

PROTEINS IDENTIFICATION BY MASS SPECTROMETRY IN TRANSGENIC CITRUS ROOTSTOCK UNDER WATER DEFICIT

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Citrus rootstocks are closely associated with orchard competitiveness by affecting various important agronomic characteristics. Swingle citrumelo plants [*Citrus paradisi* Macfad. cv. Duncan x *Poncirus trifoliata* (L.) Raf.] expressing the mutant gene *P5CSF129A* of *Vigna acontifolia*, that codes for the enzyme Δ 1-pirrolina-5-carboxilato synthetase (P5CS), accumulate high free proline. This rootstock has been shown to present greater tolerance to water deficit when compared to non transformed plants. The objective of this work was to identify differentially expressed proteins in leaves of Swingle citrumelo transgenic plants with high endogenous proline, due to the constitutive expression of the transgene *P5CSF129A*, under normal water supply conditions and when subjected to water deficit. Comparisons between non transformed plants under irrigated and stressed conditions were carried out to identify proteins expressed in Swingle citrumelo due to the establishment of water stress. The differential protein expression caused only by high proline accumulation was performed comparing control and transgenic plants under irrigated condition. Comparisons of irrigated with stressed transgenic plants were done to identify differentially expressed proteins in plants with high proline accumulation when submitted to water deficit. The level of free proline in leaf samples under normal water supply was about 2.5-fold in transgenic plants than in control plants. With the stress imposition, proline levels remained almost invariable in transgenic plants (about 350 $\mu\text{mol g. MF}^{-1}$) and increased 40% in control plants (175 $\mu\text{mol g. MF}^{-1}$). For proteomic analysis, total leaf proteins were extracted by phenol/SDS method and separated by bi-dimensional electrophoresis using IPG strips of 13 cm with pH gradient immobilized at 4-7 in the first dimension for further separation by SDS PAGE. The 2D-gel images were analyzed by ImageMaster Platinum 6.0 and by mass spectrometer MALDI-TOF-TOF. The searches made in Mascot for PMF or by direct sequence comparison of the de novo sequencing data with the NCBI database enabled the identification of 17 proteins. Subunit β ATP synthase, taxane 2-alpha-O-benzoil transferase, chlorophyll a/b binding protein, RNA binding protein glycine rich, alcohol dehydrogenase, growth promoter auxin-independent and rubisco activase were differentially expressed between non transformed plants under irrigated and drought. Over accumulation of proline under normal water supply increased the expression of a hemoglobin and miraculin proteins.