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Arabidopsis aldehyde oxidase 3, a ROS-producing key enzyme in ABA synthesis, is regulated by ABA3-dependent activation

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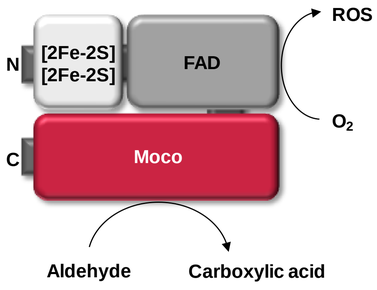
1. Introduction

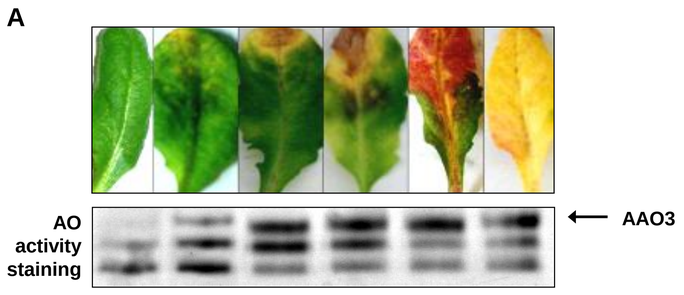
Aldehyde oxidase (AO) is a dimeric metallo-flavo enzyme whose individual subunits are composed of three domains carrying two [2Fe-2S] clusters, a FAD molecule and a molybdenum cofactor (Moco) as prosthetic groups. AO proteins are able to oxidise a variety of aromatic and non-aromatic aldehydes to their corresponding carboxylic acid with simultaneous consumption of molecular oxygen as electron acceptor.



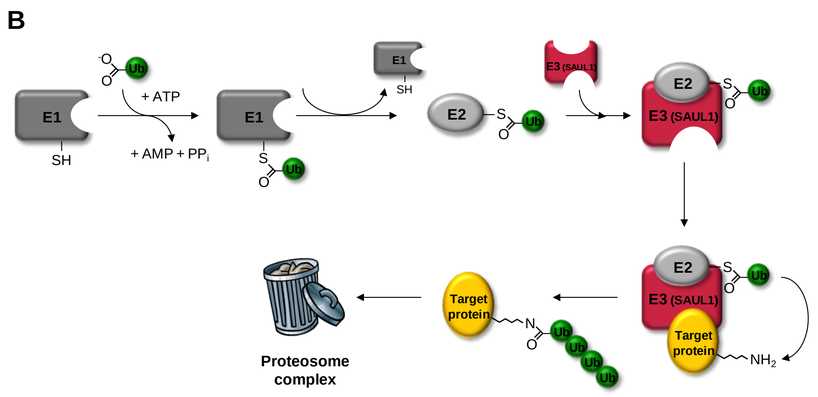
The Arabidopsis genome harbors four AO genes, AAO1 – AAO4, whose products form homodimers as well as heterodimers, thereby leading to altered substrate specificities of the respective isoenzymes[1].

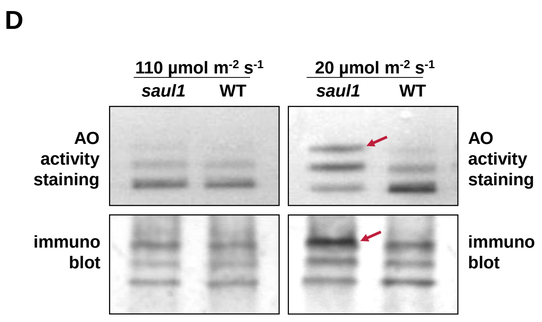
AAO1 and/or AAO2 proteins are presumed to be involved in indole acetic acid (IAA) biosynthesis. The AAO3 homodimer is characterised by a high preference for abscisic aldehyde as substrate, which is the ultimate precursor of the phytohormone abscisic acid (ABA). ABA is involved in many aspects of plant growth and development, including seed maturation, dormancy, leaf senescence, stomatal movement, and adaptation to a variety of environmental stresses. The AAO4 isoenzyme is expressed preferably in siliques and catalyses the oxidation of benzaldehyde, which contributes to the synthesis of benzoic acid for incorporation into several glucosinolate compounds.

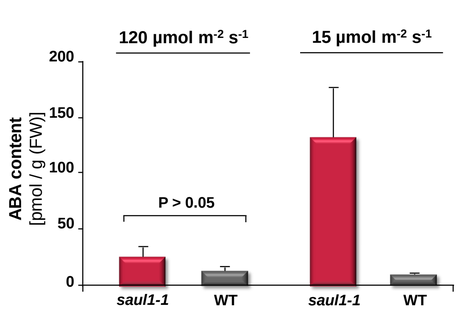
2. Importance of AAO3 for leaf senescence



AAO3 activity strongly increases at the onset of senescence in wild type plants and maintain high activities until late stages of senescence (A), indicating elevated levels of AAO3 reaction products are required for initiation and/or progress of senescence.



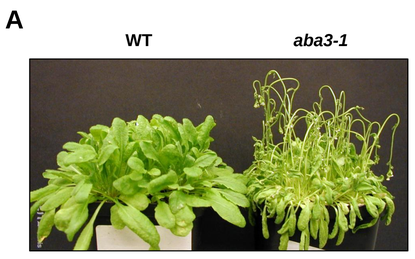


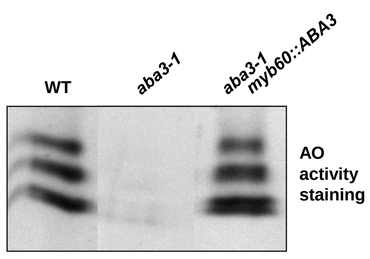


Arabidopsis plants transformed with *AAO3* cDNA do not present increased activity or protein levels. Explanation for this phenomenon is given by the *saul1* (senescence-associated ubiquitin ligase 1) mutant[2]. SAUL1 identifies ubiquitin target proteins and supports the ubiquitin transfer and thus, degradation of specific proteins (B). By continous degradation of AAO3, SAUL1 prevents premature senescence in wild type plants. In contrast, under low light conditions *saul1* mutant plants present early senescence (C), enhanced AAO3 activity and protein levels (D), and accumulation of ABA (E). In addition it was demonstrated, that AAO3 and SAUL1 are able to interact *in vitro*, which indicates that AAO3 is a natural target of the ubiquitin ligase SAUL1.

3. Importance of AAO3 in guard cell-specific ABA synthesis

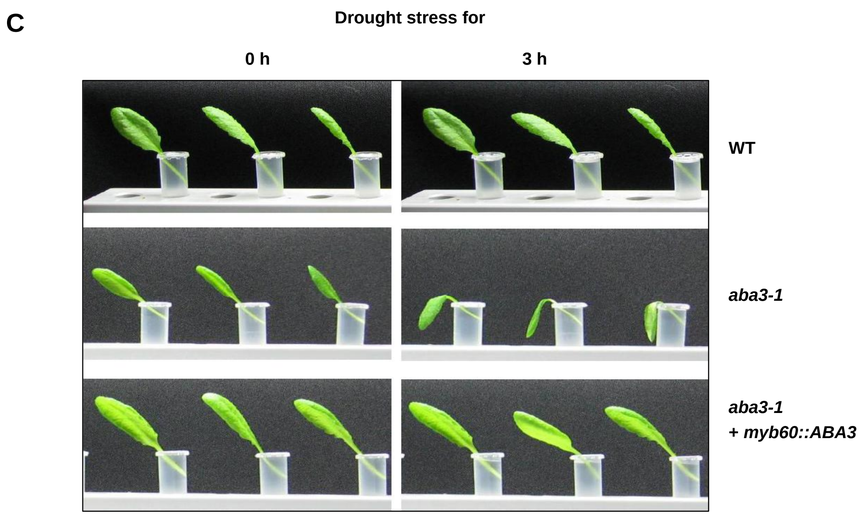
AAO3 has been shown to be localised in the cytosol of various tissues such as root tips, vascular bundles and guard cells[3]. Since the latter have been discussed extensively with respect for their contribution to ABA synthesis, we addressed the question whether guard cells indeed have the capacity to produce ABA.[4]





The ABA-deficient Arabidopsis mutant *aba3-1* is characterised by the simultaneous loss of all AO enzyme activities, incl. AAO3, a high transpiration rate and a wilty phenotype (A).

After transformation of *aba3-1* plants with a wild type cDNA of *ABA3* under control of the guard cell-specific myb60 promotor, reconstitution of AO activities (B) and of wild type-like drought stress resistance (C) was achieved.

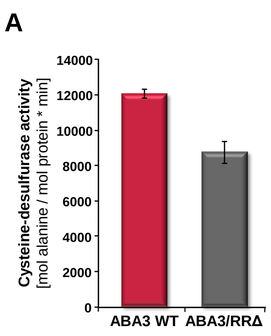


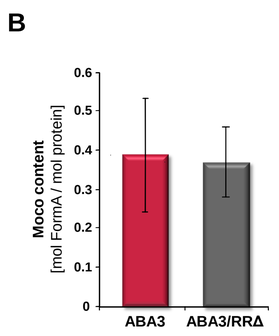
Conclusion:
ABA synthesis could be restored in the *aba3-1*/myb60::*ABA3* background, showing that guard cells have their own set of ABA synthesis enzymes and are fully equipped with all factors required for an appropriate ABA response.

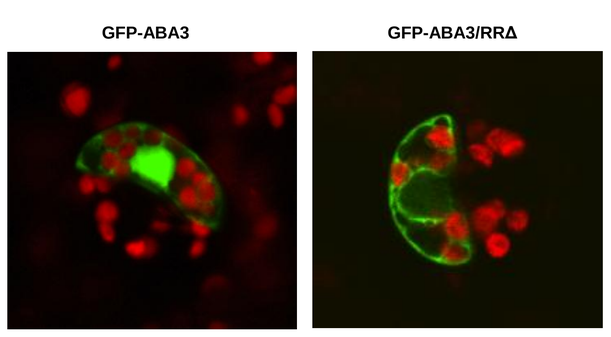
4. Unknown function of ABA3 in the nucleus

S. lycopersicum	...[764] QRFTEPLATLAGYRRAKG [781]...
flacca	...[764] QRFTEPLATLAGY--TKG [781]...
A. thaliana ABA3	...[771] KKSNEPLTTLASYRRVKG [788]...

Like the Arabidopsis *aba3-1* mutant, the tomato *flacca* mutant is deficient in the Moco-sulfurase gene[5]. In contrast to *aba3-1* mutants however, AO enzyme activities are completely abrogated in the shoot, but partially retained in the roots with simultaneously reduced ABA levels. Interestingly, the *flacca* mutation (RRΔ) resides within a putative nuclear localization signal, which is likewise found in Arabidopsis ABA3.



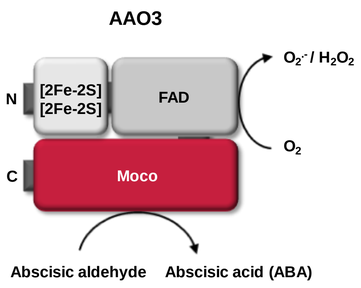


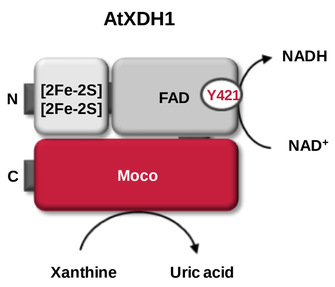


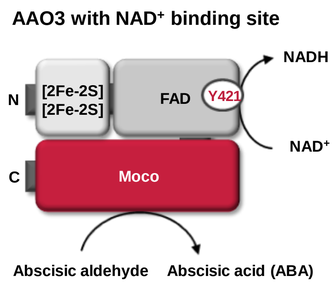
Cloning of the tomato *flacca* mutation into Arabidopsis *ABA3* cDNA and heterologous expression of the corresponding protein (ABA3/RRΔ) in *E. coli* resulted in a protein with nearly wildtype-like enzyme-activity (A) and Moco content (B), indicating the mutation does not affect these features. However, while a GFP-ABA3 fusion protein localises to the cytosol and the nucleus, a GFP-ABA3/RRΔ variant carrying the *flacca* mutation can longer be visualised in the nucleus (C).

5. Physiological importance of AAO3-generated ROS in stomata closure

Beside ABA also reactive oxygen species (ROS) such as hydrogen peroxide and superoxide anions play an important role in stomata regulation. Notably, AAO3 generates these types of ROS in each catalytical cycle during ABA synthesis[6], allowing to assume that in stomata closure AAO3 provides both ABA and ROS.







To study a possible importance of AAO3-generated ROS, we substituted the oxygen-binding motif of AAO3 for the NAD^+-binding motif of its evolutionary ancestor AtXDH1. AtXDH1 contains a conserved tyrosine residue responsible for binding the p referred electron acceptor NAD^+ (Tyr421) which is absent in AO enzymes. The resulting AAO3/Tyr421 protein will thus prefer NAD^+ rather than oxygen as electron acceptor, and be largely incapable of producing ROS. Expression of the AAO3/Tyr421 protein in *aao3* mutant plants could answer the question if e.g in response to drought stress AAO3 solely produces ABA or also ROS.

Related Literature

(1) **Bittner, F.**, Mendel, R.R. **(2010)**: Cell Biology of Molybdenum. Plant Cell Mocographs 17: 119 – 143

(2) **Raab, S.**, Drechsel, G., Zarepour, M., Hartung, W., Koshiba, T., Bittner, F., Hoth, S. **(2009)**: Identification of a novel E3 ubiquitin ligase that is required for suppression of premature senescence in Arabidopsis. Plant J 59: 39 - 51

(3) **Koiwai, H.**, Nakaminami, K., Seo, M., Mitsuhashi, W., Toyomasu, T., Koshiba, T. **(2004)**: Tissue-specific localization of an abscisic acid biosynthetic enzyme, AAO3, in Arabidopsis. Plant Physiol 134: 1697 - 1707

(4) **Bauer, H.**, Ache, P., Lautner, S., Fromm, J., Hartung, W., Al-Rasheid, K.A., Sonnewald, S., Sonnewald, U., Kneitz, S., Lachmann, N., Mendel, R.R., Bittner, F., Hetherington, A.M., Hedrich, R. **(2013)**: The stomatal response to reduced relative humidity requires guard cell-autonomous ABA synthesis. Curr Biol 23: 53 – 7

(5) **Sagi, M.**, Scazzocchio, C., Fluhr, R. **(2002)**: The absence of molybdenum cofactor sulfuration is the primary cause of the *flacca* phenotype in tomato plants. Plant J 31: 305 – 317

(6) **Zarepour, M.**, Simon, K., Wilch, M., Nieländer, U., Koshiba, T., Seo, M., Lindel, T., Bittner, F. **(2012)**: Identification of superoxide production by *Arabidopsis thaliana* aldehyde oxidases AAO1 and AAO3.