VACUOLAR DYNAMICS IN GUARD CELLS AND STOMATAL MOVEMENTS DEPEND ON POTASSIUM UPTAKE AT THE TONOPLAST

José M. Pardo, *Zaida Andrés, Javier Pérez-Hormaeche, Eduardo O. Leidi, Beatriz Cubero*

Instituto de Recursos Naturales y Agrobiologia de Sevilla, Consejo Superior de Investigaciones Cientificas, Sevilla – 41012, Spain.

The rapid uptake and loss of K^+ and of other osmolytes by guard cells, mostly in the vacuolar compartment, controls the opening and closing of stomata, and thereby gas exchange and transpiration of plants. Despite the established role of osmolyte transport accross the plasma membrane of guard cell in stomata function, osmolyte uptake into the cytosol represents only a transient step to the vacuole since more than 90% of the solutes accumulate into the vacuoles. The question addressed here is: *How is* K^+ *taken into the vacuoles of guard cells to sustain stomatal opening?* We show that tonoplast-localized K^+/H^+ antiporters in guard cells mediate the vacuolar accumulation of K^+ and that these transporters are not only required for stomatal openning but, unexpectedly, for stomatal closure as well. Arabidopsis mutants deficient in genes the two major forms of K^+/H^+ antiporters that are highly expressed in guard cells, *NHX1* and *NHX2*, were used. Double mutant lines of genotype *nhx1 nhx2* presented stomata with aberrant morphologies and a vacuolar K^+ pool in guard cells that was only half the size of the wild type. These results imply that K^+ transport by NHX proteins represents the main pathway for the K^+ uptake into the vacuoles of guard cells. Infrared thermography on whole plants revealed altered transpiration rates in the mutant. In stomatal bioassays with epidermal strips, a hypomorphic mutant exhibited impaired stomata opening and delayed closure, whereas the double knockout line was defective in both processes. These results establish that the large uptake flux of K^+ into vacuoles is not only a physicochemical requisite for stomatal opening, but also a critical component of the K^+ homeostasis that is needed for stomatal closure. Abrogation of K^+ accumulation by guard cells correlated with more acidic vacuoles in the mutant and the disappearance of the highly dynamic changes in vacuolar structure associated to stomatal movements. By using guard cell imaging, we show that K^+ transport is a critical component of the guard cell vacuole remodeling and the control of vacuolar pH that together sustain stomatal movements. Sodium supplementation partly restored stomatal opening, vacuolar dynamics and lumenal pH control. Delayed responses of stomata in the null mutant in daily cycles led to the counterintuitive finding that mutant plants survived longer under water deprivation because the plants were not only smaller but they also transpired less per leaf area unit during the day, thus consuming less soil water. Water loss at night in the mutant was greater compared to the wild type, but this was apparently compensated by diurnal water savings. In summary, this work uncovers the essential role that the active accumulation of K^+ in the vacuoles of guard cells plays in guard cell dynamics and stomatal function.