Abstract and Introduction

Phosphorus (P) is an essential macronutrient for plants as it is required to form nucleic acids, phospholipids, and energy-providing ATP (Schachtman et al., 1998; Mudge et al., 2002; Lambers et al., 2006).

The concentration of Pi in the cytoplasm of crop plants is generally greater than 10 mM or 0.2-2% total dry matter (Schachtman et al., 1998; Steen, 1998), however it is typically less than 1 μM in the soil solution (Goldstein et al., 1988; Mimura, 1999). This discrepancy has lead to a reliance of agriculture upon inefficient (10% of that applied is directly available to the crop) phosphorus fertiliser application (Morgan, 1997; Shenoy and Kalagudi, 2005; Lambers et al., 2006). Revealing two rather disturbing problems: 1) phosphorus fertilisers are non-renewable, current reserves are expected to be depleted in 50 years, or at best be half used within this time (Steen, 1998). 2) significant environmental problems are emerging, where in certain instances up to 70% of the algal available P found in freshwaters can be attributed to agricultural loss (Molen et al., 1997), causing significant eutrophication of important drinking water systems.

One way to conserve non-renewable fertiliser reserves is to increase crop plant phosphorus use efficiency (PUE), this is tactically the most promising avenue to decrease fertiliser reliance (Graham and Welch, 1996; Shenoy and Kalagudi, 2005). PUE can be improved via one of two ways; 1) increasing the plants ability to extract phosphate from the soil, or 2) increasing the plants ability to remobilise absorbed phosphate more efficiently to where it is needed (Shenoy and Kalagudi, 2005). Interestingly two different types of plasma membrane phosphate transporter, i.e. the low affinity and the high affinity transporter, have been identified that fulfil these roles in crop plants (Figure 1).

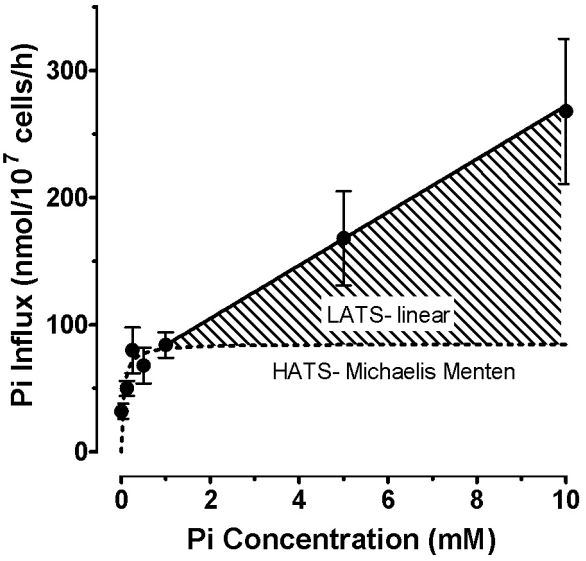

Figure 1. Native barely phosphate transport.
High (HATS)- and low-affinity transport systems (LATS) of barley (*Hordeum vulgare L.,* cv. Gerbel) mesophyll cells. The low affinity system shows linear increases in

transport as the external phosphate concentration increases; while the high affinity system follows Michaelis-Menten type kinetics, defined with K_m and V_{max} parameters, in this case 0.1 mM Pi and 85 nmol Pi/10^7 cells/h respectively. Adapted from Mimura (1999).

This transport phenomenon (Figure 1) could be governed by; 1) a single transporter that shows biphasic transport activity, such as the ammonium transporter AtAMT1.2 (Shelden et al., 2001); 2) two different transporters, one high affinity and one low affinity; or 3) a number of transporters that act in synchrony to give the barley phosphate uptake phenomena. Until now no-one has identified a barley phosphate transporter capable of this linear transport phenomenon; a transporter most likely required in Pi remobilisation.

Remobilisation of phosphate from senescing leaves is important in increasing plant PUE; it is also known that the other macronutrient anions nitrate and sulphate are remobilised from senescing leaf tissue to the seeds or other parts of the plant. This remobilisation is thought to take place via the phloem; where Himelblau & Amasino (2001) found that some 80% of P, 88% of N, and 68% of S are removed from senescing Arabidopsis leaves.

The barley phosphate transporter 1;6 was shown to be highly expressed in the phloem cells of leaves, with increased expression as leaves age (Rae et al., 2003); it is therefore likely involved in phosphate remobilisation. However, functional characterisation of HvPht1;6 and other known plant Pi transporters has been limited owing to the lack of a suitable expression system. We expressed *HvPht1;6* in *Xenopus laevis* oocytes allowing the first detailed characterisation of a plant phosphate transporter.

HvPht1;6 increased efflux of Pi near oocyte resting membrane potentials, dependent on external Pi concentration. Inward currents activated by negative membrane potentials were consistent with nH^+:HPO_4^{2-} (n>2) co-transport, and were dependant on Pi concentration gradient and pH. The large inward current (Pi influx) at -150 mV was also observed for other oxyanions including SO_4^{2-} and NO_3^-. Inward and outward currents showed linear dependence on the concentration of external HPO_4^{2-} and membrane potential, suggesting that HvPht1;6 may, in hyperperpolarised phloem cells, load Pi from the apoplast; and in depolarised cells, such as senescing mesophyll cells, unload Pi to the apoplast. The electrophysiological properties of HvPht1;6 are consistent with its suggested role in the remobilisation of Pi in barley plants. Our results demonstrate that using modified bath solution, *X. laevis* oocytes can be used for studies of electrophysiological properties of plant proton-coupled Pi transporters.

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