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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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some valuable cultivars suffer from rooting rates below 20 %. Genotypic differences can be explored for the identification of functional marker candidate genes that can assist tree breeding for higher rooting rates. How genetic variation affects capabilities for rooting is unknown. Olive rooting can be seen as stress-induced reprogramming of shoot cells (Santos Macedo et al. 2009).

The plant mitochondrial electron transport chain is characterized by presence of alternative oxidase (AOX) that competes for electrons with the standard cytochrome (Cyt) pathway (Fiorani et al. 2005). AOX branches from the main respiratory chain at the level of the ubiquinone pool and catalyzes the four-electron reduction of oxygen to water without any ATP production. Thus, AOX can use excess reductant from the Cyt pathway and thereby plays a central role in determining reactive oxygen species (ROS) equilibrium in plants (Amirsadeghi et al. 2006). *AOX* is known to be induced in response to diverse abiotic and biotic plant stress conditions or respiratory chain inhibition (Vanlerberghe and McIntosh 1996). Growing evidences suggest that under stress conditions plant mitochondria generate ROS which convey to nucleus as a signal to induce the transcription of genes such as *AOX*, whose products are needed to cope with altered metabolic conditions (Maxwell et al. 1999) leading to enhanced stress tolerance capacity (Dutilleul et al. 2003). It is likely that AOX may be to modulate the strength of a stress-signaling pathway from the mitochondrion that controls cellular responses to stress and thus maintain signaling homeostasis from the mitochondrion (Vanlerberghe et al. 2009). Based on recent development on AOX research it is presumed that AOX plays a key role in regulating the process of cell reprogramming by ameliorating metabolic transitions related with the cellular redox state and the flexible carbon balance (Arnholdt-Schmitt et al. 2006a; Rasmusson et al. 2009); however, the physiological and molecular basis of such regulation remain largely unknown. Although in most plant species AOX is expressed during normal growth and development, it is often dramatically induced at the transcript level during various stress conditions (Giraud et al. 2008) and during the process of cell reprogramming (Campos et al. 2009; Frederico et al. 2009). Supplementing salicylhydroxamic acid (SHAM), a known inhibitor of AOX, can suppress various cell-reprogramming processes such as realization of somatic embryogenesis in *Daucus carota* L. (Frederico et al. 2009), rhizogenesis in *Olea europaea* L. (Santos Macedo et al. 2009), and in *Helianthus tuberosus* L. (Hase 1987). Interestingly, the influence of AOX in cell reprogramming can be observed not only in plants but also in fungi as demonstrated during mycelial to yeast differentiation in *Paracoccidioides brasiliensis* (Splend.) F.P. Almeida (Martins et al. 2011). Despite the amount of data concerning transcript accumulation of *AOX* genes during various cell-reprogramming processes, little is known about the role of AOX in metabolic reprogramming *in planta* (Moore et al. 2002).

Phenylpropanoid derivatives, especially phenolic acids and lignin are known to be crucially involved in regulating cell division and differentiation (Tamagnone et al. 1998). It has been observed that reduced phenolic acid and lignin biosynthesis inhibits cell division and differentiation process (Cvikrová et al. 2003; Palama et al. 2010). Enhanced accumulation of phenolic acids and flavonoids was found to be highly stimulating for in vitro rooting (Fu et al. 2011). Phenolic acids, flavonoids and lignin are derived from the shikimate pathway through the common phenylpropanoid pathway (Boudet et al. 1995). Substantial allocation of carbon-energy and reducing power is required for its formation (Booker and Miller 1998). The ability of AOX to affect redox and energy metabolism may serve to maintain energy charge and constant growth under a variety of changing circumstances (Moore et al. 2002).

*AOX* was proposed as functional marker candidate for efficient adventitious rooting of *Olea europaea* L. (Arnholdt-Schmitt et al. 2006a, b). Recently, Santos Macedo et al. (2009) showed that SHAM treatment significantly reduced the number of rooted semi-hardwood shoot cuttings in the easy-to-root cv. Cobrançosa and differential expression of *OeAOX2* gene was observed during auxin-induced root formation. To study the general role of AOX in olive rooting, analyses were performed in an in vitro system developed for easy propagation of the Portuguese olive cv. Galega vulgar for commercial purposes (Peixe et al. 2007). Cv. Galega vulgar is an important cultivar in Portugal that demonstrates between 5–20 % rooting in semi-hardwood shoot cuttings. However, under optimized in vitro conditions microshoots can achieve 60–90 % rooting (Peixe et al. 2010). Our results on microshoots confirm the general effect of SHAM to suppress adventitious root formation in olive. We report the inhibition of phenylpropanoids and lignin metabolism after SHAM treatment during the process of auxin-induced root formation in cv. Galega vulgar under in vitro conditions. In addition, studies on transcript accumulation of two olive *AOX* genes (*OeAOX1a* and *OeAOX2*) during the process of root induction will be presented.

## Materials and methods

### Plant material

*Olea europaea* L. microshoots of cv. Galega vulgar (clone 1441) provided by the Superior Agricultural School of Santarém (Portugal) were used to establish in vitro cultures

by micropropagation according to Peixe et al. (2007). The derived in vitro grown plantlets were used to establish the following assays.

### Analysis of SHAM effects on adventitious rooting

Indole-3-butyric acid (IBA; Sigma-Aldrich, St. Louis, MO, USA) was used as root promoting auxin. The basal part of microshoots (±1 cm) with 3–5 nodes were dipped for 10 s into IBA solution (14.7 mM) and then quickly transferred into rooting medium (Peixe et al. 2007). To study the involvement of AOX in adventitious rooting, SHAM (Sigma-Aldrich, St Louis, MI, USA) was used as AOX inhibitor. Different SHAM concentrations (1, 10 and 100 mM) were applied for 10 s either with or without previous treatment of IBA. SHAM stock solution was prepared in dimethyl sulfoxide (DMSO, Fluka, France) and used freshly. The maximum DMSO concentration used to dissolve highest SHAM concentration was 26 % (v/v). Inhibitory effect of DMSO on rooting was previously tested using IBA-treated microshoots dipped into various concentrations of aqueous DMSO solution (0.8–26 % DMSO; v/v) and then transferred to rooting medium. In each treatment, ten microshoots were taken and at least four repetitions were performed. In all experiments visible calli and root formation was recorded 56 days after transferring into rooting medium. All data in percentage was transformed in (arc sen $\frac{\sqrt{x}}{100}$) prior to ANOVA analyses and post hoc tests.

### Analyses of phenolics, flavonoids and lignin content

In order to investigate the affect of AOX in phenylpropanoid metabolism, AOX inhibition studies were carried out using following treatments: (1) DMSO in the same concentration that had been used to dissolve IBA and SHAM (negative control), (2) IBA (14.7 mM) (positive control); (3) IBA (14.7 mM) and SHAM (200 mM). The basal part of microshoots was dipped for 10 s in each solution and immediately inoculated into rooting medium (Peixe et al. 2007). To determine total phenolics, flavonoids and lignin content the basal part (±1 cm) of 40 microshoots (0.5 g) was collected as bulked sample at different time points: 0, 12 and 25 days after treatment. The assay was repeated three times, thus, inoculating in total 120 microshoots per treatment.

To quantify the total amount of phenolics the plant material was crushed in liquid nitrogen and then extracted with 3 mL of 50 % (v/v) methanol at room temperature. The suspension was homogenized for 5 min and then centrifuged at 5,000 rpm for 15 min. The supernatant was collected and used as extract to determine total soluble phenolics and total flavonoids. Total phenolic content of microshoots were measured using Folin and Ciocalteu method (Chakraborty et al. 2008). Briefly to 0.1 mL of extract, 0.5 mL Folin–Ciocalteau reagent (dilution 1:9) was added and incubated at room temperature for 5 min to initiate the reaction; after that 0.4 mL of 5% sodium carbonate was added, and the whole mixture was incubated at room temperature for further 20 min. The absorbance was measured at 765 nm using a NanoDrop-2000C (Thermo Scientific, Wilmington, DE, USA) spectrophotometer. Total phenolic content was expressed as milligrams of gallic acid equivalents per gram fresh weight.

The extraction and analyses of total flavonoids followed the methods reported by Wang et al. (2008). In brief, 0.5 mL ethanolic solution of 2 % $AlCl_3$ was added to 0.1 mL of each sample, final volume was adjusted to 1 mL with 50 % (v/v) methanol. The mixture was incubated at room temperature for 1 h and then absorbance was monitored at 420 nm. Total flavonoid content was expressed as quercetin equivalents.

Total lignin content was estimated spectrophotometrically as thioglycolic acid (TGA) derivative. Extraction and subsequent thioglycolic acid (TGA) derivatization of lignin was carried out as described by Ali et al. (2006). The amount of lignin was determined spectrophotometrically at 280 nm. The lignin content was expressed as the absorption values ($A_{280}$) measured at 280 nm using 1 N NaOH as the blank.

### Transcript quantification by semi-quantitative RT-PCR

Two *OeAOX* genes (*OeAOX1a* and *OeAOX2*) were selected for transcript accumulation studies during adventitious rooting. For *OeAOX1a*, the primer pair was designed in the ORF (specific primers used in this work are listed in Table 1). For *OeAOX2* transcript analyses, two different pairs of primer sets were used. In a first set both primers were designed in the ORF and in a second set, the forward primer was designed in ORF while the reverse primer was designed in 3′-UTR. Induction of rooting was performed in microshoots using 14.7-mM IBA following the procedure previously described. *AOX* expression analyses were performed at selected time points: (1) before IBA treatment (0 h) and after IBA treatment at 2 h, 4 h, 5 h, 12 h, 24 h, 2 days, 3 days, 6 days and 14 days for *OeAOX1a* and *OeAOX2* analysis using both primers located in the ORF; (2) 0 h, 2 h and 14 days for *OeAOX2* transcript analysis using the reverse primer located in the 3′-UTR. The basal part of four microshoots was bulked for RNA extraction and subsequent cDNA synthesis. Plant material was collected twice to perform two biological repetitions. RNeasy® plant mini kit (Qiagen, Hilden, Germany) was used for RNA extraction according to manufacturer's

**Table 1** Genes and respective primers used for SQ-RT-PCR

| Gene | Forward primer (5′–3′) | Reverse primer (5′–3′) |
|---|---|---|
| *OeAOX1a* | GACATTCATTATCAGGGA | AGTATAGTTCAGACCATT |
| *OeAOX2* (exon 3) | CCACTAGTTGTTGGTCGTGTGC | TGCTTCGTCGGCTCGGAT |
| *OeAOX2* (3′–UTR) | CCACTAGTTGTTGGTCGTGTGC | AGTAAGTGGCATGTTTCTGTAGA |
| *OeActin* | TTGCTCTCGACTATGAACAGG | CTCTCGGCCCCAATAGTAATA |

instruction. To eliminate contaminating genomic DNA, RNase-free DNase I (Fermentas, ON, Canada) was additionally applied according to manufacturer's instructions. Total RNA content was measured using NanoDrop-2000C spectrophotometer (Thermo Scientific, Wilmingtom, DE, USA) and integrity was analyzed by electrophoresis. DNase-treated RNA (2 μg) was used for cDNA synthesis with oligo d(T)18 primer using RETROscript® kit (Ambion, Austin, TX, USA) according to manufacturer's instruction. Semi-quantitative RT-PCR (SQ-RT-PCR) analysis was performed by adding 1 μL of a diluted cDNA sample (1:9) as template to the Ready-To-Go PCR Beads (GE Healthcare, Little Chalfont, England) as described before (Santos Macedo et al. 2009); two technical repeats were performed for each biological sample. *OeActin* transcript level was used as housekeeping. The RT-PCR products were analyzed by electrophoresis in a 1.4 % (w/v) agarose gel, stained with ethidium bromide and photographed with a Gene Flash Bio Imaging system (Syngene, Cambridge, UK). To compare the level of expression of *OeAOX* mRNAs against the *OeActin* reference gene, image analysis was employed using the image J (1.36b) [http://rsb.info.nih.gov/ij/] program. Image J program can calculate area and pixel value statistics of user-defined selections. The intensity of transcripts was evaluated numerically to compare easily.

## Results

### SHAM treatment suppresses rooting on olive microshoots

As expected, IBA treatment of olive microshoots of cv. Galega vulgar-induced adventitious root formation in all assays. Root induction was always preceded by calli formation at the site of hormonal treatment (Fig. 1). A short pulse of 10 s in a solution with SHAM additionally to IBA was sufficient to reduce significantly the number of adventitious roots. In a dose–response study SHAM-mediated rooting inhibition proved to be concentration dependent with a maximum of suppressed rooting at 100 mM SHAM (Table 2). The possibility of toxic effects of the SHAM solvent DMSO on adventitious rooting could be excluded because microshoots simultaneously treated with IBA and DMSO did not present a significant reduction in rooting rates, when compared with microshoots treated only with IBA (positive control), even when DMSO was applied in concentrations higher than those used to dissolve SHAM (data not presented). Also from data in Table 2 it can be seen that SHAM treatment did not significantly inhibit calli formation, even at the highest concentrations tested. These observations argue for an involvement of AOX in root initiation, however, raises at the same time the question against the involvement of AOX in the dedifferentiation process and in calli formation.

### SHAM treatment suppresses accumulation of total phenolics, total flavonoids and lignin

In order to check the influence of AOX on phenolic biosynthesis, a time-course analysis of total phenolics was carried out in IBA-treated microshoots in presence and absence of SHAM (Fig. 2). A significant increase in the accumulation of total phenolics was observed preceding root initiation in IBA-treated explants. Compared to control (0 day), 77.5 % (4.5 mg/g fresh weight) and 107 % (5.2 mg/g fresh weight) increase in total phenolics were observed at 12 and 25 days, respectively, in IBA-treated explants (Fig. 2). When SHAM was applied in conjugation with IBA, a dramatic decrease in total phenolic accumulation was observed. Compared to 0 day, only 41 % (3.35 mg/g fresh mass) and 48 % (3.5 mg/g fresh mass) increase in total phenolics were observed at 12 and 25 days, respectively, in explants treated with IBA and SHAM together. A parallel result was observed for total flavonoid accumulation in SHAM treated explants (Fig. 3). When IBA was applied alone, a 49 and 83 % increase in total flavonoid was observed at 12 and 25 days, respectively, compared to total flavonoid content at 0 day. When SHAM was applied together with IBA, the increase in total flavonoid was only 19 % (12 days) and 28 % (25 days), compared to 0 day. In negative control explants no significant changes in total phenolic and total flavonoid contents were observed over the time-course studied. These results clearly indicated a positive influence of AOX on phenolics and flavonoid biosynthesis.

In IBA-treated explants a signification increase in lignin content was observed preceding root induction (Fig. 4).

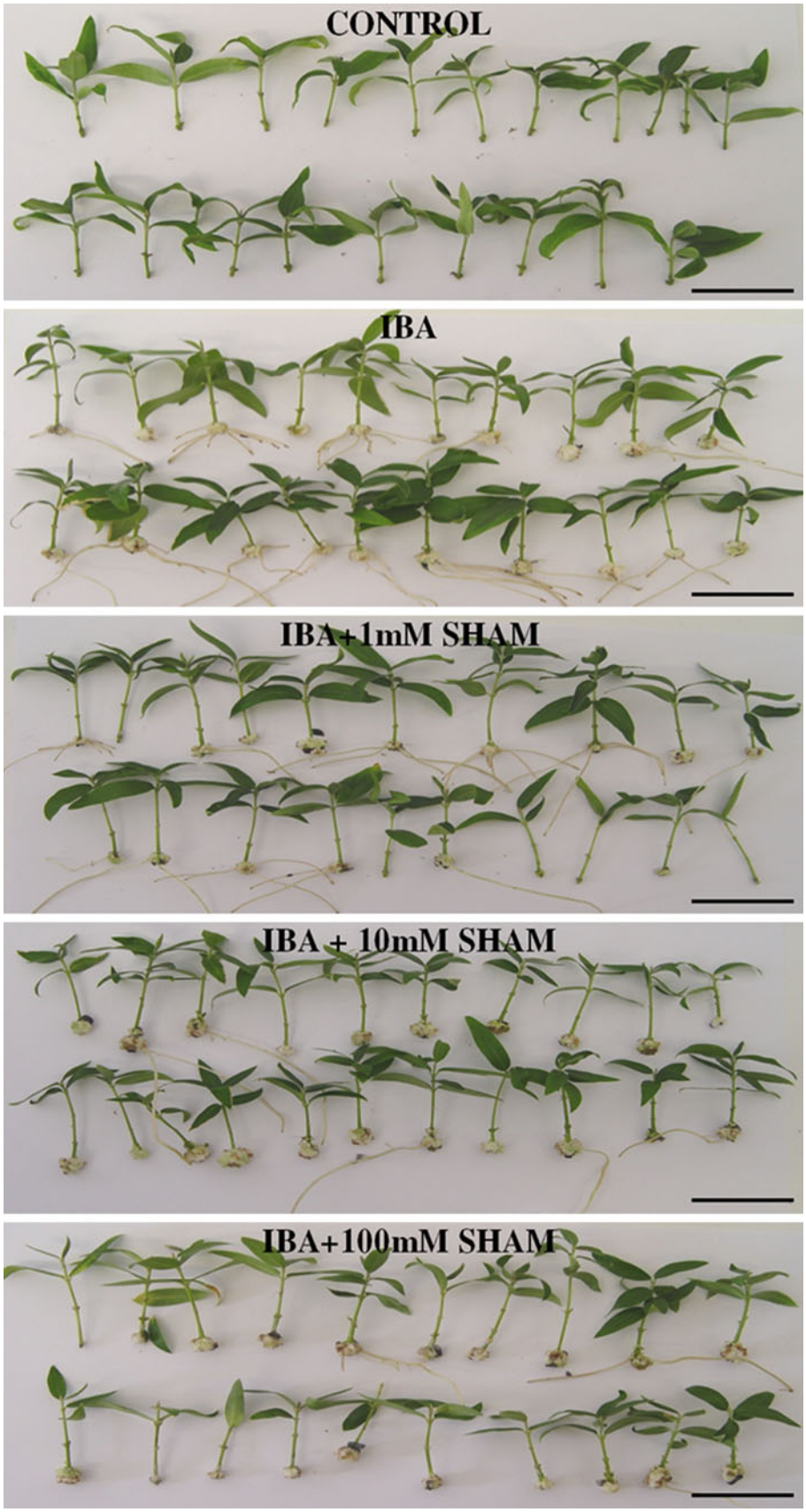


**Fig. 1** Inhibitory effect of SHAM on in vitro rooting of *Olea europaea* L. Root inhibition rate was positively correlated to SHAM concentration. IBA was applied for 10 s in a concentration of 14.7 mM. *Bar* 4 cm

**Table 2** Rooting and calli formation rates observed in microshoots during the adventitious rooting assay using SHAM as inhibitor of AOX

| Treatment | Rooting (%) | * | Callusing (%) | * |
|---|---|---|---|---|
| Control | 0 | B | 0 | D |
| IBA | 57.5 | A | 100 | A |
| 1 mM SHAM | 0 | B | 0 | D |
| 10 mM SHAM | 0 | B | 2.5 | CD |
| 100 mM SHAM | 0 | B | 22.5 | C |
| 1 mM SHAM + IBA | 47.5 | A | 85 | AB |
| 10 mM SHAM + IBA | 10 | B | 70 | B |
| 100 mM SHAM + IBA | 7.5 | B | 82.5 | AB |

Each treatment was carried out in quadruplicate sets each containing ten microshoots

* Before statistical analysis all values in percentage were transformed to (arc sen $\frac{\sqrt{x}}{100}$), being $x$ the variable value in %. Fisher (LSD) post hoc test was used for discrimination of homogeneous group and the different letters correspond to significant differences with a probability level of 95 %

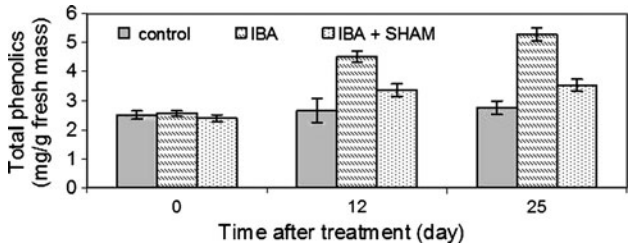


**Fig. 2** Trends in total phenolic content changes in microshoots during the process of IBA-induced adventitious rooting process. Total phenolic content was expressed in milligrams of gallic acid equivalent per gram fresh mass. Values represent the average of three measurements ± SD (three biological samples each consisting of the bulked basal parts of 40 microshoots)

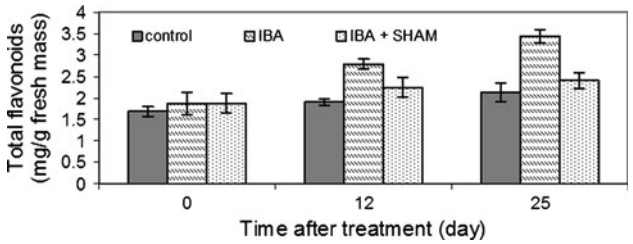


**Fig. 3** Changes in total flavonoid content in microshoots during the process of IBA-induced adventitious rooting process. Total flavonoid content was expressed in milligrams of quercetin equivalent per gram fresh mass. Values represent the average of three measurements ± SD (three biological samples each consisting of the bulked basal parts of 40 microshoots)

Time-course analyses revealed that at 12 and 25 days after IBA treatment, lignin content increased to 81 and 106 %, respectively, compared to 0 day. SHAM treatment had an inhibitory effect on lignin accumulation. Compared to IBA treated explants, 27 and 32 % decrease in lignin accumulation was observed at 12 and 25 days, respectively. Untreated explants showed no significant change in lignin content over the time course studied. This observation argues for the association of AOX activity and lignin metabolism.

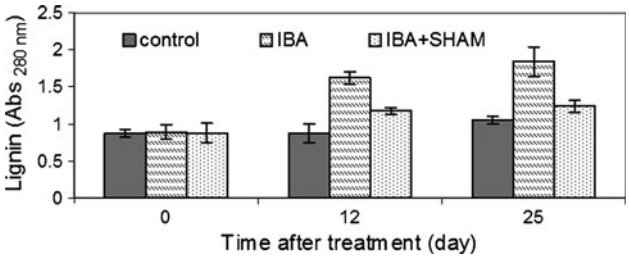


**Fig. 4** Inhibitory effect of SHAM on lignin accumulation during the process of IBA-induced adventitious root formation. Values represent the average of three measurements ± SD (three biological samples each consisting of the bulked basal parts of 40 microshoots)

### Analysis of *OeAOX1a* and *OeAOX2* transcript accumulation

Transcript accumulation of *OeAOX1a* and *OeAOX2* were studied during the process of IBA-induced adventitious rooting (Fig. 5). The amount of *OeAOX* transcripts were normalized through a ratio of integrated densities of *OeAOX* and *OeActin* amplicons. Both *OeAOX1a* and *OeAOX2* showed stable transcript accumulation during the selected time points when ORF primers were used (Fig. 5). Transcript intensity was higher for *OeAOX1a*. When the reverse primer to amplify *OeAOX2* was located in the 3′-UTR, differential transcript accumulation was observed. The level of transcript accumulation of *OeAOX2* was dramatically increased at day 14 (Fig. 6), the time when morphogenetic changes preceding root primordia formation can be observed in microscopic analyses (data not shown).

## Discussion

Adventitious organogenesis, such as root induction at microshoots, can be seen as a reaction of shoot cells upon stress (Arnholdt-Schmitt et al. 2006b; Santos Macedo et al.

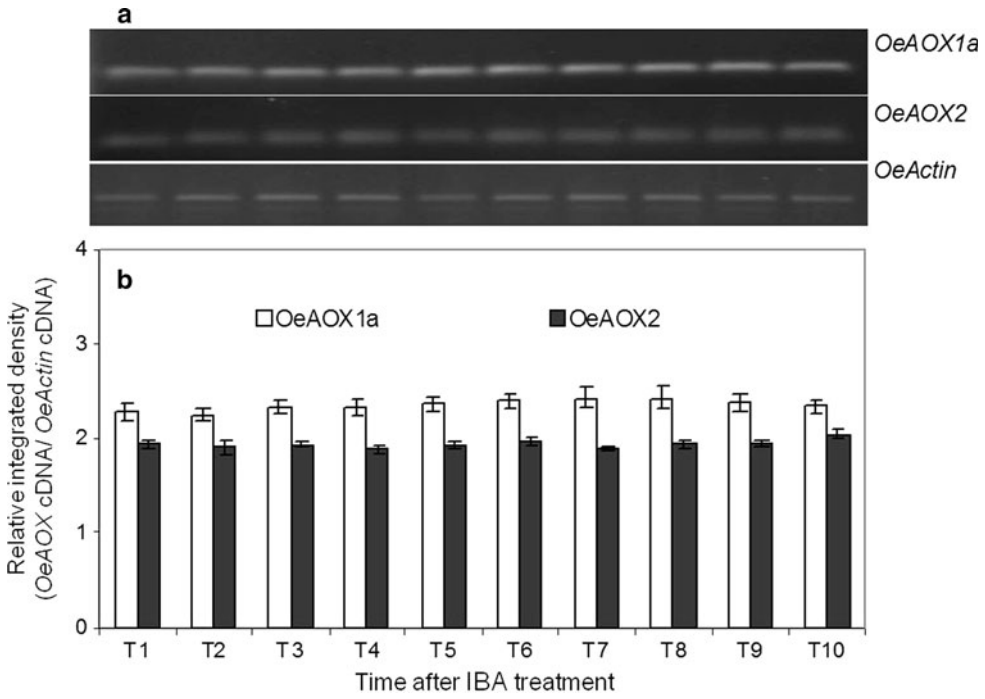


**Fig. 5** Transcript levels of two *OeAOX* genes during the process of IBA-induced adventitious root formation. **a** The gel images are representatives of the experiment and show the SQ-RT-PCR of *OeAOX1a*, *OeAOX2* and actin (*OeActin*); **b** normalization of the quantity of *AOX* transcripts through a ratio of integrated amplicon densities of the *OeAOX* and actin (*OeActin*). Data **b** are the mean values ± SD of two biological samples (bulked basal parts of 4 microshoots) and two technical repetitions. Samples were collected before IBA treatment (T1) and after IBA treatment at 2 h (T2), 4 h (T3), 5 h (T4), 12 h (T5), 1 day (T6), 2 days (T7), 3 days (T8), 6 days (T9), and 14 days (T10)

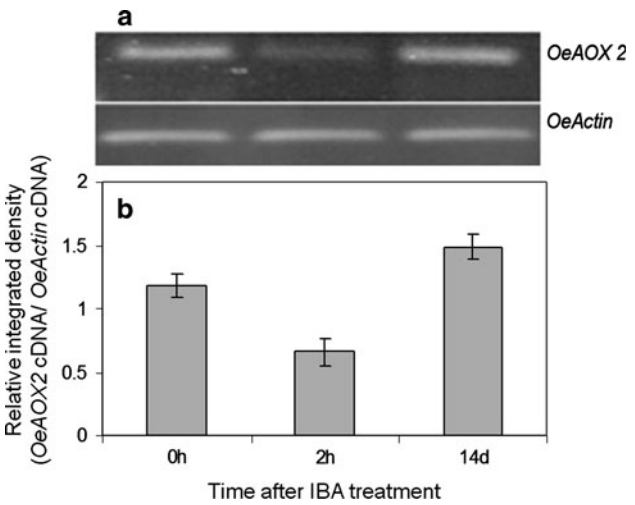


**Fig. 6** Transcript accumulation pattern of *OeAOX2* gene during the process of IBA-induced adventitious root formation in *Olea europaea* L. microshoots. **a** The gel images are representatives of the experiment and show the SQ-RT-PCR of *AOX2* and *Actin* (*OeActin*); **b** normalization of the quantity of target transcripts through a ratio of integrated densities of the *OeAOX2* and *Actin* (*OeActin*) amplicons. Data **b** are the mean values ± SD of two biological samples (bulked basal parts of 4 microshoots) and two technical repetitions. Samples were collected before IBA treatment (0 h) and after IBA treatment at 2 h and 14 days

2009) comparable to the process of stress-induced somatic embryogenesis (Zavattieri et al. 2010). Acclimation to stress can be expected to be accompanied by metabolic re-adjustment. However, the exact nature of stress-induced metabolic re-adjustment is poorly understood and represents an area of considerable research interest (Lehmann et al. 2009; Vanlerberghe et al. 2009). The present paper adds knowledge to recent state-of-the-art insights by studying association between AOX activity and secondary metabolism during adventitious rooting in olive microshoots.

The present results show that IBA-induced adventitious rooting in olive microshoots can be significantly suppressed by the AOX inhibitor SHAM. This observation points to a role of alternative respiration for the induction of adventitious roots. Induction of new cell programs covers different phases that starts from dedifferentiation and ends by the initiation of cell transforming and morphological events (Grieb et al. 1997; Zavattieri et al. 2010). However, SHAM treatment of olive microshoots failed to inhibit calli formation which is preceding the emergence of root primordia. A similar observation was reported by Hase (1987) in *Helianthus tuberosus* L. Also in this species, SHAM affected rooting but did not inhibit calli formation. These results point to a role of AOX for root initiation in the later phase during induction rather than a role for dedifferentiation in the early phase of induction.

Alternative respiration is involved in all types of abiotic and biotic stresses. AOX has been proposed to play a master role in the organization of efficient acclimation of plants to changing environmental conditions (Arnholdt-Schmitt et al. 2006a; Vanlerberghe et al. 2009). Complex interaction between AOX activity and the signaling molecule $H_2O_2$ has been reported (Amirsadeghi et al. 2006; Gray et al. 2004; Popov et al. 1997; Umbach et al. 2005; Vanlerberghe and McIntosh 1996). It is of interest to note

that in olive shoot cuttings $H_2O_2$ treatment could even substitute for the effect of added auxin as a root-inducing agent (Santos Macedo et al. 2009). In addition, SHAM has been found to interact with intercellular $H_2O_2$ in SHAM treated *Arabidopsis thaliana* L. roots, where $H_2O_2$ disappeared from the root hair zone but accumulated in the cell walls of the meristematic region. In these experiments root length was significantly reduced (Dunand et al. 2007). Hilal et al. (1998) have reported on the localization of AOX in meristem and xylem tissues in developing *Glycine max* L. roots and highlighted a link of AOX to xylem development under salt stress.

Involvement of lignin and phenolic acids in adaptive plant growth regulation, differentiation and organogenesis has been described by several groups (Ozyigit et al. 2007; Fu et al. 2011; Sircar et al. 2012). In the present olive system, reduction of root numbers in microshoots by simultaneous IBA and SHAM application was accompanied by down-regulation of the synthesis of secondary plant products from phenylpropanoid and lignin metabolism.

Phenolic acids have been suggested to play an important role in co-operation with phytohormones in altering cell wall composition and, thus, affect differentiation and morphogenesis (Cvikrová et al. 1998, 2003; Hartman et al. 1996). It is probable that phenolic acids form a complex with indole acetic acid (IAA), a key factor for root differentiation and development (Balakrishnamurthy and Rao 1988; Fu et al. 2011). Furthermore, phenylpropanoid derivatives have been reported to mimic the effects of cytokinins in regulating cell division and differentiation (Tamagnone et al. 1998). Stimulatory effects of phenolic acids and flavonoids on adventitious rooting have been observed previously in many plant species (Curir et al. 1992; Fu et al. 2011). Moreover, anthocyanin and flavonoid biosyntheses, which originate from phenylpropanoid pathway intermediates, correspond to AOX activity as has been demonstrated in transgenic *Arabidopsis* (Fiorani et al. 2005).

In our study on the transcription of two individual *AOX* genes *OeAOX1a* displayed higher transcript level than *OeAOX2*. However, both *OeAOX1a* and *OeAOX2* showed stable transcript accumulation at all selected time points when primers from ORF region were used. Lehmann et al. (2009) have pointed to a general lack of correlation between metabolites and involved transcripts. Interestingly, differential transcript accumulation of *OeAOX2* was observed when the reverse primer had been designed in the 3′-UTR. High level of *OeAOX2* transcript was observed at day 14 of culture after IBA treatment which is the initiation point of root morphogenic field formation. Furthermore, this result is in agreement with previous results where differential transcript accumulation was found for shoot cuttings of cv. Cobrançosa (Santos Macedo et al. 2009). In the 3′-UTRs of *OeAOX2* transcripts of cv. Galega vulgar sequence and length variability were identified (Santos Macedo et al. 2009). Thus, the results indicate the possibility for differential regulation of *OeAOX2* during adventitious rooting through different classes of transcripts from the same gene (Polidoros et al. 2009). The functional meaning of such type of post-transcriptional regulation of *AOX* genes in olive rooting will be explored in future experiments.

In conclusion, the data support the general hypothesis that *AOX* genes can play a key role in the process of adaptive cell reprogramming. A non-inhibitory effect of SHAM on calli formation but inhibition of rooting makes olive adventitious rooting in microshoots a highly interesting experimental system for future research. It will enable the separation of different stages in re-programming, such as re-determination and initiation of morphogenetic changes (Grieb et al. 1997; Zavattieri et al. 2010). Since phenylpropanoid and lignin accumulation was suppressed by SHAM treatment, it can be postulated that AOX control of the process of root formation associates with alteration in phenylpropanoid and lignin metabolism which in turn interact with meristematic growth (Sieger et al. 2005). A role of 3′-UTR variability in posttranscriptional AOX gene regulation is indicated and needs further clarification.

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