NADKinases-Calmodulin-dependent as driver for pollen tube tip growth

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NADP(H) is obtained by means of specialised enzymes that can phosphorylate NAD(H), named NAD-Kinases (NADKs). In *Arabidopsis thaliana* four NADK isoforms exist, one of which is activated upon Calmodulin (CaM). This class of NADKs has been named NADK-CaM-dependent (NADKc) and is present only along the green lineage. In Arabidopsis seedlings, it has been demonstrated that NADKc1 acts upstream NADPH oxidases (RBOHs), affecting the production of Reactive Oxygen Species (ROS) in response to the bacterial elicitor flagellin22.

Notably, RBOH-generated-ROS and Calcium are key players for the regulation of tip growth processes. Pollen tube tip growth relies on their concertation, that if disrupted causes abnormal pollen tube growth and even infertility. However, the extent to which NAD(P)/H and NADKs participate in such process has never been investigated in detail.

Here we display that two newly discovered putative NADKcs of *Arabidopsis thaliana*, named NADKc2 and 3, have a role in the pollen tube development and fertilisation success.

We show that *NADKc2* and *NADKc3* are exclusively expressed in mature pollen grains and pollen tubes. While single mutants were mildly affected, the double mutant showed both *in vitro* and *in vivo* reduced pollen tube elongation. Microscopy inspection of *nadkc2/nadkc3* pollen tubes showed bulged tips, compared to the wild type. Importantly, the double mutant had reduced silique length and seed number, compared to the wild type. Our results suggest that the altered pollen development of the mutant, has an impact on fertilization success, that was even more affected upon a mild heat stress.

The discovery that NADKcs are important for the fertilization process has then motivated us to study if their role is conserved evolutionary focusing on the bryophyte *Marchantia polymorpha*. We show that *Marchantia* has only one *NADKc* homolog, which is expressed in both reproductive and vegetative tissues, raising important questions on the diversification of NADKcs.