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Genome-wide analysis and expression profile of *PIN*-formed auxin carrier genes during *in vitro* IBA-induced adventitious rooting in *Olea europaea* L.

OLIVE GROVES
OLIVE OIL

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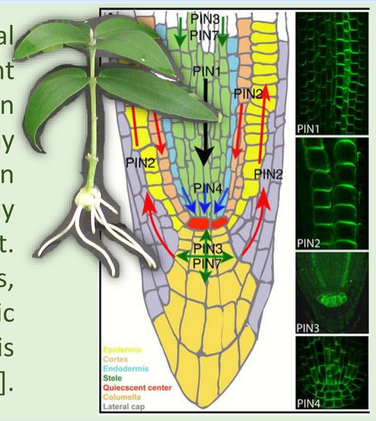
INTRODUCTION

Olive (*Olea europaea* subsp. *europaea* var. *europaea* L.) comprises several cultivars with reduced capacity to be propagated due its recalcitrant behaviour to form adventitious roots (AR). This prevents their propagation and consequently their availability in the nurseries. There are many protocols used in vegetative propagation to induce AR formation based on auxins, a group of phytohormones largely known as involved in many processes of plant development, including root initiation and development. However, most of these protocols are still based on "trial/error" approaches, where several variables need to be tested. This happens because the genetic control underlying AR formation is not completely elucidated. Auxin is mainly synthesized in the young leaves and apical meristem of the shoot [1].

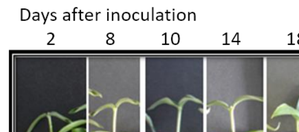


The major auxin distribution is regulated by transport from cell to cell, known as polar auxin transport (PAT) [**2**]. PAT is mediated by three main classes of membrane auxin transporters, the auxin resistant 1/like aux1 (AUX/LAX), the ATP binding cassette subfamily B (ABCB/MDR/PGP) and the pin-formed (PIN) carriers. The PIN gene family encodes a subgroup of auxin efflux carriers shown to be involved in various developmental processes, including lateral/adventitious root formation. To date, *PIN* genes have been identified in several plant species by genome-wide approaches [**3**], however, no information exists regarding their identification in olive. Our work aims to characterize *OePIN* family, as well as, to investigate the involvement of its members during AR, by studying their expression profiles in IBA-induced *in vitro* cultured microshoots of cv. 'Galega vulgar', attempting to understand whether the hard-rooting behaviour of this cultivar might be related with a disturbance in auxin transport.

MATERIALS AND METHODS

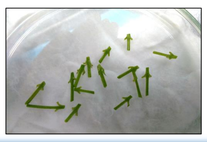
Adventitious Rooting Induction

Stem segments (microcuttings) with four-to-five nodes were prepared from the upper part of *in vitro* grown plantlets of cv. 'Galega vulgar' and all leaves were removed with the exception of the upper four. The base (approx. 1.0 cm) of each microcutting was immersed in a sterile solution of IBA (indole-3-butyric acid) for 10 s [4,5,6] .



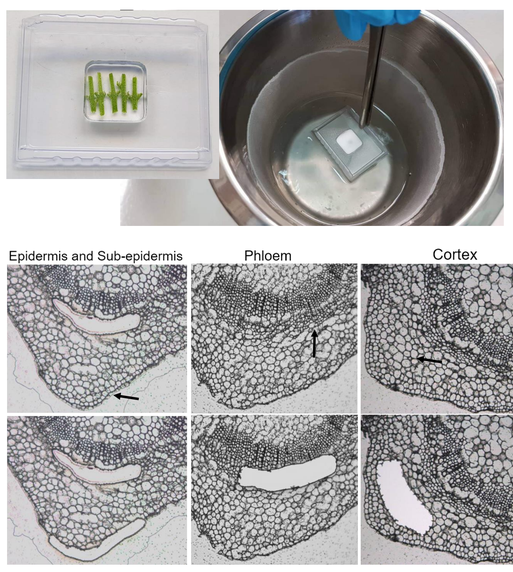
Microcuttings were inoculated in semi-solid olive culture medium (OM) and placed in growth chambers.

After several time points, microcuttings were collected and basal segments were cut.



Laser Microdissection (LMD)

Stem basal segments were placed in cryomolds containing optimal cutting temperature (OCT) compound and then freezed with isopentane and liquid nitrogen. Cryosections were placed in PEN membrane glass slides and OCT was removed with xilol, etanol 70% and 100%.



Microdissections were performed with a LMD6500 microscope (Leica).

Reactive Oxygen Species (ROS) Detection

Hydrogen peroxide (H_2O_2) and superoxide (O^{2-}) anion were detected by using 3,3'-Diaminobenzidine (DAB) and Nitrotetrazolium blue chloride (NBT), respectively.

In silico identification of OePIN gene family members

To search for *PIN* members in *Olea europaea* subsp. *europaea*, a blast search was made at the olive genome databases (http://denovo.cnag.cat/genomes/olive/ and https://www.ncbi.nlm.nih.gov/genome/?term=Olea+europaea+var.+sylvestris+genome). For classification of retrieved sequences a phylogenetic tree was constructed using PIN sequences from 13 eudicot plant species. Sequences were aligned in MUSCLE software and phylogenetic tree was constructed with MEGA 7 software [7] using the Neighbor-Joining (NJ) method. The inferred tree was tested by bootstrap analysis using 1000 replicates.

Gene Expression Analysis

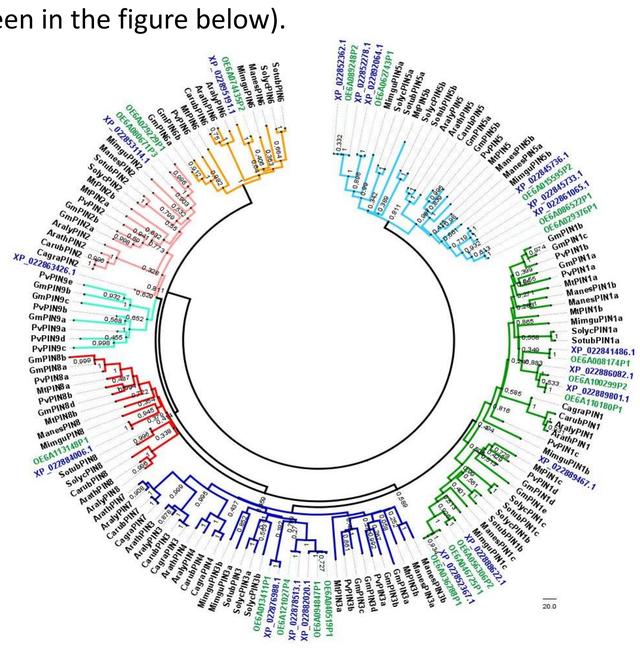
Total RNA was isolated from whole tissue (Maxwell SimplyRNA, Promega) and from microdissected tissue sections (RNeasy Micro kit, Qiagen) from 20 basal segments. Real-time PCR reactions were carried out with SYBR green chemistry and quantification cycle (Cq) values were acquired with the Applied Biosystems 7500 software.

RESULTS AND DISCUSSION

In silico identification of OePIN gene family members

✓ 21 *PIN*-homologous sequences were retrieved at the var. *europaea* whole genome databases. From those, 2 truncated loci (not considered for phylogenetic studies) and 2 duplicated loci (OE6A036288P1/OE6A046725P1; OE6A040519P1/OE6A094847P1) were identified .

✓ 17 genes were considered as the composition of *PIN* gene family in *Olea europaea* var. *europaea* (*OePIN*) (accessions shown in green in the figure below).

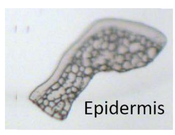


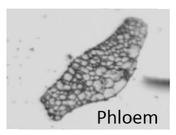
✓ The newly identified genes were named according to the cluster of PIN subfamily where they grouped. This analysis revealed that *OePIN* family is composed by members belonging to six subfamilies, named as *OePIN1*, *OePIN2*, *OePIN3*, *OePIN5*, *OePIN6* and *OePIN8*.

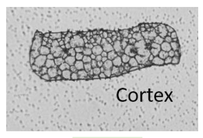
✓ Differences in the pattern of gene radiation could be seen among the different subfamilies (clusters with different colors in the phylogenetic tree).

Laser Microdissection

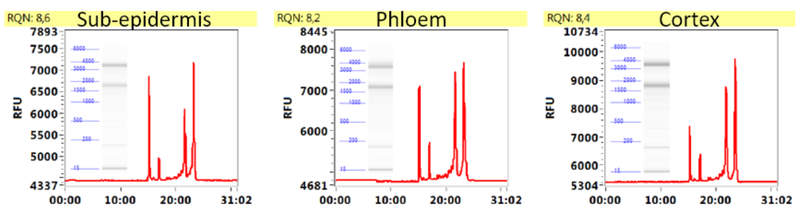
Olive tissue cryosections from epidermis (plus sub-epidermis), cortex and phloem showed high integrity allowing an efficient laser microdissection of distinct cells.





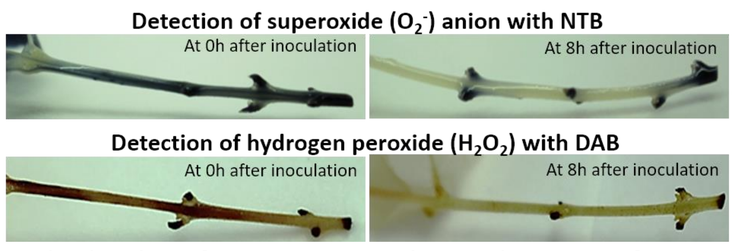


Total RNA isolated from distinct tissues showed high quality (Fragment Analyzer, Agilent) with RNA Integrity Numbers (RIN) > 8.0.



The implemented laser microdissection protocol revealed to be extremely efficient to obtain RNA of high quality to perform gene expression analysis from distinct olive cell types.

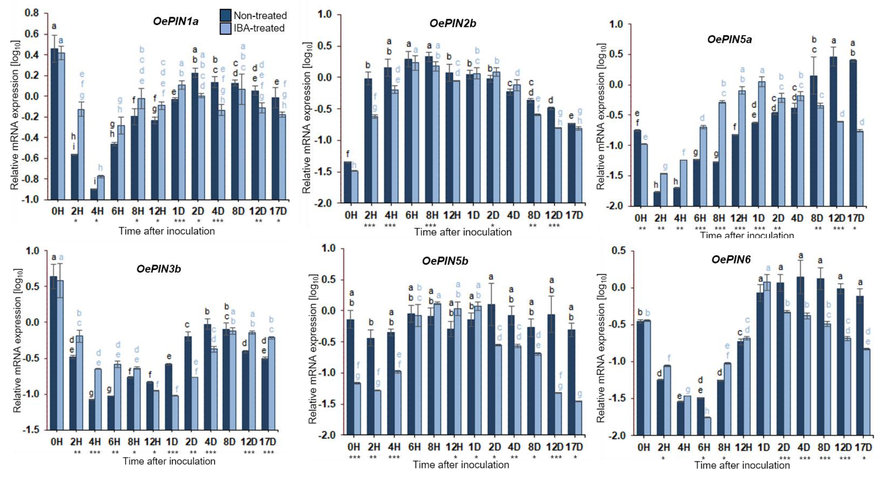
ROS levels are regulated in olive stems



High levels of ROS were observed soon after explant preparation but they decreased 8 h after inoculation in both non-treated and IBA-treated explants, probably due to increased AOX enzyme [8].

OePIN genes are differentially expressed in olive stems

The *OePIN* mRNA levels were changed throughout time within each condition (non-treated and IBA-treated explants) (different letters), however, with different expression profiles between conditions, leading to significant differences (asterisks) for most time points tested.



A disturbance in auxin transport, demonstrated by altered *OePIN* genes expression levels in the first time points, may occur after explant preparation for AR induction. When IBA is applied, *OePIN* genes are differentially regulated comparing to non-induced microcuttings.

The disturbance in auxin transport may be promoted by high levels of ROS, which are an indicator of plant response to stress conditions (cutting/mechanical damage), associated to explant preparation.

In conclusion, OePIN family members deserve to be further investigated to better understand the molecular mechanisms underlying olive adventitious rooting so that, in the future, vegetative propagation capacity will be no longer an obstacle to make any olive variety available to olive producers.

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REFERENCES

[1] Ljung, K., et al. (2001) *Plant J.* 28, 465–474; [2] Petrášek J. and Friml J. (2009) *Development*, *136*, 2675-2688; [3] Zhou J. and Lou J. (2018) *Int. J. Mol. Sci*., 19, 2759; [4] Peixe, A., et al. (2007) *Sci. Hortic. (Amsterdam)* 113, 1–7; [5] Santos Macedo, E., et al. (2012) *Plant Cell Rep.* 31, 1581–1590; [6] Macedo, E., et al. (2013). *J. Hortic. Sci. Biotechnol* 88 (1) 53–59; [7] Kumar, S., et al. (2016) *Mol. Biol. Evol*., 33, 1870-1874; [8] Velada, I., et al. (2018). *Int. J. Mol. Sci.*, 19, 597.

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