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Artificial microRNA strategy for exploring the function of Na^+ transporter gene in rice

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Soil salinity is an increasing environmental concern for agriculture. High Na^+ concentration in the soil is toxic to most plant species. Na^+-permeable HKT-type transporters have been shown to play important roles in Na^+ homeostasis upon salt stress. AtHKT1 in *Arabidopsis thaliana* and OsHKT1;5 in rice, for instance, were found by genetic approaches to be involved in leaf Na^+ and K^+ homeostasis, and thereby tolerance to salt stress, by recirculating Na^+ from shoots to roots via the phloem and/or by retrieving Na^+ from the ascending sap (Berthomieu *et al.*, 2003, EMBO J; Ren *et al.*, 2005, Nat Genet; Sunarpi *et al.*, 2005, Plant J). While only one *HKT* gene is present in Arabidopsis, nine *HKT* genes exist in rice and the role of most of them is still unknown. OsHKT1;1, which is expressed in rice in both roots and shoots, behaves like a Na^+ transporter when heterologously expressed in *Xenopus* oocytes. In order to analyze the function of *OsHKT1;1* gene in rice, in absence of loss of function mutant in the insertional mutant collections, a RNAi approach was developed to produce rice lines with low *OsHKT1;*1 transcript levels. Web MicroRNA desig (WMD2) platform was used to design two independent 21mers artificial microRNA (amiRNA) against two target sites of *OsHKT1;1* transcript. Rice was transformed with the respective cDNA sequences under the control of a strong constitutive promoter. RT-PCR analyses on transformed T0 plants validated the amiRNA strategy. Whether the low level of *OsHKT1;1* transcripts is stably inherited in next generations is under analysis.

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Functional regulation of the sodium transporter *Os*HKT1;3

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Recent characterization of HKT proteins have demonstrated that they function as sodium transporters involved in the uptake and long distance transport of this cation in plants. So far, these transporters are among the few mechanisms that show a high selectivity towards sodium, suggesting that they are involved in the control of sodium in the whole plant. In rice (*Oryza sativa*), seven HKT genes have been identified, and two of them have been shown to participate in μM sodium absorption in the absence of potassium (*OsHKT2;1;* Horie et al., 2007), or in the retrieval of this cation from the xylem in the root (*OsHKT1;5* Ren et al., 2005). Here we report the characterization of *Os*HKT1;3, by using heterologous expression system of *Xenopus laevis* oocytes, demonstrating that it functions as a highly sodium selective transporter. Moreover we investigated the possibility of post-translational regulation of *Os*HKT1;3 using staurosporine, an general inhibitor of protein kinases. The transport of Na^+ recorded in oocytes expresing *Os*HKT1;3 showed a decrease when incubated in staurosporine with respect to control oocytes. We also analyzed the possible regulation of OsHKT1;3 by pH. The results showed that changes in either extracellular or intracelullar pH did not affect the transport of Na^+ in *Os*HKT1;3.

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Signalling related to Solute and Hormone Transport II

P2-46

Abscisic acid inactivates ROP2 GTPase and accelerates the stomatal closing movement

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ROP GTPases function as molecular switches in diverse processes. Previously we have shown that ROP2 activation upon light irradiation negatively regulates the light-induced stomatal opening. Here, we demonstrated that the inactivation of ROP2 is required for stomatal guard cells to respond rapidly to ABA and CO2. In response to ABA treatment, the activity of ROP2 was decreased in the stomatal guard cells. The expression of a constitutively active form of ROP2 (CA-rop2) in Arabidopsis thaliana and Vicia faba resulted in the slower and reduced stomatal closure in response to ABA and CO2. In contrast, the expression of DN-rop2 and knocking out ROP2 (rop2) promoted the ABA-induced stomatal closure in Arabidopsis. We then investigated the mechanism by which active ROP2 suppresses stomatal closure. The endocytic membrane trafficking, which is regulated by Rho GTPases in animal cells, is known to be required for the stomatal closure. We showed that the endocytosis of plasma membrane traced by FM4-64 is largely inhibited in CA-rop2 expressing guard cells. These results lead us to conclude that the active ROP2 suppresses stomatal closure probably by inhibiting the endocytic plasma membrane uptake and the inactivation of ROP2 by ABA promotes the stomatal closure.

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Plant Methods: An independent Open Access journal for technological innovation in the plant sciences

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