S08O01

Polyamines: a key player in stress response

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Polyamines, mainly putrescine, spermidine and their diamine precursor spermine, are polycationic low-molecular-mass aliphatic amines that are ubiquitously distributed in living organisms. Polyamines have been shown to be closely implicated in plant biotic and abiotic stress response. In the last years we have carried out work to identify the role of polyamines in stress response of citrus or its closely related genus. The major work is following. First, analysis of polyamine levels in different tissues under stressful condition. Second, investigation of exogenously applied polyamines on the enhancement of dehydration tolerance. Third, molecular cloning and functional characterization of polyamine biosynthetic genes, such as *arginine decarboxylase* (*ADC*) and *S-adenosylmethionine decarboxylase* (*SAMDC*). Last, polyamine biosynthetic genes were used for genetic engineering in an effort to producing novel germplasms with enhanced stress tolerance. Our data showed that *PtADC* from *Poncirus trifoliata* was upregulated by different types of stresses, including salt, low temperature, dehydration. Ectopic expression of *PtADC* gene conferred enhanced tolerance to different stresses in transgenic plants. All these results show that polyamines are important players that can be manipulated to increase stress tolerance in citrus.

S08O02

'*Omics*' and chemical approaches used to monitor iron-deficiency in citrus rootstocks

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Two different '*omics*' approaches were performed to a better comprehension of biological mechanisms involved in citrus iron deficiency. Tips roots from 'Swingle' citrumelo and 'Carrizo' citrange (sensitive and tolerant rootstocks, respectively), growing in pots with control and chlorotic soil, were used for transcriptomic and proteomic analysis. CombiMatrix array was performed to isolate differential genes, among which *glutathione peroxidase* and *SAUR* gene showed to be the most involved ones. They were switched on 'Swingle' citrumelo grown on calcareous conditions compared to 'Carrizo' citrange (in the same soil) and to the same stock in the control soil. The over-expression of peroxidase could be the effort of plants to neutralize the oxidative environment produced by stress. The involvement of auxin in the regulation of Fe deficiency responses is also well known. Both genes, together with peroxidase and ferric chelate reductase activities and iron and chlorophyll content, were used as markers to monitor the degree of suffering on seven tolerant and sensitive rootstocks growing on the field under natural chlorotic conditions. Among differentially expressed proteins, isolated using 2D-PAGE and RP-HPLC/nESI-MSMS, a strong down-regulation of cytosolic pyrophosphate-dependend phosphofructokinase beta subunit and NADPH-isocitrate dehydrogenase could produce plant inability to sustain the energetic request of cell roots.

S08O03

Root protein interatomic network obtained from citrus seedlings subjected to water deficit

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Citrus Brazilian industry may be strongly affected by drought. Characterize rootstock behavior under different environmental conditions is crucial, since rootstocks may confer better adaption to environmental stresses. Plant responses to drought require the production of important functional and regulatory proteins. In the

present study, we identified proteins related to water deficit response in tolerant ('Rangpur' lime, *Citrus limonia*) and susceptible ('Sunki Maravilha' mandarin, *Citrus sunki*) genotypes. From 105 differentially expressed proteins, twenty-nine proteins from roots of both genotypes were identified and sequenced by mass spectrometry. Protein interaction analysis of orthologous proteins from *A. thaliana* protein expressed in the two citrus varieties was obtained by ontological and cluster analysis.Among the 16 amino acid sequences found in 'Rangpur' lime and 'Sunki Maravilha' mandarin, 13 proteins were found in *A. thaliana* via BLAST, of which, 12 showed protein-protein interaction. It is possible to group these 12 proteins into a single network interatomic, comprising 3302 proteins. Among the different biological processes found in the protein networks, regulation of response to water deficit and osmotic stress, signaling pathway of abscisic acid were highlighted. A point to take into account is the importance role of the DREB2, a protein hub/bottleneck which participate in the stimulus and the response to water stress.

S08O04

Hormone and metabolite traits related to abiotic stress tolerance in citrus

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Plants of the citrus rootstocks 'Carrizo' citrange (CC, soil flooding tolerant) and 'Cleopatra' mandarin (CM, drought tolerant) were subjected to water stress and soil flooding conditions to study root responses associated to tolerance. Responses could be classified in groups which different involvement in plant tolerance. Some of them, such as the increase in proline levels, were common to the two species and the two stress conditions assayed. Other, as the abscisic acid and jasmonic acid signals or the phenylpropanoid profiles followed similar trends in both species varying with the stress imposed. Finally, other responses as the increase in salicylic acid were specific of each genotype and stress situation. Moreover, following a metabolomics approach, it was showed that the altered compounds in response to each adverse condition had a low degree of overlapping which accounts for the specificity of the plant response to the different environmental restraints. Finally, under control conditions, different metabolites had higher levels in CM than CC which suggested that pre-existent defenses are important as a stress tolerance trait.

S08O05

The adaptation of 'Sunki Maravilha' mandarin to drought depends on the ABA accumulation balance between roots and canopy

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Abscisic acid (ABA) is an important plant hormone that participates in the adaptation to many abiotic stresses such as drought. The 9-*cis*-epoxycarotenoid dioxygenase (*NCED*) is the key enzyme of ABA biosynthesis in higher plants. The importance of ABA in two citrus varieties of rootstock, the 'Rangpur' lime and the 'Sunki Maravilha' mandarin, cultivated on hydric restriction by expression analysis of five *NCED* genes and correlation with the accumulation of ABA were evaluated. In the 'Rangpur' lime the profile of ABA accumulation ratifies that perception and induction of adaptation signals against water stress occurs from the roots and leads to a decrease in transpiration. However, this decrease is not sufficient to reduce the water flow from roots to canopy keeping its active growth even in conditions of low water availability. Instead, the adaptation of 'Sunki Maravilha' mandarin depends on the balance of ABA accumulation between root and canopy which increases with water restriction, inducing a decrease in transpiration rate and water flow from roots to canopy. Under drought, water requirement for 'Sunki Maravilha' mandarin decreases and it adapts to a lower availability of water in the soil. The use of this mandarin in combination with a scion variety with characteristics similar to its own, that responds to stress by water deficit with ABA accumulation in leaves, may have good drought tolerance under field conditions. In this study we correlated the *NCEDs* with ABA accumulation.