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Biotic and Abiotic Stress Tolerance in Plants: the Challenge for the 21st Century

BOOK OF ABSTRACTS

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S02P13

Identification and characterization of genes involved in ABA perception and signal transduction in *Coffea* spp.

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The *Coffea* genus represents a major agricultural commodity in world trade. Nowadays, drought and elevated temperatures are the major climatic limitations for coffee production. These variations also influence biochemical composition of beans, affecting directly the final cup quality. There is genetic variability within the *Coffea* genus that could be used to increase drought tolerance and generate coffee varieties better adapted to climatic variations. Abscisic acid (ABA) is a vital plant hormone acting as central regulator that protects plants against abiotic stresses such as drought. Recently, novel intracellular ABA receptors (PYL/RCARs) involved in ABA sensing and signaling have been identified in several species. A mechanism of ABA transduction has been proposed, involving PYR/PYL/RCARs receptors interacting with PP2Cs phosphatases and SnRK2 protein kinases. The goal of this study was to identify and characterize ortholog genes of this tripartite system in *Coffea* sp. For this purpose, protein sequences from *Arabidopsis*, citrus, rice, grape, and tomato were chosen as query to search ortholog genes in the coffee-sequence database. Using 51 PYR/PYL/RCAR sequences from those plant species, it was possible to identify 9 sequences for ABA receptors in coffee. Likewise, the 40 and 29 sequences query resulted in 6 and 9 similar sequences of PP2Cs and SnRK2 specific to ABA in *Coffea* sp. The 24 genes isolated, that belong to the tripartite system of the coffee's ABA pathway, showed *in silico* differential expression in tissues as leaves, seeds, roots and floral organs. Polymorphisms were found among the orthologs and homeologs genes. All analyses allowed the identification in *C. arabica* genome of sequences variations between the two ancestral diploid sub-genomes, *C. canephora* (*CaCc*) and *C. eugenioides* (*CaCe*). Further analyses will predict the functional effect of these polymorphisms in protein structure in different coffee species. All these evidences will also help us to identify the genetic determinism of drought tolerance essential to obtain molecular markers that could be used in coffee-breeding programs.

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S02P14

Physiological and molecular responses of diploid and tetraploid Carrizo Citrange under water stress

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In citrus, the use rootstock promotes productivity, improves fruit quality and may confer resistance or tolerance to biotic and abiotic stress. 'Carrizo' citrange (*Citrus sinensis* [L.] Osbeck × *Poncirus trifoliata* [L.] Raf), is one of the most popular rootstock in the Mediterranean basin. It is sensitive to drought and salt stress but confers tolerance to Tristeza virus, and promotes very good fruit quality. Previous studies have shown that doubled diploid (4x) 'Rangpur' lime (*Citrus limonia*, Osbeck) seedlings are more tolerant to water deficit than their respective diploid (2x). In the present work, we have characterized the water deficit tolerance in 2x and 4x 'Carrizo' citrange seedlings. Water deficit was applied for 35 days, followed by irrigation. Several physiological parameters were measured periodically during the experiment and samples were collected to investigate i) the activity of enzymes involved in detoxification processes, ii) the expression analysis of candidate genes involved in ABA biosynthesis, as well as iii) ABA and H_2O_2 production. Doubled diploid 'Carrizo' citrange seedlings were showed to be more drought tolerant than 2x. Water deficit caused a greater reduction in photosynthetic rates and stomatal conductance in 2x compared to 4x. Also higher ABA and H_2O_2 production were induced in 2x

when compared to 4x. The better tolerance of 4x seedlings is discussed to the light of candidate genes expression analysis and activities of enzymes of detoxification.
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S03P01
Functional analysis of a tonoplast intrinsic protein (TIP) gene involved in the response to osmotic stress in citrus
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Tonoplast intrinsic proteins (TIPs) are a subfamily of aquaporins (AQPs), belonging to the major intrinsic protein (MIPs) family, that function as transmembrane channels for water and other small molecules of physiological significance. TIPs have been implicated in the regulation of the water and nutrient balance in plants and in their adaptation to stressful environmental conditions. By mining the citrus genome-wide EST database, a key tonoplast AQP gene was identified within the large family of citrus MIPs based on its induction in response to drought, salt and/or nutritional stresses. This study aimed to characterize this TIP gene (*CsTIP2;1*) candidate for involvement in water relations and drought- and salt-stress tolerance in citrus. The steady-state mRNA levels of *CsTIP2;1* were examined in roots and leaves of irrigated and drought-stressed drought-tolerant ('Rangpur' lime) and -sensitive (Sunki 'Maravilha' mandarin) citrus rootstocks, by quantitative real-time PCR (qPCR). The complete sequence of the gene *CsTIP2;1* was also cloned, using a TA cloning system, and subsequently subcloned for generating the cassette of constitutive expression *CaMV35S:CsDTIP*. This expression cassette was then removed from the plasmid and inserted into the binary vector pCAMBIA 2301. This vector was introduced in the strain *Agrobacterium tumefaciens* EHA-105 and used in experiments of genetic transformation of tobacco. Transgenic plants were acclimatized in greenhouse and, after their complete development, the seeds of T_1 generation were collected for further analysis. The results showed that *CsTIP2;1* expression is highly and differentially induced in roots and leaves of drought-stressed drought-tolerant ('Rangpur' lime) and -sensitive (Sunki 'Maravilha' mandarin) citrus rootstocks. *CsTIP2;1* overexpression in transgenic tobacco increased the plant biomass under favorable growth conditions and significantly improved the tolerance of transgenic plants to dehydration and salt stresses. The enhanced tolerance of the transgenic plants was correlated with the inhibition of H_2O_2 accumulation. Taken together, these results suggest a physiological role for CsTIP2;1 in plant adaptation to drought and salt stresses, as a mediator of the maintenance of better water status and less oxidative damage in the plant tissues. CsTIP2;1 may thus be potentially useful for engineering drought and salt tolerant citrus rootstocks.
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S03P02
Genetic transformation of tomato mediated *Agrobacterium tumefaciens.*
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The "fusarium wilt", caused by the fungus *Fusarium oxysporum* f. sp. *lycopersici* is a disease that occurs in all regions of the world where tomatoes are grown and can manifest in any of the stages of plant development. The genetic transformation of plants is an essential biotechnological tool for breeding by the introduction of exogenous genes, maintaining of original characteristics of the variety and shortening the time required to obtain a new cultivar. One of the biggest obstacles to maintaining this resistance is the search for target genes involved in plants defense and monitoring the variability of phytopathogens.Some studies with transgenic plants had elevated expression levels of chitinase,