Plant Physiology and Biochemistry 96 (2015) 231–240

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

POLYAMINE OXIDASE2 of *Arabidopsis* contributes to ABA mediated plant developmental processes

Rinukshi Wimalasekera [a], Frank Schaarschmidt [b], Riccardo Angelini [c], Alessandra Cona [c], Parasklevi Tavladoraki [c], Günther F.E. Scherer [a, *]

[a] Leibniz Universität Hannover, Institute of Horticultural Production Systems, Section of Molecular Developmental Physiology, Herrenhäuser Strasse 2, D-30419 Hannover, Germany
[b] Leibniz Universität Hannover, Institute of Biostatistics, Herrenhäuser Strasse 2, D-30419 Hannover, Germany
[c] Department of Science, University 'Roma Tre', Viale Guglielmo Marconi 446, 00146 Rome, Italy

ARTICLE INFO

Article history:
Received 6 February 2015
Received in revised form
4 August 2015
Accepted 5 August 2015
Available online 10 August 2015

Keywords:
Abscisic acid
Ammonia
Nitrate
NO: nitric oxide
POLYAMINE OXIDASE2
Root development

ABSTRACT

Polyamines (PA) are catabolised by two groups of amine oxidases, the copper-binding amine oxidases (*CuAOs*) and the FAD-binding polyamine oxidases (*PAOs*). Previously, we have shown that *CuAO1* is involved in ABA associated growth responses and ABA- and PA-mediated rapid nitric oxide (NO) production. Here we report the differential regulation of expression of *POLYAMINE OXIDASE2* of *Arabidopsis* (*AtPAO2*) in interaction with ABA, nitrate and ammonium. Without ABA treatment germination, cotyledon growth and fresh weight of *pao2* knockdown mutants as well as *PAO2OX* over-expressor plants were comparable to those of the wild type (WT) plants irrespective of the N source. In the presence of ABA, in *pao2* mutants cotyledon growth and fresh weights were more sensitive to inhibition by ABA while *PAO2OX* over-expressor plants showed a rather similar response to WT. When NO_3^- was the only N source primary root lengths and lateral root numbers were lower in *pao2* mutants both without and with exogenous ABA. *PAO2OX* showed enhanced primary and lateral root growth in media with NO_3^- or NH_4^+. Vigorous root growth of *PAO2OX* and the hypersensitivity of *pao2* mutants to ABA suggest a positive function of *AtPAO2* in root growth. ABA-induced NO production in *pao2* mutants was lower indicating a potential contributory function of *AtPAO2* in NO-mediated effects on root growth.

1. Introduction

PAs are low molecular weight, polycationic, nitrogenous essential growth regulators present ubiquitously in both prokaryotic and eukaryotic cells. PAs are crucial components in plant growth and development as well as in abiotic and biotic stress responses (Alcázar et al., 2010; Alcázar and Tiburcio, 2014; Couée et al., 2004; Hussain et al., 2011; Kusano et al., 2007, 2008; Takahashi and Kakehi 2010, Tiburcio et al., 2014). PAs are oxidatively catabolised by amine oxidases, which include copper-binding diamine oxidases (CuAO/DAO) and flavin adenine dinucleotide (FAD)-binding polyamine oxidases (PAO) (Angelini et al., 2010; Cona et al., 2006). Plant CuAOs preferentially catalyse the oxidation of diamine putrescine at the carbon atom adjacent to the primary amino group, producing 4-aminobutanal with concomitant release of NH_3 and H_2O_2. The resulting 4-aminobutanal is further converted to γ-aminobutyric acid (GABA) via Δ^1-pyrroline. PAOs preferentially catalyse the oxidation of spermidine and spermine at the carbon atom adjacent to the secondary amino group and can be divided in two enzyme families: those catalysing terminal PA catabolism and those catalysing spermidine or spermine back-conversion. The former produces 4-aminobutanal and *N*-(3-aminopropyl)-4-aminobutanal, respectively, with concomitant production of 1,3-diaminopropane and H_2O_2 while the latter produces, respectively, putrescine or spermidine along with 3-aminopropanal and H_2O_2 (Angelini et al., 2010; Cona et al., 2006).

CuAOs and PAOs have functions in almost all aspects of plant growth and development as well as in defence responses against stresses, such as drought, heat and salinity and pathogen invasion (Cona et al., 2006; Moschou et al., 2008; Tisi et al., 2011; Wimalasekera et al., 2011a). *PAOs* are shown to be differentially regulated in response to abscisic acid (ABA), jasmonic acid, salicylic acid and wounding indicating relevance of PAOs in stress responses

Abbreviations: NO, nitric oxide; PA, polyamine; PAO, polyamine oxidase; WT, wild type.

* Corresponding author.
E-mail address: scherer@zier.uni-hannover.de (G.F.E. Scherer).

http://dx.doi.org/10.1016/j.plaphy.2015.08.003
0981-9428/

(Cona et al., 2006; Moschou et al., 2008). In *Arabidopsis* seven *CuAO* and five *PAO* genes have been identified. The cytosolic or peroxisomal localised *Arabidopsis PAOs* (*AtPAO1–AtPAO5*) show good sequence homology with *Zea mays* and rice *PAOs* (Kamada-Nobusada et al., 2008; Kim et al., 2014; Liu et al., 2014a, 2014b; Ono et al., 2012; Takahashi et al., 2010). Recombinant AtPAO1-AtPAO5 have been purified and characterized and comparative study of properties showed differences in substrate specificity and catalytic mechanism suggesting functional diversity of the AtPAO family (Ahou et al., 2014; Fincato et al., 2011, 2012; Kamada-Nobusada et al., 2008; Kim et al., 2014; Moschou et al., 2008; Takahashi et al., 2010; Tavladoraki et al., 2006). The members of *Arabidopsis PAO* gene family exhibit distinct tissue- and organ-specific expression pattern during seedling growth and development proposing specific roles of each gene (Fincato et al., 2012; Kim et al., 2014; Takahashi et al., 2010). Furthermore, ABA-inducible expression of *AtPAO1, AtPAO2* and *AtPAO3* implies their significance in ABA-dependent environmental stress responses (Fincato et al., 2012; Takahashi et al., 2010).

Despite several investigations of properties of PA catabolising enzymes and the transcriptional responses in biotic and abiotic stresses of the respective genes, investigations on the roles of *CuAOs* and *PAOs* in development are limited. The apparent close relationship of PA catabolism to ABA biology as suggested by transcriptional responses and the known role of nitric oxide (NO) as a signalling molecule in ABA action (Arc et al., 2013; Desikan et al., 2002; Neill et al., 2008; Simontacchi et al., 2013; Yu et al., 2014), PA-induced rapid NO production (Santa-Catarina et al., 2007; Silveira et al., 2006; Tun et al., 2006; Wimalasekera et al., 2011b) and implications of NO in stress biology (Leitner et al., 2009; Trapet et al., 2015) and modulation of hormonal responses (Simontacchi et al., 2013; Tun et al., 2008; Yu et al., 2014) lead us recently to investigate the role of the *Arabidopsis CuAO1* gene in NO production and ABA signalling (Wimalasekera et al., 2011b).

Data suggested that *Arabidopsis CuAO1* is a novel candidate in ABA signalling and that lead us to investigate a member of the *Arabidopsis PAO* gene family, in particular *AtPAO2*, in the context of ABA-related plant development. Since source of nitrogen nutrition, NO_3^- and/or NH_4^+, has an influence on ABA responses, root architecture, PA homeostasis and NO biosynthesis (Crawford, 1995; De Smet et al., 2006; Ho and Tsay, 2010; Manoli et al., 2014; Miller et al., 2007; Moschou et al., 2012; Patterson et al., 2010; Qin et al., 2011; Signora et al., 2001), the present study included developmental investigations in different nitrogen sources. In particular, we investigated germination, post-germination seedling growth and root growth in two *pao2* knockdown mutants and in an *AtPAO2* over-expressing transgenic line, *PAO2OX,* in different N nutrition conditions of NO_3^-, NH_4^+ or a mixture of NO_3^- and NH_4^+. Interestingly, *pao2* mutants showed phenotypes quite different from *cuao1* mutants indicating different roles of *AtPAO2* and *AtCuAO1* in plant development and ABA responses.

2. Materials and methods

2.1. Plant materials and growth conditions

Arabidopsis thaliana wild type Col-0, T-DNA insertion lines (Alonso et al., 2003) of *AtPAO2* (At2g43020), *pao2-1* (SALK_049456.42.05.x) and *pao2-2* (SALK_046281.36.95.x) of Col-0 ecotype obtained from NASC, UK and *35S::AtPAO2* provided by P. Tavladoraki, University "Roma Tre", Rome, Italy and *AtPAO2::GUS* (Col-0) provided by Y. Takahashi of Tohoku University Japan were used in this work.

The growth media either liquid or solid were modifications of the previously described medium by Wang et al. (2003). This basic growth medium contained 2.5 mM $(NH_4)_2$ succinate, 10 mM potassium phosphate (pH 6.5), 2 mM $MgSO_4$, 1 mM $CaCl_2$, 0.1 mM $FeNa_2EDTA$, 50 μM H_3BO_3, 12 μM $MnSO_4.H_2O$, 1 μM $ZnCl_2$, 1 μM $CuSO_4.5H_2O$, 0.2 μM Na_2MoO_4 and 0.5% (w/v) sucrose. Bacto agar 1.5% (w/v) was added to solid media. The seedlings were grown in different nitrogen sources and concentrations as follows **(I)** basic medium (5 mM NH_4^+) **(II)** basic medium I supplemented with 5 mM KNO_3 (5 mM NH_4^+ + 5 mM NO_3^-) **(III)** basic medium I devoid of 2.5 mM $(NH_4)_2$ succinate but supplemented with 5 mM KNO_3 (5 mM NO_3^-) as indicated under each experiment. Seeds were surface sterilized with 70% (v/v) ethanol for 5 min and then with sodium hypochlorite and 0.1% (v/v) Tween 20 for 10 min followed by 5 times of washing with sterile distilled water. Sterilized seeds were sown on respective growth media, stratified at 4 °C for 3 days and grown at 23 °C, 18/6 h light/dark cycles and 40–50 μmol m^{-2} s^{-1} light intensity for the number of days as indicated in each experiment.

2.2. Isolation of pao2-1 and pao2-2 T-DNA insertion knockdown mutants of AtPAO2 (At2g43020)

Two independent homozygous T-DNA insertion mutant lines, *pao2-1* (SALK_049456.42.05.x) and *pao2-2* (SALK_046281.36.95.x) were isolated by genomic PCR analysis. Homozygous *pao2-1* was identified by using the *AtPAO2*-specific primers pao2-1 forward (-5′-gct ctg ttt gat atg gat ggc-3′) and pao2-1 reverse (−5′-gga ggt ttc agc tac cac tcc-3′) in combination with the T-DNA-specific primer LBa1, (5′-tgg ttc acg tag tgg gcc atc g-3′). Identification of homozygous *pao2-2* was done using the *AtPAO2*-specific primers pao2-2 forward (-5′-aca gat caa taa agt cag gga tga g-3′) and pao2-2 reverse (5′-ata aga aga ggt aca gag gca ggt c -3′) in combination with the T-DNA specific LBb1.3 (5′- att ttg ccg att tcg gaa c-3′). Location of the T-DNA insertion site was confirmed by sequencing the PCR products. Total RNA was isolated (Macherey and Nagel) and cDNA was synthesised (Fermentas) from homozygous knockdown mutants and transcript null was verified by RT-PCR using the *AtPAO2* specific primers TZ PAO2 forward (5′-aac tct gat cgt caa atg cgt -3′) and TZ PAO2 reverse (5′-cct ttc gcc aga gta ttg atg-3′). PCR conditions for all were 94 °C 30 s, 60 °C 30 s and 72 °C 2 min for 40 cycles.

2.3. Histochemical GUS staining of AtPAO2::GUS plants

The effect of different N nutrition and the interaction with ABA on the expression pattern of *AtPAO2* was determined by growing *AtPAO2::GUS* seedlings in media designated **(I)** to **(III)** with different N sources and concentrations for 10 days (23 °C, 18 h light/6 h dark cycles) and subsequently transferring to respective fresh media supplemented with 50 μM ABA (Sigma Aldrich) (dissolved in DMSO) or mock treatment and incubating for 18 h at the same growth conditions. Histochemical GUS assay (Jefferson et al., 1987) was done by staining the seedlings with X-Gluc buffer [2 mM X-Gluc, 50 mM $NaPO_4$ (pH 7.0), Triton-X (0.5%), 0.5 mM K-ferricyanide, 0.5 mM K-ferrocyanide] for 16 h at 37 °C. After destaining, the documentation was done by light microscope Zeiss Axioskop MR-5.

2.4. Determination of germination, seedling establishment and fresh weights

Col-0 (WT), *pao2-1*, *pao2-2, PAO2*OX sterilized seeds were sown on solid **(I)** to **(III)** media supplemented with 0, 1.5 and 3.0 μM ABA (Sigma Aldrich) and incubated at 23 °C, 18 h light/6 h dark cycles. A seed was considered as germinated when the radicle penetrated the seed coat. Germination was scored on day 3 of incubation start and percentage of germination was calculated in three independent