The role of HKT transporters in salinity tolerance of tomato

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Summary

The Role of HKT Transporters in Salinity Tolerance of Tomato

Salinity stress is the most wide-spread and the most severe abiotic stress that plants face. More than 800 million hectares worldwide, representing more than 6% of world's land area, are affected by salinity. Tomato is one of the most important horticultural crops. However, due to the progressive salinization of irrigated land, areas for optimal growth of tomato are being reduced all over the world. To overcome this problem, many attempts have been made to increase the salinity tolerance of tomato using wild tomato species, as these species are a useful source of genes involved in salinity tolerance that can be transferred to cultivated tomato lines.

In order to identify tomato accessions to be used in breeding programmes to develop salinity tolerant tomato lines, in Chapter 2, we treated 93 tomato accessions with NaCl and we measured their Na^+ and K^+ tissue concentrations. Our results showed a great variation in ion accumulation between all accessions tested. From the data collected in this Chapter, we selected a group of 24 accessions showing either a high or a low accumulation of Na^+ in the leaves. This group of accessions was used in a more in depth analysis of salinity tolerance performed in Chapter 3.

The experiments reported in Chapter 3 aimed to find a trait or gene(s) that breeders could use to select for in new breeding programs. In this analysis, we hoped to couple gene expression to variation in levels of ions and organic molecules. In this Chapter, we analysed the expression of several genes directly or indirectly involved in Na^+ homeostasis *in planta*, such as *HKT*, *SOS*, *NHX*, *LHA* and *AVP*. We also analysed the expression of *P5CS*, a key enzyme in proline biosynthesis. Our results showed that Na^+, K^+, Cl^-, proline and sucrose concentrations did not correlate with salt sensitivity or tolerance. Nevertheless, several significant correlations between the expression of genes and Na^+ accumulation were observed. For instance, both Na^+ concentrations in the leaves and stems were positively correlated with *SlHKT1;2* expression in the roots, and Na^+ concentration measured in the roots correlated positively with *SlHKT1;1* expression also in the roots. These results suggest that Na^+ exclusion or inclusion and tissue tolerance evolved independently in tomato plants. As a

consequence, salinity tolerance can be achieved due to different combinations of Na^+ accumulations and tissue tolerance in tomato plants.

Based on the HKT expression results obtained in Chapter 3 we decided to analyze more in detail the transport characteristics of tomato HKT transporters. In Chapter 4 we present HKT1;2 protein sequences of *Solanum lycopersicum* and *Solanum pennellii* and provide evidence that both SlHKT1;2 and SpHKT1;2 are Na^+ transporters. Our kinetic studies showed that SpHKT1;2, in comparison with SlHKT1;2, had a lower affinity for Na^+. This low affinity of SpHKT1;2 correlated with higher xylem Na^+ and higher accumulation of Na^+ in stems and leaves of *S. pennellii*. Our findings demonstrate the importance of the understanding of transport characteristics of HKT1;2 transporters to improve the Na^+ homeostasis in plants.

In Chapter 5 we analysed whether single nucleotide polymorphisms (SNPs) were present in specific codons of the *HKT* coding sequence. SNPs within the *HKT* coding sequence have been reported to have an important role in the functioning of these transporters. Sequence results showed that all regions tested were conserved among all accessions analysed and SNPs affecting critical amino acids were not found. We also studied the effect of mutations in the HKT sequence on the transport characteristics of these transporters when expressed in *Xenopus laevis* oocytes. Analysis of mutations introduced in the *SlHKT1;2* gene showed that the replacement of S70 by a G allowed SlHKT2;1 to transport K^+, but at the same time resulted in a large reduction in both Na^+ and K^+ mediated currents. Stacking of mutations in positively charged amino acids in the $M2_D$ domain of SlHKT2;1 caused a reduction of Na^+ mediated currents ultimately leading to a complete loss-of-function. A double mutant of interest was the *SlHKT2;1-S70G-K477Q* mutant that we generated: this protein passes both Na^+ and K^+ ions at a reasonable rate.

To test whether transport characteristics of mutated HKT transporters as obtained with *Xenopus* oocytes had physiological importance *in planta*, we transformed *Arabidopsis athkt1;1* mutant plants with several of the mutated *HKT* constructs used in Chapter 5. The results of *Arabidopsis athkt1;1* transformed plants were shown in Chapter 6. The *AtHKT1;1* and *SlHKT1;2* wild type genes complemented the *athkt1;1* mutant growth phenotype. Intriguingly, they fully restored the accumulation of K^+ in the shoot, whereas they only partially restored the low accumulation of Na^+ as shown by WT plants. From our observation that complementation of the *athkt1;1* mutant with *HKT* genes, with a single point mutation in the first pore domain, leaves the enhanced Na^+ and reduced K^+ accumulation in the shoot unaffected, we concluded that the AtHKT1;1 protein affects K^+-loading in the xylem through membrane depolarization rather than through direct interaction with a K^+-efflux transporter.

In Chapter 7, we provide a literature review on the state of art of HKT transporters. In this Chapter, we review the latest achievements on HKT research and we discuss some remaining research questions.

In conclusion, this thesis illustrates the importance of the study of plant membrane transporters as a way to improve our knowledge on plant salinity tolerance. The knowledge gathered in these studies can provide new tools in plant breeding and in the generation of new commercially important plant lines.