Ion homeostasis: plants feel better with proper control

Plant growth and the ecologically and agriculturally important processes of biomass accumulation and seed production are affected by numerous environmental factors, including air temperature, illumination, water availability and infestation with pathogens. Inorganic ions also represent an integral part of the environmental cocktail that affects plant growth. Of the best bio-available alkali cations— Na^+ and K^+ —plants have a strong preference for K^+ . K^+ , the most abundant cation in plant cells, is an essential macronutrient; by contrast, elevated concentrations of Na^+ are generally toxic to plants. Therefore, it is not surprising that plants have evolved several strategies that enable them to keep the cytosolic concentration of Na^+ low. Only a relatively small number of species can withstand saline environments, and plant researchers are keen to unravel the underlying mechanisms to improve the salt tolerance of crops (Yamaguchi & Blumwald, 2005).

Despite the generally toxic effect of high concentrations of Na^+ , plants have transporters that allow the uptake of Na^+ from the soil. Members of the *HKT* gene family of Na^+ and Na^+/K^+ transporters seem to have a crucial role in controlling this process (Platten *et al*, 2006). Why do plants have such transporters, if Na^+ uptake is so immensely harmful? Physiological studies from the early 1980s reported that at low concentrations Na^+ can positively affect the growth of many species of plants. This indicates that, under these conditions, Na^+ acts as a nutrient rather than as a stress factor. Consequently, a model has been proposed claiming that Na^+ can, to some extent, replace its 'sibling' K^+ in carrying out crucial cellular functions. However, a transporter for Na^+ uptake at low external concentrations of Na^+ was not known in plants.

In a recent article published in *The EMBO Journal*, Tomoaki Horie and co-workers from three laboratories in San Diego (USA), Pohang (Korea) and Tsukuba (Japan) report their findings on mutant rice plants that lack a functional Na^+ transporter called *OsHKT2;1* (Horie *et al*, 2007; Fig 1A). In such lines the *OsHKT2;1* gene was disrupted through the insertion of a transposable element—*Tos17*—which undergoes local transposition events during tissue culture but remains non-mobile in regenerated rice plants. Under standard growth conditions, the knockout plants were indistinguishable from their wild-type counterparts; however, importantly, *oshkt2;1* mutants grew to much smaller sizes than the control plants when challenged with low concentrations of Na^+ under conditions of K^+ starvation. They also accumulated considerably less Na^+ in both shoots and roots. Thus, *OsHKT2;1* functions in Na^+ accumulation and favours plant biomass accumulation under such extreme conditions.

These findings clearly show the nutritional aspect of Na^+ and the involvement of a transporter in Na^+ uptake. The rice genome contains nine *HKT* genes, two of which might be pseudogenes

Fig 1 | Ion homeostasis. The model plant species, thale cress (*Arabidopsis thaliana*, right) and rice (*Oryza sativa*, left), are displayed as a chimera. (A) *OsHKT2;1* functions as a transporter for Na^+ uptake. (B) *QSO2*, a flavoprotein quiescin sulphhydryl oxidase, is a new component in ion homeostasis at the root symplast–xylem interface. (C) Downregulation of *OST2/AHA1* H^+ -ATPase activity is a prerequisite for ABA-induced stomatal closure. ABA, abscisic acid; *OST2/AHA*, OPEN STOMATA 2.

(Garcia-deblás *et al*, 2003). Although multigene families are often believed to indicate functional redundancy, fully redundant biological roles are unlikely to remain conserved over evolutionary timescales. Instead, sub-functionalization of duplicated genes often occurs, allowing the fine-tuning of physiological processes and enhancing the ability of an organism to survive in a non-stable environment. In the case of *OsHKT2;1*, the authors realized that *OsHKT2;1* is expressed in roots, its expression is upregulated under conditions of K⁺ starvation and that *OsHKT2;1* transports Na⁺. This combined knowledge allowed them to set up experimental conditions provoking a phenotype that clearly shows a role of *OsHKT2;1* in plant biology.

The results from Horie and co-workers underline that the transport of K⁺ and Na⁺ are tightly connected processes that are dependent on the nutritional status of the plant. This is also evident in another paper published recently in *The EMBO Journal*. Santiago Alejandro and co-workers from laboratories in Valencia and Málaga (Spain) discovered another new and unexpected component in plant ion homeostasis (Alejandro *et al*, 2007). Through screening of an *Arabidopsis* 'activation tagging' lines library, these authors identified a gene, called *QSO2*, which, on overexpression, confers superior tolerance to toxic concentrations of the cation nonpermidine, a non-metabolizable polyamine. By contrast, tolerance to this chemical was lowered when *QSO2* was disrupted through integration of foreign DNA—in so-called T-DNA insertion lines. A high level of expression of *QSO2* also led to tolerance against Na⁺, and Li⁺, and decreased accumulation of these toxic ions, whereas the accumulation of non-toxic K⁺ was increased (Fig 1B; the opposite was observed in the *qso2* mutant lines). The interesting thing about *QSO2* is that it encodes an 'uncommon' enzyme—the flavoprotein quiescin sulphydryl oxidase (QSO). It has been proposed that QSOs participate in oxidative folding of disulphide-containing secreted proteins in plants and animals (Coppock & Thorpe, 2006). These proteins enter the secretory pathway and are located in the endoplasmic reticulum, the Golgi apparatus or outside the cell. The domain structure of QSOs was originally identified in human fibroblasts, but their physiological functions remain only poorly defined. Although the precise mode of action of the plant enzyme is not yet known, the recent report by Alejandro *et al* (2007) indicates an important role in adjusting ion homeostasis. Experimental evidence suggests that *QSO2* regulates ion transport at the root symplast–xylem loading interface; however, the well-known xylem parenchyma-expressed outward-rectifying K⁺ channel SKOR (Gaymard *et al*, 1998) is not required for this mechanism. Future experiments will therefore have to clarify with which other proteins *QSO2* interacts to fully exploit its control function.

Another environmental stress that strongly affects plant growth is water scarcity. When exposed to drought, plants accumulate the hormone abscisic acid (ABA), which triggers the closure of stomata—the pores in the leaf epidermis that allow gas exchange for photosynthesis (CO₂ uptake) and transpiration (water vapour release). During stomatal opening under favourable conditions, activation of a plasma-membrane-localized proton pump—H⁺-ATPase—establishes a negative membrane voltage that drives the uptake of K⁺ (Fig 1C). For stomatal closure, anion channels that depolarize the membrane are activated, setting conditions for long-term K⁺/anion efflux. The question of whether deactivation of the proton pump is needed for stomatal closure, or whether the activation of anion channels is sufficient to sustain the membrane depolarization necessary to drive K⁺ efflux, has remained unsolved.

This question is difficult to address as several different H⁺-ATPases with a broad functional overlap are assumed to be expressed in guard cells. However, researchers from Gif-sur-Yvette and Cadarache in France have now found the answer, which they have published in *The EMBO Journal*. In a physiological screen, Sylvain Merlot and co-workers had previously identified *Arabidopsis* mutants called *OPEN STOMATA 1 (OST1)* and *OST2*. Both mutants show impaired guard-cell movements: stomata do not close properly (Merlot *et al*, 2002). Positional cloning of the *OST2* locus revealed its identity with the known *AHA1* gene that codes for a plasma-membrane H⁺-ATPase that is expressed in the guard cells. Two dominant change-of-function alleles of *OST2* were identified as coding for AHA1-P68S and AHA1-L169F-G867S pump variants, respectively. Both mutations disrupt the auto-inhibitory regulation of the AHA1 pump and cause its constitutive activity. Interestingly, this pump deregulation selectively abolishes the stomatal response to ABA, but to a much lesser extent the response to CO₂ or darkness (Merlot *et al*, 2007). Time-delayed downregulation of guard-cell H⁺-ATPase activity is therefore an essential component of ABA-induced stomatal closure.

These studies highlight that transport processes in plants are tightly regulated. We are just starting to understand the mechanisms that fine-tune membrane transporter proteins and therefore the basis of ion homeostasis in plants. Further insights are eagerly awaited in the near future.

REFERENCES

- Alejandro S, Rodríguez PL, Bellés JM, Yenush L, García-Sánchez ML, Fernández JA, Serrano R (2007) An *Arabidopsis* quiescin-sulphydryl oxidase regulates cation homeostasis at the root symplast–xylem interface. *EMBO J* [doi:10.1038/sj.emboj.7601757]
- Coppock DL, Thorpe C (2006) Multidomain flavin-dependent sulphydryl oxidases. *Antioxid Redox Signal* **8**: 300–311
- Garcia-deblás B, Senn ME, Bañuelos MA, Rodríguez-Navarro A (2003) Sodium transport and HKT transporters: the rice model. *Plant J* **34**: 788–801
- Gaymard F, Pilot G, Lacombe B, Bouchez D, Bruneau D, Boucherez J, Michaux-Ferrière N, Thibaud JB, Sentenac H (1998) Identification and disruption of a plant shaker-like outward channel involved in K⁺ release into the xylem sap. *Cell* **94**: 647–655
- Horie T, Costa A, Kim TH, Han MJ, Horie R, Leung HY, Miyao A, Hirochika H, An G, Schroeder JI (2007) Rice *OsHKT2;1* transporter mediates large Na⁺ influx component into K⁺-starved roots for growth. *EMBO J* **26**: 3003–3014
- Merlot S, Mustilli AC, Genty B, North H, Lefebvre V, Sotta B, Vavasseur A, Giraudat J (2002) Use of infrared thermal imaging to isolate *Arabidopsis* mutants defective in stomatal regulation. *Plant J* **30**: 601–609
- Merlot S *et al* (2007) Constitutive activation of a plasma membrane H⁺-ATPase prevents abscisic acid-mediated stomatal closure. *EMBO J* [doi:10.1038/sj.emboj.7601750]
- Platten JD *et al* (2006) Nomenclature for HKT transporters, key determinants of plant salinity tolerance. *Trends Plant Sci* **11**: 372–374
- Yamaguchi T, Blumwald E (2005) Developing salt-tolerant crop plants: challenges and opportunities. *Trends Plant Sci* **10**: 615–620

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