Plant, Cell and Environment (2002) **25,** 1325–1339

Expression analysis of putative high-affinity phosphate transporters in Chinese winter wheats

T. G. E. DAVIES[1*], J. YING[2*], Q. XU[2†], Z. S. LI[2], J. LI[3] & R. GORDON-WEEKS[1]

[1]Agriculture & Environment Division, IACR-Rothamsted, Harpenden, Hertfordshire, AL5 2JQ, UK, [2]State Key Laboratory of Plant Cell and Chromosome Engineering, Institute of Genetics, The Chinese Academy of Sciences, Bejing, 100101, China and [3]Research Centre for Eco-environmental Sciences, The Chinese Academy of Sciences, Bejing 10085, China

ABSTRACT

China's soils tend to be phosphate deficient. Application of phosphorus fertilisers to the soil is yield and cost ineffective as much of the phosphate applied is rapidly locked-in and is inaccessible to the crop. Chinese Institutes have established intensive wheat breeding programmes to generate wheat varieties that produce adequate yields and grain quality in such soils. Three such wheat cultivars have been identified with good performance characteristics in the field. These three cultivars are thought to harbour chromosome translocations that may confer enhanced phosphate scavenging abilities to the plants. The isolation and study of the expression of high-affinity phosphate transporters in tissues of these wheats, in two of the donor wheatgrasses and in another widely planted Chinese wheat variety is presented and the first full-length sequence of a wheat phosphate transporter and partial clones of several other putative phosphate transporters are reported. Relative quantitative reverse-transcription – polymerase chain-reaction was used to demonstrate that different phosphate transporters have different expression patterns within a given variety and respond differently to phosphate deprivation. The significance of the genetic background for these findings and for the different phosphate acquisition properties of the wheats under study is discussed.

Key-words: *Secale cereale*; *Thinopyrum elongatum*; *Thinopyrum intermedium*; *Triticum aestivum*; China; Lovrin 10; wheatgrass; Xiaoyan 54.

INTRODUCTION

Wheat has been cultivated in China for several thousands of years. The crop is believed to have reached China from the near East by the second millennium BC (Yang & Smale 1997). China is now considered to be one of the world's secondary centres of wheat diversity, and has abundant genetic resources including unique subspecies, local varieties and wild relatives (Baoqi, Aimin & Binjean 2001). The germplasm resource available to farmers is composed of Chinese local varieties (known for their high crossability with rye), improved cultivars or lines by Chinese wheat breeders and foreign germplasm introduced by Chinese scientists. To date a total of 40 540 accessions of wheat germplasm have been collected of which 13 233 belong to Chinese landraces (Li *et al*. 1998). All of the Chinese bread wheats (*Triticum aestivum* L.) are essentially hexaploids and are descendants of hybridized wild grass species (Harlan 1987).

China is the now the world's largest wheat producer (115 million metric tonnes per year, with average yield superior to 4t ha^{-1}). Winter wheat (autumn or winter sown with winter growth habit) accounts for nearly 90% of total production (Fig. 1); the rest is mostly low-quality spring wheat (sown March/April). Early maturity is one of the distinguishing features of the local Chinese facultative or 'winter' wheat cultivars (less than 252 d), a characteristic which is highly heritable and allows for planting of multiple crops. In order to preserve this early maturity characteristic many of the new cultivars incorporate landraces as parental material.

Predictions indicate that there will be a big surplus for phosphate (P) nutrient on farmlands of 2000 China (data obtained from Food and Agriculture Organization of the United Nations). Most of China's soils in the main wheat-growing regions of the North are however, P deficient, having an average of 6–8 ppm available P from a total of 1230 ppm total P, because they are rich in clay and very alkaline (Fig. 1) (Lindert, Lu & Wu 1996). The efficiency of P fertilizer utilization by plants is therefore low (< 20%) on these soils as a high percentage of P ends up as fixed P.

Phosphate acquisition and translocation greatly influences the quality and yield of a crop such as wheat. In soils having low availability of P, it is important for the plant to have mechanisms for acquiring the P it needs from the soil. Young cereal plants in particular need a sufficient amount of P for the development of tillers and spikelets (Romer & Schilling 1986). In developing countries with soils with unfavourable P dynamics such as those found in Northern

Correspondence: R. Gordon-Weeks.
E-mail: ruth.gordon-weeks@bbsrc.ac.uk

*These authors contributed equally to this work.
†Present address: Department of Molecular Microbiology and Biotechnology, Faculty of Life Sciences Tel Aviv University, Ramat-Aviv, 69978 Tel Aviv, Israel.

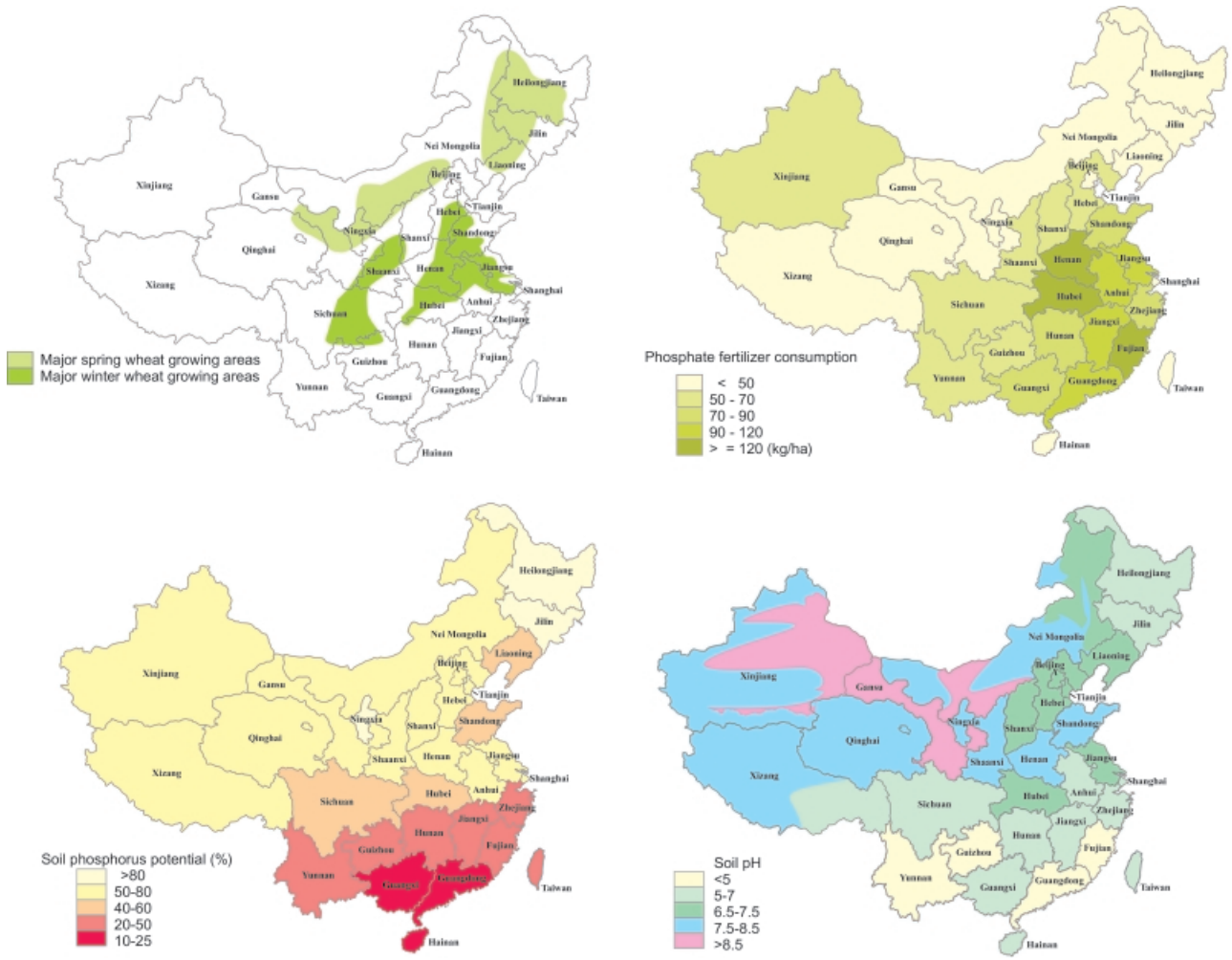


Figure 1. Maps of China showing the major provinces and (a) the main wheat growing areas – most winter wheat production is centred round Henan, Shandong, Jiangsu and Hebei provinces. (b) The consumption of P fertilizer on cultivated land per hectare – in 2000 the P requirement (effective composition) of China's crop was 5·767 million tonnes (1tonne = 1000 kg). Domestic production provided 8·7 million tonnes P fertilizer and 1·3 million tonnes of P was imported. (c) Soil phosphorus potential (% occluded phosphorus). (d) Soil pH. The maps were compiled from data obtained from the United States Department of Agriculture Foreign Agricultural Service (USDA-FAS) and the Food and Agriculture Organization (FAO) of the United Nations.

China, considerable efforts have been made to select genotypes with a low P demand capable of producing adequate yields and grain quality under such conditions. During the period 1990–92, 500 varieties (or lines) were collected and assessed and 18 of these, displaying promising agronomic characteristics, were subjected to further screening. Wheat varieties were classified as 'efficient' if grain yields at low available soil P [8 ppm Olsen (Olsen *et al*. 1954)] were similar to those at high available soil P (20 ppm Olsen), irrespective of the potential yield ceiling of a variety (Li *et al*. 1994). Three efficient wheats, Xiaoyan 54, 81(85)-5-3-3-3 and Lovrin 10 (Fig. 2) were further characterized – all are thought to harbour chromosome translocations from alien species that may confer enhanced P scavenging ability to the plants.

In this study we have examined a representative sample of Chinese wheats from diverse genetic backgrounds:

Xiaoyan 54 By means of a translocation of common wheat (*Triticum aestivum* L.) with tall wheatgrass (*Thinopyrum elongatum*), Li and colleagues obtained a super large spike wheat called Xiaoyan 6 which has a grain fill period of approximately 40 d (Li 1992; Ma *et al*. 2001). Xiaoyan 6 exhibited a steady high-yielding potential (5250–6000 kg ha^{-1}) and has been cultivated for many years in the Yellow River and Huai River valleys of China (242 000 ha planted in 1995). It has a semi-dwarf character with high resistance to lodging, is resistant to stripe rust and scab and to the effect of the China's dry hot winds. Xiaoyan 54, which is derived from Xiaoyan 6, is more cold tolerant and therefore more suited to growing in the North. It is also more able to tolerate China's dry winters. It is grown widely in Henan and Shaanxi province. Xiaoyan 54 has high quality protein content (18·25%) and gluten content (47%) and is good bread-

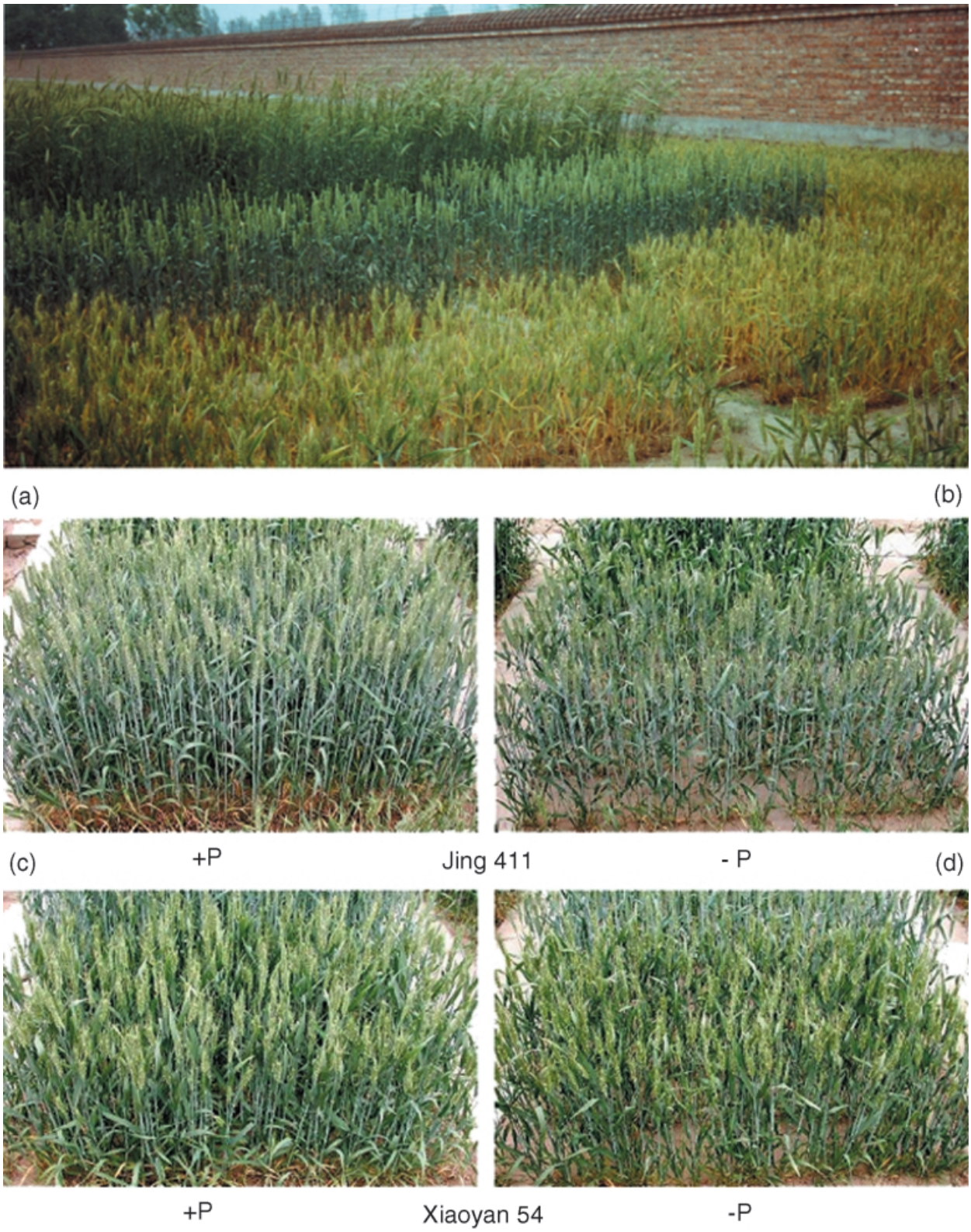


Figure 2. Field trials conducted at Changping Experimental Station, Institute of Genetics, Beijing. Top panel: furthest row: rye (*Secale cereale*) growing in soil plots containing 20 ppm Olson P (right-hand strip) and 8 ppm Olson P (left-hand strip). Middle row: Lovrin 10 growing under a similar P regime. Front row: Xiaoyan 54, again growing under high P and low P conditions. (a) and (b) Close-up of Jing 411 growing under high (20 ppm Olson) and low (8 ppm Olson) P conditions – note the reduction in growth and yield of Jing411 in (b). (c) and (d) Close up of Xiaoyan 54 growing under similar conditions.

making wheat, outperforming many quality wheat varieties from America and Canada in baking tests. It is however, prone to barley yellow dwarf virus, which is a major pathological constraint to cereal production in Northern China.

81(85)-5-3-3-3 is an experimental line. It was produced by the backcross 7336 × *Thinopyrum intermedium* × common wheat. Although a winter wheat it has a spring wheat habit. It is susceptible to freezing damage and is not cold tolerant.

Lovrin 10 is a European winter wheat introduced from Romania in the early 1970s. It carries a rye *Secale cereale* translocation. Crosses of Lovrin 10 with Chinese varieties have accounted for a significant percentage of the Chinese wheat crop since the early 1980s, as Lovrin 10 derivatives were good sources of resistance to rusts. In comparison with indigenous Chinese varieties Lovrin 10 needs to be harvested later because it is sensitive to daylength, and needs longer vernalization. It has a similar yield at low and high P to Xiaoyan 54, the latter variety however, has better grain quality.

Jing 411 is derived from a cross between Fengkang2 (developed by The Chinese Academy of Agricultural Sciences) and 74 Chang 1, and is a widely planted winter wheat cultivar in China (released in 1993, 251 000 ha planted in 1997). It gives the best yields (5900 kg ha^{-1}) when grown at high P in the Beijing region, where varieties with cold tolerance are needed. The yield however, drops by 50% when this cultivar is grown in low P conditions (see Fig. 2a & b). It was therefore used as the reference wheat in our experiments.

Three main mechanisms may account for P-efficiency in these wheat plants: first, the mobilization of P from the soil; second, a superior rate of P uptake from the soil into the plant; and third, the plants may be better at using or redistributing the P once it has been taken up. These various mechanisms involve adaptations that could include root system enlargement, arbuscular mycorrhiza establishment, increased organic acid exudation, rhizosphere acidification, increased production of phosphatases and enhanced P uptake rate. Uptake of soil P by plant roots and its subsequent translocation (as orthophosphate) throughout the plant involves transport across several different membranes. In many cases high-affinity P transporters facilitate the process. Uptake of P by plant roots from the low P concentrations that commonly occur in soil solution in particular requires high-affinity systems. A number of putative high-affinity P transporters have been cloned from various plant species (Muchhal, Pardo & Raghothama 1996; Leggewie, Willmitzer & Riesmeier 1997; Lu, Zhen & Rea 1997; Smith *et al*. 1997; Liu *et al*. 1998; Okumura *et al*. 1998; Baek, Chung & Yun 2001). The high-affinity P transporters are all comprised of approximately 520–550 amino acids and contain 12 potential membrane-spanning domains arranged in a 6 + 6 configuration around a large central cytoplasmic loop. They also contain potential sites for phosphorylation and N-glycosylation. They belong to subfamily 9 of the major facilitator superfamily of proteins and by analogy with other sequences are likely to function as H^+/PO_4^{2-} cotransporters. It is known that plants normally moderate their capacity to take up P to maintain the P concentration in their tissues within defined physiological limits (Mimura 1999). In most soils the concentration of available P in soil solution is several orders of magnitude lower than that in plant tissues, so expression of the high-affinity P transporters in the plant roots is up-regulated. Conversely, when the P concentration in the soil or growth medium is high, expression of the high-affinity P transporters is normally repressed (Muchhal & Raghothama 1999). It is thought that transcriptional control of the genes coding for these proteins is regulated by systemic signals that respond to the internal P status of the plant (Smith, Rae & Hawkesford 2000).

Here we examine the expression pattern of various putative high-affinity P transporters in the wheats described above to assess the extent of their contribution to the ability of the selected varieties to survive on low P soils. Also included in the study are the donor wheatgrasses *Thinopyrum elongatum* and *Thinopyrum intermedium*. *Thinopyrum elongatum* in particular is known to be especially tolerant of alkaline soils such as those found in Northern China.

MATERIALS AND METHODS

Plant material

Wheat varieties Xiaoyan 54, Lovrin 10 and Jing411, experimental line 81(85)-5-3-3-3 and the wheatgrasses *Thinopyrum elongatum* and *Thinopyrum intermedium* were obtained as seed from The State Key Laboratory of Plant Cell and Chromosome Engineering, Institute of Genetics, Chinese Academy of Sciences, Beijing, China.

Growth of plant material

Wheat and wheatgrasses were surface-sterilized and germinated on filter paper. When the hypocotyls had emerged they were transferred to a thin layer of quartz saturated with 0·2 mM calcium sulphate solution. Once root growth was established (normally after 5 d) the plants were transferred to hydroponic culture in a growth cabinet kept at a constant 20 °C, with a relative humidity of 75%, a photon fluence rate of 280–300 μmol photons m^{-2} s^{-1} with a 16 h day/8 h night cycle. The plants were harvested approximately 10 d after transplanting.

Basal nutrient solution

A basal nutrient solution of the following composition (according to Bollons & Barraclough 1997) was used: 3 mM nitrogen, 3 mM potassium, 1·5 mM calcium, 0·3 mM magnesium, 0·3 mM sulphur, 100 μM iron, 50 μM boron, 10 μM manganese, 1 μM zinc, 1 μM copper and 0·5 μM molybdenum (supplied as $Ca(NO_3)_2$, KCl, $MgSO_4$, FeNa – EDTA, H_3BO_3, Mn, Zn and Cu SO_4, and $(NH4)_6MO_7O_{24}$, respec-

tively). Solutions were made up in demineralized water and adjusted to an initial pH of 5·7. For hydroponic culture, solutions were continuously aerated and changed every other day.

Phosphorus treatments

For the first 5 d of hydroponic culture all plants were grown with adequate P (0·3 mM), supplied as KH_2PO_4. Seeds were then removed from all the young shoots. For P starvation plants were supplied with medium from which the 0·3 mM KH_2PO_4 had been omitted for 5 d prior to harvest.

Root hair preparation

Approximately 300 wheat seeds were surface-sterilized and plated on a square of nutrient-saturated muslin supported on a metal mesh. The mesh-supported seeds were placed over a layer made up of three sheets of Whatman no. 3 paper (Whatman plc, Maidstone, Kent, UK) soaked in nutrient solution containing 0·3 mM KH_2PO_4, in the bottom of a large plastic box under sterile conditions. Germination occurred at 22 °C, under constant darkness. On day 3 the mesh was lifted approximately 6 mm above the surface of the Whatman paper. This allowed the seedling root to grow vertically in the free space and resulted in maximal root hair formation (as described in Bucher *et al*. 1997). The mesh was similarly raised a few millimetres higher on each subsequent day. Around day 5, the seeds were carefully removed from the germinated seedlings. For P starvation, one box of seedlings was transferred to filter paper soaked in nutrient solution deprived of KH_2PO_4 following seed removal, and starved for 5 d. Roots were harvested on day 10. Root hairs were isolated from the recovered roots essentially as described by Bucher *et al*. (1997).

RNA isolation

Tissue was gently ground to a powder under liquid nitrogen and 0·2 g removed to a 2 mL tube containing 1·5 mL Trizol (Sigma Tri Reagent; SigmaAldrich Co. Ltd, Poole, Dorset, UK) and mixed for 30 s. After 5 min incubation at room temperature the suspension was centrifuged at $12\,000 \times g$ for 10 min and the supernatant was removed to a clean 2 mL tube. An equal volume of chloroform was added and the phases vortexed for 15 s and incubated for 5 min at room temperature. The phases were separated by centrifugation at 4 °C for 15 min at $12\,000 \times g$ and the aqueous layer removed to a clean 2 mL tube. An equal volume of chloroform/isoamylalcohol was added and the aqueous layer separated as before. A one-tenth volume of 3·0 M sodium acetate and 0·6 volume of isopropanol were added and after 10 min incubation at room temperature the RNA was pelleted by centrifugation at $12\,000 \times g$ at 4 °C for 10 min. The pellet was washed twice with 70% alcohol and air-dried. The RNA was subsequently re-suspended in 50 μL diethylene pyrocarbonate (DEPC)-treated water and stored at –80 °C until required.

Isolation of phosphate transporter sequences from wheat

Based on known high-affinity P transporter sequences, degenerate polymerase chain reaction (PCR) primers were designed to highly conserved regions identified by sequence alignments. A combination of one of two upstream primers (5′-TTY TTY CAN GAY GCN TAY GAY-3′ and 5′-GTN CCN GGN TAY TGG TTY CAN GT-3′) and a downstream primer (5′-GGN CCR AAR TTN GCR AAR AA-3′) were chosen for PCR. These primers were used for individual PCR reactions at a concentration of approximately 300 pmol. The Chinese Spring wheat, root-specific cDNA library was a kind gift from David Londsdale at the John Innes Centre, and was used as template for some PCR reactions. Alternatively, high-quality first-strand cDNA (generated from small quantities of total RNA isolated from roots and root hairs of Xiaoyan 54 and experimental line 81(85)-5-3-3-3) synthesized using a SMART® cDNA synthesis kit (BD Clontech UK, Basingstoke, Hants, UK) and Superscript II® RT (Invitrogen Ltd, Paisley, UK) was used as template. For *Thinopyrum* sequences genomic DNA was used. Each PCR reaction contained 10–20 ng of cDNA or 1 μg genomic DNA or 7×10^6 pfu cDNA library, 2 U *Taq* polymerase (Amplitaq; Perkin Elmer Life Sciences Ltd, Cambridge, UK), PCR buffer supplemented with 1·0 mM $MgCl_2$ (MBI-Fermentas Gmbh, St Leon-Rot, Germany) and nucleotide concentrations as recommended by the suppliers (Amersham Pharmacia Biotech UK Ltd, Little Chalfont, Bucks, UK). Reactions were performed on an Omnigene Thermal Cycler (Thermo Hybaid Ashford, Middlesex, UK) programmed to give a temperature profile of 5 min at 94 °C followed by 40 cycles of 1 min at 94 °C; 2 min at 55 °C; 1 min 30 s at 72 °C and a final 10 min extension at 72 °C. PCR products were analysed on a 1% (w/v) TAE-agarose. Fragments of the expected size were excised and recovered from the gel. The purified fragments were ligated into a linearized pCR 2·1 vector (Invitrogen), and transformed into *Escherichia coli* strain INVαF′.

5′ and 3′ Rapid amplification of cDNA ends

For 5′-rapid amplification of cDNA ends (RACE) reactions first strand cDNA was synthesized using a gene-specific primer and Superscript II® RT (Invitrogen), following the manufacturers recommended protocol. After first strand cDNA synthesis the original mRNA template was removed by treatment with RNase H, and unincorporated dNTPs, primer and proteins were separated from the cDNA using a High Pure PCR product purification kit (Roche Diagnostics Ltd, Lewes, East Sussex, UK). A homopolymeric tail was then added to the 3′-end of the cDNA using terminal deoxynucleotide transferase TdT (Invitrogen) and dCTP. PCR amplification was accomplished using *Taq* DNA polymerase (Promega, Southampton, UK), a nested, gene specific primer and a deoxyinosine-containing dG-anchor

primer (5′-GGC CAC GCG TCG ACT AGT ACG GGI IGG GII GGG GGG-3′).

For 3′-RACE reactions first strand cDNA synthesis was initiated at the poly(A) tail of mRNA using a dT adapter primer (5′-GGC CAC GCG TCG ACT AGT ACT TTT TTT TTT TTT TTT-3′). Following first strand cDNA synthesis, PCR amplification was performed using a gene-specific primer that annealed to a site located within the cDNA molecule, and an universal amplification primer (5′-GGC CAC GCG TCG ACT AGT AC-3′) that targeted the mRNA of the cDNA complementary to the 3′ end of the mRNA. The 5′- and 3′-RACE products were cloned using a TOPO-TA cloning kit and TOP10F′ chemically competent *E. coli* (Invitrogen).

Isolation of full-length clones

Reverse transcriptase (RT)-PCR, using gene specific primers designed to full-length coding sequences, was performed on cDNA isolated from P-induced roots.

Sequence analysis

Sequencing was performed using a BigDye® Terminator Cycle Sequencing Kit and an ABI Prism 310 Genetic Analyzer (Perkin-Elmer). Sequence analysis was carried out with the University of Wisconsin GCG package (Version 10.2, Genetics Computer Group (GCG), Madison, WI, USA) and ExPASy Translate Tool (Swiss Institute of Bio-Informatics, Geneva, Switzerland).

Sequence alignments

Amino acid sequences of putative high-affinity P transporters were aligned using CLUSTAL X (Thompson, Higgins & Gibson 1994) followed by manual editing of the multiple sequence (msf) file. Alignments were displayed using ESPrit2·0 [http://prodes.toulouse.inra.fr/ESPript/cgi-bin/nph-ESPript_exe.cgi].

Phylogenetic tree construction

Phylogenetic analysis was carried out using PROTDIST, FITCH, CONSENSE, RETREE and Phylodendron (D.G. Gilbert, Biology Department, Indiana University) [http://iubio.bio.indiana.edu/treeapp/treeprint-form.html]. The phylogenetic analysis was subjected to 100 bootstrap replicates.

Relative quantitative reverse-transcription-PCR

For P transporter sequences, first strand cDNA synthesis was performed on 1 μg total RNA as template using Superscript II® RT (Invitrogen) and 5 pmol of antisense primer (5′-GGN CCR AAR TTN GCR AAR AA-3′), following the manufacturers recommended protocol. For 18S RNA reactions the antisense primer (5′-CAC TTC ACC GGA CCA TTC AAT CG-3′) was used for first strand synthesis using 0·5 μg total RNA as template. PCR was carried out using one-twentieth volume of first strand cDNA, 2·5 U *Taq* DNA polymerase (Promega), PCR buffer supplemented with 1·5 mM $MgCl_2$ (MBI-Fermentas), and nucleotide concentrations as recommended by the supplier (Amersham Pharmacia Biotech Inc). Sense and antisense primers were used at a final concentration of 200 pmol. Reactions were performed on an Omnigene Thermal Cycler (Hybaid) programmed to give a temperature profile of 2 min at 94 °C followed by 40 cycles of 30 s at 94 °C; 30 s at 50–62 °C (depending on the primer annealing temperatures); 1 min or 1 min 30 s at 72 °C (depending on the length of product) and a final 5 min extension at 72 °C. PCR products were analysed on a 1% (w/v) TAE-agarose gel, using a GeneRuler® 1 kb DNA ladder (MBI Fermentas). Gels were visualized using an Eagle-Eye II system (Stratagene, La Jolla, CA, USA).

RESULTS

Growth of plant material in hydroponics

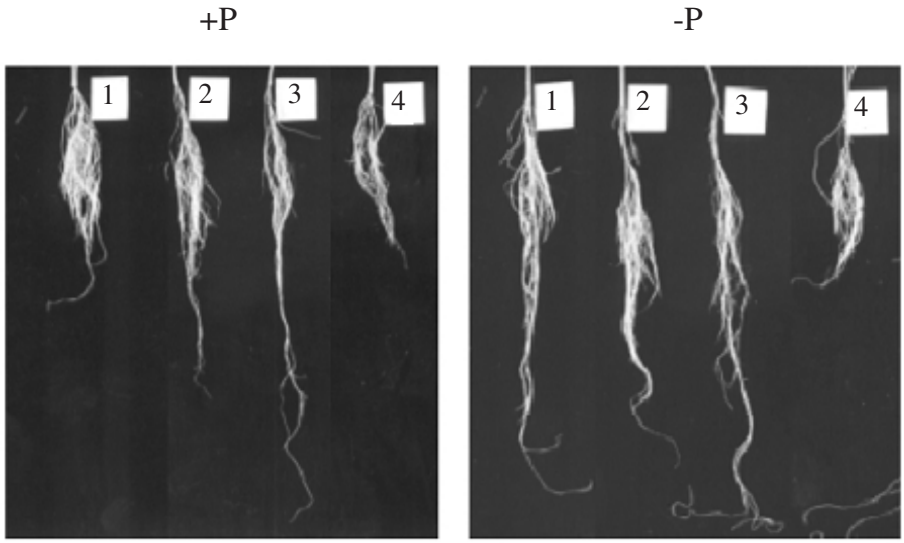
Plant high-affinity P transporters have a pH optimum of between 5·0 and 6·0 (Schachtman, Reid & Ayling 1998) indicating a preferential uptake of $H_2PO_4^-$ (predominant in media buffered below pH 6·0), but the pH of soils found in the wheat-growing regions of China are ~7·5. None the less, hydroponic studies were carried out in media buffered at pH 5·7 because a further increase in alkalinity of the medium would lead to precipitation of P (and possibly other essential micronutrients). Furthermore, there is no evidence to suggest that the pH optimum of the P transport system in the Chinese wheat plants would be affected by an alkaline soil environment. It is more likely that as has previously been reported for corn roots (Sentenec & Grignon 1985), the rate of P uptake into the roots will correlate with the pH found within the 'micro' environment of the cell wall and not of the external medium.

During growth in hydroponics at pH 5·7 the root system of Jing 411 was seen to be markedly shorter in comparison with those of the three other wheat varieties under study (Fig. 3). This observation held true for both P-replete and P-starved plants. Shoot lengths appeared to be unaffected in Jing 411. All varieties showed a comparative increase in root length on being starved of P nutrient.

Isolation of high-affinity phosphate transporter homologues from wheat and wheatgrasses

Using degenerate primers coupled with PCR we obtained a number of putative high-affinity P transporter fragments from various wheats (Accessions AJ344240 – AJ344249) and wheatgrasses (Accessions AJ413955 – AJ413964), together with a number of different isoforms for each transporter (Fig. 4). The size of the cDNA fragments obtained ranged from the approximately 150 bp (50 aa) shown in Fig. 4 to approximately 1·1 kb (370 aa).

Comparisons of the predicted protein sequences by



Variety	+P		-P	
	Roots (cm)	Shoots (cm)	Roots (cm)	Shoots (cm)
1) Xioayan 54	30.75 ± 1.71	22.52 ± 2.32	33.50 ± 1.29	21.93 ± 1.70
2) 81(85)-5-3-3-3	30.00 ± 4.08	20.13 ± 2.78	34.00 ± 2.31	19.32 ± 0.78
3) Lovrin 10	32.25 ± 4.35	24.25 ± 2.63	38.00 ± 5.66	24.25 ± 1.85
4) Jing411	12.00 ± 4.58	23.17 ± 2.02	13.75 ± 2.50	21.88 ± 0.85

Figure 3. Root lengths of phosphate sufficient (+ P) and phosphate starved (– P) plants of Xiaoyan 54, 81(85)5-3-3-3, Lovrin 10 and Jing 411 grown in hydroponic culture as described in the Materials and Methods section. Also shown is the mean of individual measurements done (with standard deviations) on four separate plants.

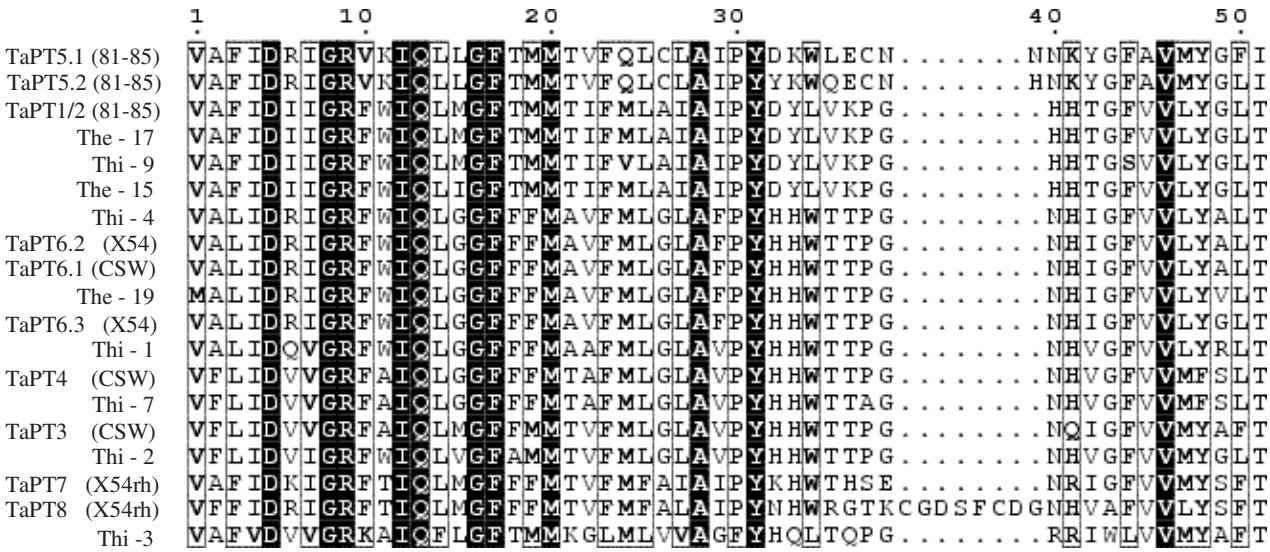


Figure 4. ESPript 2 alignment of high-affinity P transporter sequences obtained by PCR. The aligned sequences span the C-terminal end of TMS8 through to the middle of TMS10 of the protein. These fragments were obtained from a Chinese Spring wheat (CSW) root-specific cDNA library, from cDNAs isolated from roots or root-hairs (rh) of the Chinese wheats Xiaoyan 54 (X54) and 81[85]-5-3-3-3 (81–85), or genomic DNA isolated from *Thinopyrum elongatum* (The) and *Thinopyrum intermedium* (Thi).

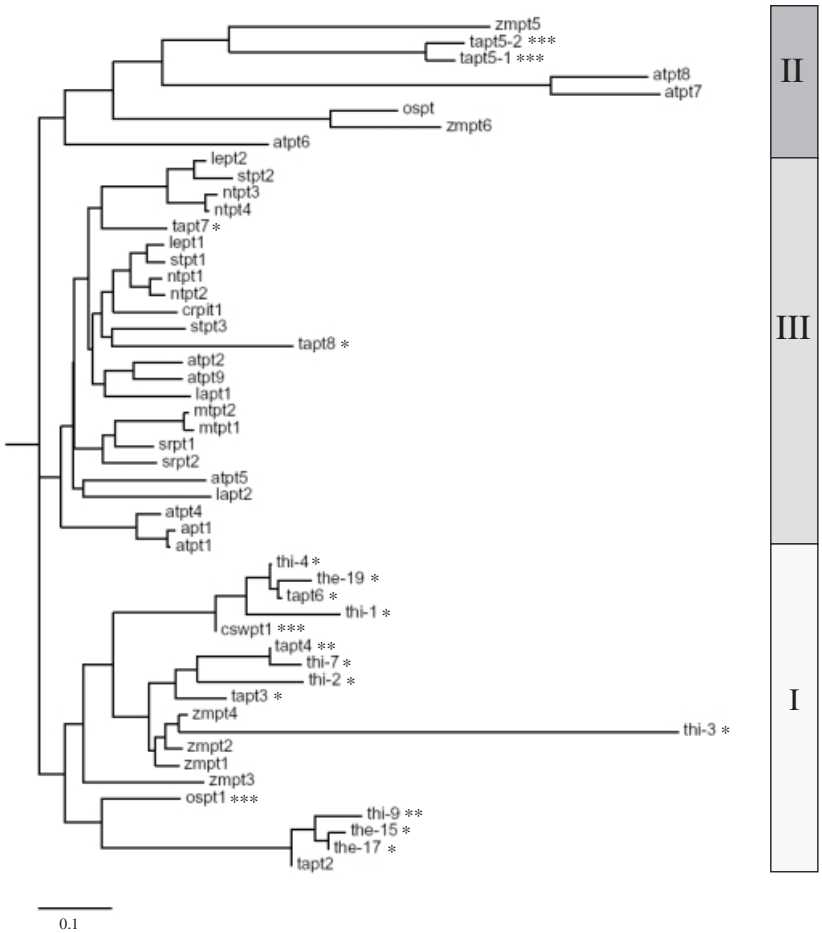


Figure 5. Phylodendrogram showing the relationship between high-affinity P transporters isolated from dicots (*Arabidopsis thaliana* AtPT1 (Q96302), APT1 (Q96243), AtPT2 (Q96303), AtPT4 (O04381), AtPT5 (O50040), AtPT6 (Q9ZWT3), AtPT7 (Q9S735), AtPT8 (unassigned) and AtPT9 (Q9MIT0), *Catharanthus roseus* (periwinkle) PIT1 (O22055), *Lupinus albus* (white lupin) LaPT1 (Q9ARI9) and LaPT2 (Q9AU00), *Lycopersicon esculentum* (tomato) LePT1 (O22548) and LePT2 (O22549), *Medicago truncatula* (Medic Barrel) MtPT1 (O22301) and MtPT2 (O22302), *Nicotiana tabacum* (tobacco) NtPT1 (Q9ST22), NtPT2 (Q9LLS5), NtPT3 (Q9AYT2) and NtPT4 (Q9AYT1), *Sesbania rostrata* SrPT1 (Q9AVR0) and SrPT2 (Q9AVQ9), *Solanum tuberosum* (potato) StPT1 (Q43650), StPT2 (Q41479) and StPT3 (CAC87043)) and monocots (*Oryza sativa* (rice) OsPT (Q94DB8) and OsPT1 (Q9M562), *Triticum aestivum* (wheat) CswPT1 (Q9XEL6), *Zea mays* (maize sequences from patent No. WO9958657)) with sequences isolated in this study. Clones marked with an * are not full-length (* approximately 50 aa, ** 50–150 aa, *** 350–400 aa). The distribution and relationships of P transporter family members on the tree are virtually identical to the tree distribution obtained using an alignment made up exclusively of the short conserved 50 aa regions of all the proteins (as shown in Fig. 4), using the same parameters for phylodendrogram constuction (data not shown). For *Arabidopsis* the nomenclature found in the database is often confusing with single clones being referred to by different names. For the purpose of this figure we have used the format AtPT1 = APT2 = PHT1; APT1 = PHT2; AtPT2 = PHT4; AtPT4 = PHT3; AtPT5 = PHT5; AtPT6 = PHT6. AtPT7, AtPT8 and AtPT9 were numbered according to database submission dates.

CLUSTAL X alignments with other known high-affinity P transporters and subsequent phylogenetic analysis revealed the existence of three distinct clusters of putative high-affinity P transporters in higher plants (Fig. 5).

Cluster I

This cluster is made up entirely of putative P transporters isolated from cereals and wheatgrasses belonging to the family *Poaceae*. A total of four cDNA fragments coding for isoforms of either *TaPT1* or *TaPT2* were isolated from roots of line 81(85)-5-3-3-3. These four cDNAs had virtually identical amino acid sequences, but varied in their coding sequence (data is not shown). The wheat P transporters *TaPT2* (Accession AJ344240, isolated from 81(85)-5-3-3-3), *TaPT3* (AJ344243, isolated from Chinese Spring wheat) and *TaPT4* (AJ344244, also isolated from Chinese Spring Wheat) have sequence identity in common with another Chinese Spring Wheat transporter *CswPT1*, rice *OsPT1* and maize *ZmPT1-4*. A further Chinese Spring Wheat isolate *TaPT6* (AJ344247) appears to be closely related to *CswPT1*. Two paralogues of *TaPT6* were also isolated from roots of Xiaoyan 54 (see Fig. 4).

The genomic DNA sequences from *Thinopyrum elong-*

atum (The) and *Thinopyrum intermedium* (Thi) are all found within this cluster. *The-15* (Accession AJ413955), *The-17* (AJ413956) and *Thi-9* (AJ413963 and AJ413964) are similar to the wheat *TaPT1* and *TaPT2* sequences. *The-19* (AJ413957), *Thi-1* (AJ413958) and *Thi-4* (AJ413961) are similar (but not identical) to *CswPT1* and *TaPT6*. *Thi-2* (AJ413959) and *Thi-7* (AJ413962) have the closest sequence homology with *TaPT3 and TaPT4*. *Thi-3* (AJ413960) is a unique sequence and is the most distantly related of all the *Thinopyrum* sequences characterized.

Cluster II

This cluster contains putative P transporter-like sequences from rice, wheat, maize, and *Arabidopsis thaliana*. The wheat sequence *TaPT5·1* isolated from experimental line 81(85)-5-3-3-3 appears to be novel. Sequencing of a 1·2 kb fragment of this clone (spanning from the 3′- end of TMS1 to the middle of TMS10) revealed a cDNA (Accession AJ344245) bearing only 50% identity with known high-affinity P transporter sequences but having several conserved structural features, including the membrane-spanning regions, the large central loop and phosphorylation motif(s) in common with other P transporters, coupled to unique external loops. Outside, loops 1 and 3 are shortened by 6 and 14 amino acids, respectively, so the N-terminal half of the protein has very little structure exposed to the outside. Two strategically placed cysteine residues which form part of an unique 16 amino acid extension HDDEV**C**KVNT**C**QVARY on external loop4 of the TaPT5·1 protein may be capable of forming disulphide bonds. Maize *ZmPT5*, which is most closely related to *TaPT5*, has a GLN/ALA rich repeat at its C-terminus; such repeat sequences are often found to be involved in transcriptional activation or repression. This region of *TaPT5·1* has not yet been characterized. *TaPT5·2* (Accession AJ344246, also isolated from line 81(85)-5-3-3-3), is a closely related isoform of *TaPT5·1* and has similar structural characteristics. The 16 amino acid extension HDDEV**C**KVNS**C**QGASY on external loop 4 is also highly conserved.

Cluster III

This cluster is made up almost entirely of known dicotyledon sequences, and includes putative high-affinity P-transporters from the legumes *Lupinus albus*, *Medicago trunculata* and *Sesbania rostrata* (all members of the *Fabaceae* family), tomato, tobacco and potato (belonging to the *Solanaceae*), and Arabidopsis (a member of the *Brassicaceae*).

The root hair isolate *TaPT7* (Accession AJ344248) isolated from Xiaoyan 54 is seen to be similar to *LePT2* isolated from P-starved roots of tomato (Liu *et al*. 1998). A second paralogue of this transporter was also isolated from root hairs of Xiaoyan 54 (not shown). *TaPT8* (Accession AJ344249, also isolated from root hairs of Xiaoyan 54) appears to be a novel fragment, having an 8 amino acid extension K**C**GDSF**C**D containing two cysteine residues on external loop5 (see Fig. 4). It appears to be most closely related in evolutionary terms to potato *StPT3*.

Isolation of full-length clones of *TaPT2* from Xiaoyan 54 and experimental line 81(85)-5-3-3-3

Full-length *TaPT2* from Xiaoyan 54 (accession AJ344240) was isolated using 5′ and 3′ RACE (primers used shown in Table 1) and encodes a novel 525 amino acid protein (Fig. 6). Southern analysis using full-length *TaPT2* as probe revealed two fragments, indicating at least two copies of this gene can be found in Xiaoyan 54 (data not shown). Two independent full-length cDNA clones were obtained from 81(85)-5-3-3-3 (accessions AJ344241 and AJ344242), using gene-specific primers and RT-PCR (see Table 1). Accession AJ344241 differed from the full-length *TaPT2* coding sequence isolated from Xiaoyan 54 by only one amino acid (L119F), whereas accession AJ344342 had five amino acid substitutions (positions L119F, I185V, M228L, T321S, T519A and V521A highlighted in Fig. 6). All these substitutions have previously been seen in other P transporters isolated from other plant species, and as such they are unlikely to have a dramatic effect on the functionality of the protein.

Expression of high-affinity phosphate transporters in Chinese wheats and wheatgrasses

Relative quantitative reverse-transcription (RQRT)-PCR was used to determine the expression of individual P transporters in each wheat variety relative to the constitutively expressed 18S rRNA (Figs 7 and 8).

Cluster I transporters

The P transporters *TaPT2* and *TaPT3* are most strongly expressed in the roots of all wheat varieties studied (Fig. 7).

Table 1. Gene specific primers used in 5′ and 3′ RACE and in RT-PCR reactions to isolate full-length clones of the putative high-affinity P transporter *TaPT2*

	Gene specific primer	Nested gene specific primer
5′- RACE	5′-GGN CCR AAR TTN GCR AAR AA-3′	5′-TCG CCG GCG GCC ATG GCG-3′
3′- RACE	5′-GCA TGT CTC TAG ACA CCA GTC GG-3′	
RT-PCR	5′-TCG CCG GCG GCC ATG GCG-3′	
	5′-GCA TGT CTC TAG ACA CCA GTC GG-3′	

```
ggggggggggacacaaacaagagagaaccagaagaagtacagaaggggcagaagtcta
gttgagagatcgccggcggccatggcgactgaacagctcaacgtgttgaaagcactcgat
                      M  A  T  E  Q  L  N  V  L  K  A  L  D
gttgccaagacgcaactgtaccatttcaaggcggtcgtgatcgccggcatgggcttcttc
 V  A  K  T  Q  L  Y  H  F  K  A  V  V  I  A  G  M  G  F  F
acggacgcctacgacctcttctgcatcgccctcgtcaccaagctgctggggcgcatctac
 T  D  A  Y  D  L  F  C  I  A  L  V  T  K  L  L  G  R  I  Y
tacaccgaccctgccctcaacgagcccggccacctcccggcaaacgtgtcggccgccgtg
 Y  T  D  P  A  L  N  E  P  G  H  L  P  A  N  V  S  A  A  V
aacggcgtggccctgtgcggcacacttgccggccagctcttcttcggctggctcggtgac
 N  G  V  A  L  C  G  T  L  A  G  Q  L  F  F  G  W  L  G  D
aagctcggccgcaagagcgtctacggcttcacgctcatcctcatggtcctctgctccatc
 K  L  G  R  K  S  V  Y  G  F  T  L  I  L  M  V  L  C  S  I
gcgtccgggctctcgcttggacacgaggccaagggcgtaatggggacgctatgtttcttc
 A  S  G  L  S  L  G  H  E  A  K  G  V  M  G  T  L  C  F  F
cgcttctggcttggcttcggcgtcggcggcgactatcctctgagcgccaccatcatgtcg
 R  F  W  L  G  F  G  V  G  G  D  Y  P  L  S  A  T  I  M  S
gaatatgctaacaagaagacccgcggcacctttatcgccgctgtgtttgccatgcagggg
 E  Y  A  N  K  K  T  R  G  T  F  I  A  A  V  F  A  M  Q  G
tttggcatcctatttggtactattgtcacgatcatcgtctcgtccgcattccgacatgca
 F  G  I  L  F  G  T  I  V  T  I  I  V  S  S  A  F  R  H  A
ttccctgcaccgccattctacattgacgccgcggcgtccattggcccggaggccgactac
 F  P  A  P  P  F  Y  I  D  A  A  A  S  I  G  P  E  A  D  Y
gtgtggcgcatcatcgtcatgttcggcaccatcccggccgccctgacctactactggcgt
 V  W  R  I  I  V  M  F  G  T  I  P  A  A  L  T  Y  Y  W  R
atgaagatgcccgaaactgcgcggtacacagcactcatcgccggcaacacgaagcaagcc
 M  K  M  P  E  T  A  R  Y  T  A  L  I  A  G  N  T  K  Q  A
acatcagacatgtccaaggtgctcaacaaggagatctcagaggagaatgtgcagggtgag
 T  S  D  M  S  K  V  L  N  K  E  I  S  E  E  N  V  Q  G  E
cgtgccactggtgatacttggggcctcttctcgcgacagttcatgaagcgccacggggtg
 R  A  T  G  D  T  W  G  L  F  S  R  Q  F  M  K  R  H  G  V
cacttgctagcgaccacaagcacttggttcctgctcgatgtggccttctacagccagaac
 H  L  L  A  T  T  S  T  W  F  L  L  D  V  A  F  Y  S  Q  N
ctgttccaaaaggacatcttcaccaagatcgggtggatcccgccggccaagactatgaat
 L  F  Q  K  D  I  F  T  K  I  G  W  I  P  P  A  K  T  M  N
gcattggaggagttgtaccgcatcgcccgcgcccaagcgctcatcgcgctctgcggcacc
 A  L  E  E  L  Y  R  I  A  R  A  Q  A  L  I  A  L  C  G  T
gtgcccggctactggttcaccgtcgccttcatcgacattattgggaggttttggatccag
 V  P  G  Y  W  F  T  V  A  F  I  D  I  I  G  R  F  W  I  Q
ctcatgggattcaccatgatgaccattttcatgctcgcaatcgccataccttacgactac
 L  M  G  F  T  M  M  T  I  F  M  L  A  I  A  I  P  Y  D  Y
ttggtgaagccagggcaccacaccggcttcgtcgtgctctacgggctcactttcttcttc
 L  V  K  P  G  H  H  T  G  F  V  V  L  Y  G  L  T  F  F  F
gccaacttcggccccaacagcacaaccttcattgtgccagccgagatcttccctgcgagg
 A  N  F  G  P  N  S  T  T  F  I  V  P  A  E  I  F  P  A  R
ctccggtccacatgccacggtatctctgccgctaccggtaaggcgggcgcgatcatcggc
 L  R  S  T  C  H  G  I  S  A  A  T  G  K  A  G  A  I  I  G
gcgttcgggttcctgtatgcgtcgcaggaccagaagaagcccgagaccggctactcacgg
 A  F  G  F  L  Y  A  S  Q  D  Q  K  K  P  E  T  G  Y  S  R
gggatcggcatgcgcaacgcactctttgtgctcgcaggcacaaacttcctgggcctgctc
 G  I  G  M  R  N  A  L  F  V  L  A  G  T  N  F  L  G  L  L
ttttccttgttggtgccagagtctaagggcaagtcgcttgaggagctctccaaggagaac
 F  S  L  L  V  P  E  S  K  G  K  S  L  E  E  L  S  K  E  N
gtcggcgacgacgacaccattgttccgactggtgtctagagacatgcaggtgtacttgca
 V  G  D  D  D  T  I  V  P  T  G  V  *
caatcgtgcattgttggtgtgcttgatttgctttttgtgttctatttttgttttctttg
ccgcgaccactttcaaaatttctagtgttgtaatggtgacaatgtaatgcgtacactgtg
acggtgattcaaattccaattctgaaaaaaaaaaaaaaaaa
```

Figure 6. Full-length sequence of *TaPT2* cDNA (accession AJ344240) isolated from Xiaoyan 54, coding for a 525 amino acid protein. HMMTOP 2·0 (Tusnady & Simon 2001) predicts 12 *trans*-membrane spanning domains (underlined), cytoplasmically orientated N- and C-terminii and a large, internally orientated central loop.

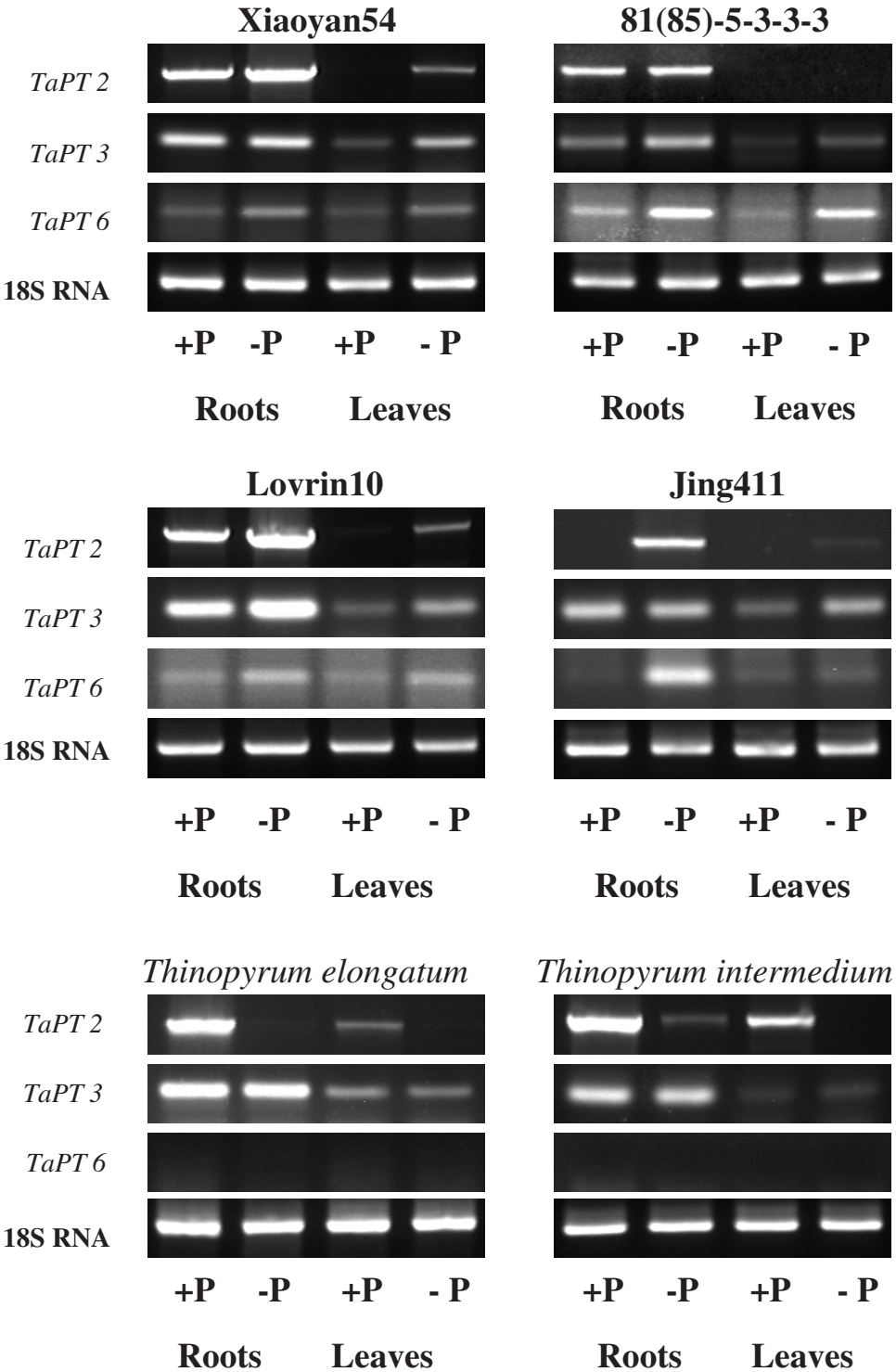


Figure 7. Expression pattern of three individual phosphate transporters *TaPT2*, *TaPT3* and *TaPT6* (and their orthologues) in wheat varieties Xiaoyan 54, Lovrin 10, 81(85)-5-3-3-3 (line), Jing 411, and in *Thinopyrum elongatum* and *Thinopyrum intermedium*. Relative, quantitative RQRT-PCR was used to determine the expression of the individual P transporters relative to the constitutively expressed 18S rRNA, using sequence specific primers and standard PCR techniques carried out under non-limiting conditions. The primers used for the individual PCR reactions were as stated in Table 2. The results shown are representative of results obtained with at least two individual sets of PCR reactions performed on each cDNA sample, and were confirmed by repeating the experiment on at least two separate sets of plant material grown under identical conditions.

TaPT6 expression in contrast is more uniformly distributed between roots and leaves in the three P-efficient varieties/lines under study.

Whereas Jing 411 only expresses *TaPT2* in roots under P deprivation, the three selected varieties also show some expression under conditions of high external P. The expression of *TaPT2* in roots of the efficient varieties does not therefore appear to be as tightly regulated by P supply. *TaPT6* expression shows some increase with P-deprivation in the selected wheat varieties, especially in line 81(85)5-3-3-3. In Jing 411 there is a significant increase of expression of *TaPT6* in roots (but not shoots) of P-starved plants, and the pattern of *TaPT6* expression is virtually the same as that seen for *TaPT2* in this variety.

Table 2. Primers used for individual RQRT-PCR reactions to determine the expression of individual P transporters in each wheat variety relative to the constitutively expressed 18S rRNA.

	Sense primer	Anti-sense primer
18S RNA	5′-GAG GGA CTA TGG CCG TTT AGG-3′	5′-CAC TTC ACC GGA CCA TTC AAT CG-3′
TaPT2	5′-ACC GAC CCT GCC CTC AAC GAG-3′	5′-GAG CAC GAC GAA GCC-3′
TaPT3	5′-GTC TTC CTC ATC GAC GTC G-3′	5′-TGA CGA CGA AGC CGA TC-3′
TaPT4	5′-CGA CCC GAG CTC AGC CGC C-3′	5′-GGC CGT CAT GAA GAA GAA GCC G-3′
TaPT5·1	5′-TCG TCG TCG ACC TCA TCG GCC AC-3′	5′-AGC TTT ATG ATA CCT AGC CAC-3′
TaPT5·2	5′-GTC GTC GAC CTC ATC AGC CAA-3′	5′-GGC TTT ATG ATA ACT GGC CC-3′
TaPT6	5′-GCG CTG ATC GAC CGG-3′	5′-CAG CAC CAC GAA CCC –3′
TaPT7	5′-GAT AAG ATC GGA AGG TTT ACC-3′	5′-AAT TCT GTT CTC ACT GTG GG-3′
TaPT8	5′-CGA CCG CAT CGG TCG GTT CAC-3′	5′-CAC CGC ATT TGG TCC CTC G-3′

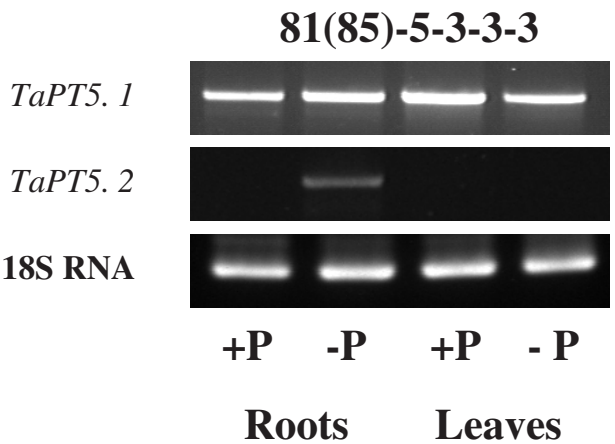


Figure 8. Expression patterns of *TaPT5·1* and *TaPT5·2* in roots and leaves of experimental line 81(85)-5-3-3-3 relative to the constitutively expressed 18S rRNA. Conditions used were essentially as described in the legend to Fig. 7.

Significantly different patterns of *PT2* expression occurred in the wheatgrasses, where the wheatgrass orthologue(s) of *TaPT2* are expressed strongly only under conditions of high external P (Fig. 7). A significant down-regulation of wheatgrass *PT2* expression must therefore occur when the plants are transferred into P-deficient nutrient solutions. This is entirely contrary to what has been observed for plant species previously studied. This strongly suggests that wheatgrass roots may adopt a different strategy for P exploitation compared with roots of cereal wheats. Expression patterns of the wheatgrass orthologue(s) of *TaPT3* mimicked those found in the wheat varieties, and were consistently the same for all the plants studied. *TaPT6* orthologues were not expressed at detectable levels in the wheatgrasses under the conditions studied.

Cluster II transporters

TaPT5·1 and *TaPT5·2* are detectable in the experimental line 81(85)-5-3-3-3, but not in Xiaoyan 54, Lovrin 10 and Jing 411. Interestingly the two homologues have distinctly different patterns of expression in 81(85)-5-3-3-3, *TaPT5·1* mRNA being constitutively expressed at similar levels in both roots and leaves of both P-replete and P-starved plants, whereas *TaPT5·2* expression is only seen in roots of P-starved plants (Fig. 8). This suggests that the homologues may be under the control of different promoters, only one of which is responsive to internal or external P concentrations.

Cluster III transporters

Neither *TaPT7* nor *TaPT8* expression was detectable in roots or shoots of any of the wheat/wheatgrass varieties grown hydroponically (data not shown). This may be due to the fact that they may only be expressed in root hairs, which are not present on plant roots grown in hydroponics. However, a subsequent attempt at amplifying these transporters from P-starved root hair isolates was also unsuccessful. One possible explanation for the absence of these transporters in the plants studied may be the timing of induction of different high-affinity P transporters in response to P starvation, which may be transient and short-lived and therefore not picked up within the narrow time window of 4 d post-starvation looked at in this study.

DISCUSSION

Phosphate efficiency in the wheat lines under study

Xiaoyan 54, Lovrin 10 and experimental line 81(85)-5-3-3-3 all appear to be able to grow in soil conditions having low available P without their yield being significantly affected. In contrast, Jing 411 suffers a major loss of yield when grown in the same soils. The mechanism(s) adopted by Xiaoyan 54 and Lovrin 10 to provide this yield efficiency is currently postulated to involve a better ability to cope with low availability of soil P at an early (critical) stage of growth, possibly through better utilization of P reserves found in the seed, or by being more efficient at acquiring P from the soil, or a combination of both. Wheat plants with high seed P reserves have previously been shown to be capable of accumulating more P from the soil, this enhanced uptake being attributed to better root system development (Zhu & Smith 2001). In the present study it

was noted that during growth in hydroponics the root system of Jing 411 was markedly shorter in comparison with those of the three other wheat varieties under study. A recent study with Jing 411 and Xiaoyan 54 demonstrated that the latter variety has higher root : shoot ratios, higher relative yields, and allocates higher percentages of assimilated carbon, soluble sugar and absorbed P to its roots under P deficiency so that it can maintain relatively high root-growth rates (Zou *et al*. 2002). This would result in an enhancement of the total surface area available for soil exploration and P acquisition. Xiaoyan 54 also releases more assimilated carbon as root exudates under P deficiency. It seems likely that shallow root architecture may also play a significant role in the ability of the efficient varieties to acquire P from the soil (Fu-Suo Zhang, personal comm.). Because soil P availability almost invariably decreases with soil depth, the shallow angle of root growth of P-deficient plants can be viewed as an adaptive response allowing increased P uptake (Bonser, Lynch & Snapp 1996; Nielsen *et al*. 1998; Ge, Rubio & Lynch 2000; Yan *et al*. 2001).

As well as good adaptation of root systems, another highly significant factor in responding to variable P availability in soils is the uptake rate per unit root (Egle *et al*. 2000). A dual uptake model for ions is generally evoked, characterized by the possession of a high-affinity transporter operating at low (μM) concentration and a low-affinity transporter functioning at high (mM) concentrations. In general, the low-affinity system appears to be expressed constitutively in plants, whereas the high-affinity uptake system is regulated by the availability of P. The results of many P uptake experiments confirm that plants adjust their P uptake based on their internal P status, particularly by increasing their maximum influx or rate of P absorption (I_{max}), whereas changes in K_m and C_{min} are of minor importance in this process. The capacity for P uptake by high-affinity P transporters can thus be enhanced either by increasing the driving force (e.g. protons or membrane potential) or by increasing the total number of high-affinity transporter molecules.

Phosphate transporter expression in the wheat lines

The existence of multiple high-affinity P transporters in plants, coupled to the size and complexity of the wheat genome means that conclusive analysis of the role of P transporters in uptake efficiency in wheat is a challenging undertaking. However in this study we have identified several putative wheat P transporters some of which are related to transporters previously isolated from maize, *Arabidopsis* or tomato, but others that are unique.

We have identified two transporters *TaPT2* and *TaPT3* that are potential homologues of the barley high-affinity P transporters *HvPT1, HvPT2 and HvPT3* reported by Smith, Cybinski & Rae (1999) in both the wheats and wheatgrasses. Significantly, the expression of *TaPT2* in the roots of the efficient wheat varieties under study does not appear to be tightly regulated by P supply. In contrast, the expression of this transporter in barley, and in the Chinese wheat variety Jing411 (which is less tolerant to external changes in P), is P-starvation dependent. This difference in the expression pattern of *TaPT2* may be a reflection of the enhanced ability of the three varieties to generate adequate yields in low P soils, and may provide a clue to the P nutrition mechanism(s) adopted by these plants to maintain their yields. Intriguingly, it has recently been demonstrated in barley that Zn plays a specific role in the signal transduction pathway responsible for the regulation of genes encoding high-affinity P transporters in plant roots (Huang *et al*. 2000). Normally expression of *HvPT1* responds to the P status of the plant. It was however, observed that under conditions of Zn deficiency this tight control is lost, leading to very high accumulation of P in plants. This observation may give an important insight into the possible mechanism whereby plants may modify expression of their P transporters in response to external nutrient stressors

In the wheatgrasses the expression of the *TaPT2* orthologues is quite different to that found in wheats, the transporters being strongly expressed only under conditions of relatively high external P. It is thought that *Thinopyrum* plants (*elongatum* and *intermedium*) may respond to heterogeneously distributed P in the soil by enhancing uptake in localized, relatively P-rich patches. This would explain the expression pattern of the high-affinity P transporter *PT2* in roots of these plants. Such a strategy would require an extensive root system able to seek out such P-enriched patches. The three wheat lines under study may possibly have acquired a similar strategy superimposed on top of a P-deficiency responsive one. This would also explain the unusual expression patterns of *TaPT2* observed in these varieties. To add further complexity to the overall picture, expression patterns of *TaPT3* (and its orthologues) were consistently the same in roots of all the plants studied.

Although we have demonstrated that the wheatgrasses have P transporter sequences that are similar to those found in the wheats from China, none of the sequences isolated from the wheatgrasses are identical at the gene level to the Chinese wheat sequences. No transfer of P transporter genes between the wheatgrasses and the wheats under study can be inferred to have taken place during the various chromosome translocation events, based on this data. The possibility of such a gene transfer having occurred however, cannot be completely ruled out as our survey of the P transporters that occur in each species has not been exhaustive. The possibility still remains therefore that transfer of a P transporter gene(s) could contribute to P efficiency in the wheats harbouring alien inserts

The root hair isolate *TaPT7 (*from P-starved root hairs of Xiaoyan 54) is similar in sequence composition to *LePT2* isolated from tomato roots (Liu *et al*. 1998) and is likely to be a *LePT2* homologue. In tomato roots, *LePT2* expression is induced during P starvation, the LePT2 transcripts being exclusively localized to rhizodermal cells. A second root hair isolate *TaPT8 (*also from P-starved root hairs of Xiaoyan 54) has similarities to the potato P transporter

StPT3. A recent study has indicated preferential expression of *StPT3* in root sectors where mycorrhizal structures are formed (Rausch *et al*. 2001). Expression of high-affinity P transporters in root hairs is a logical strategy as this would result in better rhizosphere exploitation and rapid P uptake. For wheat and rye it has been shown that root hairs can participate substantially in uptake of P from the soil, and can satisfy more than 60% of the plants P demand (Gahoonia & Nielsen 1996, 1998). This is not surprising because root hairs substantially extend the root surface area for uptake. No expression of *TaPT7* or *TapT8* in roots or roots hairs of wheat was detectable by RT-PCR within the time-window (4 d post-starvation) of this study.

Three sequences with unusual amino acid extensions were also identified. The sequences were similar in overall structure to known high-affinity P transporters. It is tempting to postulate that this sequence conservation would indicate a similar conservation of function and a role for these proteins in P uptake. This argument is strengthened by the observation that *TaPT5·2* is induced by P-starvation in roots of experimental line 81(85)-5-3-3-3. The unusual amino acid extensions, all of which contained a CX4C motif, are predicted to be located outside of the membrane. Such exposed cysteine motifs may indicate a regulatory binding site on the proteins and a role for these transporters in P regulation. The presence of an 8 amino acid extension K**C**GDSF**C**D reminiscent of a zinc-binding domain on external loop 5 of *TaPT8* and the isolation of this cDNA from root hairs indicates a possible role for this protein in environmental sensing. The constitutive expression of TaPT5·1 in leaves and roots and the induced expression of TaPT5·2 in P-starved roots may implicate the 16 amino acid extension HDDEV**C**KVN[T/S]**C**Q[V/G]A[R/S]Y (found on external loop 4 of the proteins) as being involved in internal P signalling within the plant. Further studies are clearly needed on these unusual proteins in order to understand the significance of the CX4C motifs in these amino acid extensions.

Some of the other as yet uncharacterized high-affinity transporters isolated in this study may participate in the intracellular movement of P. Others may be involved in scavenging P that has leaked into the apoplast. Under natural conditions, plant growth rate is normally adjusted to the availability of the nutrient. Reduced rate of growth leads to redistribution of P within the plant, thus allowing plants to overcome brief periods of P starvation without a major effect on growth. Transfer of P from roots to shoots is reduced and P in older leaves is remobilized to younger growing leaves and other active sinks.

CONCLUSIONS

Wheat plants probably have several different morphological and physiological adaptations that enable them to acquire and more efficiently utilize P from sparingly soluble soil fractions. The role of high-affinity P transporters in these adaptation processes remains unclear, but the current study has clearly demonstrated interesting differences in the expression pattern of such transporters in the selected wheat varieties when grown in hydroponic culture. Further studies are needed to see whether these results can be extrapolated to the field. Unusual patterns of expression of at least one P transporter in the wheatgrasses *Thinopyrum elongatum* and *Thinopyrum intermedium*, used as donors of chromosome fragments to the wheat's Xiaoyan 54 and 81(85)-5-3-3-3, also warrant further study.

ACKNOWLEDGMENTS

The authors would like to thank Dr Peter Barraclough for useful discussions on wheat physiology and cultivation, Lynda Castle for help with the artwork for Fig. 1, and Dr Tong Yiping for critically reading the manuscript. IACR-Rothamsted receives grant-aided support from the Biotechnology and Biological Research Council of the United Kingdom. Ying Jia and Xu Qi were recipients of Rothamsted International one-year research fellowships. Support was also received in the form of an ALCS (Academic links with China Scheme) grant from the British Council.

REFERENCES

Baek S.H., Chung I.M., Yun S.J. (2001) Molecular cloning and characterization of a tobacco leaf cDNA encoding a phosphate transporter. *Molecules and Cells* **11**, 1–6.

Baoqi S., Aimin Z., Bonjean A.P. (2001) Chinese wheat pool. In *The World Wheat Book: a History of Wheat Breeding* (eds A.P. Bonjean & W.J. Ang), pp. 667–701. Intercept Ltd and Lavoisier Publishing, Andover, UK and Secausas, USA.

Bollons H.M. & Barraclough P.B. (1997) Inorganic orthophosphate for diagnosing the phosphorus status of wheat plants. *Journal of Plant Nutrition* **20**, 641–655.

Bonser A.M., Lynch J., Snapp S. (1996) Effect of phosphorus deficiency on growth angle of basal roots in *Phaseolus vulgaris*. *New Phytology* **132**, 281–288.

Bucher M., Schroeer B., Willmitzer L., Riesmeier J.W. (1997) Two genes encoding extensin-like proteins are predominantly expressed in tomato root hair cells. *Plant Molecular Biology* **35**, 497–508.

Egle K., Manske G., Romer W., Vlek P.L.G. (2000) Improved phosphorus efficiency of three new wheat genotypes from CIMMYT in comparison with an older Mexican variety. *Journal of Plant Nutrition and Soil Science* **162**, 353–358.

Gahoonia T.R. & Nielsen N.E. (1996) Variation in acquisition of soil phosphorus among wheat and barley genotypes. *Plant and Soil* **178**, 223–230.

Gahoonia T.R. & Nielsen N.E. (1998) Direct evidence on participation of root hairs in phosphorus (^{32}P) uptake from soil. *Plant and Soil* **198**, 147–152.

Ge Z., Rubio G., Lynch J.P. (2000) The importance of inter-root competition and phosphorus acquisition efficiency: results from a geometric simulation model. *Plant and Soil* **218**, 159–171.

Harlan J.R. (1987) The early history of wheat: earliest traces to the sack of Rome. In *Wheat Science Today and Tomorrow* (eds L.T. Evans & W.J. Peacock). Cambridge University Press, Cambridge, UK.

Huang C., Barker S.J., Langridge P., Smith F.W., Graham R.D. (2000) Zinc deficiency up-regulates expression of high-affinity phosphate transporter genes in both phosphate-sufficient and –deficient barley roots. *Plant Physiology* **124**, 415–422.

Leggewie G., Willmitzer L., Riesmeier J.W. (1997) Two cDNAs from potato are able to complement a phosphate uptake-deficient yeast mutant: identification of phosphate transporters from higher plants. *Plant Cell* **9**, 381–392.

Li Z.S. (1992) Chromosome Engineering of wheat in China. *Critical Reviews in Plant Sciences* **10**, 471–485.

Li J., Liu X., Zhou W., Sun J., Tong Y., Liu W., Li Z.S., Wang P., Yao S. (1994) Technique of wheat breeding for efficiently utilizing soil nutrient elements. *Science in China (Series B)* **38**, 1313–1320.

Li L.H., Zheng D.S., Yang X.M., Li X.Q., Liu S.C., Song C.H. (1998) Diversity evaluation of wheat germplasm resources in China. *Genetic Resources and Crop Evolution* **45**, 167–172.

Lindert P.H., Lu J., Wu W. (1996) Trends in the soil chemistry of North China since the 1930s. *Journal of Environmental Quality* **25**, 1168–1178.

Liu C., Muchhal U.S., Uthappa M., Kononowicz A.K., Raghothama K.G. (1998) Tomato phosphate transporter genes are differentially regulated in plant tissues by phosphorus. *Plant Physiology* **116**, 91–99.

Lu Y.-P., Zhen R.-G., Rea P.A. (1997) AtPT4: a fourth member of the Arabidopsis phosphate transporter gene family (Accession, U97546). (Plant Gene Register Pgr97-082) *Plant Physiology* **114**, 747.

Ma Y.-B., Luo H.-X., Zhang A.-J., Zhao H.-L., Zhang P., Ma W.-M. (2001) Studies on the grain filling properties in super large spike wheat. *Xibei Zhiwu Xuebao* **21**, 128–135.

Mimura T. (1999) Regulation of phosphate transport and homeostasis in plant cells. *International Review of Cytology* **191**, 149–200.

Muchhal U.S. & Raghothama K.G. (1999) Transcriptional regulation of plant phosphate transporters. *Proceedings of the National Academy of Science of the United States of America* **96**, 5868–5872.

Muchhal U.S., Pardo J.M. & Raghothama K.G. (1996) Phosphate transporters from the higher plant *Arabidopsis thaliana*. *Proceedings of the National Academy of Science (USA)* **93**, 10519–10523.

Nielsen K.L., Miller C.R., Beck D., Lynch J.P. (1998) Fractal geometry of root systems: field observations of contrasting genotypes of common bean (*Phaseolus vulgaris* L.) grown under different phosphorus regimes. *Plant and Soil* **206**, 181–190.

Okumura S., Mitsukawa N., Shirano Y., Shibata D. (1998) Phosphate transporter gene family of *Arabidopsis thaliana*. *DNA Research* **5**, 261–269.

Olsen S.R., Cole C.V., Watanabe F.S., Dean L.A. (1954) Estimation of available phosphorus in soils by extraction with sodium bicarbonate. *US Department of Agriculture Circular No 939*, US Department of Agriculture, Washington, DC, USA, 19 pp.

Rausch C., Daram P., Brunner S., Jansa J., Laloi M., Leggewie G., Amrhein N., Bucher M. (2001) A phosphate transporter expressed in arbuscule-containing cells in potato. *Nature (London)* **414**, 462–466.

Romer W. & Schilling G. (1986) Phosphorus requirement of the wheat plant at various stages of its life cycle. *Plant and Soil* **91**, 221–229.

Schachtman D.P., Reid R.J., Ayling S.M. (1998) Phosphorus uptake by plants: from soil to cell. *Plant Physiology* **116**, 447–453.

Sentenec H. & Grignon C. (1985) Effect of pH on orthophosphate uptake by corn zea-mays roots. *Plant Physiology* **77**, 136–141.

Smith F.W., Cybinski D.H., Rae A.L. (1999) Regulation of expression of genes encoding phosphate transporters in barley. In *Plant Nutrition – Molecular Biology and Genetics: Proceedings of the Sixth International Symposium on Genetics and Molecular Biology of Plant Nutrition* (eds G. Gissel-Nielsen & A. Jensen), pp. 145–150. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Smith F.W., Ealing P.M., Dong B., Delhaize E. (1997) The cloning of two *Arabidopsis* genes belonging to a phosphate transporter family. *Plant Journal* **11**, 83–92.

Smith F.W., Rae A.L., Hawkesford M.J. (2000) Molecular mechanisms of phosphate and sulphate transport in plants. *Biochemica et Biophysica Acta* **1465**, 236–245.

Thompson J.D., Higgins D.G., Gibson T.J. (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22**, 4673–4680.

Tusnady G.E. & Simon I. (2001) The HMMTOP transmembrane topology prediction server. *Bioinformatics* **17**, 849–850.

Yan X., Liao H., Cao A., He Y. (2001) The role of root architecture in P acquisition efficiency of different root systems: a case study with common bean and rice. In *Plant Nutrition – Food Security and Sustainability of Agro-Ecosystems* (ed. W.J. Horst, M.K. Schenk, N. Bürkert, *et al.*), pp. 590–591. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Yang N. & Smale M. (1996) Indicators of wheat genetic diversity and germplasm use in the People's Republic of China. *CIMMYT Report*, NRG Paper 96-04, 32 pp.

Zhu Y.-G. & Smith S.E. (2001) Seed phosphorus (P) content affects growth, and P uptake of wheat plants and their association with arbuscular mycorrhizal (AM) fungi. *Plant and Soil* **231**, 105–112.

Zou C.Q., Li J.Y., Li Z.S., Zhang F.S., Tong Y.P., Chen X.P., Hou L.J., Wang X.F. (2002) Photosynthate distribution in wheat varieties differing in phosphorus efficiency. *Communications in Soil Science and Plant Analysis* **32**, in press.

Received 26 February 2002; received in revised form 2 May 2002; accepted for publication 3 May 2002