OPEN STOMATA3 – an ABC transporter implicated in ABA signalling, drought and light response

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Mutants sensitive to progressive water deficit are characterized by excessive transpiration due to the failure of stomatal closure and can therefore be detected as cold plants (Fig. 1 blue) by remote infrared imaging (Merlot *et al*., 2002).

Among the signalling mutants, three are collectively named *open stomata* (*ost*). The corresponding *OST1* and *OST2* genes encode an ABA-activated kinase and a P-type H^+-ATPase, respectively (Mustilli *et al*., 2002; Merlot *et al*., 2007).

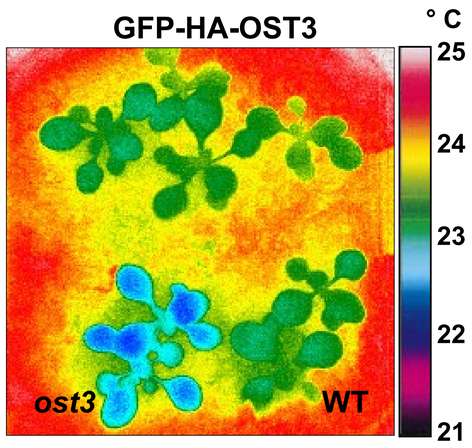
The current work deals with ***OST3*** which encodes an ATP-binding cassette (ABC) transporter. There are over 120 members of the ABC protein in the superfamily of *Arabidopsis thaliana*. Most of them are membrane-bound proteins that transport a diverse range of substances across the phospholipid bilayer.

Characterisation of the mutant phenotype confirmed that *ost3* **transpires excessively** (Fig. 1, 2). We have also shown that the *ost3* mutations **reduce seed dormancy** (Fig. 3) but seed **sensitivity to exogenous ABA seems unaffected** (data not shown). The guard cells of *ost3* are **impaired in responses to ABA and light** (Fig. 4), but are normal with respect to low level of CO_2 which stimulates stomatal opening (data not shown).

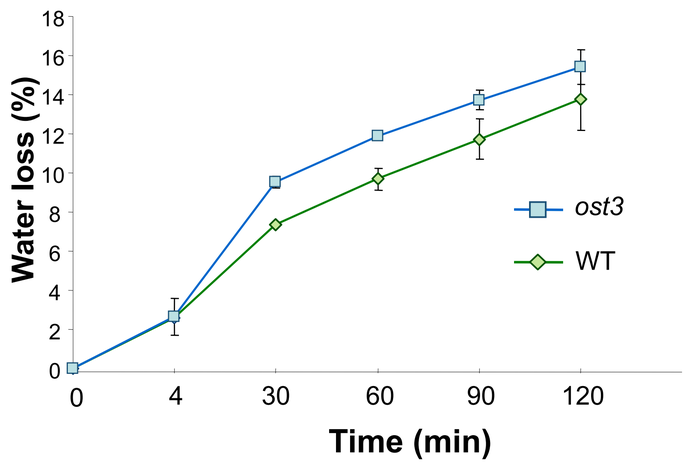
OST3 is **expressed mainly in leaves**, particularly in **guard cells** (Fig. 5), but it is low in root tissues. Transgenic expression of the OST3 protein fused to GFP in the *ost3* mutant can rescue the phenotype and moreover, the fusion **protein is targeted exclusively to the plasma membrane** (Fig. 6) suggesting that it has a role in intercellular transport required for ABA signal perception.

We found that OST3 **interacts with OST2** (Fig. 8) and **OST1** (Fig. 8 + 9). The last observation is also consistent with the fact that **OST3 can be phosphorylated by OST1** *in vitro* (Fig. 7). Therefore we suggest that the trio of proteins identified by our genetic screen may function in the same signalling complex in mediating stomatal response.

ost3 transpires excessively

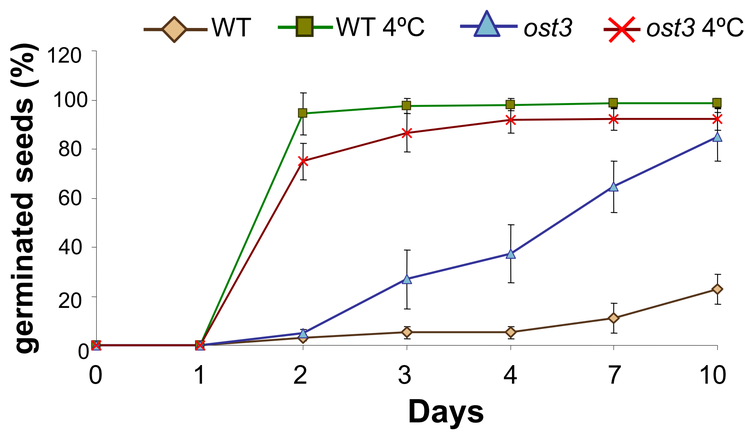


1. *ost3* mutant (blue) cannot respond to drought by closing its stomata, transpires excessively, and is thus detected as a cold plant. The mutant phenotype is restored to wild type by the transgene 35S::GFP-HA-OST3 (green).



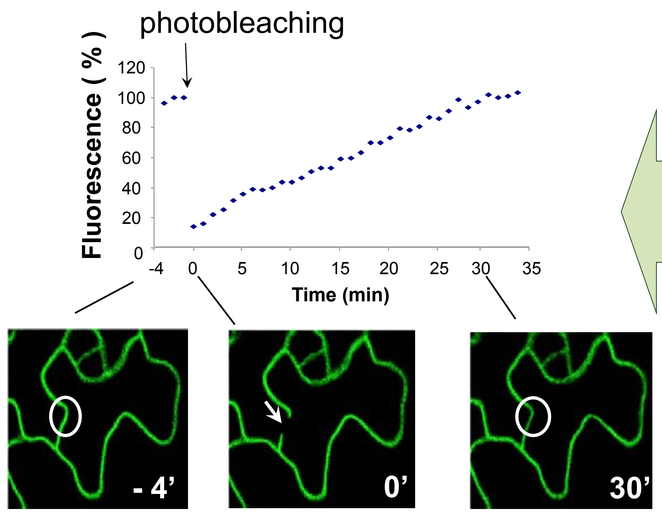
2. *ost3* transpires significantly more than the wild type 4 minutes after detaching the leaves, but this difference was maintained for the duration of the experiment.

ost3 seeds are less dormant



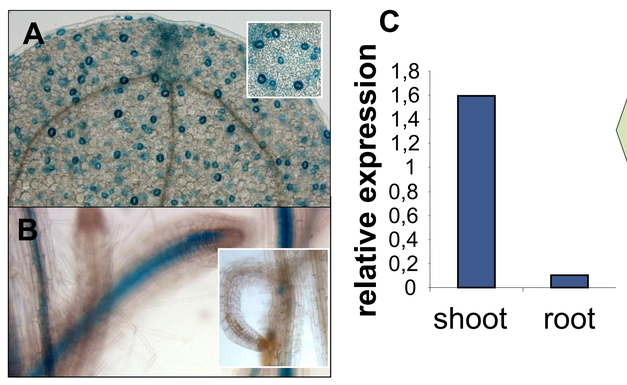
3. As compared to the wild type, *ost3* seeds germinate more readily without pre-incubation in the dark and at 4ºC to remove dormancy. In contrast, similar efficiency of germination was observed when seeds were pre-treated as above.

OST3 is a moderately mobile plasma membrane protein



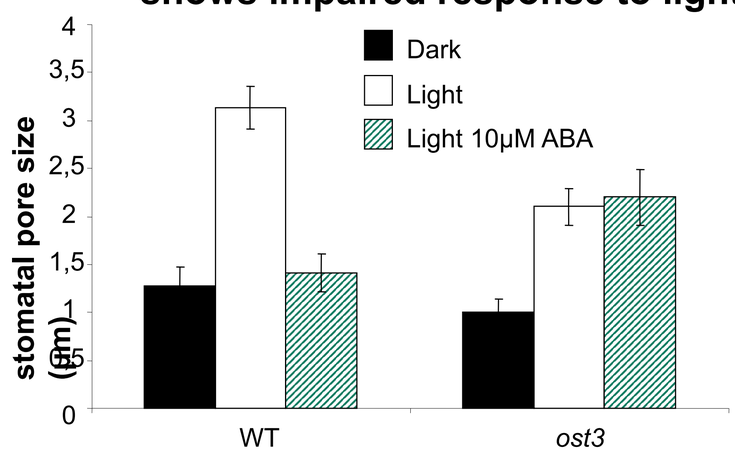
6. OST3 is targeted exclusively to the plasma membrane (controls not shown) where it is mobile and refills the 4,5 µm bleached area within 30 minutes. This is in contrast to nonmobile KAT1 and PMA2 H^+-ATPase (Sutter *et al*., 2006) also functionally linked to ABA response.

OST3 is expressed mainly in guards cells and weakly in roots



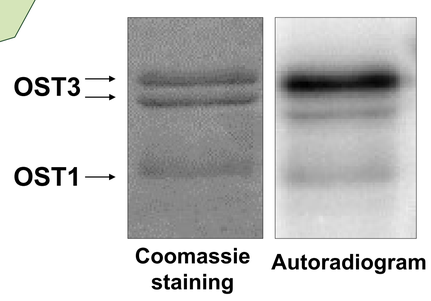
5. Expression of GUS reporter gene driven by the *OST3* promoter (A, B) and *OST3* expression profile by quantitative PCR analysis (C).

ost3 doesn't respond to ABA and shows impaired response to light

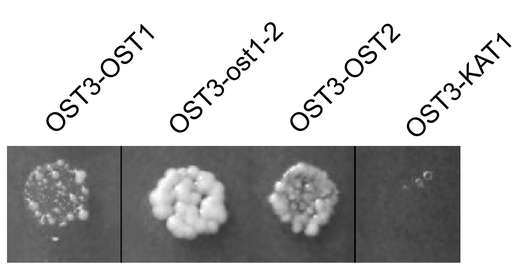


4. Response of guard cells of WT and *ost3* mutant to ABA and dark to light transition. Samples of epidermis were incubated for 0,5 hour in the dark and then for 3 hours in the light without or with 10 µM ABA.

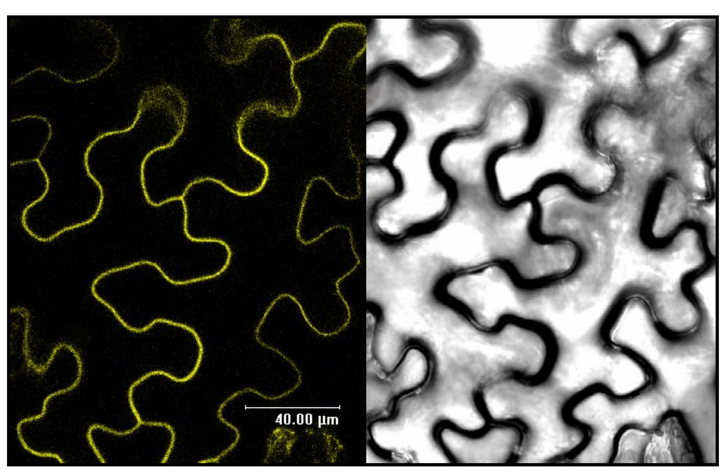
OST3 is phosphorylated by OST1 kinase *in vitro* and interacts with OST1 and OST2 H^+ATPase



7. *In vitro* phosphorylation of OST3 by OST1 kinase. 52-KDa N-cytosolic part of OST3 was incubated in presence of OST1 kinase. Activity of the kinase is confirmed by the autophosphorylation signal.



8. OST3 interacting partners in Split Ubiquitin assay in yeast. Interaction is visualised via growth selection.



9. Interaction between OST3 and OST1 is confirmed *in planta* by BiFC imaging technique (Split YFP) in transfected *Nicotiana benthamiana* leaves.

Prospects:

- Identify the specific substrates transported by OST3. Because OST3 mutant presents an ABA insensitive phenotype, we are particularly interested whether ABA is a specific substrate of OST3 transporter.
- Define interaction domains in OST3 and OST1 by Y2H and BiFC
- Verify interaction with OST2 via BiFC and colocalisation

Literature:

- Merlot S., Mustilli A-C., Genty B., North H., Lefebvre V., Sotta B., Vavasseur A., Giraudat J. (2002). Use of infrared thermal imaging to isolate *Arabidopsis* mutants defective in stomatal regulation. Plant J. 30, 601-609
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