

Expression studies of AOX during adventitious root induction in *Olea europaea* L.

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Abstract

Recently, identification of four alternative oxidase (AOX) gene sequences from olive (*Olea europaea* L.) has been reported from our group. Three genes could be classified as members of the sub-family AOX1 and one as belonging to the sub-family of AOX2. AOX has been proposed as functional marker candidate for easy-to-root olive genotypes. Validation of AOX as an appropriate marker candidate needs to highlight a close relationship between AOX expression and AOX activity to root induction and/or initiation. Here we present AOX gene expression data during IBA treatments of olive shoot cuttings and the result of studies on the effect of AOX inhibitors and stimulators. Furthermore, variable 3'-UTRs are presented as a source for differential gene regulation in the *OeAOX2* gene.

Key words: *Olea europaea* L., adventitious rooting, AOX gene expression, 3'-UTRs, functional marker

Études d'expression de l'AOX pendant l'induction de l'enracinement adventive en *Olea europaea* L.

Résumé

L'identification de quatre séquences du gène de l'alternative oxidase (AOX) d'olivier (*Olea europaea* L.) a été rapportée récemment de notre groupe. Trois gènes ont pu être classifiés comme membres de la sous-famille AOX1 et un autre comme appartenant à la sous-famille AOX2. L'AOX a été proposé comme marqueur fonctionnel pour l'identification des génotypes d'olivier à enracinement facile par bouturage. De toute façon, la validation d'AOX en tant que candidat à marqueur doit se baser sur un étroit rapport entre son l'expression et son activité, concernant l'induction et/ou le déclenchement du enracinement adventive. Ici nous présentons des données d'expression du gène d'AOX pendant des traitements de boutures d'olivier avec d'IBA et aussi le résultat des études sur l'effet des inhibiteurs et des stimulateurs d'AOX. Egalement, les variables 3' - UTRs sont aussi présentés comme source pour le réglage génétique différentiel dans le gène d'*OeAOX2*.

Mots clés: *Olea europaea* L., enracinement adventive, expression d'AOX, 3'-UTRs, marqueur fonctionnelle.

Alternative oxidase (AOX) was recently proposed as a functional marker for efficient cell reprogramming under stress (Arnholdt-Schmitt et al. 2006a). Adventitious root induction and initiation is stress-related and is being explored in olive as one example of cell-reprogramming. This approach is thus combining fundamental research related to molecular biology and cell biology and applied studies of commercial interest for cost-efficient olive tree propagation (Arnholdt-Schmitt et al. 2006b and c, see also the strategic paper on research strategies for molecular plant breeding Arnholdt-Schmitt 2005). It is thought to support the developed hypothesis in view of a functional marker candidate for easy-to-root olive genotypes to assist olive tree breeding. Validation of AOX as an appropriate source for polymorphic sequences as marker candidates that can discriminate olive trees needs to highlight a close relationship between AOX expression and AOX activity to root induction and/or initiation.

AOX is an inner mitochondrial membrane protein that functions as terminal oxidase in the alternative (cyanide-resistant) pathway of respiration where it generates water from ubiquinol (Umbach et al. 2002). AOX serves to relieve oxidative stress originating from environmental stresses by limiting mitochondrial reactive oxygen species (ROS) formation and preventing specific components of the

respiration chain from over-reduction (Popov et al. 1997, Purvis 1997) and canalizing ROS signals (Amirsadeghi et al. 2007). We hypothesize that root induction is a plant cell reaction linked to oxidative stress and that activity of stress-induced AOX is importantly involved in adventitious rooting.

Recently, Santos Macedo et al. (2006a, b, 2008a, b, and 2009) have reported the isolation of four AOX genes from olive and highlighted the existence of polymorphic sequences within the *OeAOX2* gene from diverse origins. Additionally, the group of Arnholdt-Schmitt has shown from across species studies that polymorphic plant AOX sequences have the potential to mark individual plants and varieties (Cardoso et al. 2009, Costa et al. 2009, Ferreira et al. 2009). These observations on natural AOX gene diversity and diversity in breeding lines together with supporting opinion and review papers from experts in the field of AOX research (see AOX Special Issue, 2009) encourage proceeding with this novel functional marker approach related to molecular breeding on rooting-efficient olive trees.

Experimental studies with the inhibitor of AOX protein activity, SHAM, and the two AOX stimulators, H₂O₂ and pyruvate, are giving first indications about the involvement of AOX in adventitious rooting (Santos Macedo et al. 2009). During a rooting assay with the easy-rooting cultivar 'Cobrançosa', semi-hardwood cuttings treated with SHAM (with or without IBA) revealed a significant decrease in root induction (maximal of 28%) to values observed for the negative control (without IBA treatment).

Contrarily, treatments with H₂O₂ and pyruvate revealed an increase in root induction. These physiological studies on the involvement of AOX in rooting were complemented by *OeAOX2* expression assays performed by RT-PCR. Expression was studied in tissue rings removed from semi-hardwood shoot cuttings of 'Cobrançosa' at t₀, 12h and 14 days after IBA treatment at the site of root primordial appearance during rooting assays (see Santos Macedo et al. 2009). A high expression of *OeAOX2* was observed at the stage of shoot cutting from olive trees, followed by a decrease in *OeAOX2* expression and an increase at 14 days. Two weeks after auxin treatment marks the time when first signs of root induction can be observed at histological level (Peixe et al. 2007).

Genes can be regulated at various levels. Polymorphisms in important *cis* or *trans* positions to the gene and within-gene sequences at critical positions for regulation have the potential to interfere with gene function. Thus, these positions are all candidates for functional markers that can mark different stress-related behavior of organisms if related to a gene involved in plant-environment interaction. 3'-untranslated region (3'-UTR) in genes are known to include critical sites for gene regulation, including also miRNA target sites, and can be a source for sequence signals that induce size variations at genome or transcript level (Poliodoros et al. 2009). The isolation of a partial *OeAOX2* gene sequence at gDNA level from three olive cultivars (characterized by differences of rooting behavior) allowed the identification of several polymorphisms. Insertion/deletions (InDels) and single nucleotide polymorphisms (SNPs) were identified at different gene regions – exons, intron and 3'-UTR – within and between cultivars. From the three gene regions the 3'-UTR was the most important source for polymorphisms showing 5.7% of variability. cDNA from micro shoots of 'Galega vulgar' revealed transcript length variation for the 3'-UTR of *OeAOX2* ranging between 76 and 301 bp.

The identified polymorphisms and 3'UTR length variation can be explored in future studies for effects on gene expression and stability as well as posttranscriptional and posttranslational control and a potential linkage to olive rooting phenotypes in view of marker-assisted plant selection.

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