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Oxidative browning in 'Koroneiki' olive explants as influenced by oxidative enzyme activities and endogenous phenolic compounds

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SUMMARY
Shoot tips and single-node explants from glasshouse and field growing 'Koroneiki' olive trees were assayed for their browning potential at the establishment stage *in vitro*, during the growing season. Measurements of the total phenol content, the total o-diphenol content, polyphenoloxidase and peroxidase activity were conducted, to determine the relationship of these factors to the browning of explants. High positive correlation coefficients were found between total phenol, o-diphenol content and polyphenoloxidase activity with the browning percentage of olive explants. Shoot tips from both sources of plant material exhibited higher browning rates together with higher total phenol, o-diphenol content and polyphenoloxidase activity than single node explants. Explants deriving from trees growing in the glasshouse presented higher survival rates and lower values of the three factors mentioned before, than the corresponding explants from field growing trees. Single nodes from glasshouse olive shoots proved to be superior to all other explants during the establishment stage, concerning the survival rate, as they presented the minimum phenol content and polyphenoloxidase activity.

A major problem in the *in vitro* culture of many woody plant species is the browning of explants during the establishment stage, which results in great losses of plant material. The browning reaction of explants has been attributed to the oxidation of phenolic compounds – especially o-diphenols – and the production of brown quinones, which are in most cases toxic to the explants (Thomas and Ravindra, 1997).

Many attempts to alleviate the problem of explant browning deal with the use of antioxidants, but they are not always reliably effective (Modgil *et al*., 1999; Bhatt and Dhar, 2000). Failure of antioxidant treatment can be attributed to the failure to control the interaction of the endogenous phenolic compounds with oxidative enzymes. The limiting factor for the induction of browning is not always the same, which makes its control difficult (Castaner *et al*., 1999).

In olive (*Olea europaea* L.) many attempts to culture meristems fail, due to the rapid oxidation of explants. Even the most active antioxidants fail to ensure a satisfactory survival rate (Rugini, 1986; Canas *et al*., 1992). The thorough selection of the stock material is often recommended as a solution to the browning of explant during the establishment stage (Amin Dalal *et al*., 1992). Attempts aiming to reduce the endogenous phenolic content of the explants, are often the best treatment to achieve high survival rates. Shading the mother plants or growing them in a glasshouse has often proved to be successful in limiting the problem of explant browning (Yu and Meredith, 1986).

In this study, we examine the possible contribution of phenolic compounds and oxidative enzymes in olive node and shoot-tip explant browning, as well as the possible selection of olive explants, which present the lowest browning rate, based on their endogenous phenolic composition and enzyme activities.

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MATERIALS AND METHODS

Plant material

The experiment was conducted during the growing season (March–November) of two successive years (1998–1999) with two groups of trees in each year. The first group consisted of trees growing in the field while the second group of trees was in a glasshouse. In the first group, seven 30 year old 'Koroneiki' olive trees were used. The second group consisted of 15, 4–5 year old trees. Young vigorous shoots suitable for explant sources, growing on the trunk or on pruned branches were collected from all sides of the tree in both groups, to avoid any effect of light on phenolic composition, at one-month intervals during 1998 and 15 d intervals during 1999. The shoots were transferred to the laboratory immediately after collection.

Preparation of the explants

The two kinds of explants used in this experiment were single node and shoot tip explants, from outdoor and indoor growing trees, approximately 1 cm long with excised leaves.

Measurement of browning

Approximately 30 explants from each kind – single node and shoot tip explants - per group of trees were surface sterilized with 10% (v/v) sodium hypochlorite (BDH) plus three drops of Tween 20 as wetter, for 10 min. After sterilization, the explants were washed at least three times with sterile distilled water to remove