

Diverse functions of the HvPHT1 phosphate transporters and genetic variation in the phosphate use efficiency of *Triticum aestivum*.

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B.Ag.Sc. (Hons)

Thesis presented for the degree
of
Doctor of Philosophy

In the
Faculty of Sciences
School of Agriculture, Food and Wine
Discipline of Plant Breeding and Genetics
The University of Adelaide

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Abstract

Wheat and barley are major crops both within Australia and around the world, providing a large proportion of the protein and calories required in both human and animal diets. Phosphorus (P) is an essential nutrient for all living organisms, without adequate supply both wheat and barley will produce no yield. The chemical reactions of P with metal ions in the soil are complex and often result in precipitation of this essential nutrient out of the soil solution, resulting in inadequate supply for plant growth. To ameliorate this P fertilisers are added (mostly in the developed world). The issue is that P fertilisers are a non-renewable resource and could be depleted in the next 50-100 years, after which what will we do to maintain production? Many developing countries, however, don't have access to P fertilisers; as such they suffer poor cereal yields. To tackle these problems, both those in the developed world and those in the developing world ultimately require wheat and barley crops that can produce higher yield with lower P fertiliser inputs; this is termed agronomic P use efficiency (PUE¹). Plant modifications are one way to increase PUE; this could be done via an increase in uptake efficiency or an increase in remobilisation of inorganic phosphate (Pi) within the plant.

Remobilisation of Pi within a plant is critical for sustaining growth and seed production under external Pi fluctuation. The barley phosphate transporter, HvPHT1;6 has been implicated in Pi remobilisation. Expression of *HvPHT1;6* in *Xenopus laevis* oocytes allowed a detailed characterisation of voltage-dependent fluxes and currents induced by HvPHT1;6 (see Chapter 2). HvPHT1;6 increased efflux of Pi near oocyte resting membrane potentials, dependent on external Pi concentration. Time-dependent inward currents were observed when membrane potentials were more negative than -130 mV. This is consistent with $n\text{H}^+:\text{HPO}_4^{2-}$ ($n>2$) co-transport, based on simultaneous radiotracer and oocyte voltage clamping, calculations based on SO_4^{2-} non-selective uptake, and differences found with change in Pi concentration gradient and pH.

¹Phosphorus use efficiency is most usefully defined as yield per unit phosphorus applied to the soil (Ortiz-Monasterio et al., 2001), this will be the definition used throughout the thesis, unless specified otherwise.

Time- and voltage-dependent inward currents through HvPHT1;6 were also observed for SO_4^{2-} , and to a lesser degree for NO_3^- , and Cl^- , but not for malate. Inward and outward currents showed linear dependence on the concentration of external HPO_4^{2-} , similar to low affinity Pi transport in plant studies. The electrophysiological properties of HvPHT1;6, which localises to the plasma membrane when expressed in onion (*Allium cepa* L.) epidermal cells, are consistent with its suggested role in the remobilisation of Pi in barley plants. Pi remobilisation is just one strategy that plants have to cope with external P fluctuation. An understanding then manipulation of Pi remobilisation processes could lead to improvements in plant PUE.

Unlike their low affinity counterparts, high-affinity phosphate transporters mediate uptake of Pi from soil solution under low Pi environments and could also be an important target for increasing plant PUE. The electrophysiological properties of any plant high-affinity Pi transporter has not been described yet. HvPHT1;1 was characterised in *Xenopus laevis* oocytes. A very low K_m (1.9 μM) for Pi transport was observed in HvPHT1;1 (see Chapter 3); this K_m value is similar to that found for high affinity Pi uptake into barley roots. Inward currents at negative membrane potentials were identified as $n\text{H}^+:\text{Pi}^-$ ($n>1$) co-transport based on simultaneous Pi radiotracer uptake, oocyte voltage clamping, and pH dependence. HvPHT1;1 showed preferential selectivity for Pi and As, but no transport of the other oxyanions SO_4^{2-} and NO_3^- in contrast to HvPHT1;6. HvPHT1;1 also localises to the plasma membrane when expressed in onion (*A. cepa*) epidermal cells, and is highly expressed in root segments with dense hairs. The electrophysiological properties, plasma membrane localisation and cell-specific expression pattern of HvPHT1;1 support its role in the uptake of Pi under low external Pi.

Early plant growth, after seed P depletion, is likely to be more dependent on Pi uptake rather than remobilisation. In many regions of the developing world, wheat productivity is constrained by both low moisture availability and inherently low soil P availability; interestingly these two factors interact and therefore influence plant P nutrition (see Chapter 5).

Breeding bread wheat genotypes that perform better under low P availability and dry conditions seems the most viable option for the resource poor farmers in much of the developing world. Early vigour is an important trait for improving bread wheat productivity, but little is known about the effect of P fertility on the expression of early vigour. A large genetic variation for percent early vegetative cover (%EVC) under varying P stress was found in elite bread wheats for the dry areas; where the ICARDA genotype, Hamam-4 showed only a 2 % reduction in %EVC whereas the Australian genotype, Yitpi lost 55 % of its ground cover under P stress (see Chapter 4). Phosphate fertiliser application increased %EVC by 25 % on average; and the presence of surface limestone significantly reduced %EVC. A large genetic variation in %EVC was found. %EVC was captured digitally and processed using new (free-licence) software developed by Douglas Johnson, Oregon State University. It had the strongest correlation with shoot biomass. %EVC capture is cheap, accurate and efficient, and could easily be implemented in wheat breeding programs to improve not only PUE, but also water use efficiency, weed competitiveness, and ultimately productivity.

Wheat yield production is the optimal determinate of PUE, basically because yield feeds the worlds' population. Importantly we describe the significant variation in PUE of elite Australian bread wheat germplasm. The cultivar Gladius showed the highest and most consistent PUE among the tested genotypes (see Chapter 5). This suggests that initially farmers can select varieties with higher PUE to manage the risk of rising P prices, and subsequently wheat breeders could improve the PUE of wheat by incorporating genes that confer higher PUE into their breeding programs. The next step in this project would be to define mechanisms are involved in the high PUE of Gladius, be it differences high or low affinity phosphate transporters, root traits, or root rhizosphere modifications.

Thesis Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge, contains no material previously published or written by another person, except where due reference has been made in the text.

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- **Preuss CP, Huang CY, Gilliam M and Tyerman SD (2010)** Channel-like characteristics of the low-affinity barley phosphate transporter PHT1;6 when expressed in *Xenopus* oocytes. *Plant Physiology* **152**, 1431-1441.
- **Preuss CP, Huang CY and Tyerman SD (2011)** Proton-coupled high-affinity phosphate transport revealed from heterologous characterization in *Xenopus* of barley-root plasma membrane transporter, HvPHT1;1. *Plant, Cell and Environment* (in print).
- **Preuss CP, Huang CY and Kuchel, H (2010)** Phosphate use efficiency in dry-land wheat. *Eyre Peninsula Farming Systems 2009 Summary*, 37-38.

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Acknowledgements

I would like to gratefully recognise the vital influence of my two supervisors, Dr Chunyuan Huang and Professor Steve Tyreman all the way through my PhD. Their broad range of knowledge and expertise and enjoyment in their work was emphasised by each of their ‘open door policies’ and general happiness with seeing results. Their caring nature and patience brings out the best from each of their students, and their support and ideas are highly valuable. I thank all the co-authors in the papers; Steve, Huang, Francis Ogbonnaya, Haydn Kuchel, Matthew Gilliam, and Mounir Louhaichi. They all helped mould my writing style to something better for publication. I was greatly pleased by their cooperation, willingness to help and overall enjoyment of the publications we co-authored.

I would like to extend many thanks to Sunita Ramesh, Wendy Sullivan, Nadim Shadiac, Alison Hay, Dion Bennett, Jodie Kretschmer, Stewart Coventry, Lyn Waterhouse, Grace Zurawska-Malkowska, Chris Kohler, Jan Nield, Angus Syme, Monica Ogierman, David Harris, Andrea French, Rob Wheeler, Stuart Roy, Maryln Henderson and Glenn McDonald for sharing their time, knowledge and/or equipment. Further I wish to extend my gratitude to all members of the ACPFG and Tyerman lab, and the countless others that fit under the banner of the School of Agriculture, Food and Wine at the University of Adelaide; you all greatly helped in allowing me to finish this PhD. I thank the University of Adelaide and the ACPFG for providing all of the required resources for my experimental work and thesis completion.

I thank the donor of the FJ Sandoz PhD scholarship; this financial support most importantly allowed me to focus wholly on my PhD topic- as I did not have to work to afford to live; enabling me to do the best research that I could. To the Australian Government Endeavour Awards (Australising International) for financial support, ICARDA for providing the experimental site and personnel, and my main supervisors Francis Ogbonnaya and Richard Brettell; thank you for my six month endearing experience in Syria. It was an amazing opportunity, I have grown both as a research scientist and a world citizen through this very much contrasting life experience.

For providing me the capacity to finish this PhD in 3 years of scholarship I thank my friends, family and faith; you kept me strong, motivated and a more complete person. You made finishing a PhD possible for me.