

ABERRANT STOMATA BEHAVIOUR IN *ER-ANT1*, A PHOTORESPIRATORY MUTANT OF *ARABIDOPSIS THALIANA*

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The carrier Endoplasmic Reticulum Adenylate Transporter1 (ER-ANT1) has been previously identified in *Arabidopsis thaliana* as an ATP/ADP antiporter in the endoplasmic reticulum (ER). Mutant plants lacking ER-ANT1 exhibit a photorespiratory phenotype, with an higher glycine content and a dramatic reduction in growth, when plants are grown in ambient CO₂.

Additionally, *er-ant1* knock out (KO) mutants show an enhanced stomata closure upon shift to reduced CO₂, after 3h and 6h from the light onset. This phenotype is consistent with the strongest photorespiratory mutants (*shm*, *glyk* and *pglp1*), in which the aberrant stomata closure and the subsequent reduction in CO₂ availability lead to chlorosis, stunted growth and cell death in source leaves. Moreover, epidermal peels of *er-ant1* KO mutants show defective stomata opening in a middle strength opening buffer after 2h or 4h treatment. The properly stomata response was restored in a stronger opening condition (10 mM MES; 50 mM KCl; 100 uM CaCl₂) or supplying fusicoccin (1 uM), an inhibitor of the stomatal H⁺/ATP-ase. No differences between *er-ant1* KO and wild type (WT) plants were observed in dark-inducing stomatal closing, after 3h light.

These results imply an indirect effect of ABA, which plays a major role in the inhibition of stomata opening. To assess this hypothesis, the stomata response after ABA treatment (10 uM) was evaluated in WT and *er-ant1* KO epidermal peels. *er-ant1* KO mutants show an enhanced stomata closing in comparison to the WT after 2h treatment, suggesting an hypersensitivity to ABA, putatively due to an aberrant ABA signalling and/or an increased ABA quantity.

The enhanced stomata closure observed in the *er-ant1* mutants was also confirmed by a less water loss in detached leaves during a time course, ranging from 0,5h to 8h. Furthermore, an increased survival rate after drought stress was also observed in *er-ant1* KO in respect to WT plants.

Finally, one of the most studied effect of ABA is the inhibition of seed germination. However, no significance differences in the germination rate of WT and *er-ant1* KO seeds were observed after ABA treatment (0-10 uM). This seems to imply that the ABA-hypersensitivity phenotype observed in *er-ant1* KO mutants is mainly related to the stomata closure.

Taken all together, these preliminary results suggest a possible link between ER and ABA metabolism in a photorespiratory mutant. It has been reported that a reserved of ABA in a conjugated form is stored in the ER, for activating a fast response during stress (i.e.: drought).

A depletion of ATP and an altered energetic status in the ER could activate the ABA response, through, for example, Ca⁺² or lipid signalling, key components of the ABA pathway, leading to an enhanced stomata closure and photorespiratory metabolism. However, to elucidate the role of ABA in relation to stomata behaviour and photorespiration further experiments are required, such as the quantification of ABA and the characterization of the ABA signalling in *er-ant1* KO mutants, as well as in other photorespiratory mutants.