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ORIGINAL PAPER

Involvement of alternative oxidase (AOX) in adventitious rooting of *Olea europaea* L. microshoots is linked to adaptive phenylpropanoid and lignin metabolism

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Abstract Alternative oxidase (AOX) has been proposed as a functional marker candidate in a number of events involving cell differentiation, including rooting efficiency in semi-hardwood shoot cuttings of olive (*Olea europaea* L.). To ascertain the general importance of AOX in olive rooting, the auxin-induced rooting process was studied in an in vitro system for microshoot propagation. Inhibition of AOX by salicylhydroxamic acid (SHAM) significantly reduced rooting efficiency. However, the inhibitor failed to exhibit any effect on the preceding calli stage. This makes the system appropriate for distinguishing dedifferentiation and de novo differentiation during root induction. Metabolite analyses of microshoots showed that total phenolics, total flavonoids and lignin contents were significantly reduced upon SHAM treatment. It was concluded that the influence of alternative respiration on root formation was associated to adaptive phenylpropanoid and lignin metabolism. Transcript profiles of two olive *AOX* genes (*OeAOX1a* and *OeAOX2*) were examined during the process of auxin-induced root induction. Both genes displayed stable transcript accumulation in semi-quantitative RT-PCR analysis during all experimental stages. In contrary, when the reverse primer for *OeAOX2* was designed from the 3′-UTR instead of the ORF, differential transcript accumulation was observed suggesting posttranscriptional regulation of *OeAOX2* during metabolic acclimation. This result confirms former observations in olive semi-hardwood shoot cuttings on differential *OeAOX2* expression during root induction. It further points to the importance of future studies on the functional role of sequence and length polymorphisms in the 3′-UTR of this gene.
Key message The manuscript reports the general importance of AOX in olive adventitious rooting and the association of alternative respiration to adaptive phenylpropanoid and lignin metabolism.

Keywords Alternative oxidase · Flavonoids · Lignin · *Olea europaea* · Salicylhydroxamic acid · Total phenolics

Abbreviation

AOX	Alternative oxidase
Cyt	Cytochrome
DMSO	Dimethyl sulfoxide
IBA	Indole-3-butyric acid
ROS	Reactive oxygen species
SHAM	Salicylhydroxamic acid
SQ-RT-PCR	Semi-quantitative RT-PCR
TGA	Thioglycolic acid
UTR	Untranslated region

Introduction

Olive (*Olea europaea* L.) is one of the oldest agricultural tree crops in the world and the source of olive oil which possesses health promoting properties (Bracci et al. 2011). Olive trees show high variation in rooting efficiencies and

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