

GENOTYPE-RELATED COLD RESPONSES IN LEAVES AND FRUITS OF *OLEA EUROPAEA* L.

S. D'ANGELI¹, M. MATTEUCCI¹, S. ERRICO², R. LAMANNA², G. PERROTTA², M.M. ALTAMURA^{1*}

¹ Dipartimento di Biologia Ambientale, "Sapienza" Università di Roma, P.le A.Moro, n.5, 00185 Roma.

*mariamaddalena.altamura@uniroma1.it; ²Biotec Lab., UTT Trisaia ENEA Rotondella, Matera.

Olea europaea L. is an evergreen species that ranks sixth in the world's production of vegetable oils. Since ancient times it has been cultivated in the Mediterranean Basin, where about 2600 cultivars have been identified based on morphological traits. Olive tree is a warm-temperature tree with low tolerance to frost, although cultivars which differ in terms of cold acclimation have been empirically selected. Cold acclimation involves the acquisition of freezing-tolerance by exposure to non-freezing low temperatures. It is a complex process requiring accumulation of cryoprotectants and physical and biochemical restructuring of cell membranes. In numerous species free cytosolic calcium is involved in cold acclimation. The first objective of our study was to measure cytosolic calcium signalling in the olive tree during cold acclimation and to assess the possibility of using cytosolic calcium as an early genotype-selection marker for cold susceptibility. To this end, non-cold-acclimated and cold-acclimated leaf protoplasts of cultivars empirically known for a different cold susceptibility were analysed. The results showed that olive tree protoplasts respond to rapid temperature decreases with transient increases in cytosolic calcium involving both an efflux of the ion from the organelles and an influx through the plasmamembrane. In the acclimated protoplasts, calcium transients are reduced or inhibited depending on the cold susceptibility of the cultivar (D'Angeli *et al.*, 2003). Thus, changes in cytosolic calcium is necessary for the adaptative response of this evergreen plant without vegetative dormancy, with differences among the genotypes. Moreover, it is known that cold stress causes cell membrane rigidification and depolarization, together with early cytoskeleton depolymerization, all of which associated with changes in cytosolic calcium (D'Angeli and Altamura, 2007, and references therein). We have demonstrated that osmotin, a pathogenic-related protein, is produced in the leaves of olive tree cold-resistant genotypes during the cold acclimation period, and is also involved in regulating cytoskeleton dynamics and in blocking calcium transients in leaf protoplasts (D'Angeli and Altamura, 2007). The ability to adjust membrane lipid fluidity by changing the levels of unsaturated fatty acids is another feature of cold-responsive species, and is mainly provided by the regulated activity of fatty acid desaturases (FADs). The drupe of the olive tree represents an interesting system to investigate calcium transients and fatty acid metabolism changes related to cold, because the fruits, as the leaves, have to experience cold during the winter, and at the same time have to produce the oil, whose unsaturated fatty acid fraction contributes to the quality. Presently, we are investigating the relationship among development, cold response, expression of genes coding for FADs, and oil composition in olive tree drupes belonging to genotypes differing for cold tolerance in the leaves, but producing good oil. Transient increases in cytosolic calcium levels in response to cold were observed in the mesocarp cells of all the genotypes up to the carbohydrate metabolism prevailed over the lipid metabolism in the drupe. Cold sensitivity of the same cells decreased during the oil production phase, and in a genotype-dependent manner. Differences in the expression levels of *FAD2.2* and *FAD7*, in particular, occurred after cold application during the oil production phase in response to genotype and fruit development. The unsaturated fatty acids also changed in a genotype-dependent manner in cold-shocked drupes. Taken together, the results show a relationship between fruit development and *FAD* expression and oil desaturation in response to cold stress and genotype cold-hardiness (Matteucci *et al.*, 2011). The relationship between the cold response of the pulp cells and the seed cells, and the cryoprotectants involved in seed cold responsiveness and embryo dormancy, are under investigation.

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