

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).

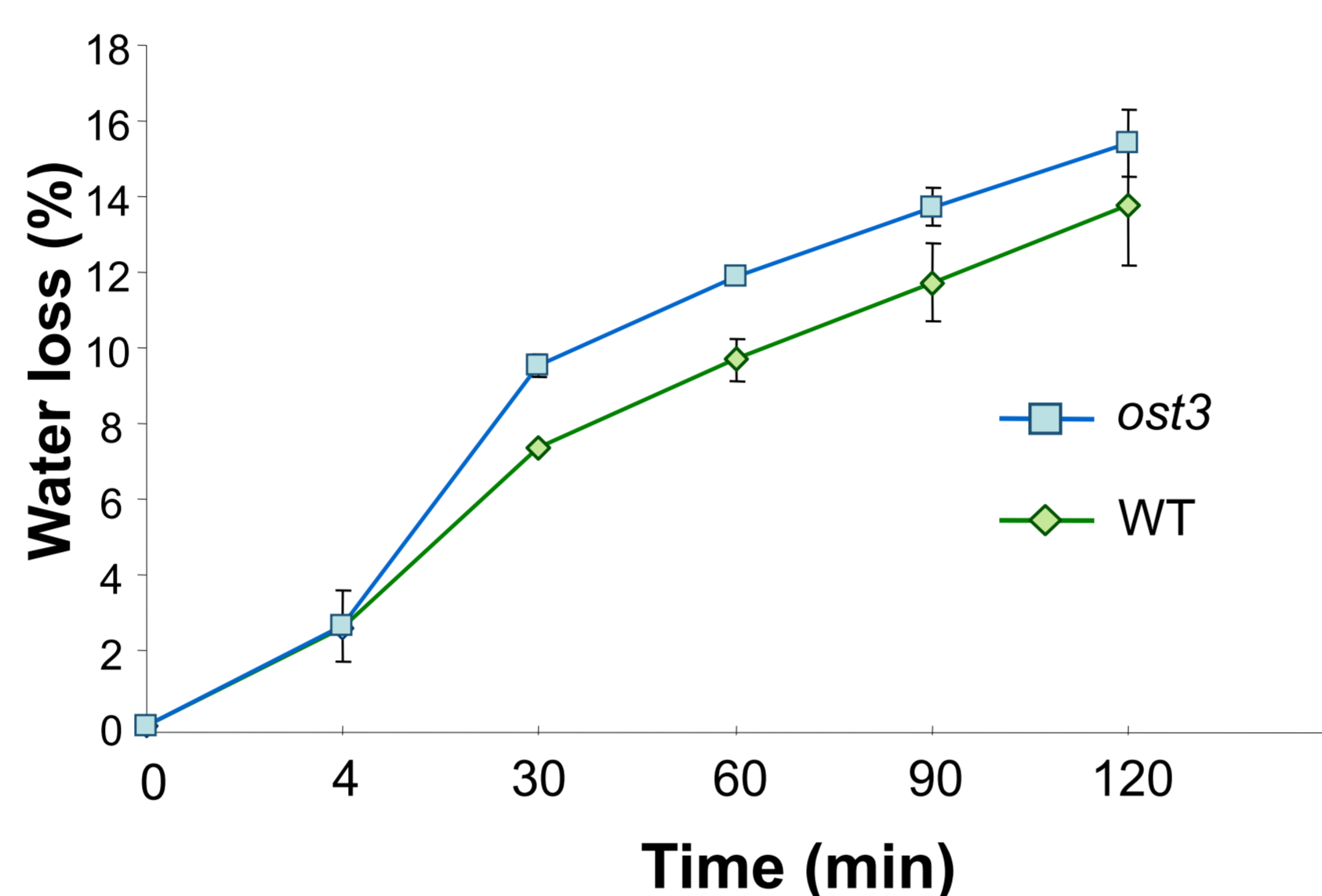
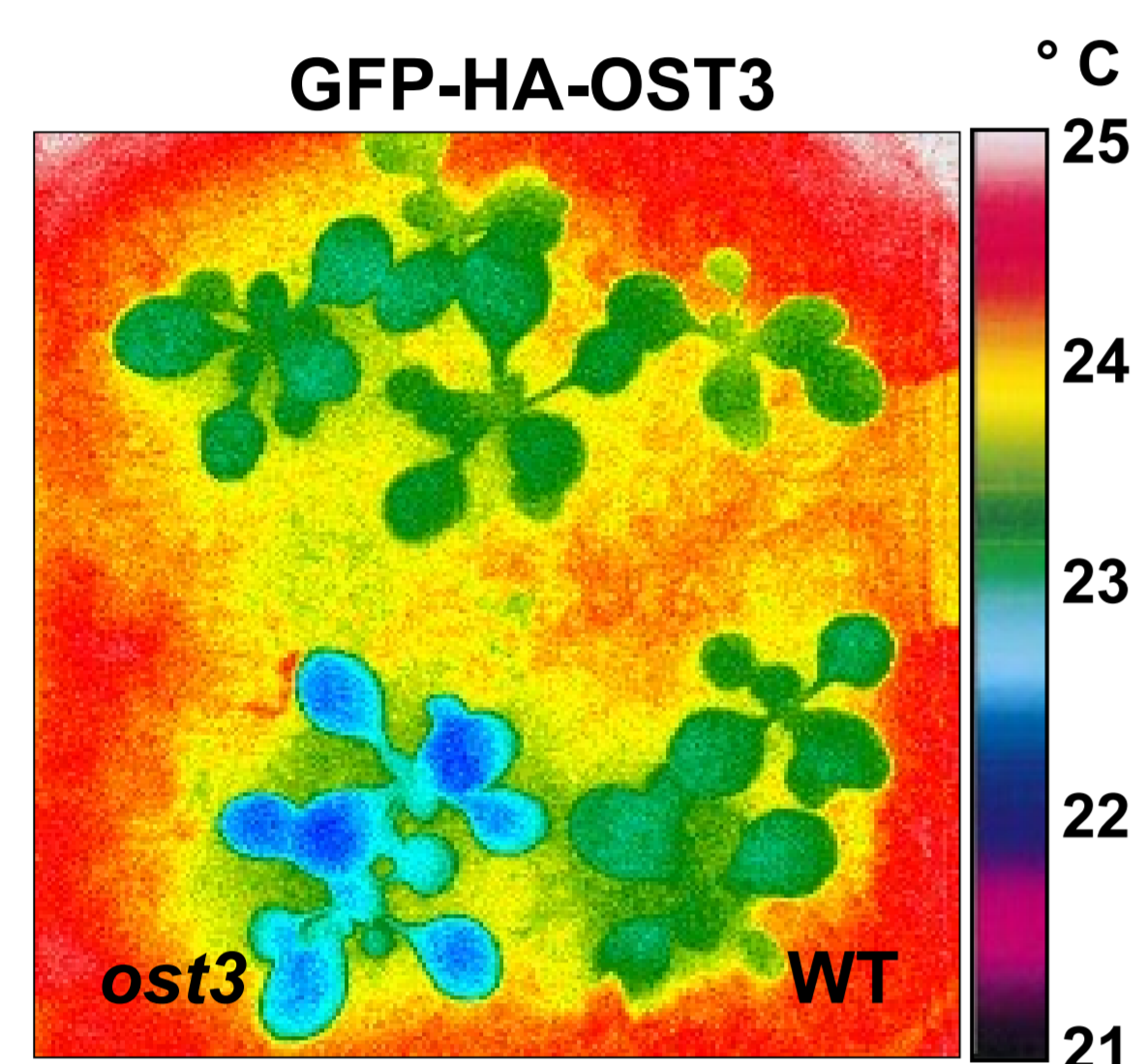
The current work deals with *OST3* which encodes an ATP-binding cassette (ABC) transporter. There are over 120 members of the ABC protein in the superfamily of *Arabidopsis thaliana*. Most of them are membrane-bound proteins that transport a diverse range of substances across the phospholipid bilayer.

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 CO₂ 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.

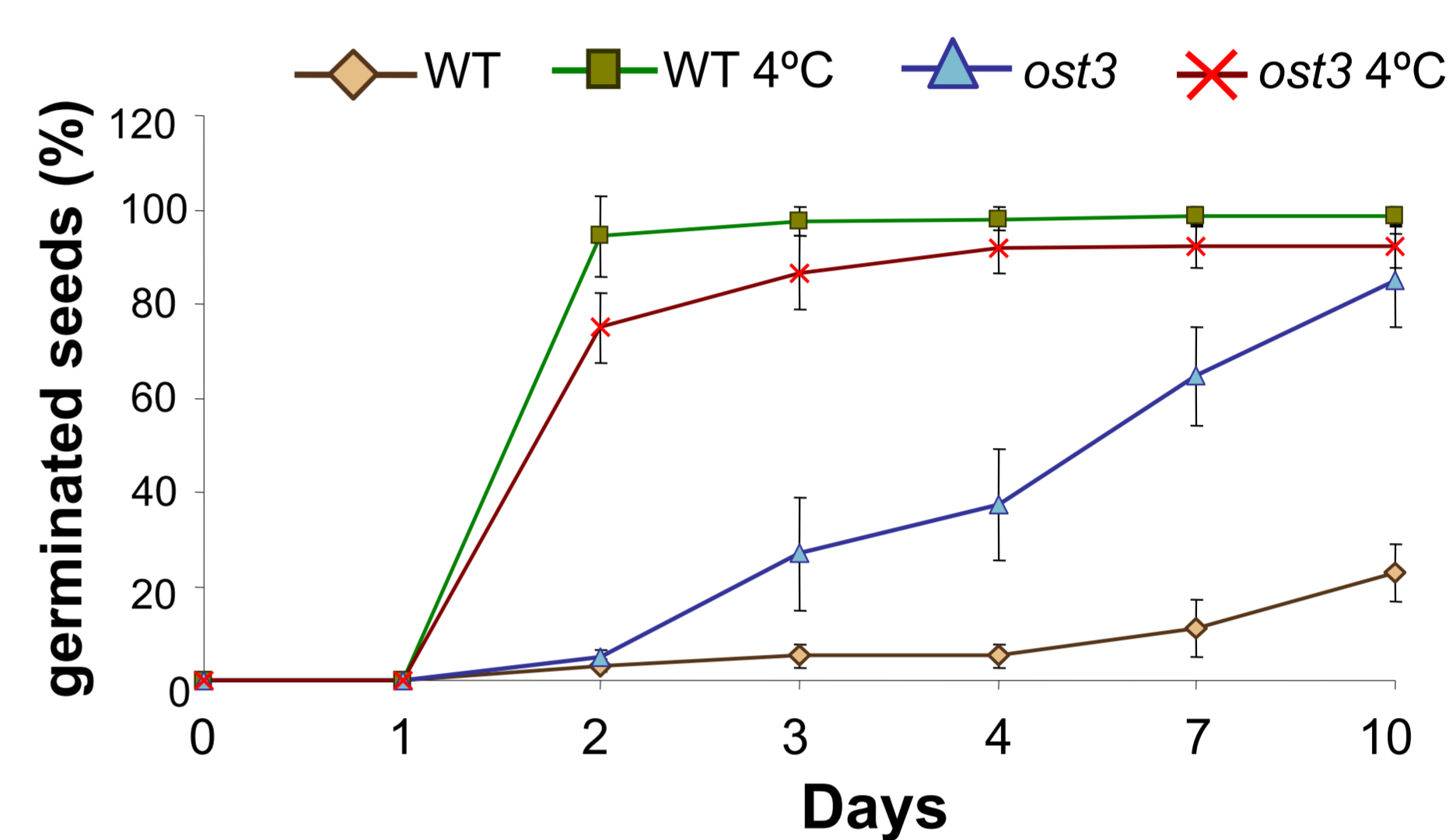
ost3 transpires excessively



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).

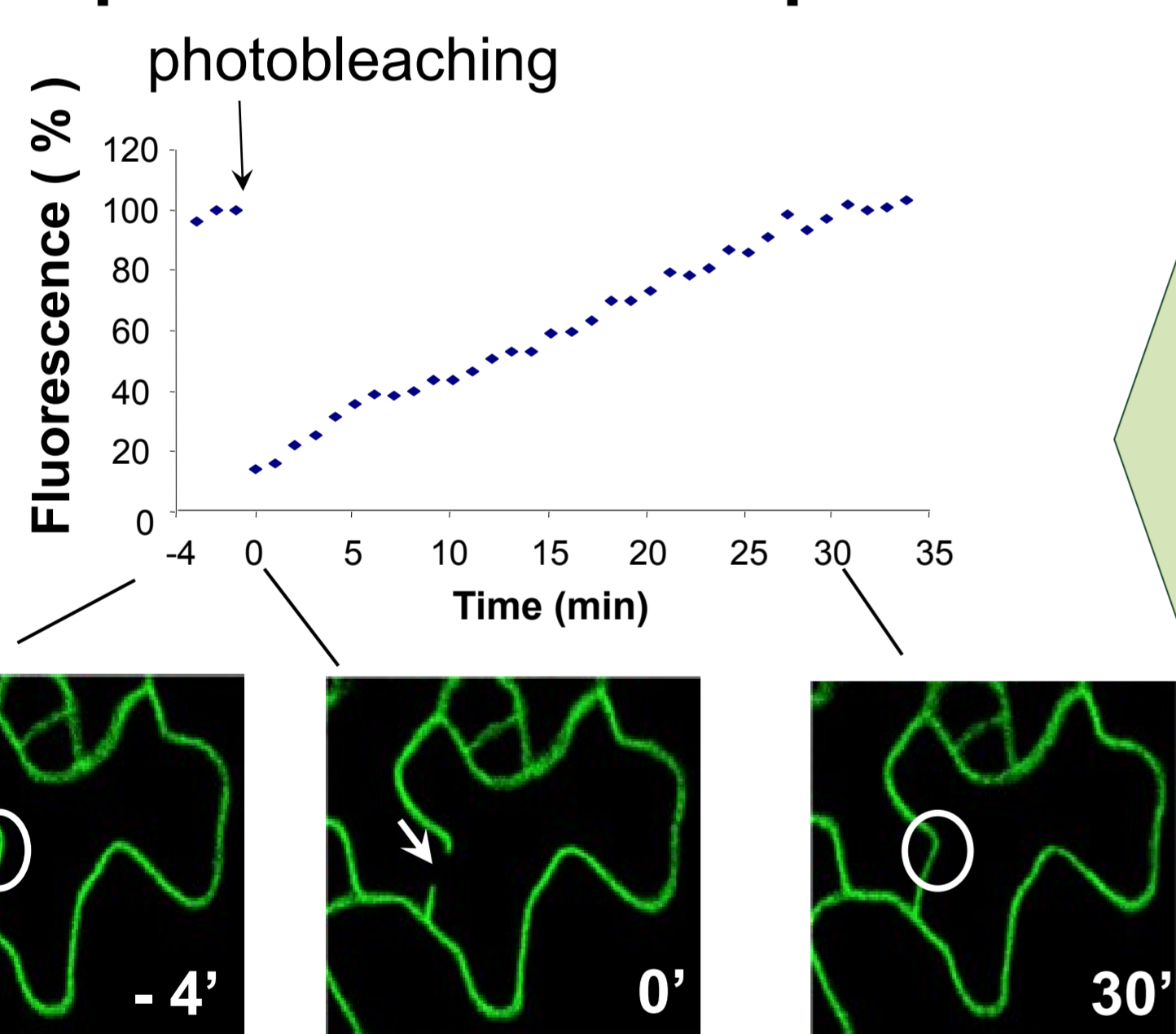
2. *ost3* transpires significantly more than the wild type 4 minutes after detaching the leaves, but this difference was maintained for the duration of the experiment.

ost3 seeds are less dormant



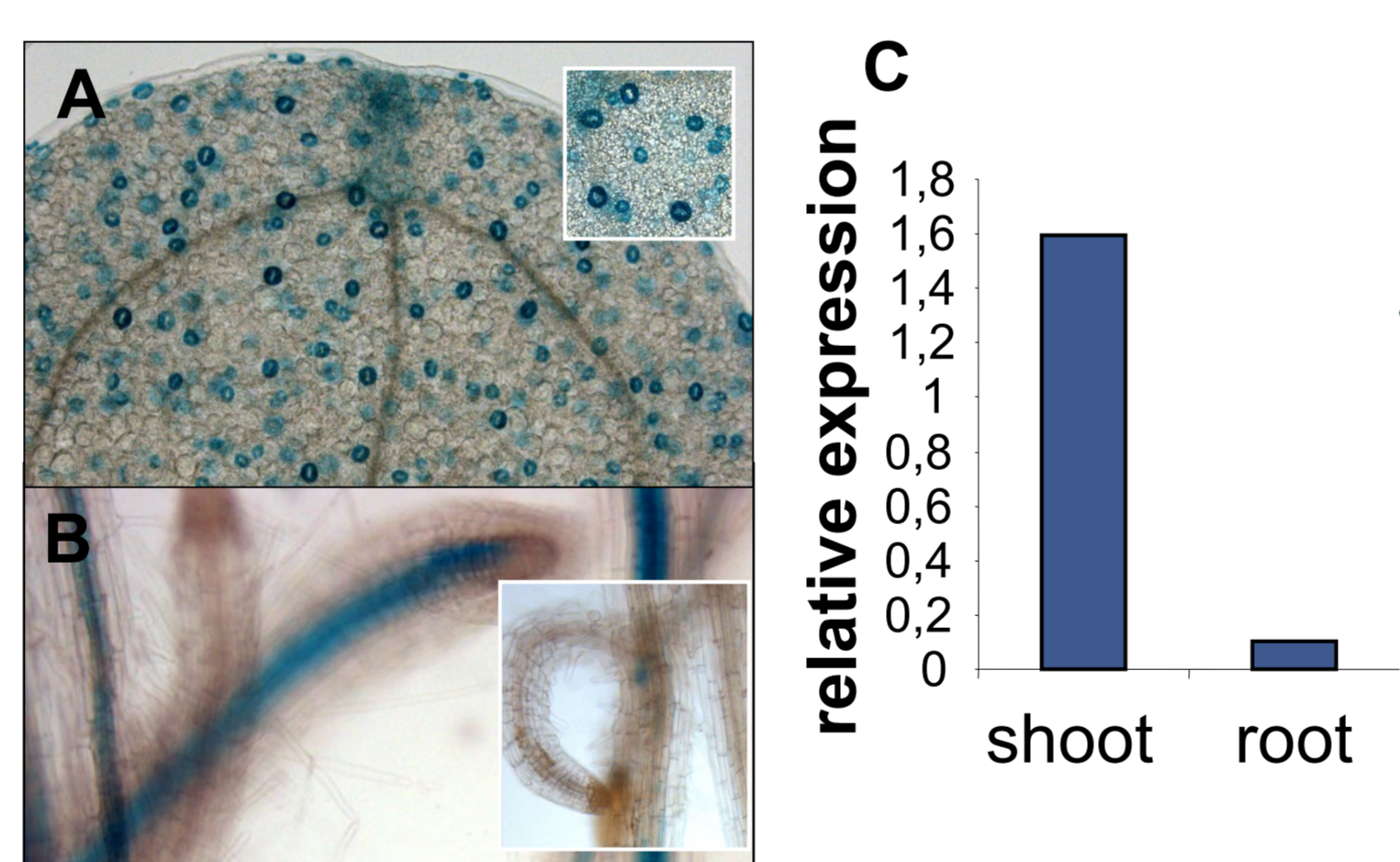
3. As compared to the wild type, *ost3* seeds germinate more readily without pre-incubation in the dark and at 4°C to remove dormancy. In contrast, similar efficiency of germination was observed when seeds were pre-treated as above.

OST3 is a moderately mobile plasma membrane protein



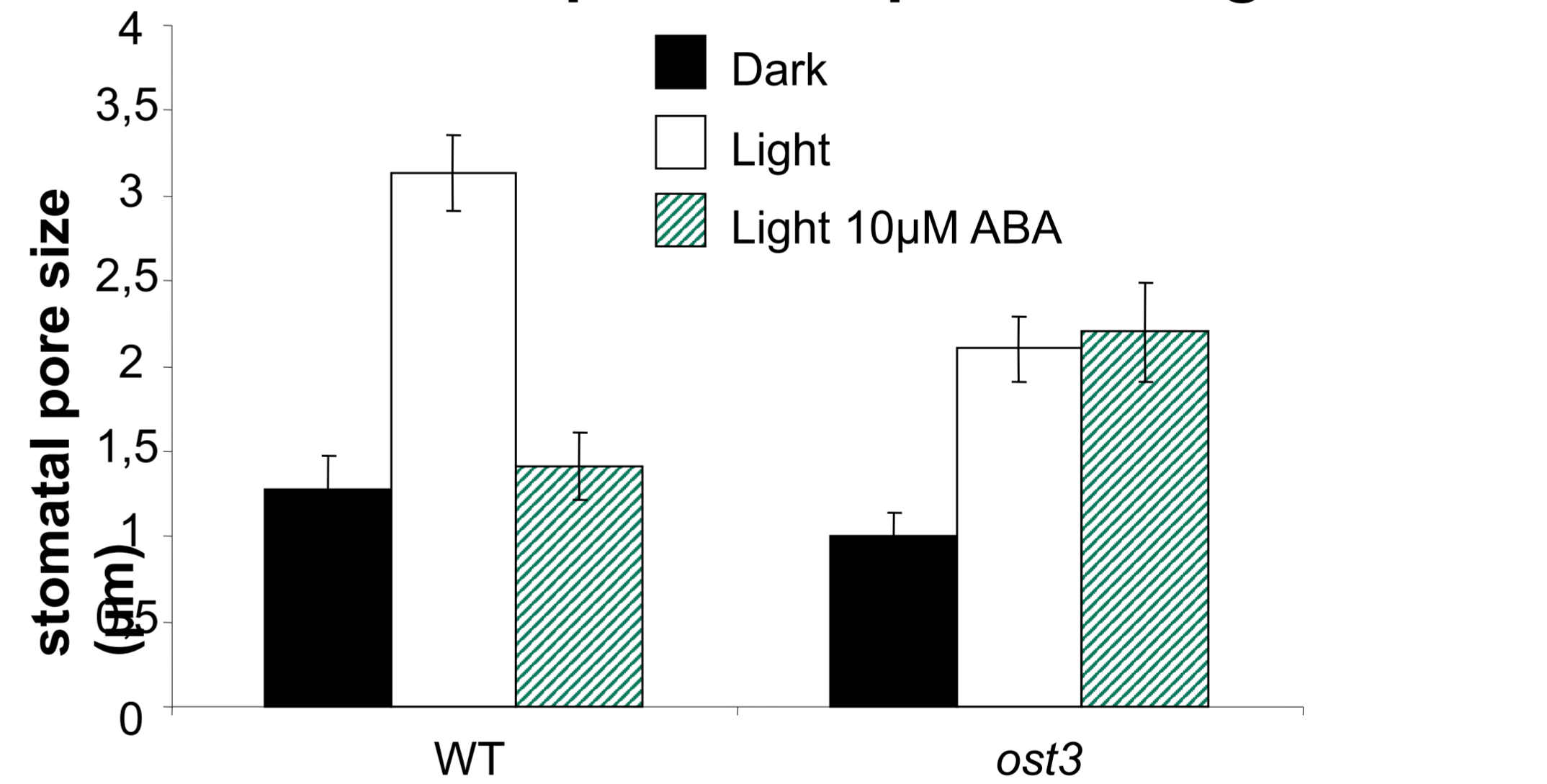
6. *OST3* is targeted exclusively to the plasma membrane (controls not shown) where it is mobile and refills the 4,5 μm bleached area within 30 minutes. This is in contrast to nonmobile *KAT1* and *PMA2* H⁺-ATPase (Sutter *et al.*, 2006) also functionally linked to ABA response.

OST3 is expressed mainly in guard cells and weakly in roots



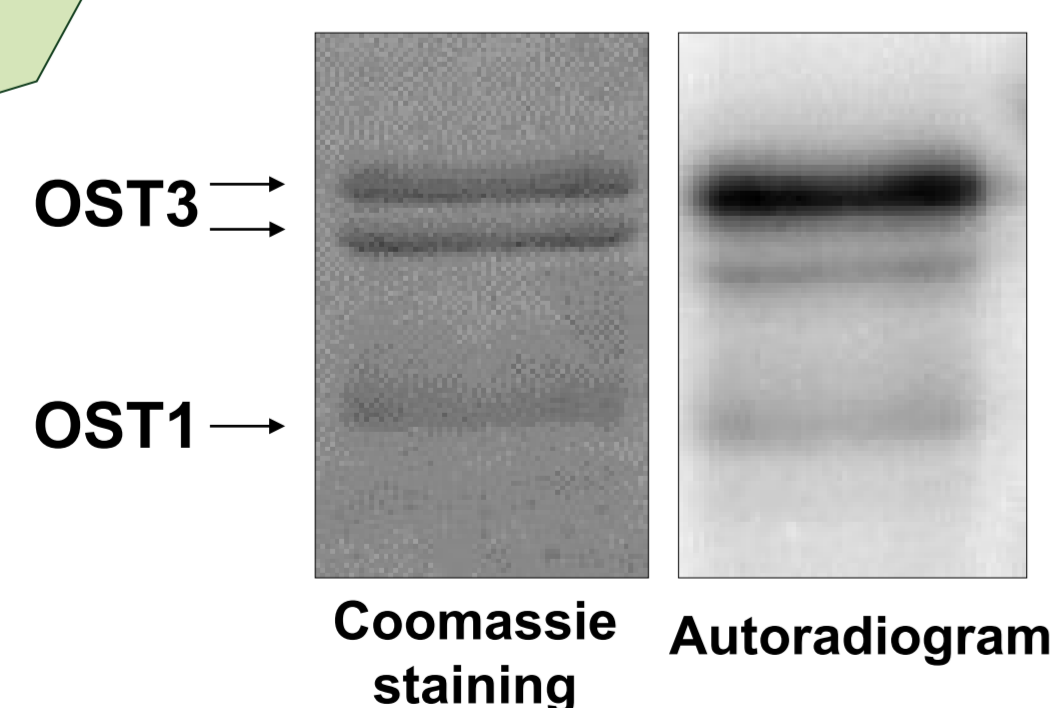
5. Expression of GUS reporter gene driven by the *OST3* promoter (A, B) and *OST3* expression profile by quantitative PCR analysis (C).

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

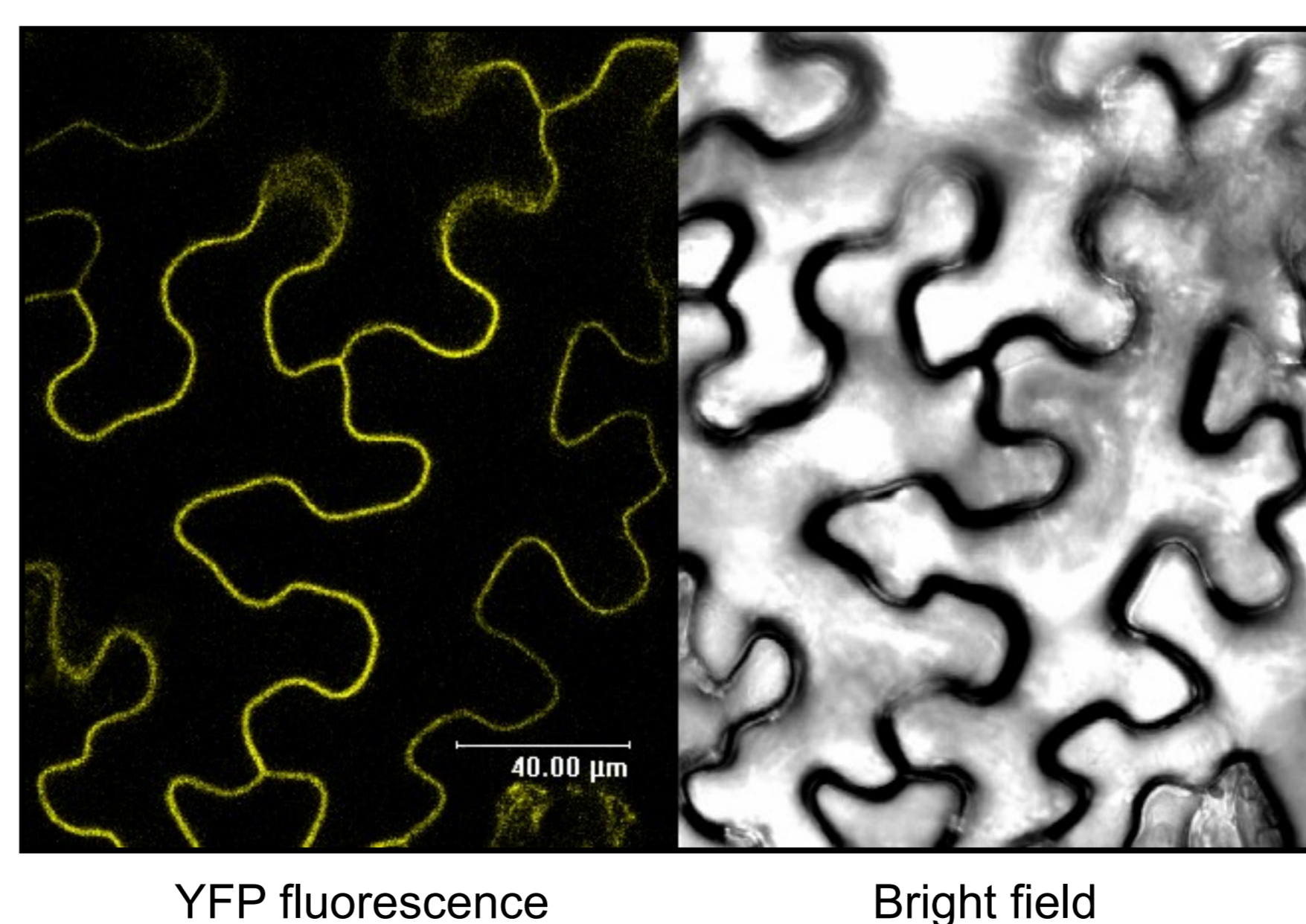


4. Response of guard cells of WT and *ost3* mutant to ABA and dark to light transition. Samples of epidermis were incubated for 0,5 hour in the dark and then for 3 hours in the light without or with 10 μM ABA.

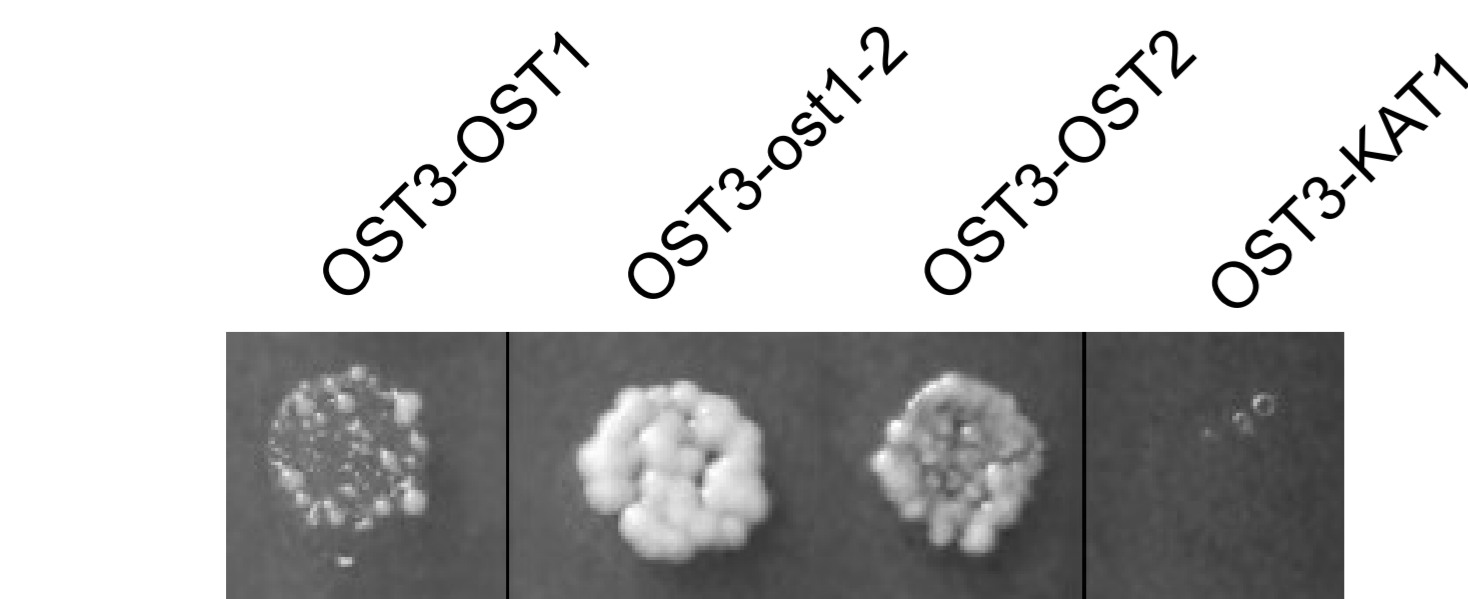
OST3 is phosphorylated by OST1 kinase in vitro and interacts with OST1 and OST2 H⁺-ATPase



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.



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



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

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:

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