethylene**,** *Oryza sativa*, semi-aquatic plants, submergence.

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COST-EFFECTIVE PHENOTYPING METHODS TO ADDRESS CROP RESILIENCE AND INTER-CULTIVAR VARIABILITY TO COPE CLIMATE CHANGE: A PRACTICAL EXAMPLE IN GARLIC

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Abstract: Climate change, the foreseeable increasing world population and current global consumption patterns, pose new challenges for agriculture and world food supply. Under this context, finding more resistant cultivars to environmental stressors, is fundamental for an effective agricultural adaptation to climate change. A good understanding on intraspecific variability patterns of the functional mechanisms involved in plant responses to the environment (ecophysiological approach), is basic for cultivar selection and enhanced crop yield. Crop phenotyping is currently in the search of improved genotypes/cultivars against abiotic and biotic stresses (Araus et al., 2014, 2008). Thus, intraspecific characterization of functional and yield responses to the environment should be a key approach in crop breeding and selection programs (Rosenqvist et al., 2019). However, phenotyping methods are sometimes expensive and time-consuming. In this sense, crop phenotyping and cultivar selection must be performed in a cost-effective manner with practical implementation (Araus et al., 2018; Costa et al., 2019; FAO, 2009). In this sense, the use of new technologies and low-cost phenotyping methods, along with enhanced agro-biodiversity, represents a host of promising opportunities to improve agriculture sustainability and crop adaptability to climate change (Deery et al., 2016; Lin, 2011; Shelef et al., 2017). In this work, we summarize the main phenotyping methods carried out in a thesis about garlic inter-cultivar variability in response to water availability. In the light of the findings and experience acquired during the thesis, We here discuss which methods were cost-effective in terms of invested time, economic cost and reliable results obtained. These findings aim to open new avenues to develop optimized protocols on cost-effective phenotyping methods for crop breeding and management in the context of climate change.

Key words: Climate change, Phenotyping, Abiotic stress, Agro-biodiversity

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COULD FRUITS MODIFY LEAF SOLUBLE SUGARS AND DETERMINE WATER-CARBON RELATIONS IN PLANTS?

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Abstract: Water scarcity is one of the main restrictions to achieve food security for a growing population. Thus, new deficit irrigation strategies are being developed based on plant physiology but surprisingly, little is known on fruit physiology. The recent results on the importance on non-structural carbohydrates (NSC) to facilitate osmotic adjustment (Blum, 2017) and their differential transport depending on the water stress level (Gersony et al., 2020) could be useful to explain the effect of fruit loading on plant water relations but it has not been studied yet. The aim of this work is to study the effect of the fruit presence or absence on the leaves water relations mediated by their effect on leaf soluble sugar dynamics and thus, on leaf osmotic potential analyzing (i) the regulation of stomatal conductance (g_s), net photosynthesis (A_N) and leaf water potential (Ψ_{leaf}) produced by fruits and (ii) determine leaf osmotic potential (π) and NSC dynamics depending on the presence or absence of fruits and water stress. The experiment was carried out between July and November 2018 on twelve olive trees. Six of them were well-watered (WW) trees and the rest were water-stressed (WS). In each of these treatments, half had the presence of fruits (+) and the other half had absence of fruits (-) (n = 3). A_N, g_s, Ψ_{leaf}, were measured weekly and π and NSC content were measured in four specific moments in each tree. The results showed that WW+ presented higher A_N and g_s values, lower leaf NSC and π less negative than WW-, indicating that carbohydrates were being exported to the fruit. Assuming that fruits behave as a carbon sink organ, we also observe that WS+ presented more negative Ψ_{leaf} values than WS-. This response would be modulated by the hydric treatments, because the effect of A_N and g_s was not as prominent in WS nor that of the hydric potential

in WW. In conclusion, our results suggested that fruits can modulate leaf NSC and maintain the carbon and water relations under water stress.

Key words: water stress, non-structural carbohydrates (NSC), stomatal conductance, photosynthesis, fruit load.

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DIFFERENT METABOLIC ROLES FOR AOX IN LEAVES OF PALUSTRINE AND TERRESTRIAL SPECIES

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Abstract: The alternative oxidase pathway (AOP) is associated with excess energy dissipation in leaves of terrestrial plants. To address whether this association is less important in palustrine plants, we compared the role of AOP in balancing energy and carbon metabolism in palustrine and terrestrial environments by identifying metabolic relationships between primary carbon metabolites and AOP in each habitat. We measured oxygen isotope discrimination during respiration, gas exchange, and metabolite profiles in aerial leaves of ten fern and angiosperm species belonging to five families organized as pairs of palustrine and terrestrial species. We performed a partial least square model combined with variable importance for projection to reveal relationships between the electron partitioning to the AOP (τ_a) and metabolite levels. Terrestrial plants showed higher values of net photosynthesis (AN) and τ_a, together with stronger metabolic relationships between τ_a and sugars, important for water conservation. Palustrine plants showed relationships between τ_a and metabolites related to the shikimate pathway and the GABA shunt, to be important for heterophylly. Excess energy dissipation via AOX is less crucial in palustrine environments than on land. The basis of this difference resides in the contrasting photosynthetic performance observed in each environment, thus reinforcing the importance of AOP for

photosynthesis.

Key words: Alternative Oxidase Pathway, Cytochrome Oxidase Pathway, electron partitioning to the AOP, primary metabolism, terrestrial species, palustrine species, heterophylly

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DIFFERENTIAL EXPRESSION OF PAL, SOD AND PPO GENES IN *VITIS VINIFERA* (TEMPRANILLO CV.) LEAVES AFFECTED BY ESCA DISEASE

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Abstract: Esca is a widespread and destructive grapevine trunk that affects grape yield and wine quality. The phenolic compounds play a role in limiting the development of this disease. The experiment was carried out in a vineyard of the Tempranillo (*Vitis vinifera* L.) variety, planted in 2001 on Richter-110 rootstock at a spacing of 2.5 m x 1.2 m. The vineyard is located at Finca la Orden (Junta de Extremadura, Badajoz, Spain). We studied the photosynthetic pigments and photosynthetic efficiency, total phenols, flavonoids and phenylpropanoids glycosides, polyphenol oxidase (PPO) activity, the compounds of ascorbate-glutathione (AsA-GSH) cycle, the total antioxidant capacity (FRAP), and the Phenylalanine ammonia lyase (PAL), Superoxide dismutase (SOD) and PPO gene expression of Tempranillo leaves. Leaves were sampled on three dates (June, August, and October) from 10 vines of each experimental condition: healthy and diseased plants.
The phenolic, flavonoid and phenylpropanoid glycosides compounds decrease, and the PAL expression increases (x2.09 and x2,83 for June and August, respectively) in diseased leaves, as compared to healthy leaves. On the contrary, the PPO activity increases, in consonance to the strong increase of the PPO expression. The chlorophylls and carotenoids drastically decrease, but only in leaves collected in June. The photosynthetic efficiency decreases from 0.781 to 0.475 for healthy and diseased leaves. The compounds of the AsA-GSH cycle and the FRAP also decrease. The membrane lipid peroxidation is not affected, except in the October samples. The SOD expression increases considerably, which can prevent the lipid peroxidation by reactive oxygen species scavenging.

Key words: Esca; gene expression; phenolic compounds; Tempranillo
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DROUGHT AND SHRUB INVASION IMPACTS ON CORK OAK WATER STRESS

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Abstract: Due to climate change, a greater intensity and frequency of droughts is expected, leading to higher tree mortality rates, including in the Mediterranean regions (Allen *et al.*, 2010). Cork oak (*Quercus suber* L.) woodlands are Mediterranean ecosystems of high economic and conservation value (Bugalho *et al.,* 2011) that are being affected by the increasing occurrence of droughts. Furthermore, the effects of drought in the cork oak trees may be aggravated by the invasion of shrubs such as *Cistus ladanifer* L. (Caldeira *et al.*, 2015). Hydraulic failure of the xylem through embolism formation is one of the main processes that can lead to tree mortality (Choat *et al.,* 2018). The main objectives of this study were to caracterize cork oak vulnerability to cavitation and to measure the impact of induced drought and invasion by *Cistus* on cork oak trees functioning.

Cork oak vulnerability to cavitation was measured using the pneumatic method (Pereira *et al.,* 2019). This method measures air discharged periodically while dehydration occurs. Also, an experiment was carried out in a cork oak woodland at Vila Viçosa, in the South of Portugal. Trees were subjeted to four treatments: cork oak control, cork oak with shrub invasion, cork oak with induced drought, cork oak with shrub invasion and induced drought. In these stands, predawn (Ψ_{Pd}) and midday (Ψ_{Md}) leaf water potencial measurements of cork oak trees and *Cistus* were carried out periodically in 2020.

Cork oak showed a more isohydric behaviour compared to gum rockrose, as cork oaks Ψ_{Md} were closer to Ψ_{Pd} values as Ψ_{Pd} decreased.

The Ψ_{50} of cork oak, i. e. the xylem water potential at which 50% of air was discharged, was -4.78 ± 0.19 MPa. Both species showed high hydraulic safety margins, maintaining midday water potential values above the threshold of 12% of air discharged.

Cistus shrubs displayed a greater water stress index, between June and October, (cumulative sum of Ψ_{Pd} multiplied by the number of days between measurements) compared to cork oak. Shrub invasion increased the cork oak water stress index significantly.

Key words: *Quercus suber*, *Cistus ladanifer*, embolism, invasion, drought.

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EFFECT OF SILICON SUPPLY ON PLANT GROWTH AND PHOTOSYNTHETIC PIGMENT ACCUMULATION IN TWO GENOTYPES OF *Vitis vinifera* L. SUBJECTED TO SALINITY STRESS

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Abstract: To assess the effects of silicon (Si) on the responses of *Vitis vinifera* L. to salt stress, two own-rooted genotypes 'Negramoll' and 'Tintilla' well-adapted to local Canary Islands conditions were subjected to NaCl stress (80 mM) for 52 days. Young plants cultivated in plastic pots filled with commercial substrate were randomly distributed in three blocks, and the established treatments were: a) Control (non-stress), b) salt stress (80 mM NaCl), and c) NaCl + Si (added to water irrigation). Data showed a significant decrease in shoot length and thickness, and total leaf number per plant induced by salt stress in both cultivars (cvs) compared to control. Nevertheless, the addition of Si to the water solution reduced the impact of salt stress on all these growth parameters. In addition, salt stress did not statistically modify leaf biomass; however, Si significantly increased this variable compared to salt stress alone and either respect to control treatment. Regarding stomatal opening, although salt stress decreased this parameter in both cvs, the application of Si slightly mitigated this decrease in 'Negramoll', but no evident effect was observed in 'Tintilla'. Concerning photosynthetic pigments, salt stress reduced total chlorophyll and carotenoid content in both cvs 52 days after treatments compared to control, and no significant effects of Si were found in the levels of pigments compared to salt stress. In contrast, it has been previously reported that exogenous Si increased the concentrations of pigments, soluble sugars, and starch in grapevines under salt stress. Furthermore, data presented here show that proline concentration was similar in stressed and non-stressed plants of both cvs; however, Si increased its level compared to control only in 'Negramoll'. The ability of proline in mitigating salt stress injuries has also been linked to the nitrogen assimilation activities. In conclusion, Si seems to be involved in decreasing salt impact on plant growth parameters in the studied cvs and could be involved in proline production at least in 'Negramoll'. Future experiments applying Si should be performed to investigate its implication in the regulation of several metabolites such as phytohormone and organic or inorganic solutes in *Vitis vinifera* L.

Key words: carotenoids; chlorophylls; leaf number; leaf biomass; NaCl stress.

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EFFECT OF THE USE OF HYDROMUCLHES ON LIPID PEROXIDATION AND TOTAL PHENOLIC COMPOUNDS DURING A SHORT-TERM DROUGHT STRESS ON SCAROLE CROP

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In this study, we investigated the effect of the use of a new type of biological mulch called hydromulch on escarole crop (*Cichorium endivia* L.), focusing on its physiology during a short-term drought stress. We tested three types of biological hydromulches, which are based on recycled additives: wheat straw (WS), rice hulls (RH), and substrate used for mushroom cultivation (MS); compared with two controls: covered control with polyethylene mulch (PE); and non-cover control (C). The objective was to determine their effects on escarole plants, specifically their ability to mitigate water stress, through the study of lipid peroxidation and total phenolic compounds. The experiment was carried out in a climate-controlled greenhouse located at the "Torreblanca" experimental field in Torre Pacheco, Murcia, Spain. Seventy-one days after transplant, the irrigation was interrupted for 4 days. Leaf samples were taken before the drought condition, during stress, and after stress. Lipid peroxidation (LP) and total phenolic compounds (TPC) were determined.

During the control, stress, and after stress condition, lipid peroxidation was significantly affected by mulch, being significantly higher in RH and C treatments in all irrigation conditions. On the other hand, after stress, plants grown with MS and PE treatment presented similar levels of LP to control conditions. There were no significant differences between the use of the mulch and the content of TPC. Nonetheless, stress caused a reduction in TPC in all mulching treatments, being this reduction very short in MS. It is noteworthy that plants grown with MS presented a similar amount of TPC under all stress conditions. In conclusion, mulch is an effective alternative to reduce the physiological effects of water

stress on escarole. In this study, the use of hydromulch with MS stands out since it produced a rapid recovery after the end of stress in terms of lipid peroxidation, besides maintaining TPC levels during stress and after stress conditions.

Key words: *Stress indicators, Water scarcity, Mulch technology, Horticultural crops, Drought stress*

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ELIMINATION OF "ACCLIMATION OF PHOTOSYNTHESIS TO ENVIRONMENT" (APE1) HAS A DELETERIOUS EFFECT ON THE GROWTH OF *Arabidopsis thaliana* UNDER MODERATE HIGH LIGHT CONDITIONS

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Acclimation of Photosynthesis to Environment (APE1) is a plastidial protein found in all photosynthetic organisms, from cyanobacteria to higher plants. It has a transmembrane domain and seems to be localized in the thylakoid membranes. *ape1* mutants show alterations on the Photosystem II (PSII)-fluorescence, changes that result from transition from low to moderate high light growth conditions (Walters et al. 2003). No other data are available for this protein and its function is largely unknown. We have analyzed two T-DNA knock-out mutants of APE1 using a pulse-amplitude-modulated (PAM) fluorimeter. Mutant plants lacking APE1, unlike wild type (WT) plants, cannot adapt the PSII performance when transferred from normal light (NL, 120 µmoles m^{-2} s^{-1}) to moderate high-light conditions (HL, 600 µmoles m^{-2} s^{-1}). They show lower electron transfer rate (ETR) and maximum quantum yield (F_v/F_m) values than WT plants, indicating light-stress mediated PSII lesions. *ape1* mutant plants germinated and grown in HL conditions show the same PSII alterations described above and lower growth rate than WT plants, indicating that APE1 is necessary for the growth of the plant under high light conditions. The ultra-structure of *ape1* chloroplasts is severely affected after one week of HL stress, with increased intra-thylakoid spaces. We are using different experimental approaches, such as study of the CO_2 fixation rates, levels of distinct proteins involved in photosynthesis (D1, NdhS, CURT1A…) in *ape1* mutants or analysis of photosynthetic complexes by Blue-Native electrophoresis, to determine the function of APE1 and the pleiotropic effect induced by its absence in plants subjected to HL stress.

Key words: *Arabidopsis*, high-light stress, PAM-fluorescence

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ENHANCING HYDROLYTIC ENZYMES PRODUCTION IN ELICITED TAMARILLO (*Solanum betaceum* CAV.) CELL SUSPENSION CULTURES

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Abstract: Plant cell suspension cultures are widely used in plant biology and biochemistry as a valuable tool for analyzing cellular and molecular processes, metabolite synthesis, and differentiation; bypassing the plant organism's structural complexity. As a biotechnological tool, they are the basis for developing technologies to produce several types of metabolites relevant for the pharmaceutical, cosmetics and food industries. Within the range of approaches used to increase metabolites' production by plant cells, one of the most recurrent is applying elicitors capable of enhancing metabolic pathways involved in stimulating cell defense mechanisms. Understanding the molecular mechanisms based on those cellular responses not only allows for a more comprehensive knowledge of plant cell stress responses but also a better modulation of those responses in different contexts. Previous proteomics analysis of tamarillo (*Solanum betaceum* Cav.) cell lines and cell suspension cultures have been used to further characterize and optimize the cell line's growth and stress-related metabolite production under *in vitro* controlled conditions. These assays showed that tamarillo suspension cultures secrete a wide range of enzymes, namely proteases and glycoside hydrolases, commonly associated with inhibiting pathogen-derived cell wall degrading enzymes (Alves et al., 2017). By testing different sucrose concentrations and the effects of two types of biotic elicitors, one directed for cell growth and protein production (casein hydrolysate and yeast extract) and the other for the promotion of plant defense mechanisms (chitosan), in two cell lines, it was found that 3% (w/v) sucrose concentration in the liquid medium affected the production of hydrolytic enzymes. Moreover, casein hydrolyzed at 0.5 and 1.5 g/l promoted protein production, whereas yeast extract (0.5 g/l) promoted glycosidase activity. Meanwhile, chitosan at 0.05 and 0.1 g/l enhanced glycosidases, alkaline phosphates, and proteases activity. These results set the basis for establishing and optimizing tamarillo cell suspension cultures as valuable tools for the analysis of stress-related metabolic networks and as a promising system for plant-derived metabolites production in scaled-up approaches, such as bioreactors.

Key words: Cell growth**,** Chitosan, Elicitation, Glycosidase activity, Hydrolytic enzymes

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EVALUATION OF PHYSIOLOGICAL RESPONSES OF TWO OWN-ROOTED CULTIVARS OF *Vitis vinifera* L. UNDER SALT STRESS CONDITIONS

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Abstract: *Vitis vinifera* L. species possess a numerous genotypes and clones cultivated at local, national, or worldwide scale. In addition, in most areas of vineyards, cultivars are usually grafted on rootstocks; however, in other regions among them Canary Islands, own-rooted cultivars are the predominant due to the absence of Phylloxera pest. In accordance with earlier studies, grapevines are believed to be moderately sensitive to salt stress. To reveal the responses of own-rooted cultivars against salt stress conditions, 'Castellana Negra (CN)' and 'Negramoll (Ne)' were subjected to NaCl stress during a relatively short-term period. The experimental design was focused on the evaluation of plant growth behaviour and the involvement of abscisic acid (ABA) and salicylic acid (SA) on stress responses of both cultivars. In general, the results showed a better response of 'CN' than 'Ne' expressed as a minor decrease of leaf abscission and a delay in salt toxicity establishment. The concentrations of ABA and SA in the leaves of control plants were the double in 'CN' than in 'Ne'. Salt stress considerably increased foliar ABA and SA content in both genotypes, in comparison to control. In conclusion, the best performance of 'CN' compared to 'Ne' exhibited as the lower decline of leaf abscission and the delay of salt injury establishment should be related to the major amounts of ABA and SA accumulated in the leaves of 'CN' under the salt stress conditions.

Key words: leaf abscission; NaCl stress; phytohormones; plant growth.

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FLORET DEVELOPMENT AND SPIKE FERTILITY IN TWO CONTRASTING WHEATS AS AFFECTED BY HEAT WAVES

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Wheat yield is frequently limited by the number of grains, and spike fertility is a critical trait for further raising yield potential. Heat waves will become more frequent and severe and may have significant consequences on wheat yield. However, most studies focused on heat during grain filling period and its effects on grain weight. There is a lack of field studies investigating the consequences of an abrupt rise in temperature during final stages of stem elongation on spike fertility, and even less, on the physiological bases for such effect considering processes like floret initiation and survival. We carried out two field experiments at two locations (Bell lloc d'Urgell and Lleida, NE Spain) in which two high-yielding cultivars, Pistolo and Sublim, were grown and subjected to heat waves imposed at booting (Zadoks 45) using portable chambers of transparent polyethylene films (placed over the canopy for 10 days with maximum temperature increased by 7.5 ºC). Pistolo is a cultivar with relatively low number of heavy grains, and Sublim is the opposite (higher number of light grains). We sampled plants frequently and dissected different spikelets across the spike and recorded the stage of development of each floret primordium within these spikelets to determine differences between cultivars and heat waves on the dynamics of generation and degeneration of floret primordia. The heat wave treatment indeed had a negative effect on spike fertility of both genotypes, reducing the number of fertile florets per spike (being the effect evident in virtually all spikelets of the spike). Exposure to the heat wave accelerated the rate of floret primordium death reducing the proportion of florets initiated that continued developing normally to become a fertile floret, and the effect was the same in all spikelets considered (averaging across basal, central and apical spikelets, there was a reduction of 13-19% in floret survival). The acceleration of developmental rates produced by the heat wave did not compensate for the reduction in time for development and labile floret primordia (florets 3-4 in central spikelets and 2-3 in basal/apical spikelets) died before progressing towards becoming a fertile floret.

Key words: Heat stress, grain number, floret survival, reproductive phase

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GRASSLANDS GROWTH AND SUSTAINABILITY UNDER THE MEDITERRANEAN CONDITIONS

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Abstract: Grasslands sustainability is highly affected by drought in the Mediterranean basin regions, where the lack of precipitations and high temperatures are mostly frequent. In this context, the selection of turfgrass varieties with tolerance to drought and low water needs is considerate as an effective approach to decrease the water requirements of natural green areas (Saunders, 2009) and can remain a satisfactory quality of grasslands under dry conditions (Jansen van vuuren, 1997). Furthermore, the precision irrigation management is also a potential tool for green space sustainability under limited conditions. In this context, remote sensing methods are widely used to improve irrigation efficiency, to estimate various plant traits, including chlorophyll content, nitrogen concentration and biomass (Saberioon et al., 2014; Yousfi et al., 2019; Marin et al., 2020;) and can provide a valuable information on plants adaptation to water stress (Araus and Cairns, 2014). This study compared the performance of different mixtures of C_3-C_4 turfgrass under rainfed condition (without irrigation) using remote sensing parameters. The choice of C_3-C_4 mixtures permit a combination of two environmental and physiological characteristics, since C_3 species are typical for cooler temperature regions and the C_4 grasses are adapted to persist in warmer environments, and both species differ in the photosynthetic system for the uptake of carbon dioxide. Field measurements were conducted in 2021 at the Madrid Institute for Rural, Agrarian and Food Research and Development (IMIDRA). Six C_3-C_4 turfgrass mixtures formed by *Festuca arundinacea* and *Poa pratensis* (both C_3) and three C_4 turfs, *Cynodon dactylon, Buchloe dactyloides* and *Zoysia japonica*, were evaluated under rainfed conditions (without irrigation). Performance of turf species was assessed using green area (GA) and greener area (GGA) vegetation indices calculated from RGB (red green, blue) images, the normalized difference vegetation index (NDVI) and the plant canopy temperature (CT). Differences among the six turf were reported through the vegetation indices (NDVI, GA, GGA) and the CT. The mixtures of *Festuca* with the three C_4 turfgrass presented higher vegetation indices and lower CT compared to *Poa* mixtures. *Festuca-Buchloe* was the mixture more adapted to the no irrigation condition, whereas *Poa-Cynodon* was the more susceptible.

Key words: Vegetation indices, Canopy temperature, Grassland sustainability, C_3-C_4 Turfgrass.

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IDENTIFICATION AND EXPRESSION OF OLEATE DESATURASE GENES IN MANDARIN FRUIT IN RESPONSE TO POSTHARVEST COLD STRESS

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Abstract: Chilling injury (CI) is a postharvest disorder developed in fruits of many *Citrus* species under low non-freezing temperature storage. CI is usually manifested as pitting and brown depressed areas in the peel of the fruit. Susceptibility to CI is highly dependent of environmental, agronomic and genetic factors, with large variations among fruit of different species. Alterations in cell membrane stability and modifications in lipid composition have long been recognized as primary events associated with CI. Lipid composition, and in particular the abundance of unsaturated fatty acids, influences membrane fluidity and its resistance to chilling stress. As a first step to understand the relationship between fatty acid metabolism and the susceptibility/tolerance of *Citrus* fruit to CI, in the current work we analysed the expression of oleate desaturase genes in mandarin (*Citrus reticulata*) fruits with contrasting tolerance to CI. The oleate desaturases, also referred to as ω-6 desaturases, are responsible for the conversion of oleic into linoleic acid. Sequence analysis evidenced the existence of two microsomal (*CsFAD2*.1 and *CsFAD2*.2) and one plastidial (*CsFAD6*) oleate desaturase genes in the genome of *Citrus* spp. The expression of these genes was studied in the flavedo (peel) of mandarin varieties with different sensitivity to develop chilling symptoms during postharvest storage at 2ºC for up to 8 weeks: Nadorcott (CI-tolerant), Nova (moderately sensitive) and Fortune (CI-sensitive). Interestingly, transcript levels of *CsFAD2*.1 at harvest were substantially higher in the CI-sensitive Fortune mandarin and decreased progressively during storage but remained unchanged in the CI-tolerant genotypes. The expression of the second microsomal isoform, *CsFAD2*.2, was more highly enhanced during cold storage in fruits of the CI-sensitive mandarin than in the other varieties. These results suggest that expression of these genes appears not to be related to the tolerance of mandarin fruit to CI. By contrast, the expression of the plastidial *CsFAD6* gene increased progressively in the fruit peel of the three varieties during cold storage, suggesting a cold regulated response.

Key words: chilling injury, citrus fruits, cold storage, desaturases, fatty acid metabolism

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INFLUENCE OF EXOGENOUS PROLINE PRETREATMENT IN *Carica papaya* L. RESPONSES AGAINST SALT STRESS

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Abstract: *Carica papaya* L. has become one of the most cultivated fruit plants in the world tropical and subtropical areas. The Canary Islands concentrate most of the Spanish papaya crop production, although a noticeable increase of cultivated area under greenhouses is taking place in south Spanish coast (Málaga, Almería…). Several papaya genotypes used in our previous experiments are well-adapted to subtropical Canary climate, and here we have used 'Sweet Mary' due its agronomic potential, which has also been recently gaining recognition by local farmers.
Soil salinity is a serious menace for global agriculture, especially in arid and semi-arid regions. The increasing incidence of salinity leads to damages in the crop growth and production. In general, papaya cultivars exhibit a moderate sensitivity to salinity, although such responses may depend, especially on the genotype. In the present study, papaya plantlets were subjected to salt stress conditions (100 mM-NaCl) during 41 days with the purpose to evaluate plant physiological variables associated to papaya stress responses. Data showed a general decrease of plant growth parameters induced by salt stress, as well as a reduction in the stomatal opening and chlorophyll content. By contrast, a significant increase was found in foliar proline levels under stress. In addition, exogenous foliar proline pre-treatment, induced a better performance of plants under salt stress at least through an improvement of stem height and biomass, stomatal movement, and chlorophyll content. Thus, the results suggest that the supply of proline as an osmo-regulator solute could increase papaya plantlets adaptation against salt stress.

Key words: salt stress, growth rate, proline, chlorophyll.

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IS IT POSSIBLE TO CLASSIFY SOIL WATER CONTENT FROM LEAF REFLECTANCE SPECTRA? MACHINE LEARNING MIGHT HELP

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Abstract: Feeding the growing population is a demanding challenge, hampered by ongoing climate change, in particular increasing water scarcity (Godfray et al., 2010). High throughput plant phenotyping is a key instrument to foster drought tolerance in new plant cultivars with agronomic interest (Henry, 2019). Currently, several methods and technologies are under scrutiny, in an effort to select the most effective and affordable to perform plant phenotyping (Costa et al., 2019). In this work, a combination of artificial intelligence with reflectance spectroscopy was tested, as a non-invasive method, for the automatic classification of soil water status in drought stress. *Arabidopsis thaliana* plants (ecotype Col-0) well-watered (S0) or subjected to water deficit by withholding watering for 7 (S1, moderate stress) or 12 (S2, severe stress) days. The reflectance spectra of fully expanded leaves were recorded with an Ocean Optics USB4000 spectrometer and the soil water content (SWC, %) of each pot was determined by gravimetry. The entire data set of the reflectance spectra (Intensity Vs Wavelength) was given to different machine learning (ML) algorithms, namely decision trees, random forests and extreme gradient boosting. The performance of different methods in classifying the plants in one of the three SWC classes (S0, S1 and S2) was measured and compared. All algorithms produced very high classification accuracies (> 90%) and seem to agree on the feature with the highest discriminative power (reflectance at ~ 670 nm). Random forests was the best performing method and the most robust to random sampling of training data, with an average accuracy

of 0.94 ± 0.09. This classification method is a promising tool to detect plant physiological responses to drought using high-throughput pipelines.

Key words: *Arabidopsis thaliana*, artificial intelligence, supervised learning, plant phenotyping, water stress

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METABOLOME DYNAMICS IN PINUS PINASTER IN RESPONSE TO COMBINED HEAT AND DROUGHT STRESS

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Abstract: Heat and drought are often concurrent stressors in nature that affects plant survival and productivity. Besides this, current projections for global climate change prognosticate an increase in heat waves and droughts intensity and frequency. Accordingly, the impact of both stressful environmental conditions can alter the composition and biogeography of forests in most of the world (Allen *et al.*, 2010). One of the most common conifer species in the occidental Mediterranean basin and sufferer of the referred adverse conditions is *Pinus pinaster.* Due to its important role in ecosystems, the development of knowledge about how this species responds to these adverse environmental conditions is essential. In the current work, we modelled the maritime pine response at molecular level by untargeted metabolomics (Valledor *et al.*, 2014) and biochemical profile (López-Hidalgo *et al.*, 2021) using a combined drought and heat stress experimental approach. For this, seedlings were exposed to two different water supply regimes (high and low hydric stresses) and two temperatures (30 ºC and 40 ºC) during 7 days. Our UPLC-QTOF-MSMS approach allowed the annotation of 403 features by MS/MS spectra using SIRIUS 4 (Dührkop *et al.*, 2019) and 1075 features by precursor within the default 5-ppm mass error. Comparative analysis of biochemical markers and annotated metabolites evidenced the hormesis phenomenon and the capacity of trees to cope with stress at 30 ºC and drought. Their metabolomes were characterized by an over-accumulation of secondary metabolites (terpenoids and polyketides), such as coniferyl aldehyde, apocynin, and dihydrokaempferol. Many of these metabolites have been previously referred in Meijón et al. (2016). Moreover, due to a great decrease in the soil water content related to evapotranspiration when the plantlets were exposed at the highest temperature, an increase in amino acids (L-tyrosine) and proteins degradation related metabolites (dipeptides) have been found. These changes revealed that the main response was related to the increase of osmotically active elements such as secondary metabolites involved in the plant protection from oxidative damage. Our results provide a benchmark in the understanding of thermal-water combined stress response in maritime pine and the basis of the knowledge for unravelling key metabolites underlying the adaptation response.

Key words: Heat, Drought, Metabolomics, Acclimation, Stress

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OSMOTIC ADJUSTMENT IN WHEAT (*TRITICUM AESTIVUM* L.) DURING PRE-AND POST-ANTHESIS DROUGHT

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Abstract:
Pre-anthesis drought is expected to greatly increase yield losses in wheat, one of the most important crops worldwide. Most studies only investigate the effects of pre-anthesis drought at maturity. The physiology of the plant before and after anthesis and how it is affected by drought is not known. Our study focussed on physiological patterns in wheat plants, subjected to drought stress. The experimental period started just before booting until two weeks after flowering and covered a large part of the elongation phase and the start of grain filling. Drought stress was induced by halting irrigation with rewatering upon turgor loss. Multiple parameters were measured every three days, three times a day: total xylem water potential, osmotic water potential, fresh weight and dry weight of different plant organs. Our results showed a delicate control of the osmotic water potential in both the leaves and the stem. During the day and under mild drought stress, osmotic water potential was maintained high by osmotic adjustment. This was more pronounced in the flag leaf than in the stem to preserve leaf functioning. Under high drought stress, osmotic water potential decreased to avoid further water loss from the storage cells. Rewatering restored xylem water potential in the leaves, but the osmotic water potential in the stem was not completely recovered. The measurements showed that by lowering xylem water potential in the flag leaf, water is redirected to the flag leaf and not used to refill the stem storage.

Key words: water potential, osmotic regulation, drought stress

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PHYSIOLOGICAL AND ANTIOXIDANT MECHANISMS TO OVERCOME WATER STRESS ON GRAFTED PEPPERS

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Abstract: Among abiotic stresses affecting crops, water scarcity rises as a major problem in many parts of the world, causing important losses in crop yield and productivity every year (Ashraf, 2010; Farooq et al., 2009; Mastrangelo et al., 2012). Grafting emerges as a sustainable technique that can be used to face this threat, particularly in Solanaceae species, usually cultivated in areas suffering from water starvation (Fullana-Pericàs et al., 2020; Kumar et al., 2017; Rouphael et al., 2018). Little has been published about the processes underlying grafted plants tolerance to water stress, especially in pepper plants. This work aimed to unravel several physiological and antioxidant mechanisms responsible for tolerance in pepper grafted plants under water stress. For this purpose, two pepper experimental hybrids with different sensitivity to water stress (H92 and H90, tolerant and sensitive, respectively), were used as rootstocks and their behaviour under a 1-month water stress was compared between them and to the ungrafted variety. These responses were analysed in terms of photosynthesis, water relations, antioxidant compounds and oxidative stress. Tolerant grafted plants (variety grafted onto H92, Var/H92) were less affected by water stress in leaf relative water content (RWC), indicating a higher capacity of water retention in leaves. Regarding photosynthesis, Var/H92 was capable of maintaining a higher rate of CO_2 assimilation (A_N) under stress conditions, compared to sensitive grafted plants (variety grafted onto H90, Var/H90) and ungrafted variety (Var). Respect to the antioxidant system, higher concentration of free radical scavengers as proline and ascorbic acid was observed in Var/H92 under water stress, which is related to a less disturbed photosynthesis. In fact, significant linear correlations for A_N with proline and ascorbic acid (negative and positive, respectively) were observed. As a consequence, Var/H92 showed lower oxidative pressure displaying equal levels of membrane lipid peroxidation in control and stress conditions, while higher values were observed in Var/H90 and ungrafted variety under water stress. To conclude, H92 could be a promising tolerant rootstock that should be tested in long-term essays to be evaluated in terms of fruit productivity.

Key words: pepper, grafting, drought, photosynthesis, antioxidants

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PHYSIOLOGICAL BASES OF GRAIN WEIGHT RESPONSE TO HEAT WAVES IN CONTRASTING WHEAT CULTIVARS UNDER FIELD CONDITIONS

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Abstract:

Wheat productivity has been challenged by rising temperatures. Transient heat stresses (heat waves), penalising wheat yield, are common during reproductive phases and will likely increase their frequency. However, it is not clear whether (i) the penalty of heat stress on average grain weight (AGW) is due to indirectly reducing source strength (due to accelerated senescence) or directly affecting the sink strength, (ii) the effect of heat stress is additive during the growing period, or if its magnitude may be pre-empted by previous heat stress. We grew under two different field conditions two high-yielding cultivars with contrasting yield components, Pistolo (of low grains/m^2 (GN) but high AGW) and Sublim (high GN and low AGW), subjected to heat waves imposed at both pre- and post-anthesis (using portable chambers with transparent polyethylene films) and different source-sink ratios by de-graining and defoliation after anthesis. We analysed in detail the AGW (considering the weight of individual grains in the population) of these cultivars and its responses to heat. Differences in AGW between cultivars were constitutive as it applied to all grains within the distribution, not just to a different proportion of large and small grains, and was not altered by changing in each cultivar the number of grains (through removing alternate rows in the plots at terminal spikelet). Furthermore, in none of the two cultivars, grain growth seemed to have been limited by the availability of assimilates (though Sublim seemed closer to that condition than Pistolo). The heat wave during grain filling did produce a significant penalty in AGW that was similar for both cultivars, while at booting did not. The effect was mostly direct on the capacity of grains to grow as the effect was not reverted by increasing assimilate availability (though reducing that availability worsened the penalty in Sublim). Also, we found a priming effect of an early heat wave on the magnitude of the effect of heat stress in post-anthesis on grain weight, showing that there might be acclimation to heat waves during grain filling if the crop would have been exposed to a heat wave in earlier phases.

Key words: yield, source, sink, grain number

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RESIN VESICLES IN *ABIES PINSAPO* SEEDS: CHARACTERIZATION AND EFFECT ON GERMINATION

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Seeds of some conifer species have resin vesicles in their coats (Kolotelo 1997). Different roles have been proposed for the liquid oleoresin contained in these cavities, such as protection of the seed against pathogens, pests and herbivores (Rubino et al. 2012; Lobo 2014; Kshatriya et al. 2018), protection of the embryo and megagametophyte from excessive drying (Gunia and Simak 1970), allelopathic effects (Dässler and Zentsch 1959; Keeling et al. 2018), and control of seed dormancy and germination (Kolotelo 2005).
In this work, the morphology, abundance and spatial distribution of resin vesicles were examined in seeds from five populations of *Abies pinsapo*, analyzing the chemical composition of resin samples by gas chromatography-mass spectrometry. The effect of resin on own seed germination was also evaluated, investigating the influence of its major constituent and the seed coat exudate.
Significant differences in the number of resin vesicles were found depending on the seeds origin, although no relevant variations were observed in their morphology and distribution. The resin from seed vesicles consisted of a complex mixture, rich in terpenes. Limonene was the major component in all populations. However, compounds present in smaller quantities showed a variable relative abundance depending on the population.
Damage of resin vesicles had a significant effect on seed germination. Injuring before germination drastically reduced the germination percentage, modifying the kinetics of the process. Seedling growth was also affected, with a significant decrease in root length and a total inhibition of shoot development. Vesicle damage prior to stratification totally inhibited the germination process. Incubation with limonene negatively affected germination and plantlet development, exhibiting a dose-dependent effect. However, the hydrophilic seed coat exudate did not exert a significant influence on the final germination percentage.

Key words: *Abies pinsapo*, germination, resin vesicle, terpenes, seed

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ROLE OF Na^+ TRANSPORTERS HKT1;1 AND HKT1;2 IN TOMATO SALT TOLERANCE. FUNCTION LOSS OF *LYCOPERSICUM* ALLELES IN ROOT AND AERIAL PART USING RECIPROCAL GRAFTING

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Abstract: In the present study, we analyse the physiological impact of function loss in *lycopersicum* alleles at the *High-Affinity K^+ Transporters-1;1* and *1;2 (HKT1;1* and *HKT1;2)* loci in the roots and aerial parts of the tomato plant in order to determine the relative contributions of each locus in each tissue to plant Na^+/K^+ homeostasis and subsequently to tomato salt tolerance. We generated different reciprocal rootstock/scion combinations with non-silenced, single RNAi-silenced lines for *SlHKT1;1* and *SlHKT1;2*, as well as doubly silenced lines, at both loci from a near isogenic line (NIL17), which is homozygous for the *S. lycopersicum* haplotype in a genomic region (31.1 Mb) containing both HKT1 loci. Grafted combinations were cultivated with a Hoagland nutrient solution supplemented with 0 and 70 mM of NaCl under natural greenhouse conditions during the fall-spring season following the usual cultural practices of commercial greenhouse tomato production. Our results show that salt treatment for 60 d sharply increased Na^+ content in leaves and flowers only in graft combinations with *SlHKT1;2*-silenced lines, either as single-/double-silenced with *SlHKT1;1* or used as rootstock and scion as compared to the non-silenced self-grafted line. Although vegetative growth in all tomato graft combinations decreased with salinity, the reduction was much more pronounced in *SlHKT1;2*–silenced and doubly silenced lines with *SlHKT1;1* in scions than in the non-silenced self-grafted line. However, only graft combinations with *SlHKT1;2*–silenced and doubly silenced lines with *SlHKT1;1* in scions showed a visible salt-hypersensitive phenotype after 60 d of treatment. Likewise, salt treatment negatively affects the yields of all tomato graft combinations, but considerably more in scions with *SlHKT1;2*–silenced lines than with non-silenced self-grafted lines. We concluded that the SlHKT1;2 Na^+ transporter prevents Na^+ over-accumulation in leaf and flower tissues and plays an important role in Na^+ homeostasis in tomato cultivated following the usual commercial cultural practices under natural greenhouse conditions. In the aerial parts, this transporter protects the flower against Na^+ toxicity and considerably mitigates the reduction in tomato productivity under saline conditions.

Key words: grafting, *SlHKT1;1* and *SlHKT1;2*, Na^+ transporters, tomato, salinity

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SECONDARY METABOLITES AND RELATED GENES IN *VITIS VINIFERA* L. CV. TEMPRANILLO GRAPES AS INFLUENCED BY UV RADIATION AND BERRY DEVELOPMENT

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Abstract: The effects of UV radiation on *Vitis vinifera* cv Tempranillo grapes were studied under field conditions as influenced by UV band (UV-A and UV-B), UV-B level (ambient vs enhanced), grape phenological stage (pea-size, veraison, and harvest), grape component (skin, flesh, and seeds), and fraction from which phenolic UV-absorbing compounds (UVACs) were extracted (soluble vs insoluble). Ambient UV-B levels caused stronger effects than ambient UV-A. These effects included increases in flavonol contents (particularly quercetins and kaempferols), the expression of flavonol synthase and chalcone synthase genes (*VvFLS4* and *VvCHS1*), and grape weight and size. In addition, the contents of flavanols and hydroxycinnamic acids increased under UV-B radiation at pea-size stage. All these compounds play physiological roles as antioxidants and UV-screens. Synergic effects between UV-B and UV-A were observed. The responses of anthocyanins and stilbenes to UV were diffuse or nonexistent. Enhanced UV-B led to rather subtle changes in comparison with ambient UV-B, but differences between both treatments could be demonstrated by multivariate analysis. Pea-size and harvest were the phenological stages where the most significant responses to UV were found, while skin was the most UV-responsive grape component. Soluble phenolic compounds were much more UV-responsive than insoluble compounds. In conclusion, UV radiation was essential for the induction of specific grape phenolic compounds. Given the physiological roles of these compounds, as well as their contribution to grape and wine quality, and their potential use as nutraceuticals, our results may have implications on the artificial manipulation of UV radiation.

Key words: Ultraviolet radiation, Grapevine, Vitis vinifera, Phenolic compounds.

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SYNERGISTIC INTERACTIONS BETWEEN VOLATILE COMPOUNDS OF *CYTISUS SCOPARIUS* AND THEIR HERBICIDAL ACTIVITIES

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Abstract: The increase in herbicide-resistant weeds together with the harmful environmental effects of synthetic herbicides have raised social concern about their use, besides stimulating the interest in discovering new herbicides based on natural products. Previous results from our lab demonstrated that the flowering biomass of *Cytisus scoparius* (L.) Link could produce and release volatile organic compounds (VOCs) capable of inhibiting the germination and growth of certain agricultural weeds. Interactions among VOCs were hypothesized to explain the inconsistency between the effects of the identified pure VOCs and those naturally released from fresh plant material. In this work, the effects of binary mixtures of verbenone, terpinen-4-ol, *α*-terpineol, and linalool were investigated, and compared to the phytotoxic activity of the pure compounds, on two agricultural weeds (*Amaranthus retroflexus* L. and *Digitaria sanguinalis* (L.) Scop.).

Powerful synergistic inhibitory effects were revealed on weeds germination and early growth. Only 3.1 ppm of verbenone was enough to inhibit *A. retroflexus* germination when paired to other VOCs. Also, the verbenone and linalool pair produced significant high synergistic effects on *D. sanguinalis* germination. The synergistic effects were predominantly irreversible for *D. sanguinalis*, since seeds exposed to the binary mixtures of VOCs were unable to recover their germination capacity after removing the phytotoxins, or produced no viable damaged seedlings.

C. scoparius has been revealed as a source of natural herbicide molecules with promising synergistic modes of action that deserve to be more profoundly studied. Potentially, the present work would inspire new eco-friendly herbicide formulations that combine lower concentrations of active principles with different target physiological processes, thus decreasing the risk of resistance development.

Key words: Phytotoxicity, Reversibility, Scotch broom, Synergism, Weed control

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THE *IN VIVO* ACTIVATION OF THE ALTERNATIVE OXIDASE PATHWAY MEDIATES THE SYNTHESIS OF OSMOTIC STRESS-RELATED METABOLITES IN *ARABIDOPSIS THALIANA*

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Abstract: Salt and drought stress induce plants to accumulate stress-related metabolites such as free polyamines (PAs) putrescine, spermidine and spermine, however, the precise molecular mechanism underlying PAs protective effects against stress is not fully understood (Alcázar et al., 2020). Their metabolism is tightly linked to tricarboxylic acid (TCA) cycle intermediates (Chen et al., 2019), which in turn, are involved in respiration. Under drought and salt stress, respiration can be restricted at the level of the ubiquinone pool, however, the activity of the alternative oxidase pathway (AOP) is thought to prevent this from occurring (Del-Saz et al., 2018). Moreover, the AOP activity is associated to a better photosynthetic performance under osmotic stress (Del-Saz et al., 2018; Vanlerberghe et al., 2020). The present research is based on the hypothesis that the accumulation of TCA cycle intermediates and stress-related metabolites under osmotic stress will depend on the response of the leaf AOP activity. To test this, the oxygen isotope-fractionation technique was used to study the *in vivo* respiratory activities of the cytochrome and the alternative oxidase pathways (COP and AOP) in leaves of wild-type *Arabidopsis thaliana* plants and of *aox1a* mutants under sudden severe drought and salt conditions (1 day, 300 mM of mannitol or salt). In addition, the levels of leaf primary metabolites and PAs were determined in parallel to photosynthetic analyses. Our results show that the lack of *in vivo* AOP response in the *aox1a* mutants coincided with lower accumulation of TCA cycle intermediates and stress-related metabolites with consequences for photosynthetic performance.

Key words: osmotic stress, alternative oxidase, primary metabolism, *Arabidopsis thaliana*

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THE ROLE OF *ARABIDOPSIS THALIANA EXT18* IN LEAD TOLERANCE AND ROOT SEQUESTRATION

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Abstract: Soil contamination by lead (Pb) has become one of the major ecological threats to the environment. Its reduction is a global challenge and understanding the mechanisms of Pb transport and deposition in plants is thus of great importance. We exposed a collection of 360 *A. thaliana* natural accessions to a Pb-polluted soil. Germination rates, growth, and leaf Pb concentrations showed extensive variations. These data were subjected to genome wide association studies (GWAs) and we found a significant association on chromosome 1 for low leaf Pb accumulation. Genes included in the LD block of the highest SNP marker were evaluated and the cell wall protein EXTENSIN18 (EXT18) was selected. Six Pb-tolerant accessions, three of them having low leaf Pb content and the rare allele and three of them having high leaf Pb content and the common allele, were screened. The relative expression of genes involved in Pb translocation was tested in the 6 Pb-tolerant natural accessions, 2 Pb-sensitive accessions, the *ext18* knockout T-DNA line, and Col-0 exposed to control and high-Pb conditions. Analysis of Darwinian fitness, root and leaf ionome, and TEM images revealed that Pb-tolerant accessions use two opposing strategies: (1) a low Pb translocation and its accumulation into root cell walls and vacuoles, or (2) a high Pb translocation and its efflux to inactive organelles or intracellular spaces. Plants using the first strategy exhibit higher expression of *EXT18* and *HMA3*, thicker root cell walls saturated with Pb depositions, and Pb vacuolar sequestration. We conclude that the upregulation of *EXT18* enhances Pb tolerance by favoring the thickening of the cell walls, improving Pb accumulation in roots and decreasing its toxicity. The possibility to cultivate crops with low ability for Pb accumulation in aerial parts is very attractive because they can be consumed safely by animals and humans. Genes involved in root cell wall structure play an important role in metal sequestration and deserve important consideration.

Key words: Lead tolerance, Cell wall, GWAs, Extensins

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USE OF PLANT GROWTH PROMOTING RHIZOBACTERIA FOR SALT STRESS MITIGATION IN CITRUS

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Abstract: Salt stress is one of the major abiotic stresses constraining vegetative growth and physiological responses of citrus trees in the Mediterranean region due to the salinization of the aquifers as a result of marine water intrusion. This adverse condition can be palliated by soil beneficial microorganisms as plant growth promoting rhizobacteria (PGPRs). In this work, six-month-old *Citrus macrophylla* plants were inoculated with the PGPRs *Pseudomonas putida* or *Novosphingobium* sp., and subjected to salt stress by supplementing the irrigation solution with 90 mM NaCl for one month. Plants were maintained at greenhouse conditions with 25±3.0 and 18±2.0 ºC day/night respectively, using a mixture of peat moss, perlite and vermiculite (80:10:10) as substrate. The assay was repeated three times using ten plants per treatment and experiment. Adverse effects of salt stress were alleviated in plants inoculated with either PGPR strains but more clearly in those treated with *Novosphingobium* sp., which is more resistant to high salinity. Plant performance improvement was correlated with a lower accumulation of abscisic acid and salicylic acid in leaves of inoculated plants, as well as with an increase of indole acetic acid concentration in leaves of stressed plants inoculated with *Novosphingobium* sp. In addition, a reduction of proline accumulation was observed in roots of stressed plants inoculated with PGPR. Regarding the photosynthetic machinery, *Novosphingobium sp*. delayed the decrease of PSII quantum efficiency (ΦPSII) under salt stress conditions, whereas stressed plants inoculated with any of the PGPR strains, maintained higher levels of maximum efficiency of PSII photochemistry (F_v/F_m) at 20 days of treatment. These results support the use of both bacterial strains as PGPRs in biofertilization or bioaugmentation programmes focused on the prevention of salt stress-induced damage in citrus orchards.

Key words: Abiotic stress**,** Photosynthesis, Phytohormones, Pseudomonas, Rhizobacteria

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WATER RELATIONS AND ORNAMENTAL QUALITY RESPONSE OF *EUONYMUS* PLANTS TO COMBINED STRESSES OF DEFICIT IRRIGATION AND SALINITY

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Abstract: Plant growth in semiarid areas is frequently limited by both high salt content and limited water availability. In this sense, osmotic stress generated by either drought and/or salinity represents the most common environmental hazard for the plants. Responses of plants to the combination of drought and salinity cannot be extrapolated from the corresponding response of plants to each of these different stresses when applied individually. In this study, *Euonymus* plants were grown in pots outdoors and subjected to five irrigation treatments lasting 12 months: control (0.8 dS m^{-1}, 100% water holding capacity; C), moderate water deficit (0.8 dS m^{-1}, 60% of water supplied in control; MW), severe water deficit (0.8 dS m^{-1}, 40% of water supplied in control; SW), saline (4 dS m^{-1}, same amount of water as control; S) and saline plus water deficit (4 dS m^{-1}, 60% of the water supplied in control; W+S). Water deficit and salinity individually decreased aerial and root growth, especially in the plants submitted to salinity. The effects of both stresses were additive in the case of biomass accumulation. At the end of the experiment, the reductions in plant height were around 6, 18, 9, and 25% for the MW, SW, S, and W+S treatments, respectively. The most negative values of the leaf water potential were found in SW plants, although plants under saline (S and W+S) exhibited slight dehydration t due to the less available substrate water. The higher chroma and lightness values and lower hue and chlorophyll content in W+S plants confirmed the more yellow and less dark green colour of the foliage. Membrane damage was not affected by the water availability of the substrate, while salt-treated plants and especially plants submitted to the combined effects of water stress and salinity increased. Our results suggest that deficit irrigation caused severe leaf tissue dehydration but did not affect ornamental quality, while salinity combined with deficit irrigation affected the plant quality and induced an excessive decrease in plant growth.

Key words: Abiotic stresses, Growth, Ornamental plant, Plant quality, Water relations

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NATURAL POPULATIONS OF *ARABIDOPSIS THALIANA*, A MINE FOR METAL TOLERANCE GENES

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Abstract: Soil is a potent selection factor shaping the plant genome. *A. thaliana* can colonize different soil types worldwide. Here we provide an overview on our recent research showing how local adaptation of *A. thaliana* populations to different soil metal concentrations allows to identifying metal tolerance genes. Two examples are depicted: 1) the alkaline metal Na^+, a main factor in local adaptation to salinity and 2) the heavy metal lead (Pb) a widely disseminated soil pollutant due to industry and traffic. 1) We characterized demes of *A. thaliana* in Catalonian coastal and inland sites. Reciprocal transplant and common garden experiments revealed local adaptation of coastal demes to high soil Na^+. Functionally different allelic variations in *HKT1* were identified. Variants with low root HKT1.1 expression had high leaf Na^+. These allelic variants better adapted to habitats with seasonal fluctuations of moderate soil Na^+ (Busoms et al., 2019). 2) Natural populations of *A. thaliana* along with knowledge on soil trace metal concentrations in their natural habitat are also useful tools for identifying trace metal tolerance genes. 360 *A. thaliana* natural accessions were exposed to Pb-rich mining soil. A GWAs approach along with root and shoot Pb analysis allowed to identify two strategies for Pb tolerance: Pb-accumulation in root cell walls and exclusion from shoots associated with higher expression of EXT18, EXT19, and ACBP1, or high Pb translocation and accumulation in vacuoles and cell walls of older leaves; strategy associated with downregulation of PSE1 and higher PINd expression (Martos et al. 2021)

Key words: Local adaptation, sodium, HKT1, lead (Pb), extensin, tolerance genes

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PLANTS NEED Fe BUT ALSO OTHER HEAVY METALS

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Abstract: Iron (Fe) is a heavy metal that participates in many essential plant functions, such as photosynthesis and respiration. It is very abundant in most soils although its availability for plants is low, especially in high pH calcareous soils (García et al. 2021). To cope with this constraint, plants induce several physiological and morphological responses in their roots, that facilitate Fe mobilization and uptake and that are tightly regulated (García et al. 2021; www.uco.es/fethyleno/). Besides Fe, plants need other heavy metals, like zinc (Zn), manganese (Mn), copper (Cu), molybdenum (Mo) and nickel (Ni; Willians 2015). Some of them also need aluminium (Al; Sun et al. 2020). Although each metal has its own transport systems, Fe can influence the acquisition of other essential heavy metals, and also of toxic metals such as cadmium (Cd), since some Fe deficiency responses can affect their mobilization and uptake. In addition, Fe can affect other plant processes, such as phosphorus nutrition and responses to pathogens, since all of them share some common regulatory components (García et al. 2021; Trapet et al. 2021).

Key words: iron, metals, pathogens, phosphorus, responses

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CADMIUM EFFECTS ON RNAI *SBPPC3* SILENCED SORGHUM LINES

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Abstract: Phosphoenolpyruvate carboxylase (PEPC) (EC 4.1.1.31) is responsible for the initial fixation of atmospheric CO_2 in C_4 and CAM plants. In addition, it has a central function coordinating carbon and nitrogen metabolisms in different physiological contexts. Six different isoforms of PEPC (*SbPPC1-6*) exist in sorghum (*Sorghum bicolor* L.). *SbPPC1* is the photosynthetic C_4-type PEPC, while *SbPPC2-5* are C_3-type enzymes, and *SbPPC6* is a bacterial-type isoform. This study is focused on *SbPPC3*. This isoform plays key roles on seeds and roots, and also in responses to abiotic stresses. We generated modified sorghum plants by silencing the *SbPPC3* isoform by RNA interference (RNAi) (*Ppc3* plants) and analysed the consequences on the responses to cadmium stress. Cadmium, decreased net photosynthesis, primarily in *Ppc3* plants, and stomatic conductance in wild-type and transgenic plants. In addition, this metal reduced shoot and root growth, although its effects were similar in all plants. Cadmium delayed seed germination, especially in seeds from *Ppc3* lines. Cadmium tolerance by plants is directly related to its ability to release organic acids to the rhizosphere. PEPC, participating in the synthesis of citrate and other organic acids, may play an important role in this process. In sorghum, cadmium increased PEPC activity in roots of wild-type plants in a dose-depentent manner, but not in *Ppc3* lines at 100 μM of cadmium. Furthermore, this increase on PEPC activity was associated with enhanced *SbPPC2* and *SbPPC3* gene expression. In *Ppc3* lines, an augmented level of *SbPPC2* transcripts, but not of *SbPPC3*, was observed in response to cadmium. Also, increased expressions of *SbPPCK2*, but not *SbPPCK3*, were detected in roots. Cadmium caused a tremendous increase in citrate and a small increase in malate exudation by roots of wild-type plants, but not in *Ppc3* lines, while succinate and lactate decreased in the presence of cadmium in both plants. Finally, cadmium reduced the number and total weight of seed in *Ppc3* plants. Altogether, these results support that *SbPPC3* has an important role in sorghum responses to cadmium stress.

Key words: Sorghum, cadmium, phosphoenolpyruvate carboxylase

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NADPH OXIDASES DIFFERENTIALLY REGULATE PHOTOSYNTHESIS, CADMIUM AND POTASSIUM FLUX IN ARABIDOPSIS PLANTS UNDER CADMIUM STRESS

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Cadmium (Cd), a highly toxic non-essential element, restricts plant growth and productivity mainly due to an excess of reactive oxygen species (ROS) production. In stressed plants, NADPH oxidases, also named respiratory burst oxidase homologs (RBOH), constitute a major source of ROS. In our laboratory, Cd was observed to accumulate mainly in *Atrboh* mutant roots, with reduced translocation to leaves compared to WT plants (Gupta et al., 2017). K^+'s status under similar conditions differed considerably in WT and *Atrboh* plants (Gupta et al., 2017), although the mechanisms involved are unknown. We assessed the involvement of RBOHs in regulating photosynthesis, K^+ and Cd flux in cadmium-challenged WT and *Arabidopsis thaliana* mutants *AtrbohC*, *AtrbohD* and *AtrbohF* using physiological, biochemical, electrophysiological and molecular approaches. Plants were grown hydroponically, supplemented or not with 50 µM Cd for 24 h. Cd differentially affects photosynthesis, stomatal conductance, density and opening, transpiration and antioxidative responses in WT and *Atrboh* mutants. Electrophysiological experiments revealed that RBOH knockout increased Cd^{2+} influx compared to WT. In mature zones, influx was highest in *rbohC* mutants and, in elongation zones, in *AtrbohD* and *AtrbohF* mutants. Under Cd stress, the *rboh* mutants were better able to retain K^+ in elongation root zones compared to WT, with *rbohD* mutant being the most efficient followed by *rbohC* and *rbohF*. In leaves, exposure to Cd^{2+} dramatically reduced K^+ influx in *Atrbohs* mutants and changed K^+ efflux to intense influx in WT. qPCR expression analysis of K^+ and Cd^{2+} transporters demonstrated differential regulation by the RBOHs tested. We show that the absence of functional RBOH proteins could enhance K^+'s retention capacity in Arabidopsis roots, while compromising their ability to minimize Cd^{2+} influx.

Key words: cadmium, potassium, NADPH oxidases, photosynthesis, metal and potassium transporters

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ZINC, AN ANTIAGING AGENT WITH POTENTIAL TO IMPROVE CROP YIELD

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Abstract: Zinc, an essential element for all organisms is a well-known antiaging agent in humans. Its role in retarding developmental senescence in plants is less clear. The aim of our study was to explore the possible influence of luxury Zn supply on the delay of developmental senescence and fruit yield using *Arabidopsis thaliana* as a model. Auxin-resistance mutant, *axr1–12*, and auxin overexpressing YUCCA6 mutant, yuc6-1D, and their corresponding background genotypes were exposed in hydroponics to either 2 μM Zn (control) or 12 μM Zn (non- toxic luxury supply) and grown to complete rosette senescence. Fruit biomass and seed number were determined for yield assessment (Cabot et al., 2021). Expressions of different senescence related markers, as well as auxin and antioxidant related genes were analysed after senescence initiation. Wild type, but not mutants responded to luxury Zn with senescence delay and enhanced yield. Enhanced expression of *SAG12* and *ARF2* coincided with decreased expression of *IAA2*. Delayed senescence was associated with high expression of *SOD3* and *CAT2*. In conclusion, senescence delay and yield increase by luxury Zn is not auxin dependent but due to enhanced antioxidant activity. Luxury Zn supply through fertilization should be further explored to favour stay green and avoid yield stagnation, especially under environmental stress.

Key words: Luxury zinc supply, Senescence marker, Auxin, Antioxidant, Stay green

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NEW INSIGHTS INTO THE REGULATION OF PHENYLALANINE IN PLANTS

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Abstract: In plants, phenylalanine (Phe) is a fundamental building block of proteins but also serves as precursor of multiple essential secondary products that account for a large extent of the total plant biomass. Within plant plastids, the shikimate pathway directly connects the central carbon metabolism, glycolysis and pentose phosphate pathway, with the biosynthesis of aromatic amino acids, and thus with the biosynthesis of Phe and its derived specialized metabolism. A comprehensive understanding of Phe biosynthesis and its coordination with the Phe-derived specialized metabolism, but also its interconnection within the plant metabolism as a whole, is fundamental to address future metabolic engineering strategies focused on the production of valuable compounds.

The final reaction of the Phe biosynthetic pathway is catalysed by the enzyme arogenate dehydratase (ADT), which is considered a key, rate-limiting step for the synthesis of this amino acid. In recent years, our group has undertaken various studies focused on the regulation of the ADT enzyme, but also on the transcriptional regulation of the corresponding genes. In this conference, a selection of these results will be presented with a particular focus on how the evolution of ADTs is directly related with its allosteric behaviour, and thus with the ability of plants to modulate the biosynthesis of Phe and fundamental derived compounds (El-Azaz et al., 2016; El-Azaz et al., 2019). Results will also be presented describing the direct relationship between ADT enzymes, their transcriptional regulation through MYB and NAC transcription factors, and lignin biosynthesis in the conifer species *Pinus pinaster* (Pascual et al., 2018; El-Azaz et al., 2020). Finally, the research that our group is currently developing on this topic, and future studies that we intend to address, will be presented.

Key words: Phenylalanine, allosteric control, transcriptional regulation, enzyme evolution.

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STRATEGIES TO PRODUCE BIOACTIVE COMPOUNDS FROM PLANT CELL CULTURES

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Abstract: Secondary metabolites have an important role in the survival of plants, and have caught the attention of the general people, since most of them have biological activity with beneficial effects on human health, so that they are considered bioactive compounds. Along with the direct extraction of these compounds from plant by-products and/or the chemical synthesis, plant cell cultures have been developed as an alternative to produce bioactive compounds that are difficult or not very profitable to obtain or when the extraction process represent a serious damage to the environment.

Amongst the different strategies that can be used to enhance the production of these compounds highlight the use of different elicitors alone or combined with magnetic nanoparticles, and plant metabolic engineering combined with elicitation. We will show how we have moved forward and made new discoveries based on the use of both these tools and the potential that plant cells have. In fact, one of the most relevant discoveries is based on the use of cyclodextrins (CD), separately or in combination, with methyl jasmonate (MJ) as elicitors, has been proven to be a very effective procedure in stimulating *trans*-resveratrol (*t*-R) production in grapevine cell cultures. However, the use of CD increases production costs, making its industrial exploitation economically unviable. Therefore, the development of recovery strategies for these molecules are needed to provide a solution to their industrial use. In fact, CD polymers coated with magnetic nanoparticles (CDP) together with MJ were able to increase *t*-R production in grapevine cell cultures, and CDP were reused during three cycles of continuous elicitation, since the induction and adsorption capacity of *t*-R remained high. On the other hand, knowing the effect of the joint action between CD and MJ on inducing maximum levels of stilbene synthase (STS) expression in grapevine cell cultures, we designed an Agrobacterium transformation strategy by which transgenic cell lines overexpressing STS were obtained and evaluated. Some of them were able to provoke a highly enhanced production of *t*-R in the presence of CD and MJ, in comparison with the non-transgenic cell lines elicited.

Key words: cyclodextrins, cell cultures, elicitors, resveratrol

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ALLELOCHEMICALS IN WHEAT (TRITICUM AESTIVUM L.): TISSUE DIFFERENCES IN THE PRODUCTION, RELEASE AND PHYTOTOXICITY OF POLYPHENOLS AND BENZOXAZINOIDS ON ACCOMPANIED WEEDS

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Abstract: Due to the health risks and hazards for humans, animals and the environment, several synthetic herbicides have been banned in the European Union. In this context, there is an urgent need to move towards alternative options such as plant based natural products (secondary metabolites) and allelopathic crop cultivars to employ them in sustainable weed management strategies. Common purslane (*Portulaca oleracea* L.) and annual ryegrass (*Lolium rigidum* Gaudin) are important infesting weeds of field crops.
Wheat is an important allelopathic crop and the present research was conducted with the aim of identifying and quantifying secondary metabolites (benzoxazinoids (BXZs) and polyphenols (phenolic acids and flavonoids) of the wheat accession 'Ursita', and to screen its allelopathic impact on *P. oleracea* and *L. rigidum*. Equal-Compartment-Agar (ECA) method (Wu et al., 2000) was employed to check the allelopathic effects of Ursita while the interference in weed leaf photosynthetic pigments was measured by HPLC. The results showed that germination, root length and weed biomass were reduced following co-cultivation of weeds with 'Ursita' wheat for 10-days. Several categories of allelochemicals have been identified as responsible for wheat allelopathy, namely phenolic acids, hydroxamic acids and flavonoids. Root exudates from 'Ursita' wheat reduced (29-60%) the seedling growth of *L. rigidum*. Moreover, allelochemicals from 'Ursita' wheat caused damage to photosynthetic pigments in weed leaves, and this effect was more prominent in *L. rigidum*. These results may be correlated with the higher content of polyphenols (such as vanillic, ferulic, syringic and *p*-coumaric acids) and hydroxamic acids (BOA, HMBOA, HBOA and DIBOA) detected in the roots, shoots, and root rhizosphere.
It can be concluded that 'Ursita' wheat is allelopathic in nature and can be used in breeding programs to enhance its allelopathic activity. These investigations also suggest that there are opportunities to explore the allelopathic effects of wheat cultivars to control *P. oleracea* and *L. rigidum* in field conditions.

Keywords: Allelopathic crops, Weed suppression, Secondary metabolites, Germination and growth bioassays, Chlorophyll pigments

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BIOTECHNOLOGICAL STRATEGY TO INCREASE TAXANE BIOSYNTHESIS AND SECRETION IN TAXUS CELL CULTURES

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Abstract: The anticancer agent paclitaxel (PTX) is found in very low amounts in the inner bark of several *Taxus* species, and its extraction from its natural source is ecologically unsustainable. An eco-friendly approach to produce PTX and derivatives is provided by biotechnological platforms based on *Taxus* spp. cell cultures. Although elicitation has proved to be an efficient strategy to improve the yields of plant cell biofactories, the limited excretion of target compounds from the producer cells to the medium hinders their extraction and purification. In this context, we studied the effect of treating *Taxus media* cell cultures with the elicitor coronatine (COR) in combination with calix[8]arene (CAL), a nanoparticle that can host lipophilic compounds within its macrocyclic scaffold. The combination of 1 μM of COR and 10 mg/L of CAL resulted in the highest taxane production (103.5 mg/L), a concentration 15-fold greater than in the control, with PTX representing 82% of the total taxane content. The study of differential expression of taxane biosynthetic flux-limiting genes, BAPT and DBTNBT, revealed a 5-fold and 15-fold increase, respectively, 4 hours after the addition of COR, confirming its action as an elicitor. In contrast, this differential expression was not found with the CAL treatment. CAL significantly enhanced taxane excretion, especially when production levels were increased by COR; 98% of the total taxanes were found in the culture medium after the combined treatment with COR and CAL. By forming complexes with PTX, which may protect cells from taxane toxicity, the nanoparticle facilitated the excretion of PTX to the medium. The combined addition of COR and CAL to *T. media* cell cultures therefore proved to be a bio-sustainable and economically viable system to improve the yield of this important anticancer compound.

Key words: Calixarenes, Coronatine, Paclitaxel; Taxane production; Gene expression.

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IDENTIFICATION AND FUNCTIONAL VALIDATION OF METHYL KETONE SYNTHASE 2 IN WOODLAND STRAWBERRY

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Abstract:
Woodland strawberry (*Fragaria vesca*, 2x) is the diploid closest ancestor of the cultivated strawberry (*F.* × *ananassa*, 8x) and the model species for genetic studies in the Fragaria genus. It is naturally distributed across Europe and it is appreciated for its delicate aroma and flavor.

Methyl ketones are compounds with demonstrated insect repellent effects (Antonious *et al*. 2003). They are highly abundant in the glandular trichomes of wild tomato (*Solanum habrochaites*), where their pathway was first described, but not in the cultivated species (*S. lycopersicum*). Their synthesis derives from fatty acids in a two-step process mediated by a thioesterase (ShMKS2) and a decarboxylase (ShMKS1) (Ben-Israel *et al.* 2009, Yu *et al.* 2010).

Higher diversity and quantity of methyl ketones are present in the volatilome of woodland strawberry ripe fruits than in those of *F.* × *ananassa* (Ulrich *et al.* 2007, Ulrich and Olbrich 2013, Zorrilla-Fontanesi *et al*. 2012, Urrutia *et al.* 2017). The aim of this study is to reveal the genetic basis of methyl ketone production in strawberry fruit.

We quantified methyl ketones (2-heptanone, 2-nonanone, 2-undecanone), their secondary alcohols (2-heptanol, 2-nonanol, 2-undecanol) and the methyl esters of their fatty acid precursors (methyl octanoate, methyl decanoate, methyl dodecanoate) by GC-MS in a natural collection of European woodland strawberry, that comprises 199 accessions fully genotyped with >1.8 M SNPs representing the continental diversity. Conducting a Genome-Wide Association Study (GWAS), we identified a candidate region linked to methyl ketones accumulation harbouring three homologues of *ShMKS2*: *FvMKS2A*, *FvMKS2B* and *FvMKS2C*. Interestingly, *FvMKS2A*, which presented two alleles in the European collection

(*FvMKS2A-1* and *FvMKS2A-2*), is the only *FvMKS2* paralog expressed in woodland strawberry fruit, being up-regulated during ripening.

Functional validation of all candidate genes and alleles by transient over-expression and silencing in both *Nicotiana benthamiana* leaves and *F. vesca* fruits has revealed that FvMKS2A and FvMKS2B, but not FvMKS2C, are capable of synthesizing methyl ketones, and point to a single SNP in FvMKS2A as responsible for the enzymatic substrate specificity, supporting FvMKS2A as the main MKS2 paralog responsible for methyl ketones in woodland strawberries.

Key words: Methyl ketone synthase 2 (MKS2), Woodland strawberry, Secondary metabolism, Volatiles.

Acknowledgments: This work was supported by the ERC Starting Grant ERC-2014-StG 638134.

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IN SILICO STUDIES AND BIOTECHNOLOGICAL TECHNIQUES PERMITTED THE CONFIRMATION OF A NOVEL HYDROXYLATION STEP OF THE TAXOL BIOSYNTHETIC PATHWAY

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Abstract: Taxanes, secondary metabolites produced by *Taxus spp.*, are a treatment of choice for several types of cancer, but an incomplete understanding of the taxane biosynthetic pathway hinders the production of these compounds by chemical synthesis. To date, only 14 of the postulated 19 steps of the pathway have been elucidated and of the five unknown steps, three are thought to involve cytochrome P450-like hydroxylases. In the current work, after an in-depth computer characterization of four candidate enzymes proposed in a previous cDNA-AFLP analysis of *Taxus baccata* cells under methyl jasmonate (MeJA) elicitation, TB506 was selected as a candidate for the hydroxylation of the taxane side chain based on an in silico analysis and comparison with previously characterized taxane hydroxylases. After the construction and optimization of a structural model, the tertiary structure of the selected candidate and the other taxane hydroxylases was studied. Docking analysis shows that TB506 is active after the attachment of the side chain based on its affinity to the ligand 3'N-dehydroxydebenzoyltaxol.

Once the suitability of the selected candidate was established, a biotransformation assay was performed within a heterologous system of *Pisum sativum L.* protoplasts expressing the candidate enzyme and the two enzymes involved in the last stage of taxane biosynthesis (BAPT and DBTNBT) incubated with 1 mM elicitor coronatine (CORO) for 6 h in the presence of 100 mg/L baccatin III and 100 mg/L β-phenylalanine-CoA. The aim was to verify whether the combination of the three enzymes would allow the transformation of these intermediate metabolites into the final product, paclitaxel. The HPLC-MS/MS analysis performed after the incubation of the transfected protoplasts showed that paclitaxel were present in all the analyzed samples, both at the extra- and intracellular level.

These results confirmed that the TB506 gene encodes a hydroxylase involved in the final hydroxylation step of taxane biosynthesis, and its identification completes the elucidation of the last unknown step at the end of the pathway. The identification and definition of this new protein opens the possibility of developing sustainable biotechnological methods, including synthetic biology techniques, to produce paclitaxel based on the abundant intermediate baccatin III, usually used in the semi-synthesis of paclitaxel.

Key words: taxane hydroxylase, paclitaxel, protoplasts transfection, cytochrome P450, biotransformation

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CHANGES IN TOMATO PHENOTYPE INDUCED BY OVEREXPRESSION OF TOMATO STEROL GLYCOSYLTRANSFERASE SGT4

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Abstract: Free sterols (FS), steryl glycosydes (SG) and acylated steryl glycosydes are essential structural components of cell plasma membrane (PM), where in combination with other membrane lipids play a key role in modulating their properties and function. Sterol glycosyltransferases (SGTs) are the enzymes that catalyze the glycosylation of the free hydroxyl group at C-3 position of FS to produce SG. Our group has demonstrated that tomato contains a small SGT gene family of 4 members (SlSGT1-4) coding for functional SlSGT enzymes that are differentially expressed. The expression of SlSGT4 gene is hardly detectable under basal conditions but is markedly enhanced in response to osmotic, salt, and cold stress, as well as upon treatment of plants with abscisic acid and methyl jasmonate (Ramírez-Estrada et al., 2017). The four *SlSGT* genes encode functional SlSGT isoenzymes, but the specific contribution of each isoform to the synthesis of glycosylated sterols and the role in tomato plant growth and development, and stress responses is far from being understood. To address this issue, we have obtained and characterized tomato (cv. Micro-Tom) plants with enhanced levels of SlSGT4. Unexpectedly, the resulting transgenic plants showed lower levels of SG, bigger fruits, and enhanced seed production compared to wild type plants. In contrast, over-expression of SlSGT4 negatively affected plant growth and development, as well as germination rate and root elongation.

Key words: Tomato, Sterol metabolism, Sterol glycosyltransferase

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COMPARATIVE ANALISIS OF ANTIOXIDANTS AND FLAVONOIDS OF TWO CULTIVARS OF KALE (*BRASSICA OLERACEA* VAR. *SABELLICA)*

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Abstract: Around 37 species belong to the *Brassica* genus (family *Brassicaceae*), and many of them are vegetable products grown for human consumption, including cauliflower, broccoli, brussels sprout, and kale, among others. They hold an enormous economic importance not only as vegetable crops but also as forages, condiments and oilseeds. Similarly, fresh vegetables are commonly believed to provide more health-beneficial constituents. Members of *Brassicaceae* family are reported to be beneficial in degenerative diseases, brain tumors, cardiovascular diseases, and several types of cancer due to the presence of such bioactive constituents i.e., phenols, flavonoids, vitamin C, carotenoids, antioxidants etc. Kale (*Brassica oleracea* var. *sabellica*) is a valuable winter vegetable that is widely cultivated in Europe and America. Considering the enormous health benefits associated with the metabolites that kale partakes, the current study was undertaken to establish a comparative phytochemical relation, particularly of antioxidant capacity and flavonoids, of its two commercially available cultivars (i.e., Dwarf Green Curled or Curly Kale, and Black Magic or Halbhoher Grüner Krauser). Both cultivars were grown in similar field conditions and three samplings were done, first 30 days after the germination and the other two were followed by a 30 days interval for each. The results showed the higher amounts of Glutation (GSH) and Ascorbic Acid (AsA) compounds for Curly Kale, being 1.06 and 1.04 times higher than that of Black Magic, during the second sampling period. In the same way, flavonoids, which bear anti-inflammatory properties, were also 1.27 times higher in Curly Kale. Likewise, similar results were obtained for Ferric Reducing Antioxidant Power (FRAP) and Trolox Equivalent Antioxidant Capacity (TEAC) tests with the highest values observed, per milligram of fresh plant weight to be 4.65±0.27 and 8.10±0.36, in the second sampling period. Briefly, these results suggest the better antioxidant and chemopreventive properties of Curly Kale over the Black Magic variety, and that during the lower temperatures, i.e., second sampling period, these vegetables exhibit higher amounts of bioactive compounds as compared to the early growth and the harvest periods.

Key words: Vegetable, Antioxidants, *Brassica oleracea,* Kale, Food

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DEEPENING ON THE MODE OF ACTION OF HARMALINE, A NATURAL COMPOUND WITH PHYTOTOXIC POTENTIAL

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Abstract:

Conventional herbicides are widely used to control weeds due to their effectiveness and low price. However, their use results in numerous environmental problems and the occurrence of resistant weeds, which make necessary the development of new strategies. Using phytotoxic natural compounds is a possible alternative, due to their *a priori* less toxic, less persistent and more easily metabolizable condition.

Harmaline is an indole alkaloid widely found in *Peganum harmala* seeds, whose phytotoxic potential and mode of action are being studied in seedlings of the model species *Arabidopsis thaliana* (L.). *In vitro* assays with harmaline showed a strong inhibitory activity on growth (IC_{50} and IC_{80} values, i.e. the concentrations of harmaline necessary for 50 and 80% inhibition, were as low as 14 μM and 29 μM, respectively), inducing shortening and thickening roots with an extraordinary increment of root hairs, all over the root.

In addition, under transmission electron microscope, plants treated with harmaline showed important aberrations on radicle development, as both, differentiation and transition zones showed strong tissue disorganization with cells of different sizes and shapes that did not maintain the characteristic organized pattern of control roots.

These results suggest a possible alteration in hormonal balance induced by harmaline. Specifically, ethylene and cytokinins could be the hormones involved in the mode of action of harmaline. For this reason, a bioassay with $AgNO_3$ (ethylene action inhibitor) was done. Plants treated with harmaline + $AgNO_3$ showed a reversal in the appearance of root hairs and root thickening, behaving almost like control plants. As well, to test alterations on cytokinins accumulation, location and transport, CKS:GFP mutants were observed under confocal microscopy, finding that cytokinins accumulate in the root apex with increasing harmaline concentrations.

In conclusion, we demonstrated that harmaline has a strong phytotoxic potential and both ethylene and cytokinins are involved in the mode of action of this natural compound.

Key words: harmaline, mode of action, root hairs, bioherbicide, ethylene, cytokinins

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DIFFERENTIAL NON-TARGET EFFECTS OF EUCALYPTUS-BASED HERBICIDES ON THE PHYSIOLOGICAL PERFORMANCE OF CHERRY-TOMATO PLANTS

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Abstract: Agri-food production is being threatened by an increasing incidence of diseases and pests, like weeds. However, currently, weed management still depends on synthetic herbicides, negatively impacting several environmental compartments. Hence, eco-friendly solutions to tackle these issues are urgently needed. Accordingly, the development of bioherbicides, by harnessing plants' specialized metabolism, is acknowledged as a promising approach. In this context, the allelopathic properties of *Eucalyptus globulus* leaf extracts are well recognized, but the options available generally depend on expensive essential oils. In this sense, new easily obtainable eucalyptus-based biocides are of special interest from a practical point-of-view. However, as herbicides can be phytotoxic not only for weeds but also for crops, when developing a new biocide it is important to evaluate their potential non-target effects. Therefore, the main goal of this work was to evaluate the environmental safety of two bioherbicides prepared from the leaves of young eucalyptus trees [EucaBio_liquid, an aqueous extract at 250 g/L for foliar-spraying; EucaBio_solid, leaf fragments to be incorporated into the soil at] for crops, using cherry-tomato plants (*Solanum lycopersicum* var. *cerasiforme*) as a model. For this, cherry-tomato seedlings were grown in artificial soils previously treated with the two biocides. After 23 days, the growth performance and redox homeostasis of tomato plants were evaluated. The results showed that EucaBio_liquid did not interfere with plant growth nor induce oxidative stress, even stimulating the activity of superoxide dismutase, catalase, and ascorbate peroxidase by 127%, 85%, and 74%, respectively, in the shoots, compared to the control, possibly priming

them for future stresses. In contrast, the presence of EucaBio_solid in the soil repressed plant growth, inducing severe redox disorders, mainly in the roots, which their antioxidant defences were not able to overcome. Thus, EucaBio_liquid seems to have the greatest potential to become a new biocide, capable of reducing synthetic herbicide application, without compromising tomato plant growth. Nevertheless, as the artificial soils used in these experiments typically have poor microbial activities, the non-target effects of both eucalyptus-based herbicides are now being evaluated on the physiological performance of tomato plants grown in natural soils contaminated with each one of the biocides.

Key words: Sustainable agriculture**,** Allelopathy, *Eucalyptus globulus*, Environmental safety.

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GRAPE SKIN COLOR RECOVERY BY INDEPENDENT MUTATIONS IN *VvIMybA1* OR *VvIMybA2* GENES, THE CASE OF *Vitis vinifera* cv ALBARIÑO AND VERDEJO

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Abstract:
Spontaneous somatic mutations produce changes in berry skin color in grapevine. V*viMybA1* and V*viMybA2* transcription factors induce anthocyanin biosynthesis pathway through the regulation of *UDP-glucose: flavonoid 3-O-glucosyltransferase* (*UFGT*) expression. The lack of berry color in white-skinned cultivars is caused by an insertion of Gret1 retro-transposable element in the *VviMybA1* promoter region preventing gene expression, and a two-nucleotide deletion in *VviMybA2* coding sequence producing a null frameshift (Walker et al., 2007). Nevertheless, occasionally, anthocyanin pigmentation capacity is partially recovered from white-skinned cultivars that then produce red berries (Azuma et al., 2009). Here we have studied the cases of Albariño and Verdejo red-berried somatic variants appearing spontaneously in the field. We used transcriptomics and targeted UPLC-QqQ-MS/MS metabolomics to reveal the molecular cause of color recovery in these somatic variant berries at véraison stage. RNA-sequencing analysis identified 39 and 225 genes that were differentially expressed in the berry skin of Albariño and Verdejo red-berried variants compared to their respective white-berried ancestors (FDR<0.05 and >2 fold-change). In both colored mutants, flavonoid biosynthesis pathway was activated, including the upregulation of UFGT, flavonone-3-

hydroxylase, and chalcone synthase genes. The anthocyanins cyanidin-3-glucoside, cyanidin-3-(6-coum)-glucoside and cyanidin-3-(6-acetyl)-glucoside, were detected in the berry skin of both red Albariño and Verdejo, respectively. Additionally, Albariño red berries also contained detectable amounts of delphinidin-3-glucoside and peonidin-3-glucoside, and of cyanidin-3-arabinoside at trace levels explaining the more intense red color observed in this variant. In addition, we identified the molecular genetic alterations responsible for color recovery. The red Albariño variant had recovered *VviMybA1* expression as a consequence of a recombination between LTRs of *Gret1*. This recombination left behind a *SOLO* LTR leading to a partially recovered expression of the gene. On the other hand, RNA-seq alignments indicated non-synonymous single-nucleotide polymorphisms and a possible homologous recombination within *VviMybA2* exon 3, which may recover the reading frame and protein function in Verdejo. This is the first time that a berry color recovery is related to a *VviMybA2* regain of function,enabling to determine the specific role of the two major transcriptional factors (*VviMybA1* and *VviMybA2*) regulating grape anthocyanin biosynthesis.

Key words: Grapevine, Berry color, anthocyanin, *VvMybA* genes

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NEW APPROACHES FOR EFFICIENT EXTRACTION OF ALLELOPATHIC COMPOUNDS FROM PLANTS: ARE WE USING OPTIMAL PROCEDURES?

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Abstract: The environmental and health concerns derived from the overuse of synthetic herbicides, and the exponential increase in herbicide-resistant weed biotypes, have accentuated the need for alternative ecofriendly products for weed control. Allelochemicals (secondary metabolites produced by plants for defense and communication) are promising substitutes, especially those from high biomass abundant allelopathic species. Nonetheless, the low efficiency of extraction procedures is still a bottleneck for implementing their use due to the different solubility in water (the natural solvent in Nature) of the most phytotoxic compounds. The development of new ecofriendly solvents opens new paths for optimizing bioherbicide formulation and application. In this work, we focused on the extraction of phenolic compounds, one of the most studied groups of allelochemicals with high but not complete solubility in water.

We used different classical and novel combinations of solvents with adequate chemical properties to extract and solubilize phenolic compounds from plant tissues: water, methanol, ethanol, and polysorbate 80, the last being a biocompatible and biodegradable surfactant. The efficacy of the different extraction procedures was tested on two target biomass-abundant allelopathic species: *Mentha suaveolens* Ehrh., and *Eucalyptus globulus* Labill., both species with proved bioherbicide capacity as well *in vitro* as at field scale (Iglesias-Rodríguez, 2016; Puig et al. 2018, 2019). The yield and throughput of the different solvents were assessed on the basis of total phenolic contents determined by Folin-Ciocalteu's method. The influence of solvent aqueous ratio of each solvent combination was also analyzed. The solvent and solvent aqueous ratio showed different efficacy of extraction between species. For *Mentha suaveolens*, the three solvents got similar yields; meanwhile, for *Eucalyptus globulus*, polysorbate 80 showed the highest yield.

The present work emphasizes the significant influence of the plant material on the extraction procedure's effectiveness. Consequently, we should consider that a generalist application of the standard procedures available from literature could lead to obtaining incomplete phenolic profiles of the target plant species, and/or underestimate their allelochemical contents. Finally, the promising potential of polysorbate 80 for future industrial extraction of phenolic compounds from alelopathic plants biomass must be highlighted.

Key words: Allelochemicals, Extraction, Phenolic acids, Polysorbate 80, Weed control

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NEW INSIGHTS INTO THE TIMELY INDUCED SPECIALIZED METABOLISM OF BRASSICA CROPS

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Abstract: In *Brassicaceae*, a large subset of stress-signaling pathways, including phytochemicals production, exhibits diurnal or circadian oscillations (Liebelt *et al*., 2019; Soengas *et al*., 2018; Goodspeed *et al*., 2013). However, to what extent the dimension of time and/or the circadian clock contribute to the accumulation of plant defensive compounds under stress responses remains largely unknown. To start to solve this questions, in the present work we studied plant responses underlying timely induced defences in two different crop species of the *Brassicaceae* family, broccoli (*B. oleraceae*) and turnip greens (*B. rapa*). Glucosinolate (GSL) content was analysed from leaves of broccoli and turnip greens following exposure to mechanical wounding in the dawn (ZT0), mid-day (ZT4) and dusk (ZT8). Several GSLs were differentially accumulated in response to wounding, and changes were dependent upon the time of day. This response was genotype-dependent. These results suggested that the circadian clock might be a key factor for inducible levels of chemical defences in Brassica crops and thereby plant resistance. Thus, in a parallel experiment, we investigated whether cyclical activation of Brassica crops phytochemicals in response to wounding might prime plants against herbivore attack. Results evidenced that maximal responsiveness of plant defense against larvae of the generalist pest *Mamestra brassicae* could be occurring at ZT0 in broccoli and ZT8 in turnip greens. Global metabolome analysis of leaves harvested at the time of maximal resistance against *M. brassicae* indicated that timely induced sulphur-containing phytochemicals, indolic GSLs breakdown products, auxin signaling components, and other metabolites activators of plant resistance such as nicotinamide and pipecolate are behind this response. Together, these data highlight a significant role for time of day in the induced plant defensive compounds affecting plant-herbivore interactions.

Key words: specialized metabolism, timely induced resistance, glucosinolates, *Brassicaceae*, *Mamestra brassicae*.

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PHYTOTOXIC AND STIMULATORY EFFECTS OF DIFFERENT CARVACROL, THYMOL AND EUGENOL OREGANO CHEMOTYPES ON *ARABIDOPSIS THALIANA* SEEDLINGS

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Abstract: The secondary metabolites carvacrol, thymol and eugenol are an alternative to synthetic herbicides thanks to their inhibitory effects on the germination and growth of different species of weeds, which is of great interest in sustainable agriculture.

Previous assays on the phytotoxic effects of carvacrol, thymol and eugenol on the metabolism of the model species *A. thaliana* showed that carvacrol induced oxidative stress, dehydration, alterations in different metabolic pathways, including photosynthesis, and cell death; thymol generated a strong reduction in root growth, tissue disorganization, torsion, reduction of root hairs and the appearance of ectopic hairs, in addition to metabolic alterations; while eugenol induced irregular and incomplete cell divisions, distorted nuclei, fragmented chromatin, alterations in the organization of microtubules and characteristic swelling of Golgi cisternae in roots of seedlings treated with this compound.

These metabolites are present in different percentage in the essential oil (EO) of *Origanum vulgare* according to different chemotypes, which are named based on their major constituent. The three main oregano chemotypes are thymol chemotype (TC), carvacrol chemotype (CC) and eugenol chemotype (EC), which show these three compounds in different concentrations in each chemotype. Therefore, the objective of this work was to test potential synergistic, antagonistic or additive effects of these three compounds when tested in the proportion they are found in the different chemotypes of oregano.

In vitro experiments on the model species *Arabidopsis thaliana* were done to test the following chemotypes compositions, using the concentrations of the major compound as positive control:

- Carvacrol chemotype (CT): 70.2% carvacrol, 2.2% thymol and 0.1% eugenol (Khan et al., 2018).
- Thymol chemotype (TC): 37.1% thymol, 9.6% carvacrol and 0.1% eugenol (Vazirian et al., 2015).
- Eugenol chemotype (EC): 7.5% eugenol, 2.4% carvacrol and 3.5% thymol (Milos et al., 2000).

To date, the results suggest the possible use of thymol, carvacrol and eugenol as a promising alternative to conventional herbicides for the control of weeds in crops. But in addition, the effects of synergies allow the use of lower concentrations of these compounds as a potential option to plant stimulants.

Key words: phytotoxicity, bioherbicide, monoterpenoid phenol, stimulant, secondary metabolites.

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PLANT BIOFACTORIES. CENTELLOSIDE PRODUCTION IN HAIRY ROOT CULTURES OF *CENTELLA ASIATICA*.

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Abstract:
The defense mechanisms of plants against pathogens include the production of secondary metabolites, many of which have biological activities of great importance for health and the pharmaceutical industry. *Centella asiatica* is a commonly used medicinal plant with therapetic effects due to its content of triterpenic saponins, commonly known as centellosides. Levels of these secondary metabolites in the plant are low, which has prompted the development of biotechnological approaches to improve their production. In this work, it was sought to increase centelloside yields by obtaining hairy root lines of *C. asiatica*. After generating various root lines, their transformed nature was confirmed by PCR amplification of rol genes from *Agrobacterium rhizogenes*. The root line with the most growth over four weeks (L1) was treated with three elicitors, methyl jasmonate (MeJa), salicylic acid (SA) and coronatine (Coro), for 2 weeks, after which the centellosides were quantified by HPLC analysis. MeJa induced a similar pattern of centelloside production to Coro, although the final levels were lower. The highest production of asiaticoside (7.1 mg/g DW) was achieved by SA at day 14, 6 hours after elicitation. The highest production of centellosides overall (123.57 mg/g FW) was observed with Coro, madecassoside predominating. Asiatic acid and madecassic acid were only found in the cultures elicited with MeJa or Coro, higher levels obtained with the latter. In summary, a genetically transformed root line of *C. asiatica* elicited with Coro produced up to 58.8 times more centellosides than the control.

Key words: Hairy roots, elicitation, centellosides, *Centella asiatica*

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RAPID DIAGNOSTIC TOOLS OF NUTRITIONAL COMPONENTS IN A PLANT SUITABLE FOR SPACEFLIGHT

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Abstract:
Aquatic plants, such as duckweeds, have tremendous potential as food crops for spaceflight applications (Appenroth et al., 2017). These floating plants can be grown rapidly and easily and are 100% edible and highly nutritious. They are rich in protein and essential micronutrients, such as β-carotene (provitamin A), ascorbic acid (vitamin C), tocopherols (vitamin E) and the xanthophylls zeaxanthin and lutein (Appenroth et al., 2017). The latter carotenoids are potent antioxidants, anti-inflammatories, and membrane stabilisers that promote ocular health and mental acuity (Demmig-Adams et al., 2020). Duckweeds can thus supply a balanced mix of essential micronutrients to a space crew. To quantify these pigments, expensive chromatographic techniques are required that are accurate but destructive and confined to laboratory environments. We introduce here a novel protocol to rapidly and non-destructively measure duckweed nutritional content via low-cost, versatile and remote sensing. To test this objective, we used a fully factorial experimental design with nitrogen and light level as treatments. We collected non-invasive reflectance spectra from paired fronds of *Lemna minnor* L. and plant container-level imagery for characterization of duckweed colonies biometrically and by their RGB (red green blue) colour as a pigment content index. These non-invasive parameters were vetted against chromatographically determined biochemical composition and plant physiological status. Our results showed: (i) a differential biochemical content (proteins, tocopherols, chlorophylls and total carotenoids) depending on treatment, which provided a database with a wide range of contents for correlations and prediction models; (ii) significant correlations between the photochemical reflectance index and the total content of carotenoids and tocopherols. (iii) A new and non-destructive method based on RGB colour image analysis (derived from the analysis of these channels) was developed for a qualitative estimation of chlorophylls and carotenoids for duckweed. Further studies are needed in microgravity environments to assess the utility of these optical properties as non-destructive indicators of nutritional components. The results presented here are promising and open the door to future research.

Key words: *Lemna minor*, nutritional quality, non-invasive,
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REGULATION OF TOCOPHEROL BIOSYNTHESIS IN *CITRUS* SPECIES DURING FRUIT MATURATION

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Abstract: Tocopherols are lipid-soluble isoprenoids synthetized in photosynthetic organisms and, together with tocotrienols, are the only natural compounds exhibiting vitamin E activity in animal cells. They play diverse functions in plants, of which their role as antioxidants is the most notable. Although the key genes regulating the main steps of tocopherol biosynthesis pathway have been elucidated in model plants, its biosynthesis and accumulation in *Citrus* remains unexplored. The aim of this work was to initiate a physiological and molecular characterization of tocopherol biosynthesis in *Citrus* fruit. To that end, tocopherol accumulation was assessed during maturation in the flavedo (peel) and pulp of fruit of four main cultivated *Citrus* species: grapefruit (*Citrus paradisi)*, lemon (*C.limon)*, orange (*C.sinensis)* and mandarin (*C.reticulata)*. Moreover, we analyzed the expression of 14 genes involved in the tocopherol-core pathway (*VTE2*, *VTE3a*, *VTE3b*, *VTE1* and *VTE4)*, as well as in the pathways supplying the tocopherol precursor homogentisate –HGA- (*TAT1* and *HPPD)* and phytyl pyrophosphate –PPP- (*VTE5*, *VTE6*, *DXS1*, *DXS2*, *GGPPS1*, *GGPPS6*, *GGDR*,). HGA provides the chromanol ring and is the common precursor for all tocochromanols, while PPP provides the prenyl tail and is specific for tocopherol synthesis. Only γ- and α-tocopherol were detected in the flavedo and pulp, and α-tocopherol was the predominant form in both tissues in all species. Tocopherol content was always higher in the flavedo than in the pulp and, while levels increased in the flavedo with maturation, they decreased or were maintained in the pulp. In the peel and pulp of mature fruit, tocopherol content ranked in the following order: lemon > grapefruit > orange > mandarin. Interestingly, the higher concentration of tocopherols in the flavedo was associated to a higher general expression of the genes analyzed, and different genes appeared to be key in regulating tocopherol content in each tissue. In the flavedo of the four species, the increase in tocopherol contents was accompanied by an induction of the gene *TAT1*, involved in the supply of the precursor HGA, and *VTE4*, which controls the conversion of γ-tocopherol into α-tocopherol. On the other hand, the expression of *DXS2*, *GGDR* and *VTE6*, involved in the *de novo* supply of the precursor PPP and through the recycling of phytol, appear to be critical for tocopherol content in the pulp. Transcriptional differences among species possibly related to the differential accumulation of tocopherols will be further discussed.

Key words: tocopherol, citrus, fruit, maturation, molecular regulation

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THE SECONDARY METABOLITE *TRANS*-CINNAMALDEHYDE CAUSES AN OXIDATIVE BURST ON *A. THALIANA*

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Abstract: The phenolic compound *trans*-cinnamaldehyde is naturally occurring in plants of the families Lauraceae like *Cinnamomun osmophloeum* (Fang et al., 2004) and *C. zeylanicum*. *Trans*-cinnamaldehyde (TC) is generally used as flavours in foods and has shown cytotoxic, fungicide and antimicrobial activity (Gill and Holley, 2004), but its phytotoxic activity was not deeply investigated.
Therefore, *Arabidopsis thaliana* (L.) Col-0 seedlings were treated with different concentrations (from 0 to 1200 µM) of *trans*-cinnamaldehyde to establish the phytotoxic potential of this phenolic compound.
Arabidopsis root growth was affected by *trans*-cinnamaldehyde treatment with an IC_{50} as low as 46 µM and a IC_{80} of 87 µM, while the germination process was completely inhibited at 800 µM. *Trans*-cinnamaldehyde-treated roots showed increased number of short root hairs and a high proliferation of adventitious roots. This morphology suggested a possible hormonal alteration at the auxin level, as demonstrated by the reversion of the effects induced by the antiauxinic PCIB, and by the high level of auxins content obtained by GC-MS. In addition, a high content of benzoic acid and salicylic acid was also observed in TC-treated seedlings.
Structural analysis by light and transmission electron microscopy of 7 and 14 days-old TC-treated *Arabidopsis* roots showed important differences compared to the control, as the thickening of the calyptra and strong tissue disorganization, in addition to an increase in the number of mitochondria, many of them undergoing division. These mitochondria presented altered membrane potential, as demonstrated by a fluorochrome JC-1 assay.
High levels of auxins, and benzoic and salicylic acid can generate oxidative stress in the plant, which was confirmed by the *in situ* determination of H_2O_2 and O_2^-. This oxidative stress would be the cause of poor mitochondrial functionality and, together with the action of auxins, of the inhibition of growth in TC-treated roots.

Key words: *trans*-cinnamaldehyde, *Arabidopsis*, auxin, oxidative stress.

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CHLOROPLASTIC THIOREDOXINS: NEW INSIGHTS FOR REDOX REGULATION OF PHOTOSYNTHESIS AND CARBON METABOLISM

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Abstract: Thioredoxins (Trxs) are essential proteins to balance the redox state in plants. Their main function is to carry out post translational modifications in target proteins. In the chloroplast it can be found *f*-, *m*-, *x*-, *y*- and *z*- type thioredoxins. Trxs *f* (*f1* and *f2* in *Arabidopsis thaliana*) are the best known being involved in the redox dependent activation of several Calvin Benson enzymes that regulate the carbohydrate synthesis. The Trxs-*m* type belong to an interesting group integrated by 4 isoforms, TRXs *m1*, *m2*, *m3* and *m4*, which function was presupposed to be similar. However, in the last years new insights have suggested that the different chloroplast TRXs have specific functions and specific protein targets. In order to unravel the role of each of the TRXs within the chloroplast, we have characterized *A. thaliana* single, double and triple mutants defective in the *m1*, *m2* and *m4* isoforms. We report in this work the physiological and developmental aspects affected by the lack of one or several TRXs by using photosynthesis determinations, metabolic analysis and microscopy studies. In addition, knowing the close relationship between the redox state and light signaling we analysed the effect on the photosynthesis response to high light (HL) in these mutants. Under HL growth conditions our results have shown that TRXs *m* function are related to mesophyll structure, stomata biogenesis, water use efficiency, maximum quantum yield of PSII (Fv/Fm), photosystem II quantum yield (Y(II)) and non-photochemical quenching (NPQ). Triple mutant (*trxm1m2m4*) sugar content was lower in relation to wild type (WT). Besides, we show different redox state of the chloroplastic fructose-1,6-biphosphatase (cpFBPase1) in the different TRXs mutants. Altogether, our results show evidence of novel functions for the plastidial TRXs linked to plant development and light adaptation under stress situation.

Key words: Thioredoxin, Redox, High-Light stress, Photosynthesis

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F-BZIP TRANSCRIPTION FACTORS AND THE MICRONUTRIENT ZINC DEFICIENCY RESPONSE IN PLANTS

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Abstract:
All living organisms require zinc (Zn) as an essential micronutrient. This is due to its structural and catalytic role in many proteins, as a cofactor in enzymes, transcription factors and protein-interaction domains. An appropriate zinc distribution and intracellular availability relies on membrane transporters and low-molecular-weight ligands, and requires a tight regulation of the zinc homeostasis network in order to avoid zinc deficiency or toxicity (Clemens, 2001). In *Arabidopsis thaliana*, bZIP19 and bZIP23 transcription factors are the central transcriptional regulators of the zinc deficiency response. They belong to the F group of basic leucine-zipper (F-bZIP) proteins (Assunção et al., 2010). bZIP19 and bZIP23 also function as Zn sensors, through a direct binding of Zn^{2+} ions to their Zn-sensor motif (ZSM), *i.e.* a cysteine/histidine-rich motif at the protein N terminus (Lilay et al., 2021). Their activity regulates the plant zinc levels through Zn-dependent changes in the expression of their target genes, which include members of the Zrt/Irt-like protein (ZIP) transporter family, involved in cellular zinc uptake (Lilay et al., 2019).

There is evidence for evolutionary conservation of the F-bZIP transcription factors and their function in the Zn deficiency response across land pants (Castro et al., 2017). Fundamental knowledge of the Zn deficiency response in the model *Arabidopsis thaliana* and in crop species, anchored at the F-bZIP transcription factors, can contribute to develop crops with improved Zn nutritional content (biofortified) and improved resilience to Zn-deficient soils. This is significant considering that Zinc-deficient soils are widespread globally and human Zn malnutrition affects about one-third of the world's population (White and Broadley et al., 2009).

Here I will present an overview of the research developed at my group to obtain functional knowledge of the F-bZIP regulatory network and its role in the Zn deficiency response in plants, from molecular to plant levels, and to integrate this knowledge in evolutionary and applied perspectives.

Key words: zinc deficiency response, F-bZIP transcription factors, *Arabidopsis thaliana*, ZIP transporters

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MOLECULAR AND BIOCHEMICAL CHARACTERIZATION OF ADENINE PHOSPHORIBOSYLTRANSFERASE FROM *PHASEOLUS VULGARIS*

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Abstract:
Adenine phosphoribosyltransferase (APRT) is one of the two enzymes involved in the salvage pathway of purines. The APRT enzyme catalyses the reaction between adenine and phosphoribosyl pyrophosphate (PRPP) to adenine mononucleotide (AMP), which can be used to get the other purine nucleotides IMP and GMP. Nucleotides are essential for any organism because they form part of nucleic acids and they are important energy and cofactor molecules[1]. Besides, in ureidic legumes, purine nucleotides are precursors of the major nitrogen transport molecules called ureides. Moreover, APRT activity is not only involved in nucleic acid biosynthesis in plants, but it does also acts in the activation and inactivation of cytokinins[2]. In common bean (*Phaseolus vulgaris*), synthesis of purine nucleotides and regulation of cytokinin functions, specifically during the formation of the nodule, are both essential in the productivity of these crops. Despite the importance of APRT, knowledge about this enzyme is very scarce in plants. In this research, molecular and biochemical characterization of APRT from *P. vulgaris* has been carried out. Four genes code for APRTs in common bean and the expression patterns of the four genes have been determined in tissues from plants grown under several conditions. Moreover, the enzymatic assay has been optimized to determine APRT activity in plant tissues. In addition, overexpression of the four PvAPRTs has been done in *Nicotiana benthamiana* leaves, recombinant proteins have been purified and their kinetic parameters have been estimated. To determine their specific function, the two genes with the highest expression levels (*PvAPRT1* and *PvAPRT5*) have been silenced in bean roots by using siRNA and CRISPR/Cas9 gene edition. The results showed that *PvAPRTs* genes have a high expression and activity in nodules of *P. vulgaris*, where *de novo* synthesis of purines predominates. *PvAPRT1* and *PvAPRT5*-silenced roots had similar number of nodules than control roots, although these nodules were significantly larger in size and weight, thus suggesting a role of these APRTs in cytokinin inactivation.

Key words: common bean, APRTs, nucleotides

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ROOT CELL TYPE MOLECULAR SPECIALIZATION IN RESPONSE TO PHOSPHATE STARVATION

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Abstract: Phosphorus (P) is one of the most important nutrients for plant growth and development. During the plant adaptation to phosphate (Pi) starvation, different cell types in the root might change their transcriptional programs to specialize and improve the response, thus sustaining plant growth and development under stress (known as "molecular adaptation"). In this work, we have analyzed the transcriptomic changes on specific root cell types such as of epidermis, cortex, endodermis, pericycle vasculature and lateral root cap during Pi starvation. Five cell type markers plants were grown in the D-Root system to cultivate them with the root system in darkness to avoid the negative light influence. Using a new improved cell sorting protocol to increase the efficiency, we were able to analyze low abundant cell types, such as lateral root cap. Our analysis indicate that more than 5000 genes were deregulated by Pi deficiency among the five cell types, but, remarkable, none of them were deregulated in all cell types. We found that pericycle was the cell layer most affected, and surprisingly, the epidermis showed fewer changes in gene expression. Gene ontology analyses reported diverse functional categories enriched in different cell layers. Among them, it should be highlighted the enrichment in the response to salt, abiotic stress, water deprivation or repose to heat. Confirming this molecular specialization, we found that plants grown at higher temperatures (28ºC) accumulated less Pi in both root and shoot, likely reducing plant nutrition. Taken together, our data show that every cell layer contributes differentially to the Pi response defining a coordinated organ adaptation.

Key words: phosphate, root development, cell type, warming

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TACKLING SUBCELLULAR COMPARTMENTATION AND VACUOLE EXPANSION TO EXPLAIN AMMONIUM STRESS IN TOMATO LEAVES

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Abstract: Ammonium (NH_4^+) fertilization is a great means to mitigate the negative effects nitrogen fertilization has on the environment. However, although NH_4^+ is a fundamental substrate for biomolecules synthesis, when present at high concentration in the soil it provokes stress symptoms, notably plant growth inhibition. The cause for this inhibition is multifactorial and includes, among others, pH disorders, metabolic alterations and cation imbalances. Moreover, an effect of ammonium nutrition on cell elongation has been reported. Ammonium stress is generally associated with NH_4^+ accumulation in cytosol and one strategy suggested for ammonium tolerance is its storage into the vacuoles. Importantly, it is known that the osmotic-driven enlargement of the vacuole is involved in the control of cell expansion. Hence, in the present study we tackled the almost neglected involvement of subcellular compartmentation in the adaptation of plants to ammonium nutrition, specially focusing on vacuoles. To do so, we grew tomato plants under nitrate and ammonium nutrition and monitored by optical microscopy vacuole expansion. In addition, we quantified pH and metabolites at the subcellular level and developed a mathematical model to predict solutes distribution across the tonoplast. The overall data provide a novel explanation for leaf growth inhibition under ammonium nutrition related to the higher energy cost of vacuole expansion as a consequence of pH alterations and organic acids depletion.

Key words: Ammonium stress, model, nitrogen nutrition, pH, vacuole

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Poster Communications



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CHLORIDE IS REQUIRED FOR OPTIMAL SEEDLING ESTABLISHMENT AND EARLY VEGETATIVE DEVELOPMENT OF PLANTS

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Abstract (350 word count):

Although defined as an essential micronutrient, chloride (Cl^-) accumulates in plants to typical macronutrient levels. Our laboratory has recently shown that, at macronutrient concentrations, Cl^- improves the plant efficiency in the use of water, nitrogen and CO_2 (Franco-Navarro *et al.*, 2016, 2019; Colmenero-Flores *et al.* 2019; Rosales *et al.*, 2020), increasing plant biomass and drought resistance (Franco-Navarro et al., 2021).

We show here that Cl^- is highly and specifically demanded during early plant development. A treatment leading to macronutrient Cl^- levels (5 μM Cl^- supplement; **CL**) is required for optimal seedling development. Growth deficiency under micronutrient Cl^- supply (75 μM Cl^- in the nutrient solution) cannot be reversed with equivalent supplies of either nitrate (**N**) or Sulphate + Phosphate (**SP**) treatments, showing the specific requirement of Cl^- to promote seedling growth in different species.

Recent reports suggest that adequate assembly/reassembly of the PS-II oxygen evolving complex require macronutrient Cl^- levels (e.g. 40 mM Cl^- in the chloroplast; Vinyard et al., 2019; Raven, 2020). In addition, macronutrient Cl^- levels lead to a higher surface area of chloroplasts exposed to the intercellular airspace of mesophyll cells, possibly through stimulation of chloroplast biogenesis (Franco-Navarro et al., 2019). To ascertain whether Cl^- is required to improve chloroplastic/photosynthetic performance during early plant development, two approaches have been addressed in tobacco plants: 1) quantification of structural and ultra-structural parameters of mesophyll and chloroplast anatomy using electron microscopy; 2) quantification of photosynthetic parameters such as the Electron

Transport Rate (ETR), the Non-Photochemical Quenching (NPQ), etc., using chlorophyll fluorescence imaging.

Our results show that during seedling establishment, 13 days after seeding (das), the CL treatment stimulates the ETR in comparison to N or SP treatments. After seedling establishment, in 24 das and 35 das plants, the CL treatment determines lower ETR and significantly higher NPQ. Interestingly, macronutrient Cl^- levels determines larger mesophyll cells containing a higher number of chloroplasts with thicker grana and much higher number of starch granules. The results confirm the impact of Cl^- nutrition on the structure and function of chloroplasts and its relevance on seedling development will be discussed.

Key words: Chloride, seedlings, tobacco, ETR(II), NPQ, Y(II), PSII,

We acknowledge: i) Financial support of the Spanish Ministry of science Innovation and Universities-FEDER grant RTI2018-094460-B-I00, European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 895613 and Spanish National Research Council grants CSIC-202040E266 and CSIC-202140E023; **ii)** Technical assistance of F. J Durán and Carlos M. Rivero-Núñez.

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CHLORIDE NUTRITION AFFECTS ROOT ARCHITECTURE DURING EARLY VEGETATIVE DEVELOPMENT IN TOBACCO AND TOMATO PLANTS

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Abstract:

Chloride (Cl^-) is an anion that participates in key processes for plants, such as photosynthesis and osmotic regulation[1]. Cl^- has recently been defined as a beneficial macronutrient for higher plants, due to the stimulation of plant growth, inducing morphological and cellular changes that include the stimulation of leaf cell size, reducing stomatal conductance and inducing additional osmolarity and turgor in adult tobacco plants. Thus, Cl^- generates bigger plants with better water and nitrogen use efficiency and, as a result, a better photosynthetic performance[2,3].

Roots are in charge of searching for water and nutrients which are essentials for plant development and growth. Nutrients distribute to the rest of plant organs via the vascular system, being regulated by complex hormonal mechanisms[4]. Although some proteins regulating Cl^- homeostasis (e.g. Cation-Chloride Cotransporter) have been reported to be important for root cell elongation[5], little is known about the effect of Cl^- nutrition on root developmental processes during early vegetative growth.

To this aim, in this work tobacco and tomato seeds were sown under greenhouse conditions and seedlings were harvested at different developmental stages, from 8 to 35 days after sowing. A Cl^- treatment (5 mM of Cl^- salts) was supplemented to the basal nutrient solution, and a treatment with a mix of sulphate+phosphate salts (SP) with the same cationic content was used as a control. Afterwards, biomass and root system architecture (RSA) parameters (primary root length, number and length of lateral roots) were analysed by using 'ImageJ' and 'Optimas' software.

Results from this work showed that the Cl^- treatment generated bigger plants with longer primary roots and shorter lateral roots during the early vegetative development. These effects may be caused by higher cell size or division during the root development, with a possible effect on hormonal regulation. Therefore, these root architecture changes are particularly relevant during early vegetative development, allowing to Cl^--fertilized crops

with longer roots to reach deeper zones in the soil with more water and nutrients and, consequently, helping to withstand drought periods that are increasingly more frequent because of climate change.

Key words: Chloride, Beneficial macronutrient, Root architecture, Early vegetative stage

Acknowledgments: Funding for the present research was provided by the Spanish Ministry of Science Innovation and Universities-FEDER grant RTI2018-094460-B-I00, Spanish National Research Council grants CSIC-202040E266 and CSIC-202140E023, and European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 895613. We would like to thank F.J. Durán for technical assistance.

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EFFECT OF NITROGEN SUPPLY AND GENOTYPIC VARIABILITY ON MINERAL NUTRIENT COMPOSITION OF PHOTOSYNTHETIC ORGANS AND GRAINS IN FIELD-GROWN DURUM WHEAT

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In C_3 cereals, preliminary studies showed that a significant part of nutrients (i.e. carbon and nitrogen) remobilized to the growing grains comes from non-laminar photosynthetic organs, such as the stems and the ears. This may be particularly relevant under water stress and poor soil fertility conditions. The aim of this work was to study the variation on the distribution of nutrient and isotope composition in laminar (flag leaf blade and sheath) and non-laminar (peduncle, glume, lemma and awn) photosynthetic organs at anthesis and grain filling stages, as well as in the grains at harvest, among four durum wheat genotypes grown under two different nitrogen supplies (105 N kg ha^{-1} and 24 N kg ha^{-1}).

The statistical analyses showed significant differences in nutrient concentration among genotypes, mainly at anthesis. In addition, N supply increased assimilation of K, Cu and Zn at anthesis, but decreased Fe content at grain filling stage.

In general, the highest nutrient concentrations in organs were determined in the flag leaf blade, particularly for N, K, Ca and Mn, at both stages. However, the concentration of some nutrients in other organs was relevant or even higher than in leaves (e.g., Fe and Zn in ear bract). Considering the changes in nutrients between anthesis and grain filling in the different organs and the values of isotope composition, we can speculate about their contribution to grain filling. Interestingly, the stems (K, Mg, P, Fe, Cu, Mn, Zn, N) and ear organs (Mg, Ca, P, Cu, Zn, N) showed a similar or even higher contribution of nutrients to grain filling than the flag leaves.

Overall, these results clearly indicate that (1) mining the genotypic variability is a good source for improving grain nutritional quality, (2) N fertilization affect the assimilation and distribution of other nutrients among the plant, and (3) other non-laminar photosynthetic organs may have a significant role in providing nutrients to the developing grains in durum wheat. More efforts are needed to dissect the effect of the fertilization manage on grain yield and nutritional composition.

Key words: Durum wheat, Grain quality, N fertilization, Ears, Field.

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EFFECT OF SEAWATER INORGANIC CARBON ENRICHMENT ON NO_3^- TRANSPORTER AND REDUCTION ENZYME GENE EXPRESSION IN *ZOSTERA MARINA* LEAVES

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Abstract: CO_2 concentration has increased over the past 200 years and is expected to continue rising. In seawater, the increase of dissolved CO_2 has led to a decrease in pH, changing the inorganic carbon chemical speciation to increase HCO_3^-. Seagrasses are the only vascular plants that colonized the marine environment, developing the capacity to use bicarbonate as a source of carbon for photosynthesis (Beer *et al*., 2002). In *Posidonia oceanica* the direct uptake of HCO_3^- through a H^+ symporter and its subsequent dehydration renders CO_2 for photosynthesis, but alters cytosolic anion homeostasis promoting the cytosolic nitrate leak (Rubio *et al*., 2017; Rubio *et al*., 2020). Therefore, under increasing dissolved HCO_3^- conditions the cytosolic NO_3^- diminution could induce the N biomass impoverishment shifting the expression pattern of nitrate transporters in seagrasses. *Zostera marina* genome, the first fully sequenced from a marine angiosperm, reveals lost of important vascular plants innovations but less than 20% as specific gene families (Olsen *et al*., 2016). Thus, key physiological adaptations to thrive in seawater, characterized by a low concentration of NO_3^-, seem to be due to molecular changes of the same family genes rather than the speciation of pre-existing genes. This could be the case for the high-affinity nitrate transporters, sodium driven high-affinity transport unique among vascular plants (Rubio and Fernández, 2019). In this work we have analysed the gene expression levels related to cytosolic nitrate homeostasis, including transporters and enzymes, in leaves of *Z. marina* plants incubated in high HCO_3^- or in the absence of N. Overall gene analysed, expression levels of *ZosmaNTR2*, the only gene quoted as a high-affinity nitrate transporter belonging to NRT2 family in *Z. marina* genome (Rubio *et al*., 2019), as well as its regulatory protein *ZosmaNAR2*, were induced in both conditions. Interestingly, expression levels of such genes were higher than the obtained for *ZosmaCHL1* (*ZosmaNPF6.3*), which expression was lower in N-replete plants, but did not vary in response to HCO_3^-. These results suggest that the induction of the expression of the NO_3^- high-affinity transporter *ZosmaNRT2* and its regulatory protein *ZosmaNAR2* are the main response for the cytosolic NO_3^- decrease in both N-deficiency or in C-enrichment.

Key words: *Zostera marina*, HCO_3^- enrichment, *ZosmaNRT2* expression levels.

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FROM MICRONUTRIENT TO BENEFICIAL MACRONUTRIENT: THE IMPACT OF CHLORIDE ON NITROGEN METABOLISM

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Abstract

Chloride (Cl^-) is an essential micronutrient recently defined as a beneficial macronutrient for plants, with new specific functions that improve the efficiency in the use of water (WUE), nitrogen (NUE) and CO_2. Nitrate (NO_3^-) and Cl^- are the most abundant inorganic anions in plants, sharing physical and osmoregulatory properties, transport mechanisms, dealing to strong dynamic interactions between the two anions[1]. Cl^- has been traditionally considered harmful for agriculture due to the belief of its competitive interaction with NO_3^- and the toxicity generated by excessive Cl- accumulation in sensitive crops under salt-stress conditions[2,3]. However, when Cl^- is accumulated in leaves at beneficial macronutrient levels (1-5 mM), the NO_3^- content is reduced while plant growth and NUE increased[4]. Therefore, the objective of this work was to determine how Cl^- nutrition affects nitrate accumulation by studying different nitrogen metabolic pathways.

To this aim, tobacco plants were grown under experimental greenhouse conditions and watered with a basal nutrient solution supplemented with 6 mM Cl^- and a control treatment containing a mix of sulphate+phosphate salts (SP) to keep identical concentration of cations. After 60 days of nutritional treatments, mature tobacco leaves were collected and, subsequently, different nitrogen forms, key enzyme activities involved in nitrogen metabolism and protein content were spectrophotometrically analysed. In addition, a free amino acids profile was determined by HPLC.

Our results showed that the Cl- treatment stimulated plant growth and increased the content of total nitrogen and ammonium in leaves, whereas the content of nitrate was reduced. The analysis of enzymes showed increased activities of nitrate reductase (NR), glutamine synthetase (GS), glutamate synthase (GOGAT) and aspartate aminotransferase (AAT) in Cl^--treated plants, supporting the Cl^--dependent stimulation of nitrate assimilation.

Interestingly, the analysis of free amino acids content showed a reduction of aspartate, glutamate, glutamine, glycine and threonine, whereas serine and asparagine significantly increased. These results suggest the implication of other metabolic pathways, such as photorespiration or urea metabolism, in the Cl^--specific response. Further studies are required to confirm the regulation of photorespiration by Cl^-, a pathway which is closely linked to NO_3^- assimilation and protein synthesis in plants.

Key words: Chloride, Nitrate, Amino acids, Nitrogen metabolism, Tobacco.

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GENETIC LOCI ASSOCIATED WITH IONOMIC AND GROWTH RESPONSES TO CARBONATES IN *Arabidopsis thaliana*

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Abstract: Calcareous soils are a major problem for crop production in Mediterranean areas. In this study, specific candidate genes potentially involved in the mechanisms of tolerance to carbonated soils were identified by means of genome wide association studies (GWAS). Screening 360 *Arabidopsis thaliana* accessions of the HapMap population grown on carbonated soil from Catalonia revealed 18 significant SNPs mapping to Zn, P and S content. Growth measurements and ionome profiling were performed on 12 selected mutant lines in order to gain insight into candidate genes to be followed-up and their role in the response to carbonates and TDT (AT5G47560) showed to be involved in enhanced Zn translocation and better growth under carbonate stress.

Key words: abiotic stress, carbonates, GWAS, ionome

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IMPACT OF MILD CHLORIDE SALINITY ON GROWTH AND NUTRITIONAL QUALITY OF LETTUCE BABY-LEAVES

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Abstract:

Chloride (Cl^-) has traditionally been considered a damaging ion for agriculture because of its toxicity under salinity conditions and antagonism with nitrate (NO_3^-). However, Cl^- nutrition at macronutrient levels (1-5 mM) has been proved as a potential strategy to improve the performance of different crops[1]. Mild salinity levels (20-40 mM NaCl) has also been formulated as a promising way to increase phytochemicals with minimal loss of yield, modulating positively quality of vegetables[2,3,4]. Among other effects, the beneficial Cl^- nutrition reduces between 25-75% the NO_3^- content in leaves of adult tobacco plants, potentially harmful to human health[1]. The study of a potential fertilization with optimal NO_3^-/Cl^- ratios is relevant to identify the best performance and nutritional quality of young lettuce plants, for reducing NO_3^- accumulation in leaves and nitrogen over-fertilization practices, meaning a more sustainable agriculture for environment and human health. Therefore, in this work we aimed to elucidate the effects of Cl^- supplied at both macronutrient and mild Cl^- salinity levels on physiological parameters, Cl^- and NO_3^- content, ascorbic acid, and different antioxidant capacity tests in lettuce baby-leaves.

Lettuce seeds were grown in semi-hydroponics under greenhouse conditions, and treated with four NO_3^- treatments: 5, 9, 12 and 15 mM of a mix of NO_3^- salts. In addition, plants from each NO_3^- treatment were treated with two Cl^- treatments: 5 (5CL) and 25 (25CL) mM of a mix of Cl^- salts, and a control treatment with a mix of sulphate+phosphate (SP) salts. Plants were harvested at 15 and 30 days after sowing, and tissues were stored for the analysis anions and antioxidants content. Interestingly, our results revealed that the 25CL treatment caused the strongest increase of biomass and leaf area at 5 and 9 mM of NO_3^- levels compared to SP treatments, whereas the loss of water and the NO_3^- content was significantly reduced in 25CL and 5CL treatments compared to SP treatments. Antioxidant determinations will be discussed on the poster. Therefore, this work demonstrates for the first time the Cl^--specific function of mild Cl^- salinity on the improvement of growth and nutritional quality in lettuce baby-leaves.

Key words: chloride, nitrate, crop performance, mild salinity, nutritional quality

Acknowledgments: Funding for the present research was provided by the Spanish Ministry of Science Innovation and Universities-FEDER grant RTI2018-094460-B-I00, Spanish National Research Council grants CSIC-202040E266 and CSIC-202140E023, and European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 895613.

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INTEGRATIVE TRANSCRIPTOMIC AND METABOLOMIC ANALYSIS AT ORGAN SCALE REVEALS GENE MODULES INVOLVED IN THE RESPONSES TO SUBOPTIMAL NITROGEN SUPPLY IN TOMATO

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Abstract: Nitrogen (N) is an essential macronutrient for plant growth and development and is usually applied in large amounts to maximize crop production. The development of high nitrogen use efficiency (NUE) cultivars under low N inputs is required for a sustainable agriculture. With this aim, in this study we analyzed the impact of long-term suboptimal N conditions on the metabolome and transcriptome of tomato (*Solanum lycopersicum* L.) to identify specific molecular processes and regulators at organ scale. Physiological and metabolic analysis revealed specific responses to maintain glutamate, asparagine and sucrose synthesis in leaves for partition to sustain growth, while assimilated C surplus is stored in roots. Comparative transcriptomic analysis showed that a major part of the transcriptomic response to suboptimal N supply is conserved between Arabidopsis and tomato, but also identified a set of processes specifically regulated in tomato, including alternative respiration, nucleoside transport, and photosynthetic cyclic electron transport. Integrative expression analyses revealed a group of nitrogen transporter genes that might be

involved in the control of remobilization and distribution of nitrogen compounds under limited N supply. Interestingly, we identified transcription factors with high identity to TGA4, ARF8, HAT22, NF-YA5 and NLP9, which play key roles in N responses in Arabidopsis. Together, this work provides a set of nitrogen-responsive genes in tomato and new putative targets for tomato NUE and fruit quality improvement under limited N supply.

Key words: nitrogen; suboptimal conditions; metabolome; transcriptome; growth

Acknowledgments: This work was supported by grants from The National Institute for Agriculture and Food Re-search and Technology (CSIC-INIA) (RTA2015-00014-C02). We also want to acknowledge the "Severo Ochoa Program for Centers of Excellence in R&D" from the Agencia Estatal de Investigación of Spain (Grant SEV-2016-0672) for supporting the scientific services used in this work.

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MOLECULAR CHARACTERIZATION OF APOPLASTIC NUCLEOSIDASES IN COMMON BEAN

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Abstract: Nucleotides catabolism implies the action of several enzymes, including nucleotidases (nucleotide phosphatases) and nucleosidases (nucleoside hydrolases, NSH), which dephosphorylate nucleotides and degrade nucleosides to nucleobases by removing the ribose moiety, respectively. Nucleotides, nucleosides and nucleobases produced in the catabolic pathway can be transported to other tissues. In addition, nucleotides and their derivatives have been identified in the extracellular space, although their origin is not clear; they may be released by wounded or intact cells, to come from the degradation of the extracellular ATP or be imported form the rhizosphere. In common bean, we have identified four genes encoding nucleosidases, two of which, *PvNSH3* and *4*, have two nucleosidase domains and are predicted to be located in the apoplastic space. Phylogenetic analysis of these sequences indicated that these genes are only present in some groups of plants, which suggests that they could have a relatively recent origin. PvNSH3 and 4 have been fussed to the eYPF which has allowed to confirm that both proteins located in the apoplastic space. *PvNSH4* have been overexpressed in *Nicotiana benthamiana* and the recombinant protein purified by affinity chromatography. The purified protein showed higher affinity with inosine, an optimum pH of 5 and a Km with inosine of 54 uM. Analysis of a fragment of 1000 pb upstream of the start codon revealed several motifs related with the response to pathogens and wounding. Therefore, we analysed the expression of *PvNSH3* and *4* in response to methyl jasmonate, a hormone that regulates the response to wounding and other biotic stresses. Results showed an increase in the levels of *PvNSH4* in radicles of seedlings, whereas *PvNSH3* levels were not altered. Concomitantly, nucleosidase activity was also induced in the same conditions, suggesting a role of PvNSH4 in the response to methyl jasmonate.

Key words: Nucleotide catabolism, nucleoside hydrolase, jasmonic acid, apoplastic space, common bean.

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NITRATE PARTIAL REPLACEMENT BY CHLORIDE TO IMPROVE GROWTH AND WATER STATUS IN TOMATO PLANTS

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Abstract:

Chloride (Cl^-) is an essential micronutrient that has traditionally been considered a harmful element for plants due to its toxicity and antagonism with nitrate (NO_3^-), which represents the major nitrogen source for crops. However, Cl^- application at macronutrient levels exhibits positive effects for plant growth, as a result of improved photosynthetic performance, whole-plant water relations, and water use efficiency[1]. Furthermore, Cl^- nutrition has been shown to improve nitrogen-use efficiency, reducing nitrate accumulation in leaves and, as a result, improving the nutritional quality of agricultural products for human consumption[2]. The agricultural use of NO_3^- in organic and chemical fertilizers is currently a major environmental concern in Europe since excessive NO_3^- concentrations cause water pollution being harmful to people and nature. Therefore, Cl^- arises as a potential strategy to reduce NO_3^- over-fertilization in agriculture by the implementation of new Cl^-/NO_3^- ratios in fertilizers.

With this aim, in this work we evaluated whether Cl- nutrition improves growth and different physiological traits in tomato plants subjected to increasing NO_3^- treatments. Tomato plants were grown in semi-hydroponics under greenhouse conditions, and watered with increasing NO_3^- treatments (3-15 mM). In addition, plants from each NO_3^- treatment were subjected to two nutritional treatments: 5 mM Cl^--based treatment (CL) and a sulphate+phosphate treatment (SP) as a control, all treatments containing a similar cationic balance. After 30 DAS, tomato plants were harvested and different growth and physiological parameters were analyzed.

Our results showed that increasing NO_3^- concentrations induced total, leaf and root growth, remaining with no changes beyond 9 mM NO_3^-. Cl^--treated plants exhibited stronger growth stimulation in all NO_3^- treatments compared to SP plants, whereas NO_3^- content was highly reduced. Interestingly, under low NO_3^- (3 mM), Cl^- showed similar values of growth, SPAD and water potential to 9 mM NO_3^- without Cl^-, whereas photosynthesis remained unchanged. In conclusion, our results demonstrated a beneficial

effect of Cl^- nutrition on tomato plant growth and water status, and more specifically when partially replacing NO_3^- by Cl^-, revealing optimal Cl^-/NO_3^- ratios with high potential to reduce NO_3^- fertilization, which could lead to a healthier and sustainable agriculture.

Key words: chloride, plant growth, leaf traits, nitrate, tomato

Acknowledgments: Funding for the present research was provided by the Spanish Ministry of Science Innovation and Universities-FEDER grant RTI2018-094460-B-I00, Spanish National Research Council grant CSIC-202040E266, and European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 895613.

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PURIFICATION AND CHARACTERIZATION OF PvRNS3, A T2 RIBONUCLEASE FROM COMMON BEAN.

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Abstract:

Salt stress decrease plant growth and is responsible for crops losses all over the world. In the last decades this problem become increasingly worst because soil salinization is predicted to be increased due to climate change. During environmental stresses, such as nutrient starvation, salt, and drought condition, the recycling of cellular components is upregulated by the mechanism of autophagy. Nucleic acids are organic compounds relatively abundant in plant tissues that contain nitrogen and phosphorous, in addition to carbon. Among nucleic acids, RNA is the most abundant molecule in plant cells, representing around 85% of total nucleic acids and 47% of total organic P (Veneklaas et al., 2012). Ribonucleases (RNases) are the enzymes that catalyse the degradation of RNA into smaller components being the RNAse T2 the most widespread family in living beings, extended from virus to eukaryotes. T2 members are classified into 3 classes (I, II, III). Previous study identified 13 potential T2 genes in common bean and the expression analyses of class I and II showed that *PvRNS3* is induced in common bean radicles in response to salt stress (Díaz-Baena et al, 2020).

In this study, *PvRNS3* was cloned into pET30b(+) and overexpressed as a recombinant protein in *Escherichia coli*. The purified protein showed RNA degrading activity but not DNA degrading activity, confirming that the identified gene encodes a functional ribonuclease. The main kinetic properties of the purified enzyme have been determined. The expression pattern of PvRNS3 gene was analysed by qRT-PCR in several tissues from common bean, and the results showed that it is poorly expressed in all of them under non-stress conditions.

Key words: Ribonuclease, Salt stress, *Phaseolus vulgaris*.

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ABA AS A SIGNALING MOLECULE IN PLANTS SUBJECTED TO HYPOXIC CONDITIONS

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Abstract: Several environmental factors reduce O_2 availability to plant cells, namely submergence of plant tissues which limits gas diffusion. Soil flooding-associated oxygen shortage induces expression of ethylene dependent group VII ERFs, which are involved in the regulation of plant anoxia survival responses, and reduces ABA levels, along with the overexpression of some PYR/PYL/RCAR ABA receptor and ABA-dependent transcription factor genes. Indeed, ABA-deficient tomato plants showed a lower ABA-dependent anoxia responses respect to wild type. Moreover, reduced oxygen availability is linked to reprogramming of metabolism of hypoxic tissues which shift to anaerobic fermentation (induction of pyruvate decarboxylase, alcohol dehydrogenase and lactate dehydrogenase) and the accumulation of Ala, which was higher in the ABA-deficient genotypes. A differential accumulation of Glu and Asp in both hypoxic and aerated tissues was associated to a different tricarboxylic acid cycle activity (in line with their involvement as sources of oxalacetate) which might account for survival of waterlogged tissues. Expression of genes involved in the anaplerotic pathways arising from Ala, Asp and Glu were more active in flooded and aerated tissues of ABA-deficient plants suggesting a systemic metabolic interaction of flooded roots with aerated shoots to ensure survival of hypoxic tissues in these genotypes. In addition, aquaporin gene expression was different in waterlogged tissues of wild type and ABA-deficient tomato, potentially influencing water relations. The relationship of these responses with ABA signalling was evidenced by the presence of ABA-responsive *cis*-elements in promoters of upregulated genes in waterlogged tissues, which might indicate that ABA plays a role in the induction of these responses. To gain more insight in the specific roles of ABA and its interaction with ethylene and NO, waterlogging experiments using Arabidopsis are being conducted and the most recent results are presented.

Key words: Anoxia, Abscisic acid, Arabidopsis, Ethylene, Flooding, Hypoxia, Metabolism, Nitric oxide, Tomato.

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IMPACT OF THE GASOTRANSMITTER NITRIC OXIDE (NO) IN PLANT HORMONAL SIGNALING NETWORKS

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Abstract: Nitric oxide (NO) is a key signaling molecule with important features in plants since it is rapidly synthesized, induces defined effects within the cells, and is also scavenged quickly when no longer required. This molecule is involved in the regulation of plant growth and development, pathogen defense and abiotic stress responses and, in most cases, this is achieved through its interaction with phytohormones (reviewed in Sanz et al., 2015; Sánchez-Vicente et al., 2019). Currently, the molecular mechanisms underlying the effects of NO in these processes and their interaction with other plant hormones are beginning to unravel. Here, we outline the described interactions between NO and phytohormones during early plant developmental processes (i.e. seed dormancy and germination, root and shoot development) and during abiotic and biotic stresses. A compilation of the NO-dependent post-translational modifications for NO action and those NO protein targets described so far will be provided, emphasizing the balanced role of NO in different developmental cues through its interaction with phytohormones during abiotic and biotic stresses.

The interplay between the phytohormone abscisic acid (ABA) and the gasotransmitter NO regulates seed germination and post-germinative seedling growth. Our previous results determined the prominent role of NO promoting seed germination through the *S*-nitrosation of the ABA master regulator ABI5, that leads to its degradation in a proteasome dependent manner (Albertos et al., 2015), and the translocation of ANAC089 to the nucleus, by changes in cellular redox status, modulating redox homeostasis, NO levels and ABA repression during Arabidopsis seed germination and abiotic stress (Albertos et al., 2021). Additionally, NO modulates root architecture by the control of auxin spatial patterns (Fernández-Marcos et al., 2011) and stem cell niche homeostasis (Sanz et al., 2014). However, NO involvement during the coordination of shoot organogenesis has an opposite effect in the root and shoot architecture, in terms of PIN1 accumulation under NO overproduction (Sánchez-Vicente et al., 2021). Finally, NO is also an essential component of the gaseous network described to modulate pre-adaptation to hypoxic conditions, a system that also comprises O_2, ethylene, and carbon dioxide (Manrique-Gil et al., 2020).

Key words: phytohormones, nitric oxide, crosstalk, molecular target, transcription factor

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ABA INTERACTS WITH OTHER HORMONES TO MODIFY ROOT ARCHITECTURE UNDER NUTRIENT DEFICIENCY IN TOMATO

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Abstract: Nutrient deficiencies cause changes in root system architecture (RSA) to increase nutrient acquisition through hormonal interactions. To study the role of ABA in these RSA responses, tomato plants overexpressing *SlNCED1* gene (key in ABA biosynthesis) and the WT cv. Ailsa Craig were grown *in vitro* under optimal conditions and N, P, K and Ca deprivation for 30 days. RSA responses to the lack of specific nutrients were studied in the root-shoot transition zone (TZ) and in the primary embryogenic root (PR) in relation to hormonal changes. Exposure of WT to nutrient deprivation revealed reduced PR development under N, P (lateral root elongation), K and Ca deprivation, while TZ development was only promoted in absence of N and P. ABA overproduction (OP) provoked a similar trade-off response between TZ and PR under nutrient sufficiency and altered that response and the hormone relations in a nutrient specific way, compared to the WT. Thus, ABA-OP intensified the PR reduction while promoted TZ in response to −K, and inhibited TZ development without altering the volume and total length and increasing the branching degree in the PR under −N. However, the main effect of ABA-OP was the promotion of TZ in response to P deprivation as a result of much more branched lateral roots, along with the length, volume and surface of the PR. Moreover, ABA-OP also improved TZ and PR branching under Ca starvation. Increased auxin IAA in the shoots and reduction in the roots occurred along with the typical TZ *vs* PR trade-off observed under nutrient starvation in the WT. ABA-OP reduced IAA levels in shoots (-N, -P, -K) and roots (all), while the ethylene precursor ACC was preferentially reduced in the shoots (-P, -K). Similarly, ABA-OP promoted JA (-N, -P, -K) and SA (-K, -Ca) in shoots and SA (all) in roots, GA4 in shoots (-K, -Ca) and roots (-P), and CKs in the shoot (all) and roots (-K, -Ca). ABA-OP also altered ABA and IAA metabolism, thus contributing to shaping RSA under each specific nutrient deficiency. Overall, ABA appears to enhance ecological functions in terms of RSA to explore the soil more efficiently by altering hormonal interactions.

Key words: ABA metabolism; IAA metabolism, root system architecture; nutrient deprivation, hormonal interactions.

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EFFECTS OF JASMONIC ACID ON MAIZE CULTURED CELLS: AN INSIGHT INTO CELL WALL MODIFICATIONS

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Abstract: Primary cell wall is a highly dynamic structure that plays an important role in the ability of plants to cope with biotic and abiotic stress. Jasmonic acid (JA) is a lipidic hormone triggering plant defense responses. It has been widely demonstrated that JA is able to impair plant cell growth enabling plants to adapt to stressful conditions (Browse, 2009).
In this work, a study focused on the effects of JA on maize cell wall composition and gene expression of selected cell wall genes was carried out. Besides, JA effects on cell growth and viability of maize cultured cells have also been addressed.
For this purpose, maize callus-cultured cells were treated with JA in a range of concentrations up to 500 µM. Growth and cell viability analysis indicated that JA promoted cell growth at low concentrations but negatively affected cell viability and growth at high concentrations. Compositional analysis of cell walls revealed that JA treatment did not significantly altered cellulose nor hemicellulose content of cell walls. Moreover, no effects on hemicellulose crosslinking were observed upon JA treatment. Quantification by HPLC-PDA of ester linked hydroxycinnamates showed that high concentrations of JA significantly promoted the incorporation of *p*-coumaric acid into the cell wall. Accordingly, JA treatment was associated to a late-time overexpression of BAHD genes codifying for enzymes that acylates hemicelluloses with *p*-coumaric and ferulic acid. These latter results would shed light into the molecular mechanism underlying the JA effect on cell wall phenolic profile. Whether or not, JA controls maize cell growth by these means deserves further investigations. Results presented here contribute to understand the cell wall basis of the growth inhibitory effect induced by JA in maize cells.

Key words: cellulose, hydroxycinnamate, maize, jasmonic acid, primary cell wall.

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GIBBERELLINS REGULATE OVULE NUMBER IN *ARABIDOPSIS* THROUGH A DELLA-CUC2 COMPLEX

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Abstract: Ovule initiation is an essential developmental process in the life cycle of plants that is controlled by a complex genetic and hormonal network (Barro-Trastoy et al., 2020). Among the hormones involved in this process, including auxins, brassinosteroids, cytokinins and gibberellins (GAs), we reported that GAs are negative regulators of ovule initiation. DELLA proteins (key repressors of GA signalling) increase ovule number, whereas GAs represses ovule formation by promoting the degradation of DELLAs (Gomez et al., 2018). The aim of this work is to deepen in the molecular mechanism of GA-mediated ovule initiation. Our data indicate that the role of DELLAs in this process is mediated by its direct interaction with the NAC transcription factor CUC2, which is needed to establish the boundaries between ovule primordia. We have identified CUC2 as an interactor of DELLA proteins. Moreover, we found that the dominant DELLA mutant *gai-1*, which usually causes an increase of the 20% in ovule number, does not increase ovule number in a *cuc2-1* background. In addition, both CUC2 and the DELLA protein GAI colocalize in ovule primordia of pistils at stage 8 of flower development. These data suggest that the GA-mediated ovule initiation requires CUC2 activity to form a DELLA-CUC2 protein complex that could be regulating the expression of genes responsible for the correct formation of ovule primordia.

Key words: Ovules, gibberellins, DELLA proteins, CUC2.

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PinAr: *Pinus* architecture and branching network

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Abstract: Plant development comprises several essential processes within its life span, characterised by a continuous growth with architectural adjustment according to the external and internal environmental stimuli, including branching (Domagalska & Leyser 2011). This complex mechanism belongs to post-embryonic development, and through the time and space, involves genetic expression changes and numerous hormonal crosstalk, including auxins, cytokinins, gibberellins, abscisic acid and strigolactones; as well as non-hormonal inputs, such as sugars and red: far red light ratio (Barbier *et al.* 2019, Kotov *et al.* 2021). In *Arabidopsis thaliana*, experiments to date have identified many of the key genes involved in these regulatory pathways, unravelling some of the signal integrators (Aguilar-Martínez *et al.* 2007, Bennet *et al.* 2016, Waldie & Leyser 2018). However, in species such as *Pinus pinaster*, a conifer widely used in the lumber industry, no further knowledge has been achieved, in spite of that control of branching can be hugely important when it comes to obtain better plantations yields. Therefore, the aim of the present communication is to set up a transfer of the knowledge obtained in model species to this conifer.
For this purpose, first a selection of key genes in the regulation of this process, belonging to the different signalling pathways involved in *A. thaliana*, was performed. Next, homologous sequences belonging to *P. pinaster* were identified, with a further protein domains analysis, selecting the most conserved proteins, following Alvarez *et al.* (2018) and Bueno *et al.* (2020). Then, preliminary gene expression profiles on a provenance assay assisted on selecting the most relevant candidates. Finally, a field assay is here presented, where a branching inducing system was set up on 2-year-old plants from same origin (France, VG06-Mimizan VF2) in the greenhouse facilities, with a rigorous selection of same ontogeny individuals, including exogenous addition of compounds (strigolactones and its inhibitor, and gibberellins), in order to modulate the branching response. Within this assay, hormone profiles, gene expression and phenotyping tracking was conducted to elucidate the branching network in this species of interest.

Key words: conifer, branching, hormonal and molecular network

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Poster Communications



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CHANGES IN SHOOT PHYTOHORMONES ARE ASSOCIATED WITH INCREASED WATER AND PHOSPHORUS USE EFFICIENCY OF VEGETATIVE RICE GROWN UNDER ALTERNATE WETTING AND DRYING

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Abstract: Sustainable approaches to rice cultivation that apply less irrigation and chemical fertilisers are required to increase crop resource use efficiency. Although alternate wetting and drying (AWD) has been widely promoted as a water-saving irrigation technique, its interactions with phosphorus (P) nutrition have attracted little attention. Vegetative rice plants were grown with two phosphorus levels, fertilised (HP) or un-fertilised (LP), and either continuous flooding (CF) or AWD irrigation. Shoot biomass and leaf area under different irrigation treatments depended on substrate P status (significant P x irrigation interaction), since LP decreased these variables under CF, but had no significant effect on plants grown under AWD. AWD increased foliar abscisic acid (ABA), isopentenyl adenine (iP) and 1-aminocyclopropane-1-carboxylic acid (ACC) concentrations, but decreased trans-zeatin (tZ) and gibberellin A1 (GA1) concentrations. Low P increased ACC and jasmonic acid (JA) concentrations but decreased gibberellin A4 (GA4) concentrations. Across all treatments, stomatal conductance was negatively correlated with foliar ABA concentration but positively correlated with GA1 concentration. Taken together, changes in shoot phytohormones in AWD mitigates the effects of substrate P deficit on shoot growth (biomass, leaf area and tiller number) thereby enhancing water and phosphorus use efficiencies compared with plants grown under CF. This study suggests that AWD may allow rice growers to decrease P fertiliser rates and irrigation volumes without diminishing vegetative growth.

Key words: Hormone balance, Irrigation strategies, *Oryza sativa* L., Phosphorus, Vegetative growth

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DOES CUC2 ACT THROUGH CYTOKININS IN ARABIDOPSIS LEAF MARGIN PATTERNING?

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The interplay between auxin and cytokinins affects many plant developmental processes (El-Showk *et al.*, 2013). However, the formation of interspersed auxin maxima has been considered sufficient to shape Arabidopsis leaf margins. These maxima form by the joint action of the PIN-FORMED1 (PIN1) auxin efflux carrier and the CUP-SHAPED COTYLEDON2 (CUC2) transcriptional regulator (Bilsborough *et al.*, 2011). Nevertheless, cytokinins favour compound leaf development in tomato (Shani *et al.*, 2010), and the *Cardamine hirsuta REDUCED COMPLEXITY* (*RCO*) gene activates cytokinins and is required for the formation of compound leaves (Hajheidari *et al.*, 2019).

In *vasculature complexity and connectivity* (*vcc*; also named *desigual1* [*deal1*]) mutant leaf primordia, the developing leaf margin and auxin maxima distribution are both aberrant (Wilson-Sánchez *et al.*, 2018). Using a split-ubiquitin yeast two-hybrid membrane-based localization assay, we found that the VCC protein interacts with components of the Very-Long-Chain Fatty Acid (VLCFA) elongation complex. VLCFAs negatively regulate leaf cell proliferation by inhibiting the expression of *ISOPENTENYLTRANSFERASE* (*IPT*) genes, which encode enzymes catalyzing the first step of the cytokinin biosynthesis pathway (Nobusawa *et al.*, 2013).

To ascertain whether cytokinins play a role in Arabidopsis leaf margin morphogenesis, we used the *RCO:ARR1ΔDDK* transgenic line (Hajheidari *et al.*, 2019), in which the *RCO* promoter drives the expression of a constitutively active form of the cytokinin effector ARABIDOPSIS RESPONSE REGULATOR1 (ARR1). The *RCO:ARR1ΔDDK* line and the *cuc2-1D* gain-of-function mutant have leaf margins with extra lobes. The *vcc-2* mutant shows ectopic expression of the cytokinin response reporter *TCSn:GFP* at leaf margins. *RCO:ARR1ΔDDK cuc2-3* and *vcc-2 cuc2-3* plants, but not *cuc2-3*, show lobes and sinuses caused by excess or lack of tissue. Our studies may pave the way for a better understanding of the action and interactions of auxin, cytokinins and VCC in leaf bilateral symmetry specification and/or maintenance.

Key words: Arabidopsis, leaf margin patterning, RCO, CUC2, cytokinins

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ETHYLENE PRECURSOR ACC PRODUCTION IN ROOTS NEGATIVELY INFLUENCES SALT TOLERANCE IN TOMATO

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Abstract: Ethylene is a highly regulated plant hormone involved in many developmental processes. However, it has been largely considered as a growth inhibitor and senescing promoter in response to abiotic stresses as salinity. Although ethylene is produced in different plant tissues, an important role has been proposed for this hormone in the root as stress sensor and root-to-shoot signalling molecule. In this study, the role of root-sourced ethylene in response to salinity has been assessed in tomato by using rootstocks altered in ethylene production. A commercial tomato cultivar was grafted onto the ethylene overproducing mutant (epinastic, Epi); a root-specific ethylene deficient transgenic line (ACCD) overexpressing an ACC-deaminase (that degrades the ethylene precursor aminocyclopropane-1-carboxylic acid, ACC) under the control of a root specific promoter (roID); and onto their wild type genetic background the cv. Micro-Tom (MT-WT). The grafted plants were cultivated in a greenhouse under control and saline stress (75 mM NaCl) conditions for 3 months. Plant yield and yield components, biomass, photosynthetic traits, changes in major plant hormones (UHPLC-MS) and ionome (ICP-MS), and expression of ethylene-related genes were analysed. Root-specific alteration of ethylene production modified growth, physiology and yield of grafted plants. ACCD rootstocks reduced the toxic accumulation of Na^+ in leaves and flowers, and improved nutritional and photosynthetic status in the scion, while the opposite was found in the high-ethylene producer Epi grafts. The enhanced salt-tolerance of ACCD-grafted plants was related to lowered ACC concentrations and enhanced accumulation of growth-promoting hormones gibberellin GA4 and cytokinins (tZ+ZR) in vegetative and reproductive structures of the scion, compared to WT and Epi. Expression of ethylene biosynthetic genes ACC oxidases (ACO1, ACO2) was constitutively and stress-inducible higher in Epi roots, while the lowest expression was found in ACCD rootstocks. However, those genes were similarly induced by salinity in the leaves of the three graft combinations, suggesting that the precursor ACC produced in the roots and transported to the shoot is influencing the putative ethylene evolution in both organs. Overall, rootstock-mediated decrease in ACC production improves plant vigour and

yield under salinity, supporting the negative role of root-to-shoot ACC signalling in salt tolerance in tomato.

Key words: aminocyclopropane-1-carboxylic acid, ethylene, graft, salinity, tomato

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INFLUENCE OF GIBBERELLIC ACID AND ITS SYNTHESIS INHIBITOR PACLOBUTRAZOL ON ADVENTITIOUS ROOTING OF CHESTNUT

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Sweet chestnut (Castanea sativa Mill.) has a key ecological and socioeconomical role, being one of the most cost-effective forest species in Europe. As disease-resistant chestnut genotypes need to be propagated vegetatively, it´s necessary to develop efficient micropropagation protocols for this species and its hybrids. For a successful acclimatization to *ex vitro* conditions, micropropagated plants have to form adventitious roots and develop functional root systems. Adventitious rooting is a post-embryogenic process through which new roots are formed in tissues different from the primary roots. Auxin is the main hormone involved in the adventitious root formation, but other hormones, such as gibberellins, appear to be related to this process by influencing both rooting rate and root growth.

In this study, experiments were carried out to determine the effects of auxin and gibberellin on the initiation of adventitious roots. The analysis was performed in two *in vitro* clones of chestnut microshoots; a juvenile-like line derived from basal shoots (P2BS) and a good-rooting clone derived from low crown branches (Loura). Exogenous gibberellic acid (GA_3) and the gibberellin biosynthesis inhibitor paclobutrazol (PBZ) were applied to determine the impact of gibberellin on the adventitious rooting process.

The effect of gibberellin was assessed by inoculating the microshoots in media with: a) Indole 3-butyric acid (IBA) 25 µM plus GA_3 10 and 40 µM; b) IBA 25 µM plus PBZ 10 and 40 µM; c) PBZ 40 µM with no IBA. Treatments were applied for 5 days under dark conditions. The explants were then transferred to hormone-free rooting medium and kept under normal photoperiod conditions during 25 days.

Our results indicate that gibberellin inhibits adventitious rooting induction whereas, on the contrary, PBZ stimulates root formation even in the absence of auxin. Root number is also

negatively influenced by gibberellin concentration. However, contrary to the hypothesis which postulates that gibberellins promote root growth, root length does not seem to have a direct relationship with gibberellin supplementation. Thus, the inhibition of endogenous gibberellin biosynthesis by means of PBZ treatment favours adventitious root development even when it is not complemented with any exogenous auxin treatment, which until now was considered essential in this plant material.

Key words: adventitious rooting, gibberellin, paclobutrazol, auxin, chestnut, *in vitro*

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OPDA (12-OXO PHYTODIENOIC ACID) MODULATE ROOT GROWTH IN OATS AS A DROUGHT TOLERANCE RESPONSE

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Abstract: Jasmonates (JAs), include a diverse group of jasmonic acid (JA) conjugated metabolites, such as jasmonoyl-isoleucine (Ile-JA) and its biosynthetic intermediate, 12-oxo phytodienoic acid (OPDA). JAs have roles in plant-abiotic stress (De Ollas et al., 2018, Kazan, 2015) but their involvement on drought tolerance responses needs to be established and in particular, whether OPDA acts as intermediate or as independent signal. We investigated dynamic changes of JA profile in roots and leaves of two oat genotypes, well-characterized for drought resistance, over a 20 days time-course. We found that jasmonic acid and Ile-JA followed a similar trend in both, leaves and roots. However, the observed pattern of OPDA was very different from that of JA or Ile-JA. No differences in OPDA were observed in leaves between the tolerant and susceptible genotype. However, a completely different behaviour was observed in roots. The tolerant genotype showed a dramatic reduction in OPDA from the earliest sampling times while the susceptible genotype showed only a slight early reduction but increases at intermediate sampling-times (from 15 days after withholding water). The overall reduction of OPDA and the other JAs is supported by the significant downregulation of the 13-LOX gene. However, increase of AOS expression in Flega roots led to a significantly higher OPDA accumulation in this genotype compared to Patones. Exogenous application of OPDA increased drought symptoms in the tolerant genotype, reduced leaf relative water content and reduced root length, in particular by delaying the production new thin roots. Treatment with phytoprostane A1-type, another reactive electrophile species, increased the drought symptoms in a way resembling the effects of exogenous OPDA. These results suggest that under drought stress, OPDA decreases favouring the growth of new roots, particularly those with smaller diameter, which are crucial for maintaining water status in the plant.

Key words: OPDA, oats, drought, roots, jasmonates

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PATTERN PRODUCTION OF 1-AMINOCYCLOPROPANE-1-CARBOXYLIC ACID (ACC) AFTER A WATER STRESS PERIOD ASSOCIATED WITH COFFEE ANTHESIS

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Abstract: Coffee flowering is one of the most important biological processes required for reproductive success and is directly associated with yield and quality (Peters and Carrol 2012). Endogenous and environmental factors such as plant hormones, temperature, photoperiod, shade, and water stress period are important for flower bud development and anthesis (Crisosto et al., 1992; Ronchi and Miranda 2020; Schuch et al., 1990b). For this study, two experiments were carried out in the UFLA's experimental area. For field experiment I, three coffee cultivars (Acaua, Oeiras, and Semperflorens), early, late and continuous for flowering, respectively were used. Roots, leaves, and flower buds were collected for ACC, ACO, Ethylene, ABA content, and gene expression of LHT1 ACC transporter on May 18th (Rainy period), August 18th (Dry period), and August 28th (Re-watering period). For field experiment II, two treatments (Covered and Uncovered plants) were used. Tissue sampling was similar to the first experiment only in the re-watering period (August 28th). The objective of this study was to evaluate the pattern production of ACC, ACO, Ethylene, ABA, LHT1 transporter gene expression involved in coffee anthesis. After 22 mm of rain, coffee anthesis occurred and for the first experiment, the results showed that ACC production increased significantly after rain (Re-watering period) for all cultivars and all tissues, whereas ethylene decreased from rainy to re-watering periods for leaf and bud tissues showing an increase only for root after rain. ACO activity was higher in the rainy period than in the dry and re-watering periods, while ABA increased in the dry period and do not decrease significantly after rain. LHT1 transporter gene expression was high in root and flower bud; however, no significant difference was detected in leaf after rain (Re-watering period). In the second experiment, covered plants did not achieve anthesis whereas uncovered plants bloomed. For uncovered plants ACC, ACO, Ethylene, ABA pattern production, and ACC-LHT1 transporter gene expression were similar that plants in experiment I after rain. In

conclusion, considering that coffee anthesis occurs after rain, ABA accumulation and low ACO activity decrease the ethylene production favoring the ACC level to be increased, and an intercellular transport in leaf, flower bud, and roots might be involved as a signaling agent in coffee anthesis.

Key words: ABA, Ethylene, LHT1, Coffee flowering, ACO, Water stress

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PHYSIOLOGICAL EFFECTS OF EXOGENOUS APPLICATION OF METHYL JASMONATE AND SALICYLIC ACID IN AVOCADO

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Abstract: Avocado is an economically important fruit crop consumed in more than 60 countries. The lack of water and the white root rot (WRR) disease caused by the soilborne pathogen *Rosellinia necatrix* are two of the main abiotic and biotic stresses affecting avocado in southern Spain. Elicitors are compounds that induce defense responses in plants similar to defense responses induced by pathogens. Elicitor's recognition triggers several signal transduction pathways resulting in the induction of plant defense genes which modulate several biochemical mechanisms and physiological responses. Methyl jasmonate (MeJA) and salicylic acid (SA) are two of the most studied elicitors because of their relevance mediating in the plant response to abiotic and biotic stresses. Therefore, the aim of this research was to evaluate the effect of exogenous application of MeJA and SA in avocado plants through physiological measurements. Plants of four-years-old 'Dusa' avocado rootstocks, susceptible to *R. necatrix* and water stress, were sprinkled weekly with MeJA or SA 5mM solutions. Following six elicitations, physiological measurements were carried out. Plants treated with MeJA showed an increase in non-photochemical quenching of fluorescence (NPQ and qN) suggesting the activation of energy dissipating mechanisms related to oxidative stress as previously described in water stressed plants. MeJA decreased leaf water content (LWC) and leaf relative water content (RWC) in consonance with an increase in the specific leaf mass area (LMA), suggesting an increase in the leaf thickness/ density, previously related to plant adaptation to stressful environments. Exogenous application of SA resulted in an increment in assimilation rates (A_N) and stomatal conductance (g_s) indicating an effect of SA at the stomatal control level. No significant differences were observed in relative chlorophyll content (SPAD index) or leaf water potential between treatments. These results at the physiological level support the operation of different signaling pathways by each elicitor previously described in genomic studies. Future research will focus on the use of elicitors against abiotic and biotic stresses such as water stress and *R. necatrix*, respectively.

Key words: Elicitors, methyl jasmonate, salicylic acid, avocado.

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PUTATIVE ROLE OF JASMONIC ACID SIGNALLING PATHWAY IN COLEOPTILE ELONGATION UNDER SUBMERGENCE.

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Abstract:

As a semi-aquatic plant, rice (*Oryza sativa* L.) has a certain ability to germinate and survive under submergence. In Asian countries, rice cultivation methods are shifting to direct seeding in flooded fields, where the capacity to rapidly germinate and elongate the coleoptiles under water are desirable traits. Submergence triggers hypoxia stress response in plants, mainly driven by the perception of variation in the levels of phytohormones. Previous lab work showed that HIGH EXPRESSION OF OSMOTICALLY RESPONSIVE GENE1 (OsHOS1) was involved in rice root curling through modulation of jasmonic acid (JA) signalling, triggered by mechanosensing. In preliminary studies we found that RNAi::*OsHOS1* mutant plants exhibited shorter coleoptiles under submergence, raising the possibility that JA signalling pathway is involved in coleoptile elongation. Furthermore, two transcription factors (TFs) binding to *OsHOS1* promotor belong to the group VII of the ethylene response factor (ERF-VII) family, that has been highly correlated to hypoxia stress response. One of these TFs is *SUB1B*, that belongs to the *SUBMERGENCE1* (*SUB1*) locus, and has the allele *SUB1A-I* considered responsible for the quiescence strategy adopted by the submergence-tolerant rice varieties. To investigate the putative role of JA signalling in coleoptile elongation, we studied the variability of coleoptile length among 10 rice accessions, submitted to aerobic *versus* submergence conditions. The treatments were performed for 4 and 8 days, and five accessions were then selected for expression analysis (RT-qPCR) of target genes, involved in JA biosynthesis, JA signalling and ethanolic fermentation. Interestingly, higher transcription levels of *SUB1B* and *OsHOS1* seemed to be related to shorter coleoptiles in submergence conditions. Furthermore, RNAi::*OsHOS1* lines exhibited similar coleoptile length to the wild-type (cv. Nipponbare) when exogenous JA was applied, likely indicating that mutant lines are less sensitive to JA.

Our results point for the involvement of *OsHOS1* on the JA signalling pathway in coleoptiles subjected to submergence, as well as for a putative role of *SUB1B* on coleoptile elongation, which had not been previously described. Further studies on this subject are still necessary and important for the development of new and thriving varieties, especially targeting submerged environments.

Key words: rice, submergence, hypoxia, jasmonic acid signalling, coleoptile

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REGULATION OF NON-SYMBIOTIC HAEMOGLOBIN EXPRESSION MODULATE NITRIC OXIDE LEVELS IN OATS AS A DROUGHT TOLERANCE RESPONSE.

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Abstract: Drought tolerance responses are extremely complex and involve a wide range of processes and intricate signalling. Understanding plant responses to drought is of fundamental importance for breeding drought tolerant crops. In a previous study, we observed that transgenic barley lines overexpressing the barley non-symbiotic haemoglobin gene, HvHb1, which oxides NO to NO_3^- were more resistant to drought. In this work, we explored whether regulation of non-symbiotic haemoglobin expression was a natural feature involving drought responses in oats. To this aim, endogenous nitric oxide (NO) production was monitored in the oat cv. Flega and Patones, previously characterized as drought tolerant and susceptible respectively (Canales et al., 2019; Sánchez-Martín 2015). Results confirmed a NO decrease in the tolerant genotype during drought stress. Expression analysis showed that this reduction was due to overexpression of the oat haemoglobin gene leading to NO scavenging. Decreased levels of nitric oxide in the tolerant cultivar were associated with low levels of ethylene as measured *in vivo*, and with a reduction of ACO5 expression. The involvement of nitric oxide in the oat response to drought, through the differential expression of haemoglobin genes, was validated in 13 additional oat genotypes previously characterised as drought moderately tolerant and susceptible (Sánchez-Martín *et al.* 2012). There was a significant correlation between the haemoglobin expression and drought symptoms, with lower expression associated with more severe symptoms. This highlights the potential of the haemoglobin gene as a candidate gene for breeding drought tolerance in oats.

Key words: Nitric oxide, oats, drought, ethylene

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ROLE OF ETHYLENE IN REGULATING ROOT SYSTEM ARCHITECTURE IN TOMATO SEEDLINGS UNDER NUTRIENT DEFICIENCY AND SALINITY STRESS

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While nutrient availability and salinity limit plant growth and development, the adaptive responses affecting root system architecture (RSA) contribute to cope with those environmental constraints. RSA manipulation is considered as a target to increase plant tolerance to abiotic stresses, but the mechanisms involved are not totally understood. Although several major plant hormones seem to be involved, the role of ethylene in root-mediated adaptive responses is little known. In this study, the role of ethylene in RSA regulation under salinity (40 mM NaCl) and specific nutrient (P and K deprivation) stress conditions has been assessed in tomato. Seedlings of the tomato cv. Micro-Tom (MT-WT), the ethylene overproducing mutant (epinastic, *Epi*), and a root-specific ethylene deficient transgenic line (*ACCD*) overexpressing an ACC-deaminase (degrades the ethylene precursor aminocyclopropane-1-carboxylic acid, ACC) under the control of a root specific promoter (roID), were grown in vitro under control and stress conditions for 30 days. RSA parameters were recorded (WinRHIZO) and changes in major plant hormones were analysed (UHPLC-MS). While ACC concentrations were not increased in the *Epi*, indicating an ACC utilization for ethylene synthesis; they decreased in *ACCD*, suggesting an ACC degradation in this genotype. Interestingly, the growth and RSA responses were related to the ethylene genotype. While the *Epi* mutant registered a growth stimulation under control conditions, compared to the WT, it was the most affected under stress. However, the growth of the *ACCD* line was stimulated under salinity and K-deprivation, suggesting that reduced ACC (and ethylene) concentration improves the plant growth under stress conditions. The *Epi* mutant registered less RSA development derived from the primary root (PR) under stress conditions and greater development of the adventitious roots in the transition zone (TZ) in all treatments. However, the *ACCD* developed a more complex RSA derived from the PR, particularly under salinity. These results suggest an involvement of ethylene in the negative control of RSA derived from the PR and a positive control in the formation of adventitious roots derived from TZ under stress conditions. The molecular basis and the agronomic impact of the ethylene-mediated changes in root

architecture under abiotic stress conditions are being investigated.

Key words: Ehylene; root system architecture; nutrient deprivation; salinity, *Solanum lycopersicum*.

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NOVEL INTERACTORS OF THE POLYCOMB GROUP (PcG) PATHWAY AND THEIR ROLE IN PLANT DEVELOPMENT

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Abstract: Polycomb Repressive Complex 2 (PRC2) represses target genes through the deposition of the highly conserved H3K27me3 epigenetic mark. In *Arabidopsis thaliana* PWWP DOMAIN INTERACTOR OF POLYCOMBS 1 (PWO1) is an interactor and regulator of PRC2, which may also act as mediator of PRC2 repression at the nuclear periphery (Hohenstatt et al, 2018, Mikulski et al, 2019). To further characterise the functions of PWO1, we analysed PWO1 putative complex(es) in planta by co-immunoprecipitation (co-IP) experiments and subsequent liquid chromatography-mass spectrometry (LC-MS/MS). The experiments yielded more than one hundred potential PWO1 interactors (Mikulski et al, 2019). The most abundant protein in our co-IP experiments was UBIQUITIN PROTEASE 5 (UBP5), which is a deubiquitinase member of the plant UBP family (March and Farrona, 2018). Furthermore, our unpublished protein-protein interaction data indicate a link between UBP5 and PRC2. Here, I will show UBP5 localisation, epigenetic and protein-protein interaction data that may help us to understand its molecular activities during plant development and its role as member of the PWO1-PRC2 protein network.

Key words: PcG pathway**,** POLYCOMB REPRESSIVE COMPLEX 2, epigenetics, plant development, chromatin

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UNCOVERING THE BIOLOGICAL FUNCTIONS OF CIRCADIAN-REGULATED LONG NON-PROTEIN CODING RNAS

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Abstract: The development of next-generation sequencing techniques revealed a dynamic transcriptional landscape rich in non-protein coding RNAs. Among those are the long non-protein coding RNAs (lncRNAs) which are over 200bp in length and classified as intergenic, intronic or natural antisense transcripts accordingly to their genomic location (Sánchez-Retuerta et al., 2018). LncRNAs were identified in multiple plant species and their biological functions range from the regulation of flowering time, auxin signalling, abiotic stress responses to photomorphogenesis (reviewed in Chekanova, 2015; Fonouni-Farde et al., 2021). Recently, we identified *FLORE* (*CDF5 LONG NON-CODING RNA*) a natural antisense lncRNA (NAT-lncRNA) which partially overlapped with the transcriptional regulator *CDF5* (*CYCLING DOF FACTOR 5*) (Henriques et al., 2017). *FLORE* and *CDF5* are circadian-regulated and display mutual inhibition, which is reflected in their opposite effect on photoperiod-dependent flowering, an indication that oscillating lncRNAs could link the clock to relevant agricultural traits. This robust timekeeper allows organisms to anticipate and adjust their biological processes to 24h cycles, which increases their fitness and biomass (Dodd et al., 2005). To further understand the biological relevance of circadian lncRNAs, we characterized *lncCDF1*, a novel NAT-lncRNA to *CDF1* (*CYCLING DOF FACTOR 1*). Under long days, *lncCDF1* transcript accumulation peaks between ZT6-9 when *CDF1* levels decrease. Similarly to *CDFs*, *lncCDF1* was detected in the vasculature of roots and shoots (Fornara et al., 2009; Song et al., 2015); however it also accumulated in

cotyledons and leaf blades of newly emerging leaves, and decreased in older, fully expanded leaves. Modulation of *lncCDF1* levels either by overexpression or insertional mutation did not affect flowering significantly, although we observed an increased tolerance to drought stress in *lncCDF1-promoter* mutant lines. Moreover, *lncCDF1-promoter* mutant seeds showed higher germination rates at different ABA concentrations, suggesting crosstalk between *lncCDF1* and this signalling pathway. Taken together our findings uncover a role for the *lncCDF1*/*CDF1* module in adjusting drought stress responses. This is in agreement with recent reports expanding the biological function of CDFs beyond flowering time regulation and including a relevant role in the control of plant development in unfavourable environments (Renau-Morata et al., 2020).

Key words: circadian clock, long non-coding RNA, flowering, abiotic stress

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A NOVEL FUNCTION OF THE HISTONE DEMETHYLASE LSD1 IN REGULATING THE TRANSCRIPTOME LANDSCAPE DURING ADVENTITIOUS ROOT FORMATION IN TOMATO

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Abstract: Excision-induced adventitious root (AR) formation is a bottleneck for plant survival. We have established a model for wound-induced AR formation in tomato hypocotyls where polar auxin transport from young leaves activates the expression of specific auxin-responsive genes required for cambial cell division and root primordia specification in the vascular region near the wound (Alaguero-Cordovilla *et al*., 2021). By screening a collection of highly mutagenized population of the 'Micro-Tom' cultivar (Alaguero-Cordovilla *et al.*, 2020), we identified a mutant, dubbed as *more adventitious roots1-1* (*mars1-1*), with an enhanced AR response that is affected in a gene encoding the conserved lysine-specific histone demethylase LSD1. We confirmed its AR phenotype with two additional null alleles of this gene (*mars1-2* and *mars1-3*) generated by CRISPR/Cas9. Quantitative histology of hypocotyl cross-sections and studies using known auxin transport inhibitors or auxin signalling inhibitors confirmed that LSD1 is upstream of the auxin signal required for AR initiation. Besides, the *mars1* mutants displayed altered responses to exogenously applied CKs. To determine whether the *mars1* phenotype could be caused by global alterations in the epigenome of the cambial initials, we studied H3K9me2, H3K4me2 and H3K27me3 levels during AR formation. We were able to alter the AR phenotype of *mars1* mutants by chemically inhibiting histone modifications. Finally, a time-course RNA-seq transcriptome analysis of protein coding genes and long non-coding RNAs (lncRNAs) in the *mars1* mutants suggest that LSD1 might be involved in the establishment and maintenance of silencing in specific genomic regions required for tissue-specific reprogramming.

Key words: epigenetics, cellular reprogramming, RNA-Seq, lncRNAs, histone demethylase

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INVESTIGATING MIRNA-DEPENDENT AUXIN RESPONSES DURING SOMATIC EMBRYOGENESIS IN CONIFERS

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Abstract: Somatic embryogenesis (SE) is being used as a tool for clonal propagation of several forest tree species in breeding programs for commercial purposes. Additionally, SE is often the model system of choice to better understand the molecular regulation of plant embryo development especially in angiosperm model species, like *Arabidopsis*. Based on previous studies in our lab (Rodrigues *et a*l., 2019), a set of candidate miRNAs and corresponding target transcripts have emerged as potential embryogenesis regulators. However, the roles of specific miRNAs in the regulation of the genes involved in SE, in particular those encoding transcription factors with an essential function during SE including *AUXIN RESPONSE FACTORs (ARFs*), remain mostly unknown in conifers. Centered on the differential expression analysis of identified miRNAs throughout embryo development, we hypothesize that specific miRNA-target regulatory nodes potentially involved in auxin responses are crucial developmental regulators. Based on its expression pattern, *miR160,* putatively targeting an *ARF10* has been selected for further characterization using a conifer *in vitro* embryogenesis model system, and a combination of cell, molecular biology and genetic tools. As a first step, we used a transient expression system in *Arabidopsi*s mesophyll protoplasts for *in vivo* validating the miRNA-target interaction. We overexpressed the selected pine miRNA isoform and target mimics to manipulate miRNA levels. We also used luciferase-based sensors for quantifying miRNA activity that respond specifically to both endogenous and overexpressed miRNAs and target mimics. These miRNA sensors allowed us to confirm the *in vivo* interaction between the pine *miR160* isoform, and the predicted target transcript homolog to Arabidopsis *ARF10*/*ARF16*/*ARF17*. In Arabidopsis, the miR160-*ARF10/ARF16/ARF17* node was suggested to contribute to the LEC2-mediated pathway of embryogenic transition in induced somatic cells (Wójcik *et al*., 2017). Expression analyses of this miRNA-target pair

and putative associated transcripts has been performed by qPCR revealing the expression dynamics throughout somatic embryo development in *Picea abies*. We will present and discuss the regulation by miR160 of SE in *Picea abies* used as a conifer model system.

Key words: AUXIN RESPONSE FACTOR, Embryo development, *Picea abies*, sRNA transcriptome

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miRNA-MEDIATED PHOSPHATE STARVATION MECHANISMS INVOLVED IN EMBRYOGENIC COMPETENCE MAINTENANCE IN TAMARILLO (*SOLANUM BETACEUM* CAV.) CALLI

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Abstract: Somatic embryogenesis (SE) is a developmental process by which somatic plant cells reprogram acquiring competence to develop into embryos, and later on into whole plants. It is a valuable tool for the rapid and large-scale micropropagation of numerous economically important species, an efficient regeneration system for genetically modified plant production and an important experimental system for studies on plant embryogenesis and cellular developmental plasticity. Due to the relevance of this regeneration process, there is a great interest in the analysis of the gene regulatory networks underlying it. Recently, the generation of genome-wide profiles of microRNAs (miRNAs) and their target genes has pointed out several of these molecules as key factors controlling SE induction and embryo development. We used previously obtained high-throughput sequencing data (RNAseq and small RNAseq), generated from SE-induced cell lines of tamarillo (*Solanum betaceum* Cav.), to guide our investigation into the functional characterization of specific miRNAs-target pairs that showed differential expression in cell lines and tissues with distinct embryogenic ability. We found that while specific miRNA-target regulatory nodes were indicated as involved in the acquisition and expression of embryogenic competence, others seem to be related to the loss of that capacity during subcultures. So far, miR399 and miR827 showed to be up-regulated in calli that lost their embryogenic capacity throughout subcultures comparatively to embryogenic ones. Concomitantly their targets, involved in phosphate-transport responses in the cell (*PHOSPHATE2* and *PHOSPHATE TRANSPORTER 5*), are down-regulated in long-term calli. Furthermore, since sugar mediates phosphate starvation responses, our hypothesis is that the long exposure to high sucrose rates is responsible for the loss of embryogenic competence in tamarillo throughout subcultures. More results are being obtained on the functional validation of both miRNAs. Nevertheless, the results so far obtained corroborate the hypothesis that phosphate metabolism is determinant for embryogenic competence commitment and expression during SE.

Key words: embryogenic competence, miRNAs, phosphate starvation, subcultures

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SYSTEMIC SILENCING OF A MAGENSIUM CHELATASE-ENCODING *SULPHUR* GENE *NICOTIANA BENTHAMIANA* BY A 21-NT ARTIFICIAL SMALL RNA

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Abstract:

Eukaryotic small RNAs (sRNAs), such as microRNAs (miRNAs) and small interfering RNAs (siRNAs), are responsible for the sequence-specific degradation of highly sequence complementary RNA molecules, a process known as gene silencing. In plants, gene silencing can be amplified through the production of secondary sRNAs, for instance upon the targeting of RNAs by 22-nucleotide (nt) sRNAs (Cuperus et al. 2010). sRNAs are mobile, they can spread from initially silenced sites to neighboring cells, and to distal tissues in a process termed systemic silencing (SS) (Molnar et al. 2011). The possibility of intentionally inducing the SS of a desired RNA should facilitate the control of gene expression and/or induce antiviral resistance at the whole-plant level. Unfortunately, the molecular bases governing the induction and spread of SS are not clear, what has hampered the development of efficient methods for triggering SS in plants.

Here we report that the transient expression in *Nicotiana benthamiana* leaves of the 21-nt artificial microRNA (amiRNA) amiR-NbSu-2, designed to silence the magnesium chelatase-encoding *SULPHUR* gene (*NbSu*), induced *NbSu* local silencing in agroinfiltrated tissues, as predicted. Silenced tissues displayed a characteristic bleached phenotype and accumulated low levels of *NbSu* mRNA. Surprisingly, amiR-NbSu-2 also induced strong SS characterized by the yellowing of the areas surrounding leaf nerves in younger non-agroinfiltrated leaves. We used artificial sRNA (art-sRNA) high-throughput tools (Carbonell 2019) for generating multiple amiRNA and synthetic trans-acting siRNA (syn-tasiRNA) constructs to evaluate the role in SS of the art-sRNA class, sequence, size and accumulation level. Our results show that strong SS is obtained with those 21-nt (and not 22-nt) amiRNAs and syn-tasiRNAs that target *NbSu* mRNAs more efficiently in agroinfiltrated tissues. To determine the nature of the mobile sRNAs and the genetic requirements for their production, we are using a collection of *N. benthamiana* RNAi plants

with reduced expression of genes involved in secondary siRNA biogenesis and analyzing the identity of the sRNAs produced in both local and distant silenced tissues by high-throughput sequencing. The identification of the molecular mechanisms governing the induction and spread of SS will serve to improve art-sRNA designs for enhanced RNAi at the whole-plant level.

Key words: small RNAs, systemic silencing, artificial microRNA, *Nicotiana benthamiana*, magnesium chelatase

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Poster Communications



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A GENE REGULATORY NETWORK FOR TISSUE REPROGRAMMING IN TOMATO HYPOCOTYLS AFTER WOUNDING

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Abstract: Plants regenerative capacity allows them to overcome tissue damage caused by different environmental stressors. In a previous study (Alaguero-Cordovilla *et al.*, 2021), we characterized wound-induced adventitious root (AR) formation in young 'Micro-Tom' tomato explants by means of physiological, genetic and molecular approaches. Besides, we performed detailed transcriptome analysis and targeted metabolomics approach, and found metabolic differences and divergent developmental pathways that contributed to tissue-specific *de novo* organ formation (Larriba *et al.*, 2021). By comparing two sets of bulk RNA-Seq data with a specific bioinformatics pipeline, we investigated the transcriptional networks involved in AR formation in this species. By combining these results with those of PlantRegMap, TF2Network. Cistome and PlantPAN 3.0, several hub genes were selected for functional studies. We will present here our results on the AINTEGUMENTA-LIKE (AIL) network. In conclusion, our results identified key network transcription factors, which could be used as potential regulatory targets for enhancing plant regeneration in recalcitrant species.

Key words: *Solanum lycopersicum* L., *de novo* organ formation, gene regulatory network (GRN), integrated bioinformatics analysis

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AUXIN INDUCES DIFFERENT TRANSCRIPTOMIC RESPONSES IN CHESTNUT ACCORDING TO THE ONTOGENETIC ORIGIN OF THE TISSUES

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Maturation imposes several changes in plants, which are particularly drastic in trees. In recalcitrant woody species, such as Chestnut (*Castanea sativa* Mill.), one of the major shifts relates to the inability to form adventitious roots in response to auxin treatments as the plant ages. To analyze the mechanisms underlying this phenomenon, an *in vitro* model system of two different lines of microshoots derived from the same field-grown tree was established. While BS shoots (basal sprouts) exhibit a juvenile-like character and root readily when treated with exogenous auxin (~90% rate), microshoots established from the crown of the tree (CR, adult) rarely form roots. Distinct profiles of metabolite, hormones and gene expression have been reported for each type of shoots.

In the present work, a transcriptomics approach has been accomplished to dissect the genetic basis underlying the responses to auxin of easy- and difficult-to-root microshoots. For this purpose, long-read sequencing technology has been used (Oxford Nanopore Technologies). Shoot basal sections (1 cm) from BS and CR microshoots were collected for RNA extraction 24 h after they were treated with indole-3-butyric acid (1 mg/mL) for 60 s. Three equivalent samples were analyzed for each of the two lines.

A total yield of 5.9 Gbases was obtained for the 6 samples, with a mean sequence length of 606 bp. The files were mapped with Minimap2 against the *Quercus suber* draft genome. Approximately 80% of the reads were successfully mapped. Files were assembled using Stringtie, and the R software Ballgown was used for differential expression analysis.

We detected around 800 transcripts with significant differential expression between juvenile-like and mature tissues, with a notable amount of uncharacterized genes, pseudogenes and long non-coding RNAs. In adult-like tissues, significant expression was found for genes related to stress responses and specific hormone signaling pathways (ethylene, abscisic acid). Besides, transcripts related to auxin responses showed contrasting results between both types of tissues. We highlight relevant expression results of transcription factors and hormone-related genes. These findings require further confirmation but open interesting possibilities for the analysis of the genetic basis of adventitious rooting and maturation.

Key words: Auxin, Adventitious rooting, Chestnut, Long-read sequencing, Maturation, Transcriptomics

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CHARACTERIZATION OF RIPE FRUIT EPIDERMIS-SPECIFIC TRANSCRIPTION FACTORS IN STRAWBERRY

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Abstract: The epidermis is the external cell layer in direct contact with the environment, and it plays essential biological roles. Transcriptome analysis (RNA-seq) of *Fragaria vesca* fruit receptacles at four ripening stages (green, white, turning and red) and of different tissue types of receptacles (pith, vascular bundles, cortex and epidermis) at two ripening stages (green and red) allowed us to infer tissue- and stage-specific Gene Regulatory Networks (GRN). Due to the potential role of the epidermis in defense and in the differential anthocyanin accumulation pattern that shows *F. vesca* fruits at the ripe stage (the skin is red, while the inner part is white), we have focused on the GRN of the epidermis of red receptacles. In this study, we aim at the functional characterization of two transcription factors (TFs) that constituted the main hubs of this GRN: a MYB-like gene, and a member of the NAC family of TFs. MapMan analysis of the genes constituting the GRN in ripe epidermis showed that wax and flavonoid biosynthesis were significantly overrepresented functions in the epidermis of red receptacles. Using the Luciferase/Renilla (Luc/Ren) system, the interaction of the MYB and NAC TFs with their wax-related putative targets was validated. To experimentally validate the target genes of these two TFs, their binding sites were mapped genome-wise using DAP-seq analyses. Consistently, MYB bound to a set of genes involved in cuticle formation and flavonoid biosynthesis, while a number of genes involved in solute transport were enriched among the NAC direct targets. Finally, protein interaction assays showed that MYB physically interacts with two members of the bHLH TF family specifically expressed in the red epidermis of receptacles, leading to the subcellular relocalization of one of them from the cytoplasm to the nucleus. Currently, we are phenotyping transgenic overexpressing lines of MYB and generating CRISPR/Cas9 mutant lines to functionally characterize these two TFs.

Key words: Strawberry, Epidermis, Transcription Factor, Gene Regulatory Network.

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EXPANSION OF THE CUPULIFORMIS (CP) FAMILY IN BRASSICACEAE SUGGESTS SUBFUNCTIONALIZATION OF CP3, CP4 AND CP5

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Arabidopsis INCURVATA11 (ICU11) is a H3K36me3 demethylase that acts as an accessory protein of the Polycomb Repressive Complex 2 (PRC2) (Bloomer *et al.*, 2020). ICU11 belongs to the CUPULIFORMIS (CP) family of 2-oxoglutarate and Fe(II)-dependent dioxygenases, together with CP2, CP3, CP4 and CP5 (Mateo-Bonmatí *et al.*, 2018; Nadi *et al.*, 2018). Whereas loss-of-function alleles of *ICU11* cause mild, pleiotropic effects such as leaf incurvature and early flowering, the *cp2*, *cp3*, *cp4* and *cp5* null mutants are indistinguishable from wild type. The *icu11 cp2* double mutants, however, exhibit a seedling lethal phenotype: they skip vegetative development and flower immediately after germination, in a way reminiscent of strong single *embryonic flower 1* (*emf1*) and *emf2* mutants. We also obtained *cp2 cp3 cp4 cp5* and *icu11 cp3 cp4 cp5* quadruple mutants, which were indistinguishable from wild type and the *icu11* single mutants, respectively.

Micrographs of transgenic plants expressing translational fusions of *CP4* and *CP5* with a gene encoding GFP suggested that CP5 is a nuclear protein, whereas CP4 is both nuclear and cytoplasmic. *CP2* overexpression, but not that of *CP3*, *CP4* or *CP5*, fully restores the wild-type phenotype in *icu11* plants. The $35S_{pro}$*:CP3*, $35S_{pro}$*:CP4*, and $35S_{pro}$*:CP5* transgenic lines, in a Col-0 background, show no morphological alterations. Since the CP family seems to be of ancient origin, we studied the effects of the heterologous expression in Arabidopsis of putative members of this family, taken from the moss *Physcomitrella patens* (*PpCP1* and *PpCP2*), the monocot *Oryza sativa* (*OsCP*), and the dicot *Brassica rapa* (*BrCP*). Heterologous expression of the $35S_{pro}$*:OsCP* or $35S_{pro}$*:BrCP* transgenes rescues all leaf morphological defects of Arabidopsis *icu11* plants, indicating that at least some functions of ICU11 and its orthologs are conserved among angiosperms. Phylogenetic analysis of the Brassicaceae orthologs of Arabidopsis *CP* genes suggest that *CP3*, *CP4* and *CP5* are suffering a process of subfunctionalization.

Key words: CUPULIFORMIS family, phylogeny, functional redundancy, epigenetics

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MOLECULAR CHARACTERIZATION OF A WAX ESTER SYNTHASE/ACYL-COA:DIACYLGLYCEROL ACYLTRANSFERASE FROM SUNFLOWER (*HELIANTHUS ANNUUS*)

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Wax esters (WEs) are neutral lipids formed by esterification of long-chain fatty alcohols and CoA-activated fatty acids (acyl-CoA). WEs are found on the surface of many plant organs contributing a protective function from biotic and abiotic stresses. The formation of wax esters involves two sequential enzymatic steps, where an acyl-CoA reductase (FAR) catalyses the reduction of fatty acids to primary alcohols in a NADPH-dependent reaction. Primary alcohols are then esterified with acyl groups, a reaction catalysed by a wax synthase (WS).
Three unrelated families of wax ester synthases have been identified, all of which catalyse wax ester formation: mammalian WS, jojoba-type WS and bacterial WS/DGAT bifunctional enzymes. The genome of *Arabidopsis thaliana* contains 11 homologues of the *Acinetobacter* WS/DGAT, with one of these genes, WSD1 (*At*5g37300), displaying high expression level in the stem epidermis.
In the present work, we identified WSD-like genes in *Helianthus annuus* related to the *Arabidopsis* WSD1 one. In so doing, we studied the expression of some sunflower WSDs in different development stages, paying attention to the expression of these genes in relation with the wax ester accumulation profile in the seed hull The form named *Ha*WSD8 showed a strong expression in that organ, matching the seed WE accumulation curve. This candidate gene was cloned and characterized. Its structure was modelled and its phylogenetic relationship with other plants WS forms was discussed.

Key words: Wax esters, Wax synthase/diacylglycerol acyltransferase, *Helianthus annuus*

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MOLECULAR SPECIALIZATION: TWO MAIZE SEED-SPECIFIC RESPONSE REGULATORS INTERFERE WITH CYTOKININ AND AUXIN SIGNALLING

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Abstract: *ZmTCRR1* and *2* are atypical type-A response regulators (RRs) expressed in the maize endosperm transfer cells (TC). Type-B RRs control the transcription of canonical type-A RRs as part of the cytokinins' signal transduction mechanism. The *ZmTCRRs* are regulated instead by *ZmMRP1*, a non-RR transcription factor involved in the definition of TC identity. In addition, the corresponding proteins are not detected in the TC but accumulate in the inner endosperm cells in a region called the transmitting tissue. These features suggest both molecules are not involved in classical, cell-autonomous, cytokinin signaling pathways. To understand the role of these genes on kernel development, we have produced transgenic maize plants with RNAi or overexpression constructs for *ZmTCRR1* and *2*. These lines show significant alterations in the endosperm´s developmental status and expression of nutrient storage genes.
We are obtaining maize lines with edited alleles of the genes. However, we envisage that the large number of experiments required to explore the effect of the ZmTCRRs on hormone perception would be impractical in maize developing seeds. To circumvent this, we have produced transgenic Arabidopsis lines overexpressing (35S promoter) ZmTCRR1 or 2 to facilitate testing many experimental conditions regarding hormone concentration and hormone combinations. Here we show the results obtained on the characterization of these lines and their response to hormone treatment. We found that the expression of ZmTCRR2 blocked, almost entirely, cytokinin perception. The transgenic plantlets show a dramatic dwarf phenotype and can only develop further in media supplemented with cytokinin. In contrast, ZmTCRR1 did not affect cytokinin perception but displayed a consistent resistance to auxin instead. We discuss these findings in connection with the delayed differentiation shown by the maize endosperm domain, the transmitting tissue, in which these proteins accumulate. Our results support the existence of a regulatory mechanism centered on the TC-specific transcription factor *ZmMRP1*, which coordinates the kernel development rate with the availability of nutrients from the mother plant.

Key words: response regulators, cytokinin perception, auxin, seed development

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NUCLEO-CYTOSOLIC SHUTTLING OF ARGONAUTE PROTEINS

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Abstract:
Plant miRNAs undergo stepwise nuclear maturation before engaging cytosolic, sequence-complementary transcripts in association with the silencing effector protein ARGONAUTE1(AGO1). Since their discovery, how and under which form plant miRNAs translocate to the cytosol has remained unclear, as has their sub-cellular AGO1 loading site(s). We show that the N-termini of all plant AGO1s contain a nuclear-localization(NLS) and nuclear-export(NES) signals that, in Arabidopsis thaliana(At), enables AtAGO1 nucleo-cytosolic shuttling in a Leptomycin-B-inhibited manner, diagnostic of CRM1(EXPO1)/NES-dependent nuclear export. Nuclear-only AtAGO1 contains the same 2'O-methylated miRNA cohorts as its nucleo-cytosolic counterpart, but it preferentially interacts with the miRNA loading chaperone HSP90. Furthermore, mature miRNA translocation and miRNA-mediated silencing both require AtAGO1 nucleo-cytosolic shuttling. These findings lead us to propose a revised view of the plant miRNA pathway in which miRNAs are matured, methylated, loaded into AGO1 in the nucleus, and exported to the cytosol as AGO1:miRNA complexes in a CRM1(EXPO1)/NES-dependent manner. We proposed the NES possibly exchanges between docked/undocked configurations into the RNA channel of unloaded AtAGO1, suggesting a regulation of AGO1 subcellular localization depending sRNA loading status. Canonical eukaryotic AGOs contain four main domains: a variable N-terminal domain and three highly conserved PAZ, MID, and PIWI domains. AGOs fold into a bilobal structure displaying a central groove for sRNA binding. Their functions range from cytoplasmic post-transcriptional gene silencing to nuclear processes such as transcriptional gene silencing, splicing modulation and DNA repair. Although the mechanisms behind AGO subcellular transport routes remain largely unknown in all organisms. Exploring the AGO1 shuttling hypothesis to other AGOs, including in metazoans, we study different examples where AGO proteins switch subcellular locations depending on sRNA loading status.

Key words: ARGONAUTES proteins, RNA pathways, Nuclear shuttling processes.

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PHYSIOLOGICAL AND MOLECULAR RESPONSES OF 'DUSA' AVOCADO ROOTSTOCK TO WATER STRESS

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Abstract: Avocado (*Persea americana* Mill.) consumption increases year by year and its cultivation has spread to many countries from the tropics to the Mediterranean region. Like many other crops, avocado cultivation must cope with low water availability environments that threaten the sustainability and profitability of avocado orchards. However, to date, there is not much information on the behaviour of commercial avocado rootstocks against drought. The aim of this research was to evaluate the physiological and molecular responses of 'Dusa' avocado rootstock to different levels of water stress. Plants were subjected to water deprivation until soil water content reached 50% (mild-WS) and 25% (severe-WS) of field capacity. Once these levels were reached, plants were rewatered and physiological response during recovery was monitored. Leaf water potential (Ψw), net CO_2 assimilation rates (A_N), transpiration rate (E), stomatal conductance (g_s) and plant transpiration rates significantly decreased under both WS treatments, reaching significantly lower values in plants subjected to severe-WS. After rewatering, mild and severe-WS plants showed a fast recovery in most of physiological parameters measured. To analyse root response to different levels of drought stress, a cDNA avocado stress microarray containing transcripts from *de novo* sequencing of 'Dusa' was carried out. The total number of differentially expressed genes

(DEGs) was 549 in mild-WS and severe-WS. Plant showed a wide transcriptome response linked to the higher degree of water stress, with 189 DEGs specific to mild-WS and 706 to severe-WS. Functional enrichment of DEGs revealed abundance of common sequences associated with water stress as well as specific categories for mild-WS (abscisic acid binding, endopeptidase inhibitor activity) and severe-WS (water transport, calmodulin binding). Overexpressed DEGs previously linked to drought tolerance were induced under both water stress levels, i.e., NAC, MYB, WRKY and ethylene-response transcription factors, genes related to ABA response, redox homeostasis, osmoprotection and cell wall organization. Taken all together, physiological and molecular results are pointing out a good performance of 'Dusa' rootstock under low water availability conditions, although further water stress experiments must be carried out under field conditions.

Key words: Drought, water stress, microarray, avocado.

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PLANT PROXIMITY IS MODULATED BY THE CHROMATIN REMODELER LHP1

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Abstract: Plant adaptation to environmental cues is regulated by a highly dynamic and responsive chromatin structure, that is a significant component of the modulation of gene transcription. Plant exposure to plant proximity, also referred as shade, triggers the regulation of the expression of a set of genes that prepares the plant to compete for light. In Arabidopsis seedlings, one of the most evident responses to shade is the promotion of the elongation of the hypocotyl. Genetic analyses have involved the chromatin remodeler LIKE HETEROCHROMATIN PROTEIN 1 (LHP1) in the shade-promoted hypocotyl elongation. Indeed, the mutant *lhp1*, which presents a short hypocotyl in shade, has a reduced expression of many of the rapidly shade-induced genes, alongside with a decrease of the shade-generated auxin burst when compared with wild-type plants. This set of impaired responses in the mutant are explained by the participation of LHP1 in the regulation at different points of the shade signaling cascade, from perception to transcriptional activation. Importantly, we found that LHP1 significantly localizes to many shade-induced genes. Moreover, according to the LHP1 binding capacity to the Histone 3 lysine 27 trimethylation (H3K27me3), we also detected an enrichment on those genes of this repressive histone mark. Consequently, we have established a link between the epigenetic regulation by LHP1, H3K27me3 and the control of gene expression in the shade. This work deepens the involvement of chromatin in the plant response to environmental changes.

Key words: Plant proximity, shade response, LHP1, H3K27me3

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SPECIFIC GENE EXPRESSION AND SPLICING EVENTS DEFINE TISSUE IDENTITY IN *PINUS RADIATA*

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Abstract: The seed plants today are represented by five lineages: one corresponding to the species-rich angiosperms and four to gymnosperms, among which conifers are the most widely distributed group (Yang et al., 2018). Although conifers are a clear plant molecular diversity hotspot, their tissue determination pathways remain underrepresented. One of the most robust methodologies currently available consists of alternative splicing/expression comparative analysis in a large number of tissues which allows the determination of master events that lead to tissue identity. To fill this gap, we collected all public *Pinus radiata* RNA-seq datasets available so far and investigated how steady-state gene expression and alternative splicing are coordinated in defining different classes of tissues. Our tool generated a large amount of basic knowledge that probably will make applied research easier in this non-model organism. Our comparative approach revealed that both transcriptional regulatory layers are involved in tissue identity; however functional divergent tissues-related variation, like needle and xylem, is mainly linked to expression. On the other hand, bud identity variation is slightly more associated to splicing. This analysis allowed us to decipher tissue definition crucial functions such as: photosynthesis, redox homeostasis, phytohormone action and secondary metabolism. In addition, we propose robust multi-genotype and multi-age tissue identity markers which involve alternative splicing events of the aforementioned categories and others as *PROTEIN EARLY FLOWERING 4*. More generally, these findings open the door to include plant species with unique features, like conifers, to determine the extent of convergence and divergence in tissue definition by gene expression regulation.

Key words: Splicing, Expression, Atlas, Conifers, Tissues

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CRISPR-CAS9 BASED *INCURVATA11* MUTANT SHOWS MILD PHENOTYPE BUT STRONG GENETIC INTERACTIONS

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Arabidopsis INCURVATA11 (ICU11) and CUPULIFORMIS2 (CP2) are members of the CP family of the 2-oxoglutarate/Fe(II)-dependent dioxygenase superfamily. ICU11 and CP2 are unequally redundant paralogs, as shown by the seedling lethal phenotype of the *icu11-1 cp2-1* double mutant, which is reminiscent of the strong *embryonic flower 1* (*emf1*) and *emf2* single mutants (Mateo-Bonmatí *et al.*, 2018; Nadi *et al.*, 2018). ICU11 also functions as an accessory protein of the Polycomb Repressive Complex 2 (PRC2) (Bloomer *et al.*, 2020). To exclude any contribution of the genetic backgrounds of *icu11-1* (S96) and *cp2-1* (Col-0) to the morphological and molecular phenotypes of the *icu11-1 cp2-1* double mutant, we obtained novel *icu11* alleles in the Col-0 and S96 (as controls) genetic backgrounds via CRISPR/Cas9 editing (Tsutsui and Higashiyama, 2017).

The novel *icu11-5* and *icu11-6* mutants that we isolated in this way exhibited similar fertility defects and early flowering, but milder leaf aberrations than those shown by *icu11-1*. All double mutant combinations of *icu11-5* or *icu11-6* with *cp2-1 or cp2-2* rendered lethal seedlings with an embryonic flower morphological phenotype. However, when grown on culture medium supplemented with 3% sucrose (instead of the usual 1%), 38% of these double mutants developed a stem with disorganized flowers, 33% of which produced siliques with seeds. A 15% of the mature seeds obtained germinated, yielding embryonic flowers. Culture on medium supplemented with 3% sucrose allows us to study the effects of the loss of function of the whole ICU11-CP2 epigenetic module on postembryonic development, for which we are using our novel *icu11 cp2* double mutants with a nonhybrid genetic background.

Key words: CUPULIFORMIS family, epigenetics, ICU11, CP2, PRC2, Cas9, mutagenesis

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UNISEXUAL FLOWERING IN THE FAGACEAE, A MOLECULAR APPROACH

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Abstract

The temporal separation of male and female flowering events is a highly adaptive trait that enhances cross-pollination as a means to avoid inbreeding. This trait has been observed in several species of the *Fagaceae* family such as *Castanea sativa* (chestnut tree) and *Quercus suber* (cork oak). Both species are monoecious species that present male flowering before female flowering. Despite the overall importance of these species, the mechanisms that control flowering induction and unisexual flower development are still elusive.
In the present work, a characterization of members of the ABCDE MADS-box gene family, recruited in the flower meristem to specify flower organ identity, was performed, and the sex-biased gene expression and identification of novel protein-protein interactions suggest that unisexuality in these species might be related to a redeployment in the dynamics of the ABCDE model. A phylogenetic profiling in both species led to the identification of homologs of genes known to be involved in flowering induction and repression, and their role in flower development was accessed with functional studies in Arabidopsis. The expression analysis of these homologs throughout several seasons in *Q. suber* suggests that male and female flowering may be triggered in separate induction events, a pattern that may be conserved in *C. sativa*. Furthermore, transcriptomic analysis also shown that the flower induction events and flower development might be intrinsically dependent on specific climatic conditions, and that the current climate change scenario poses a serious threat for fruit productivity in these species as flowering becomes more inconsistent.
Overall, the results here presented constitute a step forward in the elucidation of the mechanisms responsible for unisexual flower development in monoecious species of ecological and agronomic interest. This knowledge may be applied in the maximization of forestry practices, and consequent increase in production yield.

Key words: unisexuality, *Fagaceae*, FLOWERING LOCUS T, PISTILLATA, monoecious



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MEMBRANE SOLUTE TRANSPORT IN GRAPEVINE AND PLANT ENVIRONMENT INTERACTION

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The transport of solutes across cell membranes, including organic nutrients, osmolytes, ions, or water, is pivotal in all living systems. In higher plants, membrane transport is also required for long distance transport of the photoassimilated carbon from mature leaves throughout the phloem to sink tissues. In grapevine (*Vitis vinifera* L.) sugar unloading impacts grape berry chemical composition and organoleptic properties (sugar/acid balance, phenolics, etc) and thus wine quality. Sugar transport systems account for the compartmentation up to 1 M glucose and fructose into the grape berry mesocarp cells that starts at veraison and continues until the harvest. Thus, the unlocking of the transport mechanisms involved in phloem loading and unloading, and how they are regulated, either endogenously or in response to environmental cues, has an important basic and applied relevance. Moreover, in the context of the ongoing climate changes, grapevine, like many other important crops, faces a continuous battle against adverse environmental factors, including extreme climate events and water scarcity. We observed that the production, transport and accumulation of compatible solutes like polyols play an important role in grapevine and olive response to drought and impacts fruit composition. The understanding of copper homeostasis in grapevine is also particularly relevant because copper-based fungicides are intensively used in viticulture. The initial uptake of copper is performed by the family of COPT transporters belonging to a highly conserved Ctr-like copper transporter family. Our results showed that the application of Bordeaux mixture induces a significant increase in copper content in berry tissues which is either a cause or a consequence of the observed transcriptional reprogramming of the expression of the

VvCTrs. In grapevine different studies have also linked aquaporin activity to the vine water status, being involved for instance in stomatal conductance. Up until now, 23 MIPs were identified with genome sequence analysis in a single grapevine cultivar. Like animals, where important diseases such as depression and hypertension are commonly treated with drugs targeted to specific transporters, plants have also benefited from the extensive and ongoing study of membrane transport.

Key words: grapevine, sugar transport, mineral transport, aquaporins

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PLANT AQUAPORINS FOR SOLVING AGRICULTURAL PROBLEMS

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Abstract: Aquaporins are transmembrane proteins that function as membrane channels, facilitating the transport of water and small non-charged molecules across biological membranes. They appear in plants in multiple isoforms, reflecting a high diversity of cellular localizations, transport selectivity, and regulation properties. Forasmuch as the importance of water for plants and the involvement of aquaporins in its transport, among all the other compounds that they are able to transport, it is not surprising that the regulation of these proteins is associated with tolerance to numerous stresses and physiopathies. From our team, we work to respond to real problems in agriculture based on the study and characterization of aquaporins in crop plants, the analysis of their implications in resistance to environmental stress, the development of biotechnological systems such as bio-vesicles for controlled foliar fertilization or the relationship with physiopathies such as melon splitting, broccoli blindness or loss of turgor in stone fruit storage.

Key words: Aquaporins, environmental stress, physiopathies, crop plants.

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Oral Communications



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AtPGAP1 IS AN ENZYME INVOLVED IN GPI ANCHOR LIPID REMODELLING AND IS NECESSARY FOR A CORRECT AND EFFICIENT TRANSPORT OF GPI-ANCHORED PROTEINS TO THE CELL SURFACE

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Abstract:

In *Arabidopsis thaliana*, around 300 proteins have been predicted to be GPI-anchored proteins (GPI-APs) and among them there are cell wall structural proteins, proteases, enzymes, receptor-like proteins (RLPs) and lipid transfer proteins. They play an important role in a variety of plant biological processes including growth, stress response, morphogenesis, signalling and cell wall biosynthesis. Disrupting GPI-anchor synthesis in *Arabidopsis* is lethal as it happens in yeast and mammals indicating the key role of these proteins. The GPI-anchor contains a lipid-linked glycan backbone that is synthesized in the endoplasmic reticulum (ER) where it is subsequently transferred to the C-terminus of proteins containing a GPI signal peptide by a GPI transamidase. Once the GPI anchor is attached to the protein, the glycan and lipid moieties need to be remodelled. In mammals and yeast, this remodelling is required for GPI-APs to be included in Coat Protein II (COPII) coated vesicles for their ER export and subsequent transport to the plasma membrane/cell surface. The first reaction of lipid remodelling is the removal of the acyl chain from the inositol ring by the enzyme Bst1p (yeast)/ PGAP1 (mammals) (Fujita et al., 2006; Maeda et al., 2007). In this work, we used a loss-of-function approach to study the role of *PGAP1*/*Bst1* like genes in plants. *AtPGAP1* is the only *Arabidopsis* ortholog found. We have shown that AtPGAP1 localizes to the ER and probably functions as the GPI inositol-deacylase which cleaves the acyl chain from the inositol ring of the GPI anchor. In addition, we have shown that PGAP1 function is required for efficient transport of GPI-APs to the cell surface.

Key words: GPI anchored proteins, GPI-anchor remodelling, cell wall biosynthesis, signalling, plant growth.

Acknowledgments:

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TRANSLATIONAL REGULATION BY UPSTREAM OPEN READING FRAMES IN AN ARABIDOPSIS VACUOLAR TRANSPORTER

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Upstream Open Reading Frames (uORFs) are protein-coding regions in the 5' untranslated region (UTR) of mRNAs. They are known to affect translation of the main ORF (mORF), usually reducing the levels of protein production due to a decrease in translation efficiency. Around 35% of *Arabidopsis thaliana* genes contain uORFs, but in plant genes very few uORFs have been examined for their functional significance. These elements can inhibit translation in different ways, with ribosome stalling being the most frequent in plants, but they can also be translated and act in *trans*, prevent translation reinitiation or indirectly target the mRNA to degradation by Nonsense-Mediated Decay (NMD). We have identified three uORFs, named uORF1, uORF2 and uORF3, in the Arabidopsis thaliana *ZIF2* (Zinc-Induced Facilitator 2) gene. ZIF2 is a membrane transporter that mediates vacuolar compartmentalization of zinc in root cortical cells, thereby conferring plant tolerance to the heavy metal. Using a reporter system in isolated arabidopsis protoplasts, we show that simultaneous disruption of all *ZIF2* uORFs results in a marked increase in the activity of luciferase (LUC), demonstrating the ability of at least one uORF to inhibit mORF translation. Individual disruption of the *ZIF2* uORFs revealed that uORF2 is the main player in the observed translational repression and that uORF1 does not affect translation, while uORF3 inhibits mORF translation only in the absence of uORF2. We therefore postulate that uORF3 acts as a fail-safe mechanism to repress translation, to our knowledge the first observation of such a mode of regulation in plant systems. Using reverse genetics, we demonstrated that these uORFs are also inhibiting *ZIF2* translation *in planta*. Both uORF2 and uORF3 are translated *in vivo*, with uORF2 acting in a peptide sequence dependent manner, and neither uORF2 nor uORF3 inhibit mORF translation in *trans*. These data, together with the fact that *ZIF2* mRNA is not targeted to NMD degradation, point to ribosome stalling as the mechanism underlying *ZIF2* uORF-mediated translational repression. We are also addressing a potential key role for *ZIF2* uORFs under different stress conditions.

Key words: translational regulation, upstream ORF, membrane transporter, abiotic stress

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Poster Communications



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SMP-CONTAINING PROTEINS AT MEMBRANE CONTACT SITES: SUBCELLULAR LOCALIZATION AND CHARACTERIZATION.

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Abstract: Membrane contact sites (MCS) are microdomains where two membranes of two different organelles are in close apposition, but they do not fuse. MCS are essential for non-vesicular transport of lipids. This lipid transport is mediated by several families of proteins which all of them contain a lipid transport domain, as the synaptotagmin-like mitochondrial lipid-binding (SMP) domain. SMP domains assemble into a tubular like structure with hydrophobic lipid transport cavity.

The most studied SMP protein is *Arabidopsis* SYT1 which is known to be involved in tolerance to multiple abiotic stresses, including wounding, cold or high salinity. Later studies in other SMP proteins of the same family have shown that SYT1 and homologous such as SYT3 or SYT5 gave similar results. However, little information is available about the role other SMP proteins in plants: their localization, or their implication in abiotic stress tolerance.

We have studied the occurrence of additional SMP proteins in *A. thaliana* and *S. lycopersicum*. In order to identify these proteins, SMP sequences from human and yeast were used to identify their remote orthologues in *A. thaliana* and *S. lycopersicum*, allowing the identification of several putative encoding SMP domains. We have found that some of the identified proteins are exclusive of plants as they do not have direct orthologs in yeast nor human. Transient expression in *N. benthamiana* leaves followed by confocal microscopy was used to study the subcellular localization of these proteins. Our results show that some of these proteins are localized at ER-Golgi contact sites (SYT6, NTMC2T6 and TEX2) and two other proteins at ER-Chloroplast sites. In addition, we have studied their interactions by using FRET. Finally, to determine whether these proteins

are involved in abiotic stress tolerance, we have analysed the root growth and seed germination rates of Arabidopsis mutants for these genes under different conditions. Some of these mutants have shown different germination rates in media supplemented with NaCl and different rates of expanded cotyledons in media supplemented with ABA. These results suggest that some these proteins may be implicated in abiotic stress signalling through an ABA-dependent pathway.

Key words: SMP, contact sites, lipids, membrane

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THE β'-COP SUBUNIT OF THE COATOMER COMPLEX IS REQUIRED FOR SALT TOLERANCE

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Abstract: Vesicular transport is essential for maintaining the homeostasis of the endomembrane system as well as for biosynthetic transport of newly synthesized proteins and lipids and for the uptake and intracellular transport of exogenous macromolecules. In addition, it has been involved in the regulation of stress responses. The formation of transport vesicles is mediated by cytosolic coat proteins. Three main classes of coated vesicles have been described: COPII vesicles for ER export, COPI vesicles for retrograde Golgi-ER and bidirectional intra-Golgi transport and clathrin-coated vesicles operating in the late secretory and endocytic pathway. The key component of COPI vesicles is the coatomer complex, that is composed of seven subunits (α/β/β'/γ/δ/ε/ζ), which are recruited *en bloc* onto Golgi membranes in the presence of membrane-anchored ARF1-GTP (ADP-ribosylation factor 1). In *Arabidopsis*, several isoforms of all coatomer subunits, except for γ- and δ-COP, have been identified. In addition, two morphologically different types of COPI vesicles have been detected in electron tomography studies, raising the possibility that different COPI vesicles, involved in different trafficking steps like Golgi-ER transport or intra-Golgi transport, are made of different coatomer isoforms. Therefore, we decided to functionally characterize *Arabidopsis* coatomer subunit isoforms. We have previously characterized the two isoforms of the coatomer α subunit (α1-COP and α2-COP) and the two isoforms of the coatomer β subunit (β1-COP and β2-COP). We found that loss of α2-COP (but not of α1-COP), and loss of both β-COP isoforms, affects the expression of secretory pathway genes and Golgi morphology and leads to growth defects. Moreover, we observed that the loss of each β-COP isoforms induce hypersensitivity to salt stress. We have now also used a loss-of function approach to characterize the isoforms of the coatomer β' subunit (β'1-COP, β'2-COP and β'3-COP). The data obtained indicated that all β'-COP isoforms are required for salt tolerance.

Key words: β'-COP, Coat protein I (COPI), Salt stress, Arabidopsis

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PHOSPHOENOLPYRUVATE CARBOXYLASE (PEPC): ONE PROTEIN FAMILY FOR MULTIPLE PLANT RESPONSES

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Abstract: Phosphoenolpyruvate carboxylase (PEPC) is a ubiquitous cytosolic enzyme carboxylating phosphoenolpyruvate (PEP), using bicarbonate as a substrate, to yield oxaloacetate (OAA) and Pi. *In vivo*, OAA can either be reduced to malate by malate dehydrogenase (MDH), or converted to aspartate by aspartate aminotransferase (AAT). PEPC has been extensively studied in C_4 and CAM plants, where it carries out the first carboxylation step of atmospheric CO_2. However, PEPC also participates in other non-photosynthetic metabolic contexts such as C/N interactions and anaplerotic flux, stomatal opening control, root exudation, or seeds development and germination (O'Leary *et al*., 2011). To fulfil such different roles, PEPC protein is composed by a multigene family, with 6 members (Sb*PPC1-6*) in the C_4 cereal sorghum (*Sorghum bicolor*) (Paterson *et al*., 2009). PEPC isozymes show different patterns of expression, location and posttranslational modifications. SbPPC1-5 are referred as plant-type PEPCs (PTPCs), characterized by a conserved Ser residue susceptible for regulatory phosphorylation, in opposition with SbPPC6, referred as bacterial-type PEPC (BTPC), lacking this Ser residue. SbPPC1 is the isozyme participating in C_4 photosynthesis, and the major PEPC protein in leaves, whereas SbPPC2-5 are C_3-type PEPCs carrying out non-photosynthetic functions described above, and found in lower amounts. PTPCs are phosphorylated by a calcium-independent kinase named PEPC-kinase (PEPCk). Phosphorylated PEPCs show higher Vmax and lower inhibition by allosteric effectors. In sorghum, PEPC-ks are coded by small gene family with 3 members (Sb*PPCK1-3*). In this talk, we analyse the specific roles of PEPC isozymes and their implications in growth, stress responses or plant yield, and show recent findings from our lab regarding these key features of plant metabolism. In particular, we have generated modified sorghum plants with the Sb*PPC3* gene silenced by RNA interference (*Ppc3* lines), and characterized them under control or abiotic stress conditions (salinity, phosphate starvation, cadmium or aluminium toxicity) (de la Osa, 2020). Finally, to unravel the significance of PEPC phosphorylation, we have generated sorghum plants with Sb*PPCK1-3* genes silenced (*Ppck* lines), and started characterizing them under the same conditions mentioned above. Altogether, our results demonstrate that PEPC and its regulation are important factors for plant growth, productivity or stress

responses.

Key words: Phosphoenolpyruvate carboxylase, abiotic stress, plant yield, primary metabolism

Acknowledgments: This research was supported by the Junta de Andalucía (P12-FQM-489 and PAI group BIO298), and by Ministerio de Economía, Industria y Competitividad (AGL2016-75413-P). Thanks are due to all scientific collaborators, PhD students, master students, and under-graduated students, that had contributed in some way for more than 20 years with results showed here.

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WHAT DOES LIMIT GROWTH AND YIELD, PHOTOSYNTHESIS OR WATER STATUS?

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Abstract: Successful growth, both vegetative and reproductive, are the final goals for plants. In the case of crops, knowing how plants make use of the available resources to convert them into biomass, and especially, what is the main limiting factor for growth, is of paramount importance. Although much effort has been dedicated to study how different agronomical practices affect crop water relations and leaf gas exchange, we still lack the knowledge on the precise conditions that favour or limit plant growth. The classical view, shared among most scientists, is that carbon limit plant growth. This conception makes sense since photoassimilates are the bricks to build the biomass. However, other processes are as important as CO_2 uptake. In particular, the maintenance of turgor is as critical for growing as photosynthesis. This misconception on the key limiting factor for growth, giving a major role to the source activity instead of the sink activity, has emerged from the limitation and availability of technical methods to evaluate both. In our research group we have developed novel methods to overcome these limitations, and we have made them suitable for their use under field conditions and long period of time during the growing season. Our results confirm some opinions found in the literature and support, now with empirical evidence, that photosynthesis, in most cases, is not the main limiting factor for growth but turgor. The new concept "hours of turgor" emerges and will help us understand the performance of a species under a given environment and the functional physiological traits that make the difference in growth among species.

Key words: crop growth, photosynthesis, water relations, turgor, stomatal conductance

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FLORET DEVELOPMENT AND SPIKE FERTILITY IN BARLEY AS AFFECTED BY PPDH1 AND VRNH1 GENES

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Abstract: Heading time in barley is critical for adaptation. VrnH1 and PpdH1 are the major genes controlling time to heading through vernalization and photoperiod sensitivity, respectively. They are known to affect the duration of pre-heading phases when numerical components of yield are being produced. Consequently, these genes have been reported to affect not only phenological development but also spike fertility. We aimed to determine whether PpdH1 and VrnH1 genes affect spike fertility through (i) indirectly modifying the duration of the period in which spikelets and florets develop, or (ii) directly affect the development of florets, and then altering the number of fertile florets per spike beyond the effects these genes have on the duration of the phases. We grew four near isogenic lines (NILs) of barley (produced by CSIRO, Australia) to study the performance of plants carrying different combinations of PpdH1 and VrnH1 genes under natural conditions as well as under a 24 h daylength (artificially extending with low-intensity lamps) in field conditions (where they were naturally vernalized) as well as in growth chambers at 12 ºC after 29 days of vernalization and 12 or 24 hours of photoperiod. In natural photoperiod, differences in floret survival seemed positively related to those in the length of the period of floret development and the maximum number of florets initiated. However, in extended photoperiod no difference in the maximum number of differentiated florets was observed, the survival of initiated floret primordia being the main cause of the increased number of fertile floret. In general, the longer the duration to heading due to genetic factors or to photoperiod imposed the greater the spike fertility. The extended photoperiod treatment allowed the thermal time to anthesis to be uniform, in this condition a greater number of fertile flowers was observed in the spring insensitive genotype over the winter insensitive as well as over the spring sensitive. This would imply that the spring Vrn and the insensitive Ppd alleles may promote a greater survival of floret primordia resulting in higher spike fertility even in absence of major effects on time to heading.

Key words: Hordeum vulgare, spike fertility, photoperiod, vernalization

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INSIGHTS INTO THE BIOCHEMICAL AND GENETIC RESPONSES OF STRAWBERRY TO MORE SUSTAINABLE NUTRITIONAL REGIMES

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Abstract:
Strawberry (*Fragaria* × *ananassa* Duch.) is one of the most consumed berry fruits worldwide. The increased interest of the industry and consumers in this crop has risen the need for the development of novel sustainable production practices leading to higher yield and fruit quality. A preharvest regular application of algae-based extracts (Bio) and minerals, such as calcium (Ca) and silicon (Si), have been suggested as possible strategies to increase crop performance but little is known about their way of action at plant level. Herein, four nutritional regimes were applied to strawberry plants grown in soilless conditions, and the modulation of oxidative stress, expression of nutrient uptake related genes, and mineral accumulation were monitored during the main harvest growth stage.
Plants were grown in a greenhouse using a commercial strawberry substrate (10% peat, 40% coconut fiber and 50% composted pine bark) and were fertirrigated with a complete nutrient solution (control) and supplemented with a commercial algae-extract (3L/ha, every 15 days), Ca (CaO: 5L/ha, every 7 days), Si (SiO_2; 1L/ha, every 15 days) or a mix of Ca and Si (1:1 Ca and Si treatments mixed every 15 days). All treatments, except for the Bio application, led to a significant increase in oxidative stress related genes (*LOX*, *OMT*) when compared to control and to the downregulation of the photosynthetic activity-related gene *TK*. These effects might be a result from a priming activity by Ca and Si. A synergy between Ca and Si was found, as the mix treatment revealed a greater impact on the upregulation of Ca, N and other minerals' uptake and transport related genes (*CaM*, *MYB59*, *NRT1*, *MOT1*, *PHO1*). The application of Ca induced the accumulation of Ca (31%) and manganese (35%) in the leaves as compared to the control. Gene expression patterns obtained with Si, Ca and Mix treatments suggest that the nutritional regime differently regulates strawberry´s responses, with potential to elicit the oxidative and photosynthetic machineries which may increase plant endurance.

Key words: biostimulant, calcium, *Fragaria* x *ananassa*, gene expression, silicon

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MOLECULAR AND BIOCHEMICAL CHARACTERIZATION OF A MULTIPLE RESISTANT POPULATION OF *AMARANTHUS PALMERI* TO GLYPHOSATE AND ALS-INHIBITING HERBICIDES

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Two amino acid biosynthesis enzymes are important targets for herbicide action, belonging to two different biosynthetic pathways; 1) the enzyme 5-enol-pyruvate-shikimate-3phosphate enzyme synthase (EPSPS) in the biosynthetic pathway of aromatic amino acids, which is the target for the most used herbicide worldwide glyphosate. 2) The enzyme acetolactate synthase (ALS), which participates in the branched-chain amino acid biosynthetic pathway, is the target for ALS inhibitors, another popular herbicide class, pyrithiobac being the herbicide used in this study. Unfortunately, owing to the enormous selection pressure that has been exerted on weeds through the repetitive use of the same herbicides, some populations have developed multiple resistance (MR) to both types of herbicides, like some populations of *Amaranthus palmeri*, one of the most troublesome weeds in United States.

The objective of this study was to unravel the resistance mechanisms of a MR population to glyphosate and ALS inhibitors. To this purpose, whether resistance was evolved due to mechanisms related to the target enzymes was investigated. Leaves of MR plants were compared with plants of a standard sensitive (S) population. EPSPS and ALS gene copy numbers were evaluated by qRT-PCR, ALS gene was sequenced and genotyped by dCAPS. ALS activity sensitivity to pyrithiobac was determined by *in vitro* incubation.

All plants analyzed from the MR population were glyphosate-resistant due to EPSPS gene amplification, as previously described (Gaines et al., 2010). Resistance to ALS inhibitors was more variable among individuals and was related to point mutations at five positions in the ALS gene sequence. All MR plants were heterozygous for W574, the most abundant mutation. The other four mutations were S653 (homozygous), A122, A205, and G654. In plants with double mutation, the point mutation W574 was always present. The presence of mutations did not affect ALS functionality as plants with one point mutation presented similar ALS basal activity profiles than S plants, while plants with W574L + S653N showed higher basal ALS activity. Plants with the mutation W574L (simple or double) showed a

less sensitive ALS extractable activity to the herbicide, as has been reported in other resistant populations (Yu et al., 2010).

Key words: glyphosate, pyrithiobac, Palmer amaranth, multiple resistance.

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PO212 CONTROLS SOILBORNE PATHOGENS BY INDUCED RESISTANCE KEEPING FRUIT YIELD AND QUALITY

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Abstract: According to the policies of European Union, the use of chemicals to control crop diseases has to be reduced as much as possible. In order to keep a sustainable plant disease management, the development of other control tools, as biological control and induced resistance, is encouraged. In this work, we show the effectiveness of *Penicillium rubens* strain PO212 (a biological control agent) to reduce disease symptoms of Verticillium wilt (caused by *Verticillium dahliae*) and Phytophthora Root Rot (caused by *Phytophthora capsici*) in phytotron assays. These two soilborne plant pathogens are responsible of important yield losses in pepper (Capsicum annuum L.) crops (García et al., 2018; Veloso et al., 2020; Veloso and Díaz, 2021). Besides results in phytotron, PO212 was also effective in greenhouse assays against *Phytophthora capsici*. Pepper plants treated with PO212 showed an increase in both biochemical (PR proteins) and structural (lignin) plant defences, both before and after inoculation with the pathogens. Such a response was accompanied by changes in gene expression, determined by qPCR (before and after pathogen inoculation) and RNA-seq (after pathogen inoculation). All these results suggest the involvement of induced resistance as one of the modes of action of PO212 in pepper. Finally, fruits from greenhouse experiments were collected and analysed in order to check whether fruit yield and/or quality were affected by PO212 treatment. These later results demonstrate that PO212, besides being effective to control plant disease, it does not cause any negative effect in pepper fruit production.

Key words: Fruit yield, Induced resistance, Pepper, *Phytophthora capsici*, *Verticillium dahliae*

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Poster Communications



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A METABOLOMICS APPROACH TO ELUCIDATE THE EFFECTS OF ABSCISIC ACID TREATMENT ON PRIMARY METABOLISM OF ZUCCHINI FRUIT STORED AT LOW TEMPERATURE

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Abscisic acid (ABA) is a phytohormone involved in the regulation of different abiotic stresses including low temperature. In fruit of cold-tolerant zucchini varieties an accumulation of ABA has been reported during the first days of exposition to cold stress at postharvest. The aim of this work has been the use of a metabolomic approach to characterize compositional changes of primary metabolites in zucchini fruit treated with ABA and their response to cold stress. Zucchini fruit of the cold-sensitive variety "Sinatra" were used. Fruits were submerged 20 min at 20 °C in 0.5 mM of ABA, dried 2 h, and stored in a controlled environmental chamber in darkness at 4 °C and 85–90% RH during 1, 5 and 14 days. Through a metabolomic analysis (GC–MS) a total of 60 metabolites were identified. A principal component analysis (PCA) was performed to explore possible grouping patterns in relation to cold storage time and ABA treatment. PCA of the whole dataset allowed to clearly discriminate between treated and untreated fruit at 1 and 5 days of cold storage. By contrast, the PCA analysis reveals no differences in the metabolomics profile between the control and ABA-treated fruit after 14 days of storage at low temperature. The same response was observed when amino acids, sugars and organic acids were grouped and analyzed by PCA. Primary metabolic profiling revealed that organic acids, amino acids and sugars were up-regulated in ABA treated fruit at 5 days of cold storage, which coincides with high levels of endogenous ABA. In conclusion, the exogenous application of ABA improves the postharvest quality of zucchini fruit stored at low temperature accelerating the primary metabolism of the exocarp of fruit.

Key words: Cold stress, Zucchini, Postharvest, ABA

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ACCLIMATION OF MELISSA OFFICINALIS AND MENTHA SPICATA IRRIGATED WITH WATER AT DIFFERENT SALINITY LEVELS DURING A SHORT TIME PERIOD

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Abstract: During the acclimatization period, the selection of plant species and the use of adequate conditioning techniques are of vital importance to improve plant yield and tolerance to environmental abiotic stresses after transplantation. *Melissa officinalis* L and *Mentha spicata* were submitted during 15 days to three irrigation treatments in a controlled chamber: Control with EC< 0.9 dS m^{-1} (Control), NaCl solution with EC: 1.5 dS m^{-1} (CE 1.5) and NaCl solution with EC:3.5 dS m^{-1} (CE 3.5). Plant water status, stomatal and hormonal regulation as well as growth were evaluated. Both species irrigated with saline water reduced leaf water potential in comparison with control plants from the beginning of the experiment. Nevertheless, Mentha plants irrigated with treatments CE 1.5 and CE 3.5 recovered these values, which were close to control plants at the end of the acclimation period. Only treatment CE 3.5 affected negatively gas exchange in both species, especially Melissa, which could be related to plant dehydration and hormonal changes. As a consequence, Melissa plants reduced leaf number, aerial and root dry weight as salinity increased in the irrigation water, while Mentha plants were able to maintain growth, by increasing root DW and reducing leaf area with similar leaf number. These results indicate that, despite a short-time period, physiological and morphological characteristics of Mentha were less affected than Melissa by salinity, since Mentha was able to cope with saline water up to CE: 3.5 dS m^{-1}.

Key words: Aromatic plants, Salt stress, Growth, Water relations, Gas exchange.

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ALLELOPATHIC POTENTIAL OF ORGANIC-SELECTED BUCKWHEAT VARIETIES AGAINST COMMON PURSLANE AND ANNUAL RYEGRASS

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Abstract: In view of our interest in weed suppressive crops and further evaluation of allelochemical interactions in their rhizosphere, germination bioassays and metabolic profiling studies were carried out with selected buckwheat genotypes. Experiments were conducted to evaluate the allelopathic interaction of 15 buckwheat genotypes (Billy, Lada, PI481671, PI451723, PI476852, 290, 01Z5000012, RCAT068749, Gema, Kora, Jianzui, Zita, Eva, Westwod Ican and Sarasín a Ployes) on two economically important weed species - common purslane (*Portulaca oleracea* L.) and annual ryegrass (*Lolium rigidum* Gaudin). After growing together (buckwheat and weed), germination percentage, total weight, shoot and root invasion capacity (total space occupied by the weed), shoot and root length, and photosynthetic pigment content were measured for the two target weeds. As well, polyphenols determination was done in the buckwheat varieties to find a correlation among the chemical profile of buckwheat and the allelopathic potential of the different varieties.

Results show that germination of *P. oleracea* was significantly reduced for more than 50% by Kora, RCAT 068749, PI481671, PI476852, and 01Z5000012. However, the total weight was reduced only with Kora. An increase in leaf length was more significant with Westwod Ican, followed by Sarasin a Ployes, PI481671, PI476852, 01Z5000012 and Eva. As for root length, different buckwheat varieties produced stimulation such as Sarasin a Ployes, Gema, Westwood Ican, and PI481571. This suggest longer but thinner leaves and roots that can make the weed weaker to survive in the ecosystem.

With respect to germination of *L. rigidum*, almost all the varieties tested induced inhibition of germination on annual ryegrass. As well, regarding growth parameters, eight varieties induced more than 50% significant inhibition. This reduction of total weight was especially pronounced when grown with Lada followed by Gema, Billy, Westwod Ican, PI481671, Eva, and Zita. Total weight was reduced between 52 and 88% in most of these varieties.

From these data, we can conclude that Kora is the most inhibitory variety against *Portulaca oleracea* and *Lolium rigidum*. The polyphenol determination of these varieties

shows differences in the chemical profile.

Key words: buckwheat, allelopathy, germination, growth, polyphenols

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ALLELOPATHIC POTENTIAL OF WHEAT VARIETY TOPKAPI ON MONOCOT AND DICOT WEEDS

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Abstract:
Allelopathy of wheat (*Triticum aestivum*) has potential for pest and disease management. Both, wheat crop residues and root exudates can be used for weed management, including resistant biotypes. Wheat varieties differ in their allelopathic potential, indicating that allelopathic variety selection could be a useful strategy for integrated weed management in an organic farming system, and for improving the production of this worldwide crop. Several categories of allelochemicals have been identified for wheat allelopathy, namely, phenolic acids, hydroxamic acids, flavonoids, and short-chain fatty acids.
The present investigation aims the allelopathic activity screening and the identification and quantification of secondary metabolites (benzoxazinoids (BXZs) and polyphenols (phenolic acids and flavonoids)) of one wheat accession named *Top Kapi*. This variety was previously selected for its beneficial characteristics for organic farming (disease resistance, nutritional value, cold resistance, etc.). Following Wu et al. (2000), we tested the effect of *Top Kapi* against two weeds, one dicot (*Porulaca oleracea* L.) and one monocot (*Lolium rigidum* L. (Gaud)) comparing the germination, total weight, leaf and root length, and shoot and root invasive capacity of the weed. The allelochemicals analysis was carried out in *Top Kapi* growing alone and *Top Kapi* growing in the presence of *L. rigidum* or *P. oleracea*. After the harvest, photosynthetic pigments extraction was done to evaluate the effect of *Top Kapi* on the physiological status of *L. rigidum* and *P. oleracea*, and HPLC analysis to identify and quantify benzoxazinoids and polyphenols.
The results of germination bioassays show a very significant reduction in germination, total weight and invasiveness of shoots and roots of *L. rigidum* when grown with *Top Kapi*. By contrary, when *P. oleracea* was grown with *Top Kapi*, germination was not reduced. However, there was a significant decrease in fresh weight, suggesting that the plants grow weaker.
These results may be correlated with the high content of polyphenols (such as 4-CBA, FA, *P*-CA, LU) and hydroxamic acids (DIMBOA, BOA, MBOA, HBOA, HMBOA and DIBOA) detected in the agar medium.

Key words: allelopathy, biochemical analysis, organic agriculture, weed suppression, wheat**.**

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CHARACTERIZATION OF EGGPLANT LANDRACES: A KEY FOR ENSURING NUTRITIONAL QUALITY AND BIODIVERSITY CONSERVATION

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Abstract: The genetic erosion caused in crops by prioritizing production over quality has led to a loss of biodiversity (Brush, 1992; Castillo, 2008), which compromises global food security. As for eggplant, there is little morphological variability among commercialized eggplants (Hanson et al., 2006), and it is therefore necessary to have a source that helps to increase crop diversity, as are the landraces. Traditional varieties are those that have been differentiated by farmers through a historical process of selection, and are considered an important source of agricultural biodiversity (Jain & Gupta, 2013), which can be used for the development of new varieties while supporting the local economy (Ebbert, 2014). In order to take advantage of the landraces, thus helping to preserve them, a detailed description of the agronomic, phenotypic and nutritional quality characteristics is required. In relation to these statements, this work elaborates a characterization of 15 valencian eggplant landraces, by using 22 quantitative and 14 qualitative conventional phenotypic descriptors. The selected varieties correspond to the collections of the Institute for the Conservation and Improvement of Valencian Agrobiodiversity (COMAV) and the Valencian Institute for Agrarian Research (IVIA). Although traits corresponding to the plant, stem, leaves, flowers and fruit were taken into account, landraces were mainly differentiated based on their fruit colour and morphology. In this sense, varieties were grouped based on their fruit skin colour (black-purple, striped and white) and landraces B14, B16 and B17 were chosen, as being representative of the three groups, to analyse fruit quality and to, thus, provide verified information on their added value. Knowing the benefits provided by antioxidants for human health (Hussain et al., 2014; Kaur et al., 2014), total phenolic, ascorbic acid and flavonoid contents were determined in fruit pulp. Significant differences appeared among these three landraces, with B14 standing out for its antioxidant properties, followed by B17 for its high vitamin C content. The information reported here indicates the wide variability existing

among eggplants, which reinforces the importance of traditional varieties as the main source of agricultural biodiversity today.

Key words: Antioxidants, biodiversity, landrace, nutritional quality, phenotypic.

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CHARACTERIZATION OF PRODUCTIVITY, NUTRITIONAL AND PHYSIOLOGICAL TRAITS IN LANDRACE AND COMMERCIAL VARIETIES OF SORGHUM (*Sorghum bicolor (L.) Moench*).

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Abstract: Sorghum (*Sorghum bicolor* (L). Moench) is a worldwide distributed C_4-type crop used for food and fodder purposes. Currently, it is the fifth major cereal crop in the world but the most important in the semi-arid regions of sub-Saharan Africa. Sorghum exhibits high levels of drought tolerance and presents a high water use efficiency (WUE). It suggests that it could be a good alternative in soils affected by drought which will become more frequent as consequence of global climate change.
Exploring the genetic diversity of sorghum germplasm in the Iberian Peninsula, we have selected six landrace cultivars from different regions available in the Plant Genetic Resource Centre (CRF-INIA) belonging to the Spanish Government. Also, two commercial hybrid cultivar from Pioneer Hi-breed were included in the study. Several landrace cultivars come from Andalusian, in the south of Spain, which may be better adapted to semiarid climate conditions.
The purpose of this study was to characterize the development, biomass production, phosphoenolpyruvate carboxylase (PEPC) activity and nutritional parameters (starch and cyanogenic glucosides) in eight sorghum genotypes, growth in control conditions for one month, with the objective to identify genotypic variations for these traits. The highest values of biomass production were found in Zahina Gigante cv and the lowest in the commercial varieties (PR87 and PR88). The absolute growth rate revelled a wide genotypic variation not only in the peak growth rate but also in the time to achieve the maximal rate. Commercial varieties presented the highest PEPC activity, but surprisingly, high level of cyanogenic glucosides were also found in these cultivars. Significant genotypic variations were also found for protein and starch level in leaves. These results suggest a potential application in future breeding programs for improvement the yield in sorghum crops.

Key words: Sorghum, landrace cultivar and genotypic variation.

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COMPARISON OF TWO LIGHTING REGIMES ON CUCUMBER (*C. SATIVUS* L.) FRUIT MINERAL CONTENT

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Abstract:

Light and vapour pressure deficit (VPD) are the major environmental drivers of plant gas exchanges and nutrient uptake, and therefore compromising both crop production and fruit quality. Nowadays, supplemental lighting is essential to grow cucumbers in the north of Europe, where solar light during winter is constrained. The traditional high-pressure sodium (HPS) lamps require higher amounts of energy consumption in comparison with light-emitting diodes (LEDs) technology. Although LED is a promising technology, its influence on yield and cucumber quality is still not well known, and certain aspects like the absence of heat emission, normally caused by HPS lamps, could modify the VPD of greenhouses. Nowadays, there is a trend in the use of hybrid HPS-LED lighting by cucumber growers to ensure their crops. To this end, the present work was aimed to compare the effect of both hybrid HPS-LED and complete LED-LED lighting treatments on quality parameters of cucumbers. Hence, cucumber size, fresh weigh, dry matter content, colour, Brix, firmness and mineral concentration of 5 cucumber commercial cultivars (n = 6) were analysed at the end of the growing season. Additional SPAD values, fresh and dry weigh of leaves (n = 6) were also recorded. We hypothesized that hybrid HPS-LED lighting would impose certain level of plant stress through an increase of air water demand (i.e. VPD) that might reduce the quality of cucumbers in comparison with a complete LED-LED treatment. The effects on fruit fresh and dry weight; and fruit mineral concentration will be presented.

Key words: LED, high pressure sodium lamps, minerals, fruit quality, VPD

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CULTIVAR CHOICE AS A WATER SAVING STRATEGY IN CULTIVATED STRAWBERRY

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Abstract: Water availability for irrigating strawberries (*Fragaria x ananassa* Duch.) in the main production areas worldwide is dwindling under the current climate change scenario. Strategies for efficient water-use and for saving water are essential for long-term sustainable strawberry cultivation. Water savings can be achieved by improving irrigation water productivity (WP_I), but also, by using productive strawberry cultivars with low water requirements and/or tolerant to water shortage. In this study, water consumption and the agronomical and physiological response of 'Sabrina', 'Splendor' and 'Fortuna' strawberry cultivars (different yield efficiency, YE; g fruit cm^{-2}) to sustained and increasing water stress, were assessed to identify their water-use strategies and their relative water stress tolerance. Plants were grown in containers for estimating water balance (ETc) on each cultivar and were subjected to two water treatments during the entire productive cycle: 120% and 75% ETc following a randomised block design. 'Fortuna' and 'Splendor' used 46% and 38% less water than 'Sabrina', respectively. These differences were associated with smaller plant size and transpiration area. However, fruit yields in 'Sabrina' and 'Fortuna' were similar (~1150 g/plant), resulting in higher crop water productivity (WPc, g fruit L^{-1} consumed water) in 'Fortuna'. This cultivar displayed lower leaf-level CO_2 assimilation (*A*) and transpiration rates (*T*) and similar *A*/T but partitioned more biomass into fruits (higher harvest index, HI). Water shortage of 25% decreased fruit yield ~ 200g $plant^{-1}$ in 'Fortuna' (19%) and 'Sabrina' (13%) but not in 'Splendor'. Fruit quality parameters and antioxidant capacity were unaffected by the water treatment. Photosynthetic response to soil desiccation revealed that photosynthesis impairment occurred at different soil-moisture thresholds (55%, 50% and 45% of Fc, in 'Sabrina', 'Splendor' and 'Fortuna', respectively), decreasing gradually in 'Fortuna' and 'Splendor' but sharply in 'Sabrina', suggesting different water stress tolerance among cultivars. Thus, important water savings (more than 50%) can be achieved by combining deficit irrigation up to 20% ETc with the use of drought-tolerant strawberry cultivars with high WP_C and HI. In-depth studies of anatomical, metabolic and molecular traits are necessary to reveal the basis of their differential water stress tolerance for its use in breeding programs.

Key words: Drought, strawberry, water productivity, water stress tolerance.

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DYNAMICS OF PHYSIOLOGICAL PARAMETERS DURING TOMATO GRAFT HEALING

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Abstract: Tomato grafting is a well-known technique due to its important applications that support crop health and yields. Although some studies have been carried out on certain basic physiological parameters, to the best of our knowledge, none of them incorporate a wide range of days after grafting (DAG), and especially, merge several techniques in an autograft model. Here, we present a comprehensive study about: (1) graft junction mechanic resistance (by traction assays), (2) scion photosynthetic performance (by quantum yield determination) and (3) scion water status (by water potential and foliar transpiration analyses).
By traction assays, it was found that effective graft adhesion was measurable from 6 DAG. At 16 DAG no differences between grafted and non-grafted tomato plants were found. Quantum yield of chlorophyll fluorescence is a reliable method to assay photosynthetic performance and stress status in plants. Quantum yield of chlorophyll markedly decrease at early grafting. From 6 DAG a steeply increase in quantum yield was clearly measured. This trend would indicate the recovery of grafted plants after an initial phase of wound and water-induced stress. Besides, quantum yield results allowed us to endorse for this technique as a useful tool for monitoring graft functionality. Before vascular reconnection occurs, scions have to overcome water stress associated with grafting. Scion water potential decreased immediately after grafting keeping a steadily status during graft healing. Putatively, this effect could be due to the high humidity conditions of the healing chamber, where the grafts are kept during grafting. On the other hand, estimated transpiration by thermography of leaf surface showed a dramatic decreased in transpiration up to 2 DAG. Then, transpiration quickly increased between 4 and 6 DAG, allowing us to suggest for this interval, the time set for water column recovery.
A wide set of techniques have been used to follow the graft healing process by a physiological point of view. These findings could be crucial in order to understand the graft union process and it would also allow insights in the early detection of graft failure.

Key words: graft junction, mechanic resistance, quantum yield, thermography, water potential.

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EFFECT OF THE REDUCTION OF THE WATER AVAILABLE IN THE SUBSTRATE ON THE WATER USE EFFICIENCY, MORPHOLOGY AND PHYSIOLOGY OF HEBE SPECIOSA GROWN IN POT

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Abstract

The availability of water in the substrate in potted plants affects the physiological and morphological behavior of the plants, since the amount of water available in the root zone is limited and sudden changes in humidity take place frequently under these conditions. The present study was carried out on *Hebe speciosa* plants, a species with great interest for ornamental horticulture, with a certain tolerance to water stress, from February to September 2020. The proposed objective was to study the response of this plant to three set thresholds (49.01, 38.45 and 31.77%) of volumetric water content (VWC) in the substrate, using dielectric soil sensors, applying very shorts irrigation events to avoid water losses due to percolation. Plants grown with the lowest VWC reduced their growth, while chlorosis and leaf abscission was not observed. In all treatments, the water use efficiency (WUE) was high due to the absence of drainage, a value that increased when the VWC was lower, since the reduction in evapotranspiration (ET) was greater than the reduction in growth. Photosynthesis (P_n) was more sensitive to high summer temperatures than VWC. The stomatal conductance showed a strong reduction as a consequence of a decrease in the leaf water potential due to the drop of the VWC. Hebe promotes photochemical dissipation different from CO_2 assimilation, when carbon assimilation is limited. The nutrient content in Hebe tissues increased as VWC decreases, inducing an accumulation of solutes in the leaves and promoting osmotic adjustment, which helped to maintain the hydration of the plants under conditions of lower humidity in the substrate. We conclude that *Hebe speciosa* can grow successfully with a low moisture content in the substrate and an irrigation regimen without drainage, which improves considerably the water use efficiency.

Key words: ornamental plant, irrigation, VWC, water status, photosynthesis

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EVALUATING THERMOGRAPHY IN DIFFERENT CROP CONDITIONS AND WATER SOURCES IN CITRUS TREES

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Abstract: Canopy thermography allows the study the temperature of a large population leaves remotely, allowing to adjust irrigation scheduling (Bellvert et al., 2016), and saving important amounts of water. In this work, we evaluate several thermography-based indexes in different *Citrus* species, by comparing them against the robust and well-known plant water status indicator, the stem water potential, in order to determine the optimal index. The plant material consisted of: (I) young lime trees (*Citrus latifolia* Tan., cv. Bearss) grown in two crop conditions: shade and open-air, and (II) adults grapefruit trees (*Citrus paradisi* Macf., cv. Star Ruby) irrigated with two water sources: Tajo-Segura transfer and regenerated saline water. Along one growing season several diurnal evolutions of stem water potential (Ψ_{stem}) and canopy temperature (T_c) were carried out. In grapefruit, the diurnal values of Ψ_{stem} were closely related with T_c (r^2=0.84**) and T_c-T_a (canopy to air temperature difference) (r^2=0.80**) but not with the crop water stress index (CWSI) (r^2=0.11ns). Therefore, T_c and T_c-T_a reflect well the water status of grapefruit, as well as occurs in other cultivated species (Ru et al., 2020). Moreover, the plants irrigated with reclaimed saline water showed in general lower values of Ψ_{stem} and higher T_c values. This could be due to high concentration of salts in the soil of these plants make difficult the water uptake with negative repercussions in its water status (Mounzer et al., 2013). In lime trees, Ψ_{stem} *vs.* T_c showed also higher r^2 values than *vs.* T_c-T_a in the two crop conditions studies. The plants cultivated in shade conditions showing a better correlation for the two thermography indexes (r^2 = 0.91*** and 0.55* for T_c and T_c-T_a, respectively). It should be noted that shade conditions created an optimal microclimate for crop development (Mira et al., 2020). In conclusion the absolute T_c value resulted to be a robust index in both *Citrus* species and in the crop conditions and water sources studied. The grapefruit trees irrigated with reclaimed saline water and lime trees grown in shade conditions showing a better Tc vs.Ψ_{stem} relation.

Key words: Canopy temperature, Stem water potential, Plant water status.

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EVALUATION OF THE IMPACT OF DIFFERENT ENVIRONMENTAL CONDITIONS ON THE NUTRITIONAL TRAITS OF QUINOA SEEDS

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Abstract: *Chenopodium quinoa* Willd. (quinoa), is still, in many different areas of the world, an underutilized crop known for its capacity of adaptation to different environments and the remarkable nutritional properties of its seeds, including a high protein content, a balanced amino acid profile, and because of its high antioxidant and mineral contents (Abugoch James, 2009). These characteristics explain why quinoa has gained worldwide recognition in the last few decades. However, little efforts have been made to identify the nutritional changes in quinoa in response to differences in environmental conditions, even though it is known that these traits can be determined by the environment. Thus, in the present work nutritional seed traits were analyzed in six different quinoa cultivars sown during three consecutive years, aiming to evaluate the effect of the environment on the seed nutritional parameters. It was found that quinoa quality traits were determined mainly by the environment, but also by the genotype, and the GxE interaction, being 2018 the year that showed lower yields, germinative rates, and antioxidant activity, but higher protein content. We also found interrelation among important nutritional variables, like a negative correlation between protein content and yield or between protein content and antioxidants, and that the seed potassium, manganese, and magnesium concentrations can determine their germination capacity. Overall, this work provides new insights into the environment, genotype, and GxE impact on quinoa nutritional properties.

Key words: Quinoa, Nutritional traits, Seed quality, Genotype x Environment interactions
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EXTENSIN AND HEMICELLULOSE DISTRIBUTION AND RELATED GENE EXPRESSION DURING OLIVE FRUIT ABSCISSION

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Abstract: Although cell wall modification is believed to underlie changes in organ abscission, information concerning the changes in cell wall proteins and hemicellulose polysaccharides is still limited. The aim of this work was to analyze the spatio-temporal patterns of the distribution of different extensin proteins and hemicelluloses in the abscission zone (AZ) during natural ripe-fruit abscission in olive (*Olea europaea* L.). In this study, we employed immunogold labeling in the ripe-fruit AZ during olive AZ cell separation, using an expanded set of monoclonal antibodies that recognize different types of hemicelluloses (LM11, LM15, and LM21), callose (anti-(1,3)-β-D-glucan) and extensin (JIM19) epitopes, and transmission electron microscopy imaging. Our data demonstrate that AZ cell separation was accompanied by a loss of the JIM19 extensin epitopes and a reduction in the detection of the LM15 xyloglucan epitopes in AZ cell walls, whereas AZ cells were found to be enriched with respect to the xylan and callose levels of the cell wall during olive ripe-fruit abscission. By contrast, AZ cell wall polysaccharide remodeling did not involve mannans. Moreover, in ripe-fruit AZ, quantitative RT-PCR analysis revealed that *OeEXT1*, *OeEXT2*, *OeXTH9*, and *OeXTH13* genes were downregulated during abscission, whereas the expression of *OeXTH1*, *OeXTH5*, and *OeXTH14 g*enes increased during abscission. Taken together, the results indicate that AZ cell wall dynamics during olive ripe-fruit abscission involves extensin protein and hemicellulose modifications, as well as related expressed genes. This is the first study available demonstrating temporal degradation of extensin protein and hemicelluloses in the AZ at the subcellular level.

Key words: callose, cell wall, extensin protein, fruit abscission, hemicellulose

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GENOTYPE-DEPENDENT VARIATION OF NUTRITIONAL QUALITY-RELATED TRAITS IN QUINOA SEEDS

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Abstract: Exploiting the relationship between the nutritional properties of seeds and the environment (E), genotype (G) and the GXE interaction, is an essential process which contributes to broaden our knowledge regarding the control of the nutritional quality of seeds or of any other edible plant structure. This constitutes an important aspect when aiming at improving the nutritional characteristics of plant species, including those of *Chenopodium quinoa* Willd. (quinoa) which is intended to be one of the main nutrient sources ensuring food security worldwide. This crop has gained popularity in the last decade, therefore achieving a fast-global expansion, due to its excellent nutritional and agronomical properties together with the excellent adaptation shown to a wide diversity of agroclimatic conditions. The influence exerted by the environment, genotype or their interaction produces changes in the nutritional properties of quinoa seeds. These changes have been already described in previous works, but the analyses carried out had important limitations, including the outcomes that can be translated into agronomical practices by which quality can be improved. In this study, fifteen quinoa cultivars grown in a particular environmental context were analysed, obtaining several seed nutritional-related parameters such as compounds that can be determinants of seed quality which varies with the genetic background. Important nutritional and agronomical differences were found among quinoa varieties, such as differences in the levels of protein content ant antioxidant capacity of the seeds. In this regard, cultivars such as A-SE-15 showed high levels in both parameters meanwhile other cultivars like A-SE-6 showed low levels. Important differences were also found in the mineral contents depending on the cultivar. Furthermore, strong correlations between total phenol content and protein content in germination rates were observed, highlighting the importance of choosing a proper genotype when cultivating quinoa. In line with this, a path coefficient analysis was performed to determine the direct and indirect effects of these nutritional and agronomical differences in yield and germination rates of quinoa seeds. The implications of all these aspect on this crop establishment and performance is discussed.

Key words: Quinoa, Seed quality, Nutritional traits, Seed yield.

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HORMONAL GENETICS OF ADVENTITIOUS ROOT FORMATION IN DIVERSE *PRUNUS* GENOTYPES

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Abstract: The effective propagation of elite cultivars in many plant species is highly dependent on adventitious rooting, a process that is highly regulated by hormonal crosstalk (Mhimdi and Pérez-Pérez, 2020). Several species of the *Prunus* genus are among the most important stone fruits in the Mediterranean basin, and there is an urgent need to obtain rootstocks with specific adaptation to a changing environment. However, some *Prunus* rootstocks remain difficult to propagate and a better understanding of the molecular signatures of the available genotypes is of great importance for the establishment of the new breeding programs. As a first step towards the understanding of the genetic determinants involved in adventitious rooting in *Prunus*, we evaluated the rooting of hardwood cuttings of five peach-based rootstocks: four peach-almond hybrids (*Prunus amygdalus* × *Prunus persica*): Adafuel, Adarcias, Garnem and GF 677; and one *P. persica* × *P. davidiana* hybrid: Cadaman, grown in hydroponic culture. We found that auxin-induced callus formation and rooting responses were strongly genotype-dependent. To investigate the molecular mechanisms involved in these differential responses, we performed a time-series study of adventitious rooting in microcuttings of Garnem and GF 677 rootstocks and of two commercial *Prunus dulcis* genotypes with and without the auxin treatment. Garnem and GF 677 rootstocks displayed dynamic changes in endogenous hormone homeostasis during rooting that might account for the observed differences. An integrative approach combining targeted metabolomics and selective transcriptome profiling (Justamante *et al.*, 2019) will allow us to identify the key regulatory pathways involved in adventitious rooting performance in *Prunus* species, which could be used to breed *Prunus* rootstocks with enhanced rooting ability to increase production under challenging environmental conditions, such as drought.

Key words: vegetative propagation, rootstocks, hormone profiling, auxin homeostasis.

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IS THERE STRENGTH IN NUMBERS? *SECALE CEREALE* L., *TRIFOLIUM SQUARROSUM* L., AND THEIR MIXTURE FOR WEED CONTROL

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Abstract: Cover crops are essential tools in agroecosystems for reducing the reliance on synthetic inputs and associated environmental risks. Alongside their benefits to soil fertility, cover crops can control weeds by their competitive and allelopathic attributes. Laboratory and field experiments were conducted to assess the allelopathic potential of two cover crop species, rye (*Secale cereale* L.) and squarrose clover (*Trifolium squarrosum* L.), alone or intercropped, on seed germination and growth of arable weeds. Aqueous extracts of the two cover crops and their mixture were tested in a bioassay on *Conyza canadensis* (L). Cronq., *Amaranthus retroflexus* L. and *Digitaria sanguinalis* (L.) Scop. All three extracts were able to reduce the germination of *A. retroflexus* considerably (-87% of control). Inhibitory effects by rye and the mixture extracts on radicle growth of the weed species ranged between 51 and 82% of control. Rye extract was the best at reducing shoot length of *C. canadensis* and *D. sanguinalis* (-39 to 44% of control), while squarrose clover was more effective on *A. retroflexus* (-79% of control). The chemical characterization of cover crop residues, both shoots and roots, revealed a notable richness of allelopathic phenolic acids and flavonoids, especifically when both species were intercropped. Accordingly, in the field experiment, the mulch consisting of a mix of rye and squarrose clover under no-till management demonstrated a higher potential for weed suppression over its single counterparts. Such effectiveness could be partially due to interspecific competitive interactions when growing together, leading to an increased release of diverse allelochemicals from plant tissues to water. Our work demonstrated the allelopathic activity of the studied cover crops as potential natural herbicides and the benefits of their inclusion as mixtures in weed management strategies according to cropping system needs.

Key words: flavonoids, mixtures, phenolic acids, phytotoxicity, positive interaction

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MEALWORM FRASS AS A POTENTIAL ORGANIC FERTILIZER IN SYNERGY WITH A BIOSTIMULANT BASED ON PGPS IN LETTUCE PLANTS

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Abstract: One of the modern agriculture challenges is to propose alternatives to the use of mineral fertilization, fitting with the concept of circular economy. In this context, the application organic residues joint with efficient microorganisms can accelerate the decomposition process, consequently releasing nutrients, and resulting in an efficient tool to enhance nutrient uptake and crop production. In this work, a biostimulant formulated with plant growth promoters (PGPs), more exactly a mixture of *Bacillus* (*B. licheniformis*, *B. pumilus*, *B. safensis* and *B. velezensis*) was jointly applied with frass, the organic residue of the rearing of mealworm (*Tenebrio molitor*). Frass has a great potential to be used as organic fertilizer due to its rapid mineralization and its high content in readily available nutrients. In a pot experiment with lettuce (*Lactuca sativa* L.), increasing rates of frass (0%, 1%, 2.5%, 5% and 10%) were tested applied alone or combined with a PGP-based biostimulant with the aim to explore the combined use of these inputs as fertilization tool. Biostimulant application to unfertilized pots significantly increased plant yield and quality in terms of lettuce fresh weight, juiciness or chlorophyll content. The application of 1% of frass significantly increased plant growth, chlorophyll content, and P and K extraction by the plant. The jointed application of biostimulant doubled lettuce biomass whatever the frass dose, suggesting a positive synergy between frass and biostimulant, leading to plants with a better quality also in terms of chlorophyll, P and K content. The joint application also showed to induce a great impact in rhizospheric microbiota, where cultivable fungi decreased in general with biostimulant application. On the other hand, higher doses of frass affected negatively lettuce root growth. Responses were toxic above 5% of frass, due to the presence of potential pathogenic species like *Fusarium*.

Key words: *Bacillus*, *Tenebrio molitor* faeces, efficient microorganisms.

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METHYLGLYOXAL: THE DARK SIDE OF AMMONIUM TOXICITY IN PLANTS

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Abstract: Plants acquire inorganic nitrogen mainly as nitrate (NO_3^-) and ammonium (NH_4^+). Excess NH_4^+ in the soil leads to severe growth retardation and other toxicity symptoms in many plants commonly referred to as *ammonium syndrome* (Britto and Kronzucker, 2013; Esteban et al., 2016). The effective incorporation of NH_4^+ into amino acid structures requires high activity of the mitochondrial tricarboxylic acid cycle and the glycolytic pathway (Foyer et al., 2011). An unavoidable consequence of an overflow in glycolytic pathway is the production of methylglyoxal (MG), which is very toxic and inhibits cell growth in all types of organisms (Allaman et al., 2015). When applied together, NO_3^- alleviates the toxic symptoms of NH_4^+ in plants and can even improve their development (Garnica et al., 2012; Hachiya et al., 2012). However, information related to the influence of NH_4^+ and NO_3^- nutrition on glycolytic and respiratory activity is still limited, despite is are the most important metabolic pathways for producing cellular energy sources from sugars. Here, we approach the mechanisms of ammonium toxicity from another perspective, exploring the impact of high NH_4^+ nutrition and the counteractive role of NO_3^- on MG accumulation. We studied two plant species with different sensitivities to NH_4^+ and capacities for accumulating NO_3^- in their leaves. Spinach (*Spinacea oleracea*) is sensitive to ammonium nutrition and is nitrate-accumulating, while pea (*Pisum sativum*) is a non-NO_3^--accumulating species which is more tolerant to NH_4^+ (Cruz et al., 2006; Ariz et al., 2011). We grew spinach and pea plants under 5 mM of nitrogen as NO_3^-, NH_4^+ and both (NH_4NO_3 1:1) and at two different pH levels (6 and 8) because plant tolerance to NH_4^+ and acidity are related (Zhu et al., 2011; Wang et al., 2016). We show that strict NH_4^+ nutrition leads in spinach and pea the increased sugar content and enhanced glycolysis that promotes MG production, together with the less optimal growth pH, 6 or 8 in spinach and pea respectively. Overall, our studies indicate that MG content could be a reliable indicator of plant stress originated by NH_4^+ nutrition, and NO_3^- is a counteracting agent against glycolytic MG induced damage.

Key words: ammonium toxicity, glyoxalase, methylglyoxal, nitrate.

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MORPHOCHEMICAL CHARACTERIZATION OF HOP STROBILES THROUGHOUT THEIR DEVELOPMENT AND RIPENING

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Abstract: Hop (*Humulus lupulus* L.) is a crop of great interest to the brewing industry. Only female inflorescences or strobiles are used for this purpose due to their high concentration of lupulin, which includes essential oils and resins (prenylphloroglucinols, that are the sum of α- and β-acids, and prenylflavonoids). These metabolites provide the bitterness and the aroma of the beer and each hop variety has a different concentration of these compounds (chemical fingerprint). Thus, in order to study the evolution of resins and characterize the strobiles of two commercial varieties grown in León (Nugget and Magnum) and two ecotypes (L1 and L2), the female inflorescences were analysed during their development and ripening. First, a morphological assay was carried out following the guidelines of the International Union for the Protection of New Varieties of Plants (UPOV, 2006) and afterwards, a quantification of resins by HPLC-PDA was performed.

The development of the inflorescences showed two phases. Firstly, the increase of width and length caused an increment of dry weight (DW); secondly, the size remained constant and the increase of DW was due to the accumulation of different compounds related to the strobile ripening. These changes were more prominent in Nugget and Magnum than in the ecotypes. The metabolites analysed in lupulin were present since the earliest harvest dates of the inflorescences, increasing their concentration mainly during the growth period, in the case of commercial varieties, or remaining almost constant in the ecotypes. The highest amount of the metabolites per strobile, which determines the optimal harvest time, was different for each variety. The lupulin analysis also allowed us to classify the hop varieties according to their chemical fingerprint on the last harvest day. In this sense, Magnum and Nugget are high-α varieties; however, a lower percentage of α-acids and β-acids was detected in these strobiles, in comparison with the same varieties grown in other countries (Krofta, 2003; Patzak *et al.*, 2015). The ecotypes L1 and L2 were classified as dual-purpose and fine aroma varieties, respectively.

Key words: lupulin, *Humulus lupulus*, chemical fingerprint.

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PRELIMINARY STUDY OF THE PHYSIOLOGICAL RESPONSE OF SPIKE LAVENDER (*Lavandula latifolia* Medik) AGAINST DROUGHT STRESS

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Abstract: Spike lavender (*Lavandula latifolia* Medik) is an aromatic-medicinal plant, native to the Mediterranean region. It is cultivated for the production of essential oil, Spain being the largest producer with 150–200 t per year (Lesage-Meessen et al., 2015). The possible socio-economic importance of this crop, with its minimal water requirements, is reflected in the fact that it could be used for revaluing rainfed marginal lands, contributing to the re-settlement of rural areas. In the Mediterranean area, the negative effects of climate change, with increasingly frequent extreme heat waves and droughts, selecting plants with greater tolerance to drought is crucial for the development of this rainfed crop.

To the best of our knowledge, the physiological response of this species to drought stress is unknown, a fact that explains the aim of this preliminary work. In the study, seeds of spike lavender from local growers (northwest area of the Region of Murcia, SE Spain) were germinated and the plantlets grown under greenhouse conditions for eight months. Subsequently, twenty plants were assigned randomly to two homogeneous groups. One group was irrigated to field capacity as a control and the other was submitted to water stress for 21 days. The relative water content (%RWC), osmotic potential (Ψh), proline,

abscisic acid (ABA), photosynthetic pigments (chlorophyll a and b, carotenoids) and α-tocopherol concentrations were measured in the leaves of control and stressed plants. As expected, the plants grown under drought stress exhibited higher concentrations of ABA, proline and α-tocopherol than the control. Although the level of photosynthetic pigments was higher in stressed plants, the chlorophyll a/chlorophyll b ratio and total chlorophyll/carotenoids ratio were similar in both treatments. The %RWC and Ψh were lower in stressed plants than the control due to dehydration of the plants. These findings suggest that spike lavender is affected by drought, although it shows some strategies to overcome the negative effects of lack of water. Knowledge of the levels of these physiological parameters in incipient plants could be a tool for the pre-selection of more drought tolerant spike lavender individuals.

Key words: Drought stress, *Lavandula latifolia*, α-tocopherol, proline, photosynthetic pigments

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REVISITING LANDRACES VARIETIES TO IMPROVE SORGHUM CROP

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Abstract: Sorghum (*Sorghum bicolor* L. Moench) is a C_4-type cereal highly resistant to drought and salinity. It is used in human and livestock feed and has the additional feature that it is gluten-free cereal. An increasing need for food, in parallel with a decrease in water resources for agriculture due to climatic change, is one of the most compelling challenges to society in the near future. Sorghum, therefore, has great potential as a key carbohydrate source for the future.

In this study, we used the plant genetic resources available in the CRF-INIA and selected several landraces cultivars with different characteristics and from different Spanish regions. Although we concentrated on cultivars from Andalusia (southern Spain), probably better adapted to drought characteristic of this region, we have also included cultivars from the north and center of the peninsula subjected to different climatic conditions. This implies not only the characterization of autochthonous varieties but also the enhancement of plant genetic wealth as a potential tool in future breeding programs.

We have analyzed the growth rate and size of the different cultivars, the germination speed, and nutritional and biochemical parameters of its seeds and studied their relation with water stress tolerance. Thereby, we have made a first progress in the identification of relevant plant characteristics important for the use of these sorghums within a future and adapting agricultural program. Among all the varieties analyzed, we propose Zahina, particularly tolerant to water deficit, as an interesting candidate for grain and forage production.

Key words: sorghum landraces, cereal, water stress, grain, forage

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UNFUNCTIONALITY OF TOMATO GRAFTS: TISSUE DEVELOPMENT, CELL WALL MODIFICATIONS AND ASYMMETRY OF SUGAR DISTRIBUTION

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Abstract: Grafting is a widespread horticultural technique with applications in numerous crops due to its great advantages for yield and crop health. In tomato cultivation, this technique is well recognised and it has a huge economic impact. Nevertheless, this technique is limited by the occurrence of non-grafted grafts, which decreases the success rate and thus the benefits achieved. Here, we inquire in the reasons of this failure. For these purposes, tomato (*Solanum lycopersicum* L.) intraspecific grafts of different cultivars (Minibel and Marmande VR) were evaluated for graft failure, focusing on the tissue development, cell wall modifications and sugar balance in the graft interface. Success rate was determined, and a wide set of microscopic techniques were performed to study the graft union tissues and cell walls. Finally, total sugars determination was carried out in autografts to study sugar balance of the graft union. The success rate in autografts was higher than in homografts and heterografts. Unfunctional homografts and heterografts showed similar responses: absence of vasculature and lack of adhesion between scion and rootstock, even though callus cells clusters and differentiation of new vascular tissues were produced. The unfunctional scions accumulated more amyloplasts and soluble sugars than the rootstocks, showing a strong asymmetry in the response. Three types of deposits were observed in the cell walls of unfunctional grafts: lignin, suberin and callose, being frequent the combined accumulation of more than one of them, particularly lignin and suberin. These deposits apparently prevent adhesion and seem to be a major cause of graft unfunctionality.

Key words: callose, grafting, lignin, starch, suberin, sugars.

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A PROBLEM-BASED LEARNING APPROACH FOR THE TEACHING OF PLANT PHYSIOLOGY SUBJECT IN THE DEGREE OF BIOLOGY AT THE UNIVERSITY OF THE BASQUE COUNTRY (UPV/EHU)

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Abstract: The principles of plant physiology are best learned in an environment in which students have the need to use those principles. To accomplish this purpose in a way that is exciting and enriches the student's learning process, a problem-based learning pedagogy was used. With this methodology, the student learns through the experience of solving an open-ended problem. This approach is being used since the 2015/16 academic year, in students attending to the subject of Advanced Plant Physiology, which is taught in the third year in the Degree of Biology.

At the beginning, the professor provides different papers and news to engage the students into the different problems that society has to overcome (food security and quality, soil pollution, climate change, and so on). Once the students are aware of the problems, groups of three to four students choose which problem want to solve and make a list with all the learning results they require to solve it. The problem and the associated learning results are shared with the professor and the other groups in order to receive feedback. Then, by reading books, scientific papers, with the theoretical lessons and with the guidance of the professor the students develop those principles. Finally, the group writes the solution to the problem based on an adequate reasoning by using the developed principles. The professor reviews the proposed solution and gives feedback if needed.

To know how adequate the approach was, a survey was conducted for four years in which 181 students participated. Compared to more traditional methodological approaches, the followed methodogy helped the students to 1) better understand the theoretical contents, 2) relate the contents of the subject, 3) be more proactive towards their learning, 4) develop autonomous work skills, 5) properly analyze situations of professional practice and 6) make decisions around a real scenario. In adittion, during the four year period analysis, more than 90% of the students expressed that they would like to repeat this approach in other subject. Therefore, the data demonstrates the problem-based learning approach to be an adequate pedagogical technique to teach and learn plant physiology.

Key words: learning results, problem-based learning, plant physiology, real situation.

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AWAKENING INTRINSIC MOTIVATION

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Abstract:

Fostering student motivation is a difficult but essential issue of the teaching and learning process. University students are generally motivated, but their motivation primarily relies on extrinsic motivation driven by external rewards, as getting good grades in order to obtain the degree. However, in education, extrinsic motivation resulted in more superficial learning, produces only short-term effects, and could represent a severe problem for future professional development and life-long learning. In fact, extrinsic motivation will be only helpful, if sufficient intrinsic motivation is behind. Intrinsic motivation, as to satisfy their curiosity, seek for novelty, the need to develops their own capabilities and potential, are internal aspects that do not need external rewards, increase student satisfaction and lead to long-lasting learning. In any a case, both motivations could co-exist and complement each other. Here, we present strategies based on active and service-learning methodologies employed in Plant Physiology and Plant Biotechnology courses from the grades of Environmental Science or Biotechnology of the University of the Basque Country to enhance and maximize student´s learning experience based on intrinsic motivation. Experiences in the frame of service-learning by combining objectives with societal needs will develop the cognitive and emotive aspects of learning resulting in better learning outcomes during the University period and therefore as life-long learners. The main strategies we employed are: (i) we are (the professors) the first highly motivated towards the subject and educational process, showing our own enthusiasm and passion; (ii) the course is coherently organized highlighting the relevance to the grade and their future profession; (iii) we clearly define a "road-map" to succeed in the course; (iv) we connect each study topic to societal real problems and challenges, media news, including the latest and more relevant research in the topic; (v) the students design their own project that will involve creating and performing strategies to cope with the selected societal challenge; (vii) we use e-learning methodologies as moodle environment and e-mails as reminders to boost their work and for improving motivation towards plant physiology. We conclude that by focusing on these basic tools, instructors and teachers will succeed in awakening intrinsic motivation.

Key words: Curiosity, motivation, strategies.

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HOMEMADE EXPERIMENTS IN PANDEMIC TIMES: A RESOURCE TO SUPPORT SELF-DIRECTED LEARNING IN PLANT PHYSIOLOGY

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Abstract: The COVID-19 pandemic situation has considerably limited the possibilities of carrying out practical teaching in the laboratory, but it has boosted creativity for a more autonomous learning process. To this end, students of three subjects taught by docents from the area of Plant Physiology in the Biology Degree at the University of León - Biology I (Plant Physiology) in the first year, and Secondary Metabolism of Plants and Environmental Physiology of Plants, optional subjects in the fourth course- were asked to carry out different experiments or models with the resources available at their homes. The results had to be presented as a task posted in a "Moodle dropbox" opened for a period of about a week, according to each case. The assignments could consist of a "pdf" document or a video showing the materials, the procedure and the results of the research. Some of these experiments were: the prevention of fruit browning (by inhibiting polyphenol oxidase activity); demonstrating the hydration capacity of a poikilohydric plant; making models of turgid cells, or of extensible cell walls, or of functional stomata by changing turgor. In general, the activities were optional, were part of the ongoing assessment, and contributed to increase the final score by up to one point.
Surveys indicated a positive feedback from the students. They stated that a) carrying out this type of activity contributed to a better understanding of the subject, and b) that the methodology was highly motivating and helped them to stay "stuck" to the subject.

Key words: autonomous learning, experiments with plants, plant models.

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Abstract: Since the beginning of the implementation of Bologna Process and the launching of the European Higher Education Area, teaching strategies had changed in the Universidade da Coruña (Spain), and particularly in all the subjects about Plant Physiology taught both in the bachelor and master degrees offered by this University. We were more or less well adapted to this new concept of teaching and learning, but suddenly, in 2020 Covid-19 pandemic arrived and we had to face a new challenge: use teaching tools that allow, in the first months, non-presential (distance) learning, and later on a combination of both presential and distance learning. Many people said that this was a chance to improve the learning process with the use of Information and Communications Technology (ICT or, in Spanish TICs). Indeed has been not only a chance but also a challenge made worst because of economic issues and no increase of budget. Here, we explain our experience using ICT tools as Microsoft Teams, Moodle, Camtasia Studio, OBS, webcams, video recording cameras, Chromakeys, and so on. All these tools have been useful to achieve the goal of keeping a good learning and teaching process, but lecturers have been overwhelmed with a lot of additional job. We show here experiences regarding live videoconference lectures, pre-recorded lectures and practical classes, and the adaptation of some previous seminar experiences to the new situation (e.g.: "The Big Plant Theory", "The Hunger Games: Plantajo" and "Stranger Plants"). The assessment of this experience shows both negative and positive results that will be discussed in the communication.

Key words: Covid-19, ICT, Plant Physiology, Video.

Poster Communications



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ADAPTING LABORATORY CLASSES TO PARTIAL FACE-TO-FACE LEARNING USING IMAGE ANALYSIS TOOLS

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Abstract: Universities have had to adapt quickly to the new situation imposed by current sanitary conditions, implementing changes in the methodologies used until now. Laboratory learning is particularly affected by the current health situation since the manipulative training that it involves can only be acquired "by doing". However, given the great development of information and communication technologies (ICT) and their easy adaptation to the educational environment, it is possible to move the laboratory from the university facilities to the student's home. To enable full compliance with the practical program of Plant Physiology without increasing the demand for teachers or more laboratory space, we opted to adapt half of the laboratory classes so that students can do them autonomously. For that, we provided them with the corresponding protocols and some minimal basic materials. Some of the experiments, however, require the use of more complex and expensive laboratory devices, such as spectrophotometers, that students cannot afford. For this, image analysis can offer a suitable solution. However, obtaining quantitative color intensity data from photographic images does not provide comparable values to measurements made with a spectrophotometer. On the one hand, the photographic data do not represent transmittances, but rather reflectances. Furthermore, the values obtained after logarithmic transformation of the color intensity data necessary for linearization show much lower slopes than the spectrophotometric data, which limits the quantification of low concentrations of the analyte and makes it difficult to properly discriminate between samples of close concentrations. To minimize these problems, we propose: i) taking the photographs with the samples inserted between the light source and the camera lens, ii) using a computer screen to illuminate the sample with light equivalent to the wavelength that would be used to the spectrophotometric measurement (https://academo.org/demos/wavelength-to-colour-relationship), and iii) quantify only the data of transmitted color corresponding to the illuminating light, instead of color intensities averaging the entire RGB histogram. In this way, slopes much closer to the spectrophotometric ones are obtained.

Key words: Image analysis, laboratory classes, manipulative skills, RGB color space, spectrophotometry.

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CITIZEN SCIENCE OBSERVATORIES: TOOLS FOR PLANT BIOLOGY TEACHING

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Abstract:
Citizen science consists of the participation of the general public in scientific research activities, in which citizens actively contribute, either with their intellectual effort, with knowledge of their environment or by contributing their tools and resources. The Observatory for Citizen Science at the Public University of Navarra (http://www.unavarra.es/unidadculturacientifica/ciencia-ciudadana?languageId=100000) is an initiative derived from the CENEDUCA3/2019 project funded by the Department of Education of Navarra Government. This initiative aims to promote and disseminate citizen science activities carried out in Navarra, creating a repository of projects fundamentally related to biodiversity. Citizen science can be a valuable educational resource to develop critical competencies, especially scientific competencies, in an integrated way (Bonney et al., 2009; Kobori et al., 2016). In addition, participation in citizen science projects may be attractive and motivating for students (Doyle et al., 2017; Steinke et al., 2017). In Navarra, several citizen science projects linked to the study of plant biodiversity have been launched to directly focus on environmental education contributing to biodiversity knowledge and care of the environment by future citizens. Among the projects launched, a virtual herbarium of the Basaula Nature Reserve (https://www.inaturalist.org/projects/basaula-puy) carried out in collaboration with the El Puy Secondary School in Estella, Navarra (Echevarría et al., 2021). A similar initiative study, the Arga river park (https://www.inaturalist.org/projects/arga) running through Pamplona's city, makes accessible a valuable natural environment to formal and informal educational institutions. Finally, a project focused on the Public University of Navarra campus (https://www.inaturalist.org/projects/campus-upna-pamplona-nupeko-iruneko-campusa) is offered as an accessible and practical tool for different plant biology subjects and scientific campus activities stimulating the student interest in plant biology discipline.

Key words: citizen science, iNaturalist, biodiversity, plant biology

Acknowledgments: CENEDUCA3 / 2019 Department of Education of the Government of Navarra.

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COMBINED ONLINE-TEACHING WITH LABORATORY PRACTICES ADAPTED TO THE COVID-19 REGULATIONS IN A SUBJECT OF THE BIOLOGY DEGREE

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Abstract: The subject "Response of plants in adverse conditions" presents to the students the mechanisms that plants have to survive unfavourable environmental conditions. This subject corresponds to the 4th year of the Degree in Biology at the University of Coruña (UDC). The laboratory practices of this subject focus on analysing a problem present in the crop fields of Galicia. The excessive use of certain agrochemicals causes large concentrations of heavy metals in the soil, like copper in the case of copper sulphates. To analyse this problem, the students carry out a laboratory experience where they apply a high concentration of copper to plants grown in trays and subsequently measure biometric and biochemical parameters of the plants. During this experience, the students become aware of the problems that exist around anthropogenic soil contamination with heavy metals. The final objective of the practice is to meet one of the sustainable development goals promoted by the UN: Responsible consumption and production (goal number 12). In this sense, students will realize the need to replace the use of agrochemicals, many of them currently prohibited (such as some formulations of copper sulphate), with other types of compounds that are more respectful with the environment. The use of information and communication technologies (ICTs) is combined with laboratory work as a learning technique and to adapt to the COVID-19 regulations. The use of ICTs has been applied in regulated education for several years now (Bravo *et al.* 2018). In the last year, this incorporation has been accelerated by the pandemic situation caused by COVID-19 throughout the world. At the UDC, the Microsoft Office 365 application package has been made available to students. Specifically, in this activity, the TEAMS, FORMS and EXCEL applications will be used within the Office 365 package. These applications can be installed on smartphones as required, greatly facilitating and motivating its use by students.

Key words: heavy metals, sustainable agriculture, online teaching, COVID-19.

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IMPLEMENTATION OF EDUCATIONAL QUIZZING GAME 'KAHOOT!' FOR TEACHING PLANT PHYSIOLOGY: PRELIMINARY RESULTS

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Abstract: gamification is a didactic technique consisting on adding gamelike elements to a task to encourage participation. Currently, there is a panoply of digital options to make easier and funnier the acquisition of new knowledge. Amongst the existing tools, the platform 'Kahoot!' has gained popularity since its foundation in 2012, being nowadays used by millions of people all over the world. Briefly, it allows teachers to share with their students a quizzing game to be performed in the classroom in real time via their mobile phones. Despite its popularity, in practice the employment of this tool in higher education is less usual than in primary or secondary education, and even less in scientific subjects.
The objective of this communication is to give some tips for an optimal 'Kahoot!' implementation in scientific teaching, based on a case study performed during the laboratory sessions of 'Genetic and Plant Breeding', a 3rd year subject of the 'Agricultural and Rural Engineering Degree'. The students were randomly divided into two groups, which were examined via a traditional paper-and-pencil two-choice questionnaire or by means of the same exam but employing 'Kahoot!'. Groups and evaluation techniques were exchanged during the period of practical sessions. Statistical analysis of data showed neither significant differences in the academic performance nor in the perception of difficulty of the exams. By contrast, it was found a striking preference for the 'Kahoot!' format. This preliminary finding is truly encouraging for continuing performing and evaluating these dual tests in teaching of subjects related to the area of Plant Physiology.

Key words: Kahoot!, gamification, trivia, Genetic and Plant Breeding.

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LOOKING INTO THE POTENTIAL APPLICATIONS OF DRONES AND SATELLITES IN PLANT PHYSIOLOGY TEACHING

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Abstract: Drones and satellites are paving the way towards a sustainable and precision farming: by adding a substantial value to crop data acquisition, devising yield simulations, predicting the harvesting and irrigation periods, eradicating the metrological data hindrances etc. The continuous technical and pragmatic improvements of the two, over the past three decades, have considerably contributed towards the maturity of these technologies. The application areas and domains of these are vast, and range from crop monitoring, disease surveillance, irrigation, soil analysis, fertilization, crop harvest, and crop yield simulations to even determining water stress, leaf area index (LAI), and leaf canopy temperature. Nevertheless, they both have their own assets and liabilities. For example, satellite imagery is freely available by various government bodies i.e., European Union, National Aeronautics and Space Administration (NASA) etc., bearing a significant spatial and spectral resolution. Whereas, attractive features like flying time flexibility, manoeuvrability, and economical benefits make drones superior over satellite technology, but both can be regarded as alternatives, and are indispensable in the achievement of sustainable agriculture. Likewise, both serve for developing vegetation indices (VIs) that provide stress, disease and growth-related information of the crop. Few of the commonly calculated VIs, among others, include normalized difference vegetation index (NDVI), soil-adjusted vegetation index (SAVI), chlorophyll index – green (CI-G), normalized green red difference index (NGRDI), and photochemical reflectance index (PRI) etc. Out of these the NDVI, provides physiological information about the crop health i.e., biomass production, canopy coverage and density, water stress detection etc. Therefore, the scope of the study is to entail the interest and basic understanding of the latest advances in emerging technologies into the students. It is important that the students become familiar with the different VIs and know about the different open access databases for satellite data collection applied in Plant Physiology. Given the significant importance of these sustainably proactive and novel technologies, it is

indispensable to educate and reinforce the students, so as to maximize the potential of today´s agriculture: thereby ensuring sustainability.

Key words: Digital agriculture, drones, satellite, precision farming, vegetation index (VI)

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MAKING SHORT VIDEOS BY STUDENTS TO LEARN PLANT PHYSIOLOGY

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Abstract: The use of short videos can be a useful tool to study Plant Physiology at different scholar levels and acquire transversal skills. For this reason, the elaboration of a series of videos about different aspects of plants was proposed to 2nd year biotechnology students. This voluntary activity was performed during the quarantine in 2020. All students selected a topic and wrote a script, which was corrected by the professor, and then recorded a 3 min video at home. These videos were uploaded to the University of Leon website (A) and to the Savia Sabia YouTube channel (B) under the slogan #YO ME QUEDO EN CASA ESTUDIANDO FISIOLOGÍA VEGETAL, available to all students. To assess the activity, 2 types of questionnaires were developed: a) for students who participated developing the activity, and b) for their classmates. In both, a 5-point Likert scale was used. In all cases the assessment exceeded 3.5 points, but the students highlighted that they had developed their ability to communicate orally and disseminate science. They also considered that the activity was very appropriate to promote analysis and synthesis of information.
In 2021, part of these videos was processed by the professors with Edpuzzle, which is a free online video editing tool. Using this, some questions were included across each video, which had to be answered to continue watching it. After that, the videos were visualized by 12 secondary school students and 65 biotechnology degree students. The degree students obtained scores ranging from 8 to 9 out of 10. The secondary school students scored an average of 6 to 7, with their worse results being obtained in photosynthesis videos. Apart from this activity, student groups elaborated a new 2 min video for explaining two problems proposed by the teacher about (1) capillarity and xylem transport, and (2) photosynthesis improvement. In this case, they searched for additional information and made an informative explanation that they showed in a video. After evaluation, we concluded that these videos were very useful for developing oral skills and studying the subject in a fun way.

Key words: short videos, oral skills, informative tools, plant physiology.

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TEACHING FROM A FASCINATION FOR PLANTS. SOME EXPERIENCES OF THE INNOVATIVE EDUCATIONAL GROUP "SAVIA SABIA"

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Abstract: In teaching, a novel idea is didactically useful when it is surprising, concrete, visualisable and generates a powerful fascination for the topic of study, therefore fascinating contents are an excellent motivational resource. In the science of plant biology, fascination through experimentation stimulates curiosity and interest and creates an ideal environment for the transfer of knowledge and skills.
Over the last ten years, a team of Plant Physiology teachers at Universidad de León, under the suggestive name of "Savia sabia", have developed a series of innovative teaching activities, covering a wide range of tasks and aimed to the acquisition of different skills, depending on the degree and course of the students.
These activities include the participation through scientific experimentation workshops and gymkhanas in the events programmed for the international Fascination of Plants Day (FoPD); the collaboration with the "Bachillerato de Excelencia en Ciencias" of the IES Sánchez Albornoz (León, 2013-2021) making available by using a set of experiments under the title "the plant factory", and by tutoring of introductory research works; the creation of the student-run radio programme "Hablando en verde" and a theatre play that told the story of Plant Biotechnology; the implementation of scientific congresses in the classroom for the subjects "Applied Plant Physiology" and "Plant Biotechnology"; the launch of scientific writing workshops for students; the setting up of mini-videos produced by students; the release of gamification projects such as "El desafío" or the quiz "Dolly vs. Bt"; the promotion of service-learning experiences for students at science fair presentations (Expociencia); as well as problem-based learning (PBL) through plant biotechnological challenges. Together these activities have led to the publication of the book "Experimentos fascinantes con plantas" (2019) and "Fascinating experiments with plants" (2021), the creation of a website and a Youtube channel named "Savia Sabia" and the launch of the university journal of scientific divulgation "AmbioCiencias", hosted by Universidad de León.
In short, this methodology based on fascination and experimentation is helping to generate

an ideal environment for the transmission of knowledge and skills in plant biology.

Key words: experiments, Fascination of Plants Day, gamification, PBLs, service-learning.

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THE TAKE-HOME EXAM IS A VALUABLE ASSESSMENT TOOL IN MASTER DEGREES' COURSES IN PLANT SCIENCES

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Abstract: The Take Home Exam (THE) has been used in Anglo-Saxon universities for the last decades as an evaluation tool in subjects of bachelor and master degree. In a THE, the fill-up time is usually extended from overnight to several weeks, in which students do a non-proctored exam at any location they choose. THE involves taking home a questionary in which we request to explain the concepts related to those in the bibliography. Although the limiting factor is the availability of time that students have to complete the exam, the extended time limit allows to test more complex and open-ended questions and alleviate stress on the students. As opposed to other home assignments, THEs have a decisive impact on the students' grade.

In our master's course called *Agro-environmental Factors of Abiotic Stress in Plants*, the THE methodology was implemented in 2010 with a value of 40% of the final mark of the course. Our evaluation system entails monitoring students in theoretical and practical sessions, as well as in the conduction of an oral presentation of both a laboratory report and a research article. With these assignments, along with the written THE, we evaluate the knowledge that every student acquires about the subject. In this communication, a five-year follow-up study of the marks is performed. Each assessed part evidenced a strong correlation of THE results with the final subject mark (0.85). Accordingly, THE mark shows the highest correlation with the global punctuation of the master´s degree (0.76), followed by the practical report (0.58) and the individual article assessment (0.45). A principal component analysis (PCA) revealed that the PC1 explained 71.4% of the total variation, where THE (-0.54), the practical report (-0.63) and the individual assessment (-0.56) were balanced driven factors. Moreover, in this communication, some student suggestions for improvement of the course are presented along.

We can conclude that THE is an advantageous methodology for master degrees since it promotes higher-order thinking skills and reduces student stress, while it represents a realistic way of evaluating since THE results are in accordance with the global course mark of students.

Keywords: Take-home exam, evaluation methodology.

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THE WHOLE WORLD IN A GLASS JAR: THE USE OF PLANT MICROPROPAGATION AS AN EDUCATIONAL TOOL

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Photosynthetic organisms perform unique biochemical processes which sustain life in our planet. Besides, they form part of special ecosystems such as forests, which play essential roles in carbon sequestration, soil and water quality preservation and biodiversity conservation.

The group of Biotechnology of Woody Plants of the Galician Institute of Agrobiological Research is committed to promoting basic scientific knowledge and environmental awareness by performing educational and dissemination activities. We use micropropagated trees to address scientific concepts such as photosynthesis, mixophotoautotrophy, genetic diversity and gene expression, as well as current social challenges as climate change mitigation and germplasm conservation.

Our educational activities are classified in 5 groups:

1) Scientific workshops for students from primary and secondary schools (in our labs and in rural schools), with the specific goal of stimulating vocational interest in science.
2) Training courses (3 months) for students of vocational training.
3) Training courses (1 month) for undergraduate and master students.
4) Supervision of doctoral, master and degree projects.
5) Participation in actions promoted by international organizations, such as the Fascination of Plants Day (18 May, EPSO), or the International Day of Women and Girls in Science (11February, UNESCO and UN-Women).

For dissemination activities, we take our research to the "real world", seeking the interaction with people of all ages and scientific background. We use "travelling forests": glass containers with *in vitro* cultures of trees as oak, cork oak, birch, chestnut, willow, cherry... We use them for talking about plant physiology, photosynthesis, light, oxygen and carbon dioxide, soil, water, ecosystems, genes, biotechnology and the conservation of biodiversity, etc. The activities are designed to promote the participation of people, as they

can touch the plants, the culture medium, laboratory recipients, and can share their opinions and feelings about forests, nature, trees... We prepare games for adults and children, using seeds, leaves, balloons…

We take our travelling forests to popular festivals, performing “scientific events” at the street, to exhibition fairs, to book presentations and even to bars. Our aim is to show the wonder of nature to all people, since we think that we only preserve what we know and love.

Key words: Biodiversity conservation, Biotechnology, Dissemination, Micropropagation, Woody species

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