

Adventitious root formation in olive (*Olea europaea* L.) microshoots: anatomical evaluation and associated biochemical changes in peroxidase and polyphenol oxidase activities

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SUMMARY

Trials were performed using *in vitro*-cultured microshoots of the olive (*Olea europaea* L.) cultivar ‘Galega vulgar’, as initial explants, to identify histological events and modifications in peroxidase and polyphenol oxidase activities during adventitious root formation. Explant bases were submitted to a 10 s quick-dip treatment to promote rooting, using a sterile solution of 14.7 mM indole-3-butyric acid (IBA). Samples for histology and quantification of enzyme activities were collected at pre-established periods from 0 to 720 h. The first signs of modifications in stem cell morphology were observed 96 h after explant inoculation on olive culture medium (OM), with some cortical cells showing a dense cytoplasm and a large central nucleus, with visible nucleoli. The first mitotic events were observed after 144 h and evolved *via* two different pathways: non-specific cell division, leading to callus formation; and organised cell division, leading to the formation of root meristemoids. After 456 h, the first organised root primordia became visible. No root formation was achieved without earlier callus development, and 89% of root primordia originated from tissues other than cambial/phloem tissue. Peroxidase and polyphenol oxidase activities were recorded throughout the whole rooting process. The first significant modification in enzyme activity, with a drop from 0.19 to 0.14 ΔA_{490} units min^{-1} 50 mg^{-1} of explant material, was observed for peroxidase within the first 4 h after IBA treatment. Subsequent changes in both enzyme activities could be correlated with different phases of the adventitious rooting process.

Considerable progress has been made in the last 20–30 years towards understanding rooting by characterising it as an evolutionary process consisting of a successive series of interdependent phases (i.e., induction, initiation, and expression), each having specific physiological and environmental requirements (Moncousin *et al.*, 1988; Gaspar *et al.*, 1992; Rout *et al.*, 2000).

Adventitious roots originate *via* the redifferentiation of several cell types such as those from sub-epidermal tissues, the cortex, cambium, secondary phloem, pericycle, or vascular bundles. In olive, the capacity to develop adventitious roots has proved to be extremely variable among cultivars (Salama *et al.*, 1987; El-Said *et al.*, 1990; Fouad *et al.*, 1990). Differences in the anatomical structure of cuttings were proposed to explain this dependence on genotype, with several authors stating that the presence of a continuous ring of sclerenchyma, between the phloem and the cortex, may act as a mechanical barrier to root emergence (Salama *et al.*, 1987; Qrunfleh *et al.*, 1994). Nevertheless, other reports have provided evidence that the difficulty in rooting olive cuttings could not be correlated with the

anatomical structure of the cutting, and that genetic, biochemical, or physiological causes, rather than anatomical ones, could be related to the incapacity of several olive cultivars to form adventitious roots (Bakr *et al.*, 1977; Fabbri, 1980).

Several studies on adventitious root formation have highlighted the important role that oxidative enzymes such as peroxidases (POX) and polyphenol oxidases (PPO) play in this process (Moncousin and Gaspar, 1983; Berthon *et al.*, 1989; Gaspar *et al.*, 1992; Rival *et al.*, 1997; Rout *et al.*, 1999; Cheniany *et al.*, 2010; Fu *et al.*, 2011).

Plant peroxidases (POX; E.C. 1.11.1.7) are haem-containing enzymes that catalyse the oxidation of a diverse group of organic compounds. Studies on adventitious root formation have shown that POX isoenzymes play a fundamental role in the rooting of cuttings, with changes in POX activity often being used as a biochemical marker for the rooting process (Gaspar *et al.*, 1992; Metaxas *et al.*, 2004; Syros *et al.*, 2004; Hatzilazarou *et al.*, 2006).

Typically, the minimum POX activity appears at the root induction phase, while a subsequent increase, reaching a peak of activity, marks the end of root initiation and the start of the root development phase (Gaspar *et al.*, 1992). Chao *et al.* (2001) reported that a

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