Phosphate transport in Mycorrhizal Plants: Cloning and Characterisation of Genes Encoding Phosphate Transporters

by

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Abbreviations and Symbols

Abbreviation	Full title
~	Approximately
%	Percentage
>	Greater than
#	Catalogue number
°C	Degrees Celsius
bp	Base pair of nucleic acids
cDNA	Complementary DNA
cm	Centimetres
cv	Cultivar
DNA	Deoxy ribonucleic acid
eg.	Example
et al.	And others
g	Gram
gDNA	Genomic DNA
h	Hours
kDa	Kilodalton, molecular mass
kg	Kilogram
K_m	Affinity of a substance for an enzyme – Michaelis-Menton constant
L	Litres
lb sq.in.$^{-1}$	Pounds per square inch, a measure of pressure
m	Minutes
M	Molar
mg	Milligram
mg kg^{-1}	Milligram per Kilogram
mL	Millilitres
mm	Millimetres
mM	milli Molar
m^2s^{-1}	Metres squared per second
ng	Nanograms
nm	Nanometres
N	Normal = 1 Molar
P	Phosphorus, phosphate

Pi	Inorganic phosphate
rpm	Revolutions per minute
RNA	Ribonucleic acid
s	Seconds
µg	Microgram
µL	Microlitres
µm	Micrometres
µM	micro Molar
UV	Ultra violet light
V	Volts
Vmax	Maximum velocity of a reaction

Abstract

Many Australian soils are phosphate deficient. This has encouraged the use of fertilisers for profitable agricultural production. However, the inefficiencies, expense and environmental issues associated with high fertiliser use have led to a search for technologies that improve phosphate (P) uptake and utilisation.

Most crop plants are adapted to low soil P through symbiotic relationships with mycorrhizal fungi that enhance P acquisition. Mycorrhizal plants have two possible routes for P uptake from soil a) the direct uptake pathway via the root epidermis and root hairs, and b) a mycorrhizal pathway. In the latter mycorrhizal fungi deliver P from the soil to the interfacial zone between the symbionts, where plant P transporters in the cortical cell membranes acquire the P provided.

This project has successfully identified four plant P transporters that are expressed in mycorrhizal roots of the major cereal crop species barley (*HORvu;Pht1;8*), wheat (*TRIae;Pht1;myc*), maize (*ZEAma;Pht1;6*) and rice (*ORYsa;Pht1;11*) and are implicated in the mycorrhizal uptake pathway. The information on barley, maize and wheat is new; *ORYsa;Pht1;11* from rice was reported in 2002 with further information presented here. In barley the expression of *HORvu;Pht1;8* and two other barley P transporters, that appear to be involved in the direct uptake pathway, has been compared in plants grown in high and low P soil and in the presence and absence of mycorrhizal colonization. The expression pattern of these genes is indicative of the mycorrhizal P uptake pathway being utilised by the plant.

It is known that cereals are not highly responsive to mycorrhizal colonization in terms of either growth or P uptake and it might be expected that the mycorrhizal P uptake pathway is relatively unimportant. However, it has been recently shown that other non-responsive plants can receive 100% of their P via the mycorrhizal pathway, implying that P transporters in the direct pathway (epidermis and root hairs) are switched off. This hypothesis can now be tested with cereals. The finding that field grown (and hence mycorrhizal) barley and other cereals may acquire P via mycorrhizal fungi and not directly via the epidermis and root hairs would have significant implications for improvement of P efficiency.

DECLARATION OF ORIGINALITY

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 and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

D.Glassop

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1 Introduction

Plants must have access to the macronutrients nitrogen (N), phosphorus (P), potassium (K), magnesium chloride (Mg), calcium (Ca) and sulphate (S), as well as micronutrients, for optimal plant growth. P is involved in many plant biochemical processes as a primary energy source, adenosine triphosphate - ATP; as an essential structural component in cell membranes and nucleic acids, as well as buffering cell solutions. P is often the limiting factor in plant growth because of the low concentrations or poor availability in soils worldwide. In order to overcome this limitation the use of P fertilisers has become common practice.

Crops do not access applied P fertilisers effectively. Only 5-30% of soluble P applied is recovered in the first crop and the remainder of the P (70-95%) accumulates in the soil as inorganic and organic P compounds (Bolland & Gilkes R.J., 1998). This inefficiency has been managed by increasing the application of fertilisers, a wasteful practice which is further magnified by the diminishing stock of high grade rock P and the increasing cost of fertilisers (Trolove *et al.*, 2003). Because soils worldwide are often either P deficient or contain P in inaccessible forms, research into the processes of P acquisition by plants is required in order to improve P uptake and increase P acquisition from less available sources.

Improved P acquisition is achieved by plants using one or more of the following mechanisms (Comerford, 1998; Trolove *et al.*, 2003; Hedley *et al.*, 1994; Lynch & Brown, 1998):

- Alterations in root morphology (development of long, fine, hairy roots; increased root growth in regions of high P; cluster roots)
- Changes in the soil chemistry, which increases P availability (e.g. release of inorganic P by changing pH or releasing chelating agents; accessing organic P by releasing hydrolytic enzymes). These mechanisms will increase the solution concentration of P in the rhizosphere and expose P transporters expressed in epidermal cells of the root to higher concentrations of P.
- Formation of associations with arbuscular mycorrhizal fungi (AM fungi) that provide the plant with an alternative avenue of P uptake by accessing P beyond the range of the plant root system and delivering the P past the root epidermis to the cortical cells.
- Altering the expression of P transporters so that they are present at sites of available P. The expression of P transporters in root epidermal cells, associated with acquiring P from the soil, is reduced when the plant is colonised by AM fungi. AM fungi deliver P to root cortical cells where P transporters (different from root epidermal P transporters) are able to acquire the P (Liu *et al.*, 1998b).

The genetic manipulation of P transporters to alter their regulation may increase the P acquired by plants. Before this approach can be taken it is important to identify which P transporters are associated with P acquisition at the interfaces between roots, AM fungi and soil, particularly those that are activated when plants are grown in the field. Field experiments or at least experiments in soil-based systems are particularly important as soil has many complexities (physical, chemical and biological) that will influence the way in which a plant acquires P.

Plants acquire P against a steep electrochemical gradient (Mimura, 1999). P concentration in cytoplasm and soil have been measured at 10 mM and 1 μM respectively, which together with the negative potential difference across the membrane equate to energy demanding conditions for uptake of negatively charged $H_2PO_4^-$ ions (Mimura, 1999). In order to accumulate negatively charged $H_2PO_4^-$ ions in higher concentrations than the external environment the plant utilises active $H_2PO_4^{-}/ H^+$ symporters (referred to as P transporters throughout this thesis). Many plant P transporters have now been identified/cloned (mainly from various crop species). P transporters are membrane-associated proteins involved in P acquisition from soil or from the AM fungal interface and in mobilisation and redistribution of P within the plant. The P transporters work by an energy-mediated co-transport process, whereby protons (H^+) and $H_2PO_4^-$ ions move through the membrane together into the cell (Clarkson & Grignon, 1991; Muchhal & Raghothama, 1999). The proton motive force for H^+ transfer is supplied by a membrane bound H^+-ATPase pump that pumps H^+ ions to the outside of the membrane (Smith *et al.*, 2003a). Once P is acquired by the plant from the soil or from AM fungi it is translocated throughout the plant where it may be found in various chemical forms, inorganic ion or as organic derivatives, depending on function and location (Mimura *et al.*, 1996).

P uptake and translocation are regulated by the concentration of P available for uptake and the P concentration within plant cells, particularly the vacuolar concentration (Schachtman *et al.*, 1998). An example of loss of this regulation occurs in the *Arabidopsis* mutant pho2 that is unable to down-regulate the P uptake system and thus continues to accumulate P within leaves to a point of toxicity (Delhaize & Randall, 1995). Regulation of P uptake and mobilisation is reflected in the transcription levels of P transporters; eg the barley P transporters (*HORvu;Pht1;1* and *1;2*) have reduced transcription levels when plants have a sufficient supply of P compared to when P is limiting. Understanding the regulation of P transporters and P content within plants, as well as the mechanisms of P acquisition, is important if these systems are to be manipulated in order to overcome low concentrations of available P in soils.

The research presented within this thesis investigated the effects of the symbiosis between plants and AM fungi on P acquisition, with particular emphasis on the expression of P transporters and their

regulation in response to mycorrhizal colonisation and P nutrition. Mycorrhizas form in the roots of most plant species, with associations being found in fossils dating back 500 million years (Redecker *et al.*, 2000). It is the interaction with AM fungi that is of particular interest in this project as these fungi have been shown to increase the uptake of P by plants, including many crops (Howeler *et al.*, 1987; Smith & Read, 1997). The symbiotic relationship involves the exchange of nutrients. The plant provides a source of reduced carbon to the AM fungi while receiving P and other nutrients from the fungi (Marschner, 1995). The external hyphal networks of AM fungi acquire P from the soil. P transporters expressed in external mycelium have been identified from two AM fungi (Harrison & van Buuren, 1995; Maldonado-Mendoza *et al.*, 2001). The P is then translocated along the hyphae to the internal AM fungal structures (Smith *et al.*, 2001). Within the root cortical cells, AM fungi form finely branched intracellular structures known as arbuscules. The fungi are also able to form hyphal coils and arbusculate coils within cortical cells, dependent on fungal species (Cavagnaro *et al.*, 2001). Generally plants benefit from increased P uptake and plant growth is increased when associated with AM fungi, although considerable variations in P uptake and growth responses have been reported (Smith & Read, 1997). This variation is associated with factors such as plant species, plant cultivar, AM fungal species and growth conditions (Johnson *et al.*, 1997). AM fungi are able to enhance P uptake in plants by accessing P supplies not accessible by plant roots due to soil conditions, distribution of soil P and by delivering the P directly to colonised root cortical cells, bypassing the P depletion zone formed around the plant roots.

There are several lines of evidence implicating arbuscules as the main site of P transfer to the plant:

- The arbuscule/peri-arbuscular membranes have a large surface area (Dickson & Kolesik, 1999),
- There is no evidence of fungal P transporters expressed within colonised roots. Consequently the AM fungi cannot take up P that it has originally made available for plant uptake at the arbuscule/peri-arbuscular membranes. (Harrison & van Buuren, 1995),
- H^+-ATP-ase activity is high in cortical cells containing arbuscules. This would provide the proton motive force necessary for plant $H^+/H_2PO_4^-$ symporters to acquire P. (Gianinazzi-Pearson *et al.*, 2000) and,
- Prior to the start of this research a P transporter cloned by Rosewarne *et al.* (1999) from tomato (*LePT1/LYCes;Pht1;1*) was found to be expressed in cortical cells containing arbuscules and at the root apex and root hairs of non-colonised roots (Rosewarne *et al.*, 1999; Liu *et al.*, 1998a).

While initial evidence points to the arbuscule as being a site of P delivery to the plant other mycorrhizal associations such as internal hyphae (intra- and inter-cellular), arbuscular coils and hyphal coils could also be involved with P delivery.

I investigated how cereal crops access phosphate (P) from the soil. Plants are able to acquire P via two pathways. One pathway (the direct pathway) utilises the root system in contact with the soil, while a second pathway is via AM fungi. The main focus of this research was on the P transporters used by the plant to acquire P via both pathways, and how the colonisation of the plant with AM fungi influences the expression of plant P transporters. Chapter 2 reviews the current literature to explain in detail the importance of P for plant growth, strategies for accessing P from the soil, how mycorrhizas are one of these strategies and how P transporters are involved in P uptake. This is followed by Chapter 3, which details the methods and materials that are common to more than one experimental chapter.

Chapter 4 describes two experiments that identify barley cultivars that may acquire P with different efficiencies and secondly determines if they vary in colonisation by AM fungi. Prior to determining pathways of P acquisition an experiment was designed to establish if there was any variation in P efficiency between barley cultivars. Barley cultivars of low and high P efficiency were then assessed to determine if their ability to acquire P caused any variation in colonisation by different AM fungal species. Within the literature the interaction between plant species or plant cultivar with AM fungal species is highlighted with respect to the overall benefits to the plant.

Chapter 5 reports on several experiments looking at P transporters in barley, wheat and maize. My research had access to several P transporters that had been identified in barley. Three of these transporters had been identified as being expressed within roots cells, one was expressed in shoots and the others had unidentified tissue expression patterns. This suite of transporters allowed further research into how these P transporters interacted when barley plants were colonised by AM fungi and which pathway of P acquisition they may be involved in. As well as identifying barley P transporters involved in acquiring P from AM fungi, other cereal crops including maize and wheat have been assessed for analogous P transporters.

During the time I was identifying cereal P transporters involved with P acquisition from AM fungi the rice genome was published. Screening of the rice genome with a rice P transporter (Godwin, 2002) revealed 13 putative P transporters. Chapter 6 reports the expression pattern of these putative P transporters in AM colonised and un-colonised rice roots.

A general discussion of the results and future work that could be continued is presented in the final chapter.

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2 Review of the Literature

2.1 Supply of soil phosphorus and its importance to plants

2.1.1 Importance of P

Phosphorus (P) is one of the essential major nutrients required by plants. Essential nutrients have been determined by three criteria: - 1. the element is required for normal growth and reproduction in several different plants, 2. it cannot be replaced by another element, and 3. its role is direct and not involved with biochemical reactions to correct other substance imbalances (Keeton & Gould, 1986). These essential macronutrients are nitrogen (N), phosphorus (P), potassium (K), sulphur (S), magnesium (Mg) and calcium (Ca). Table 2.1 lists the optimal nutrient levels and functions associated with these major nutrients in the cereal crop, barley, the plant used in most of the experimental work described in this thesis.

Table 2.1. Functions of major essential nutrients in plants and the concentrations as % dry matter required for optimal growth of barley. Information extracted from compiled references in (Reuter *et al.*, 1997).

Element	% dry matter	Functions
N	1.75 - 5	Amino acids, proteins, nucleic acids, nucleotides, chlorophyll
P	0.2 – 0.68	Energy storage and transfer - ATP, membrane integrity, nucleotides
K	1.5 - 5	Translocation, water and energy relations, cellular pH, osmoregulation, cation-anion balance
S	0.13 – 0.4	Protein synthesis and function, amino acids, energy transfer, structure
Ca	0.25 – 1.2	Membrane maintenance, cell division and elongation, cell wall stabilisation, cation-anion balance, osmoregulation, signalling
Mg	0.12 – 0.5	CO_2 assimilation, cellular pH, cation-anion balance, protein synthesis, carbohydrate partitioning.

The importance of P lies in the low concentrations in most soils worldwide, causing P to be a common growth-limiting factor in plants and crops. P deficiency results in fewer leaves, reduced leaf expansion and low leaf surface area (Marschner, 1995). P deficiencies may be recognised by tips of leaves turning yellow (as seen in barley), reddening or purpling, with symptoms affecting older leaves first (Grundon *et al.*, 1997). Symptoms of P toxicity can be seen in many plant species when P is accumulated to concentrations > 1% of the dry matter, with barley demonstrating symptoms at levels > 0.7% (Marschner, 1995; Weir & Cresswell, 1994). P toxicity is defined as interveinal chlorosis in younger leaves; necrosis and tip die back, marginal scorch, interveinal necrosis, and shedding of older leaves (Grundon *et al.*, 1997). P deficiency is more common than toxicity, as the majority of world soils are P deficient, including most Australian soils. Under low P conditions plants conserve the use of P by initiating improved and alternate acquisition strategies or pathways, and farmers apply phosphatic fertilisers to ensure profitable agricultural production. Those countries that utilise P fertilisers have the added complication that a large percentage of P applied in fertiliser is unavailable to the crops due to the soil chemistry and localisation of applied P within the soil.

The P cycle in soils involves several interacting pools (Figure 2.1). P is acquired via P transporters in the roots as inorganic orthophosphate ions ($H_2PO_4^-$) from the soil solution at an optimal pH of 4.5-5.0 (Smith & Read, 1997; Raghothama, 1999b). The amount of available Pi in soils is dependent on pH, ionic strength, concentrations of P (organic matter and fertilisers) and binding metals (Fe, Al, Ca, Mg) and anions (Vance *et al.*, 2003). As much as 50% of the P present in soils is unavailable due to it being strongly absorbed onto Fe and Al compounds and locked in organic forms, which need to be mineralised before the soluble inorganic P becomes accessible to plants (Figure 2.1) (Horst *et al.*, 2001).

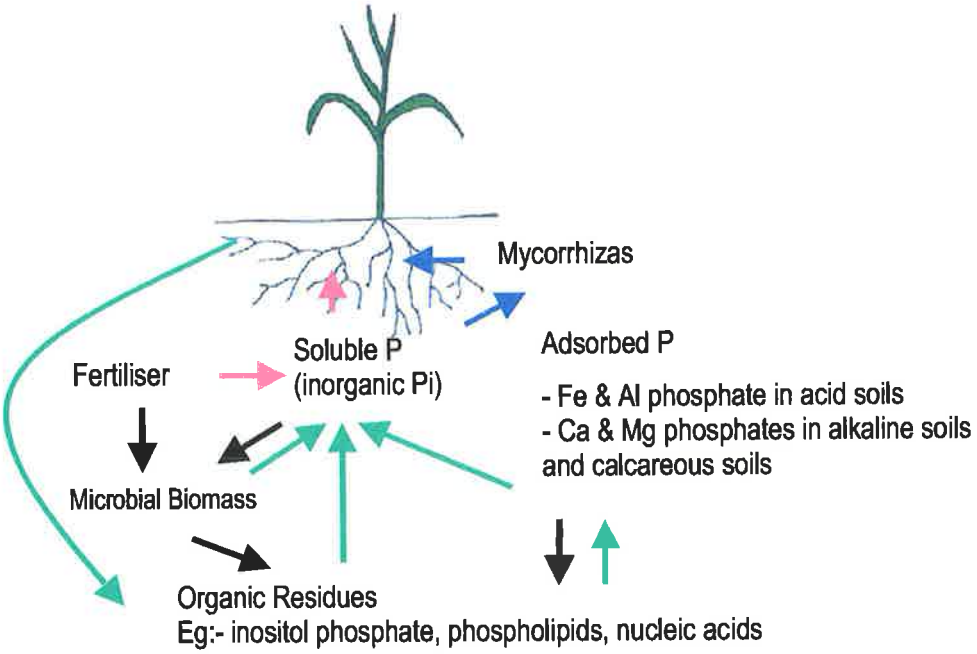


Figure 2.1: Schematic representation of supply of phosphorus to plant roots in soil systems. Black arrows indicate the immobilisation of P, green arrows illustrate the mineralisation of P, pink arrows explain the diffusion of P in solution and the blue arrows depict P pools accessed via mycorrhizal fungi and transported to roots (Moody & Bolland, 1999; Bolan, 1991).

2.1.2 P in soil and acquisition

There are three ways that Pi reaches the surface of roots and AM fungal hyphae (Bolan, 1991). These are: -

1. Root or AM fungal hyphal interception - where the root or hyphae comes into close contact with the nutrient,
2. Diffusion of ions from areas of high concentrations to low – accessed by roots and AM fungi hyphae, and
3. Mass flow where the nutrient is brought to the "root surface in the convective flow of water during the absorption of water by the plant".

The diffusion of P in soil has been found to be more important than mass flow (Barber, 1962). Because of the low concentration of Pi in the soil solution (usually < 10 μM, (Mimura, 1999; Schachtman *et al.*, 1998) and a low diffusion rate (D_{soil} 10^{-12}-10^{-15} m^2s^{-1}, (Jungk, 1996)) a P depletion zone of ~1.5 mm forms around actively absorbing roots within a few days. Pi uptake is greatly reduced due to the lack of

available Pi at the root surface. In order to overcome these depletion zones, and access previously inaccessible Pi supplies, plants have evolved strategies to enhance Pi acquisition by more effective exploitation of the soil. These strategies include alterations in root morphology and root hair production, effecting changes on the soil chemistry to increase bioavailability, by developing symbiotic relationships with AM fungi and regulating plant P transporters (Comerford, 1998).

Changes in root morphology are regulated by auxins, ethylene and cytokinins (Nissen, 1996) resulting in: -

- Topsoil foraging. This occurs where shallow root systems show enhanced lateral root production in the topsoil, exploiting the localised higher concentrations of Pi. This change in morphology is seen in *Arabidopsis* grown in low P conditions (0.1 mM P), resulting in a decrease in the primary root length and increased number and length of lateral roots when compared to plants grown in high P conditions (2.5 mM P) (Williamson *et al.*, 2001). Variations of this topsoil foraging morphology occurs between *Arabidopsis* accessions (Narang & Bruene, 2000). Altered root morphology has also been observed in beans and displays variation of top-soil foraging morphology between bean genotypes (Lynch & Brown, 2001; Liao *et al.*, 2001). Grass species also have top-soil foraging strategies, producing roots that are able to access Pi from treated high Pi areas (Jackson *et al.*, 1990).
- Increased root hair density and length. This results in increased exploration of the soil volume close to the roots. Increases in root hair length and/or root hair density have been observed in rape, spinach, tomato, *Medicago* and *Arabidopsis* as the plants become P deficient (Fohse & Jungk, 1983; Vance *et al.*, 2003; Ma *et al.*, 2001; Bates & Lynch, 2000). Variation in barley cultivars in root hair density and length has been associated with differences in Pi uptake by Gahoonia and Nielsen (1997).
- Formation of cluster roots (proteoid roots). These are groups of densely packed lateral roots (with abundant root hairs) formed on a parent axis, which results in an increased surface area but not always a large increase in soil volume explored. Cluster roots form in pockets of soil rich in nutrients (Vance *et al.*, 2003). They also release exudates containing organic acids, H^+ and acid phosphatases that release bound Pi from inorganic and organic complexes (Marschner, 1995). The major groups of plants that produce cluster roots are the Proteaceae, some members of the Brassicaceae and *Lupinus*. Further information on cluster roots is contained in the recent review of Vance *et al.* (2003).
- Another adaptation which helps plants to overcome problems associated with the development of Pi depletion zones is the formation of a symbiotic relationship with AM fungi. These fungi access Pi from the soil beyond the depletion zone, translocate it considerable distances (up to 25 cm) through

external hyphae to roots and, at plant-fungus interfaces within root cortical cells, exchange it for organic carbon produced by the plant (Smith and Read, 1997).

2.1.3 The role of AM fungi in supplying P to plants

AM fungi (classified in the order Glomales of the Zygomycota) form normally beneficial associations with the plant root, based on exchange of nutrients whereby the plant receives mineral nutrients while the fungus receives organic carbon compounds (Smith and Read, 1997). The outcome of this exchange can be beneficial, mutualistic or parasitic, depending on whether the plant has an increase, no change or a decrease (respectively) in nutrient uptake or plant dry matter (Johnson *et al.*, 1997; Baon *et al.*, 1993b; Marschner, 1995). The majority of AM fungi/plant relationships are beneficial or mutualistic. Barley and indigenous mycorrhizal fungi were observed by Khaliq and Sanders (1998 & 2000) to have a mutualistic association in field and field/pot experiments that displayed no increase in yield.

When a plant is colonised by AM fungi the plant is then able to access P via the root system and/or the mycorrhizal fungi. Colonisation of plants with mycorrhizal fungi generally results in increased plant growth and health due to the supply of Pi from the fungi (Azcón-Aguilar & Barea, 1997). Pi influx in mycorrhizal plants can increase to rates of 10^{-11} $mol^{-1}m^{-1}s$, 3 to 5 times higher than non-mycorrhizal plants (Smith and Read, 1997). Results of Pearson and Jakobsen (1993) support the supply of P via AM fungi. Data that directly assessed the P uptake of cucumber plants determined that P acquisition via the root was reduced when colonised with mycorrhizal fungi (Pearson & Jakobsen, 1993). P transporters associated with P acquisition via roots were assumed to be down regulated, thus encouraging the acquisition of P via the mycorrhiza (Pearson & Jakobsen, 1993). It was also observed that there was a difference in the amount of Pi supplied to the plant via the mycorrhiza when colonised by different mycorrhizal fungi. This may be due to differences in the rate of growth and length of the hyphae and differences in the P transport capacity of the hyphae (Pearson & Jakobsen, 1993). Hyphal length may not always be important, with research by Smith *et al.* (2000) showing that, despite extensive hyphal length, different AM fungi will access P from different distances from the plant root. *M. truncatula* also displayed variation in the amount of P acquired via mycorrhizal fungi when colonised by various AM fungi (Burleigh, 2001). Pearson and Jakobsen (1993) observed that increased P status of colonised plants was also associated with the apparent down regulation of plant P transporters. The regulation of the *Medicago* P transporter *MEDtr;Pht1;2* is linked to the P status of the plant (Burleigh, 2001). *MEDtr;PhT1;2* is up regulated when the plant is P deficient and is down regulated to varying degrees when colonised with AM fungi. The degree of down regulation is dependent on the species of mycorrhizal fungi colonising the roots (Burleigh, 2001).

2.1.4 AM fungal structures

AM colonisation can be classified into two main types, *Arum*- and *Paris*-AM. *Paris*-type colonisation is characterised by intracellular coils (hyphal coils) formed in cortical cells (Figure 2.2). *Arum*-type mycorrhizas are the most commonly investigated and are characterised by the formation of a well-developed mycelium of intercellular hyphae within the root cortex and branched structures (arbuscules) within the cortical cells (Figure 2.2). The arbuscules are short-lived, about 4-12 days (Cox and Tinker, 1976). The highly branched arbuscule invaginates the host cell plasma membrane (peri-arbuscular membrane), forming a symbiotic interface with a very large surface area (Schematic diagram, Figure 2.2). This is thought to be the site of nutrient transfer from fungus to plant. The coils in *Paris*-AM (which also present a very large area of interface) are being implicated in this role as well (Smith and Read, 1997).

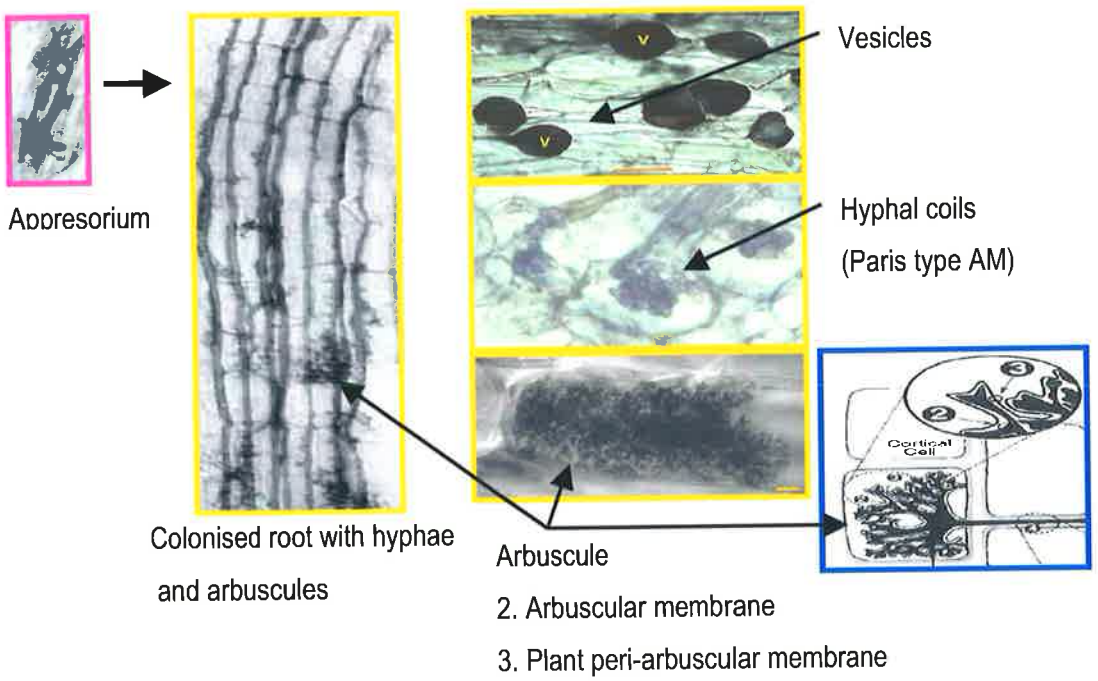


Figure 2.2: Internal AM fungal structures. AM fungal hyphae can penetrate the root epidermal cell or enter via intercellular spaces and subsequently penetrate the cortical cells. The point of entry into the root by the fungus is called the appressorium (pink bordered box; reproduced from Brundrett, 2000). From the appressorium internal colonisation spreads via hyphae, followed by the formation of arbuscules, hyphal coils and vesicles (yellow bordered boxes; reproduced from Brundrett, 2000). The schematic diagram of an arbuscule (blue bordered box, reproduced from Harrison, 1999) emphasises the apoplastic space between the arbuscular membrane and the plant peri-arbuscular membrane; indicating that the fungi remain separate from the plant cortical cell and transfer Pi across the arbuscular membrane into the apoplastic space, from which the plant uses P transporters within the plant peri-arbuscular membrane to absorb that Pi. (Harrison, 1999; Brundrett, 2000)

The AM fungi extend a fine network of hyphae into the soil, accessing Pi up to 25 cm from the root (Figure 2.3) (Smith and Read, 1997). Two P transporters, with a similar structure and function to plant P transporters, have been identified from two mycorrhizal fungi, *G. versiforme* (Harrison & van Buuren, 1995) and *G. intraradices* (Maldonado-Mendoza *et al.*, 2001). These fungal transporters are not expressed in the plant, suggesting that they are involved with the acquisition of Pi from the soil. Once taken up, the Pi is translocated along the hyphae to the mycorrhiza/plant interface.

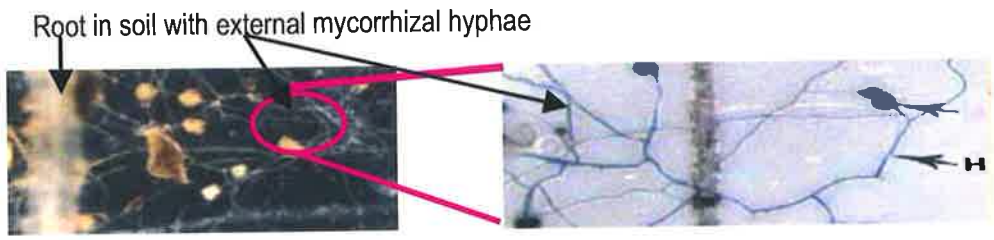


Figure 2.3: Images of AM fungal external hyphal network. Spores (arrowed) of AM fungi in the soil initiate hyphal (H) growth towards the plant root. (Images reproduced from Brundrett, 2000)

The fungus and plant remain separated by an apoplastic space, with the fungus topologically outside the plant. It is probable that the apoplastic space is the site of nutrient transfer, with a high activity of ATPase present at the peri-arbuscular membrane (thereby providing protons for coupled transport, discussed in section 2.3.1, (Gianinazzi-Pearson *et al.*, 2000)). This is further supported by research performed by Ayling *et al.* (2001) which showed that the root membrane electric potential was ~10 mV higher in non-mycorrhizal leeks than in leeks colonised by *Scutellospora calospora* and forming intracellular coils. The reduced electric potential difference is due to Pi being supplied by the fungal hyphae (Ayling *et al.*, 2001). There is supporting research that measured the amount of P (via cryo-analytical scanning electron microscopy) revealing a higher concentration of P in colonised cells than non-colonised cells (Ryan *et al.*, 2003). While these measurements account for total P (poly-P, Pi and all other forms) present at the site of examination, there was a higher concentration of P in fungal structures than in plant cells, and plant cells containing arbuscules had a higher concentration of P than those cells with no fungal structures. This provides further circumstantial evidence that arbuscules are a site of exchange with the plant. Some root intercellular spaces contained a liquid that was observed to include P when associated with fungal hyphae (Ryan *et al.* 2003). There is no reported evidence that any plant P transporters are specifically expressed around these intercellular spaces to acquire P from these pools. It is unclear as to whether this is a site of active P exchange (i.e. Are the AM fungi exporting Pi at this site?) or diffusion (movement from high to low concentrations). Either way the plant

would be able to scavenge this source of Pi with alternative P transporters located within the cortical cell membranes. The model of Pi acquisition via AM fungi may be more complex than initially thought.

2.1.5 Plant/AM fungi interactions

The involvement of AM fungi with plants is further complicated by the fact that major effects of AM fungi on plant P uptake may not be reflected in benefits at the whole plant level. Recently, work done by Smith *et al.* (2003b & 2004) demonstrated that *G. intraradices* supplied flax, *Medicago truncatula* and tomato with all of the Pi measured in plant tissues, with noticeable increases in plant dry weight only in flax and *M. truncatula*. Tomato showed decreased dry weight when colonised by AM fungi compared to non-colonised plants, which may be due to the amount of carbon supplied to the AM fungi. This research also demonstrated that different mycorrhizal fungi supplied different percentages of Pi to plants. For example tomato plants received 100% of their total P from *G. intraradices*, ~77% from *G. caledonium* and ~7% from *Gigaspora rosea*. The outcome of the interaction was also plant dependent. *Gigaspora rosea* provided flax with ~13% of its P, *Medicago truncatula* with ~44% and tomato with ~7%. These findings are particularly important to agricultural practices since, based on our earlier understanding, the role of AM fungi is likely to have been dismissed in non-responsive crops such as many cereals. Regardless of growth response this symbiosis can be the major pathway of Pi acquisition for the plant, depending on plant-fungus combination.

2.2 *The importance of phosphate transporters in P uptake*

2.2.1 The Pht1 family of P transporters

In response to growth requirements, various membrane associated P transporter proteins are involved in the acquisition of Pi from the soil and movement of Pi between tissues. The genes encoding these transporters have been classified into gene families depending upon their topology. The majority of P transporters isolated to date are found within the Pht1 family. These proteins consist of 12 hydrophobic membrane-spanning regions that are separated into two groups of six by a large intracellular hydrophilic, charged domain (Figure 2.4-A). They are approximately 58 KDa in size and contain 520 – 550 amino acids (Smith, 2001; Pao *et al.*, 1998; Raghothama, 2000; Smith *et al.*, 2003a). This topology is similar to other solute transporters that are members of the major facilitator super-family (Marger & Saier, 1993; Smith *et al.*, 1999).

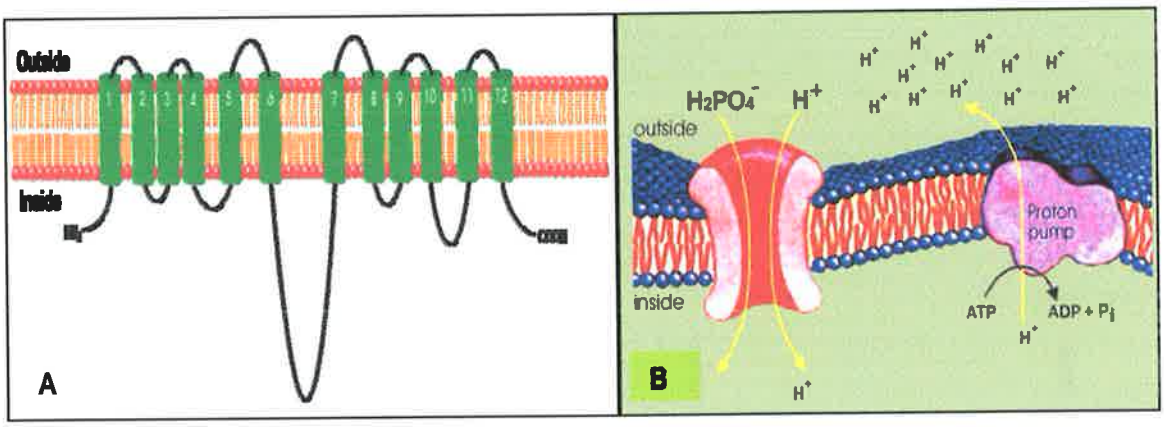


Figure 2.4: Schematic images of the PhT1 family phosphate transporters demonstrating: A. The 12 membrane spanning regions (composed of 17 – 25 amino acids) and the central intracellular hydrophilic loop, with the N and C termini intracellular; and B. The processes involved in co-transport of $H_2PO_4^-$ ions across the cell membrane, with H^+ ions supplied by a proton pump. (Figure A reproduced from Smith *et al.*, 2003a; figure B reproduced from Smith 1999)

Another group of P transporters, the Pht2 family, is found in mammalian, bacterial and algal systems where they are usually associated with Na^+ co-transport. Two plant P transporters with Pht2-like topology have been isolated from *Arabidopsis* (Daram *et al.*, 1999) and *Medicago* (Zhao *et al.*, 2003). Unlike the Na^+ coupled transport that is typical of animal systems, the plant members of the Pht2 family are H^+ coupled (Daram *et al.*, 1999; Zhao *et al.*, 2003). Topology of the Pht2 transporters differs from the Pht1 family by having a long extracellular hydrophilic N terminus and a hydrophilic loop between trans-membrane regions 8 and 9 (Daram *et al.*, 1999). Chloroplast transit peptides are featured in the

N terminus of the *Arabidopsis Pht2;1* transporter and when the *Pht2;1* gene was fused with green fluorescent protein the resulting Pht2;1 protein was localised to the chloroplast envelope. While other P transporter families have been identified from plants (eg. mitochondrial P transporters) the research described in this thesis concentrates on the Pht1 family of P transporters because these proteins are likely to be more significant in strategies aimed at enhancing plant Pi uptake and mobilisation efficiencies.

Pi acquisition is an energy requiring process, because of the large electrochemical gradient between the soil solution and the cytoplasm of plant root cells. Uptake therefore requires both a transporter and an energy source. ATPases are needed to generate proton motive force. Pi uptake is achieved by an energy-mediated co-transport process, whereby protons (H^+) are coupled with the $H_2PO_4^-$ ions to move into the cell (Figure 2.4-B) (Clarkson & Grignon, 1991; Muchhal & Raghothama, 1999). The requirement for co-transport is evidenced by the depolarisation of the plasma membrane associated with the influx of positively charged protons (Poole, 1978). If $H_2PO_4^-$ ions alone crossed the membrane a hyperpolarization would be observed. Further support for co-transport with H^+ is the restricted uptake seen when H^+ inhibitors/uncouplers are present (Lin, 1979). Cations other than H^+ have not been successfully associated with plant Pi transport.

2.2.2 Regulating P uptake

The strategies that enhance Pi acquisition include regulation of P transporters to access available Pi from soil and facilitate transport within the plant. P transporters located in root epidermal cells are involved in acquisition of Pi directly from the soil and P transporters located in the cortex acquire Pi from the apoplast and move it symplasticaly through cell plasmodesmata connections to the xylem parenchyma for distribution. The use of active transporters to acquire and distribute Pi is essential because intracellular Pi can be measured in concentrations of 5 – 17 mM (Mimura *et al.*, 1996; Mimura, 1999), compared to external concentrations that are in the range of 1 – 8 μM (Barber, 1962; Reisenauer, 1966). There can be as much as 1,000- 10,000 fold difference between internal and external Pi concentrations (Bieleski & Ferguson, 1983; Schachtman *et al.*, 1998). The Pi status of plants and their rate of growth determine the requirement for influx and net absorption of Pi by the roots (Clarkson & Grignon, 1991). Due to the uneven distribution of Pi in the soil, plants have the ability to increase rates of acquisition in those areas with high Pi via those methods discussed in section 2.1.2, thus compensating for Pi deficient areas. It is thought that overcoming uneven Pi supply is done by Pi cycling in the phloem between shoots and roots via P transporters (Clarkson & Grignon, 1991).

2.2.3 P transporter kinetics

Kinetic studies of P transporters have yielded results that indicate differences in K_m between species, cell types and growth conditions, demonstrating the control of the Pi transport systems, see Appendix 1. Studies involving excised barley roots identified that there were two systems of uptake in plants, dependent upon the external concentration of the ion (Figure 2.5) (Epstein & Hagen, 1952; Fried & Noggle, 1958). P transporters are active depending on the Pi nutritional status of the plants and level of Pi supply (Zhu *et al.*, 2001). The K_m for high-affinity transporters ranges from 3 - 30 μM, whereas for low-affinity transporters the range is 50 - 668 μM, see Appendix 1.

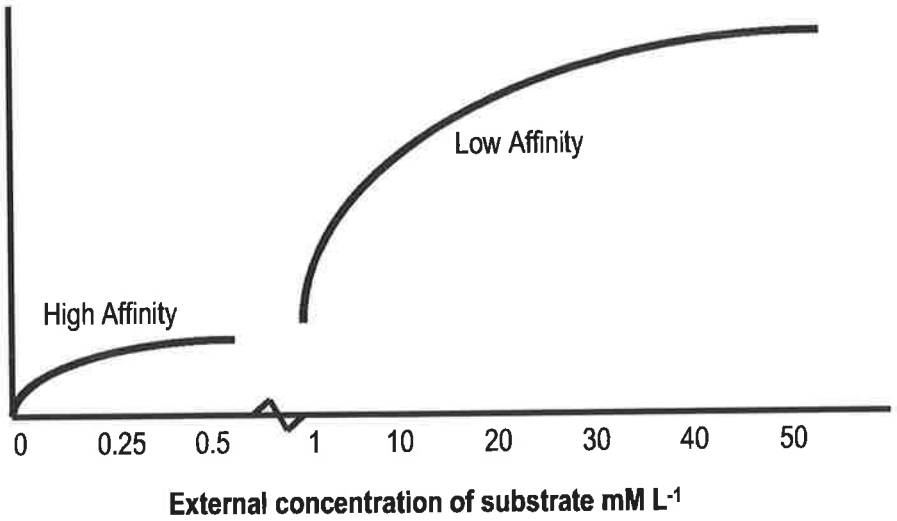


Figure 2.5. Concentration-dependence of ion uptake by plant cells, illustrating the dual-isotherm Michaelis-Menten kinetics. High affinity has a low Michaelis constant, high affinity for the substrate and a low maximal rate. Low affinity has a high Michaelis constant, low affinity for the substrate and a high maximal rate. Reproduced from Lüttge and Higinbotham 1979.

When *Lemna gibba* is P-deficient the V_{max} of P transporters involved in high and low affinity systems has been reported to increase (Ullrich-Eberius *et al.*, 1984), while experiments with *Catharanthus roseus* only exhibited an increase in V_{max} for the high affinity system (Furihata *et al.*, 1992). The increased activity (V_{max}) observed by both laboratories was not associated with a change in K_m. Increases in the rate of Pi uptake associated with Pi starvation is a result of increased transcription of P transporter genes, resulting in increased translation, increased density of transporters in the membrane in question and enhanced Pi uptake (increase in V_{max}) (Muchhal & Raghothama, 1999; Raghothama, 1999a; Furihata *et al.*, 1992). The transcription rate of P transporter genes increases soon after a change in cellular Pi concentration and long before the appearance of any visible Pi-deficiency symptoms (Liu *et al.*, 1998a; Yao *et al.*, 2001; Dong *et al.*, 1998). This finding suggests that signals that

induce gene expression are initiated by internal concentrations of Pi. This will be discussed later in this chapter.

2.2.4 Verification of function of P transporters and kinetics by yeast complementation

There is controversy about the determination of the affinities of P transporters. Most data has been obtained using heterologous expression in yeast, which has yielded higher K_m values than expected from physiological studies (Smith *et al.*, 2003a). Initial studies utilised *Saccharomyces cerevisiae* yeast mutants defective in the high-affinity P transporter *pho84* gene (Bun-ya *et al.*, 1991). The introduced plant P transporter genes complemented the mutations and restoration of Pi uptake was taken as evidence for Pi transport via the plant transporter. The K_m's determined with *pho84* yeast mutants were of the order of 110 – 493 μM. For example *ARAth;Pht1;1* complemented the yeast mutant NS219 grown on 110 μM P medium (Muchhal *et al.*, 1996), and could therefore be interpreted as being a low-affinity transporter, while plant cell suspension cultures determine that it is a high-affinity transporter (discussed in further detail in the following section). Another yeast P transporter, *pho89*, is involved with Na^+ coupled P transport in yeast. The development of a second yeast mutant, PAM2, defective in both *pho84* and *pho89* P transporters, (Martinez & Persson, 1998), yielded lower K_m values for the same genes that were expressed in the *pho84* mutants. The plant P transporters tested in the PAM2 yeast mutant were not Na^+/H_2PO^{-4} symporters yet provide K_m values that came closer to expectations of high and low -affinity P transporters. This is seen with *SOLtu;Pht1;3* K_m 64 μM - high affinity (Rausch *et al.*, 2001) and *MEDtr;Pht1;4* K_m 668 μM - low affinity (Harrison *et al.*, 2002). Interestingly Harrison *et al.* (2002) measured the K_m of *MEDtr;Pht1;4* in both single and double yeast mutants, and obtained values of 493 μM and 668 μM respectively, which confirms low-affinity kinetics but further demonstrates the variability of the yeast mutant system.

The K_m's of plant P transporters when measured in mutant yeast strains may not accurately represent K_m's in the plants for several reasons. Firstly, analysis of the plant P transporter genes suggests that putative phosphorylation sites in the sequence may require activation and may prevent the plant P transporters from fully complementing the yeast mutants. Secondly, N-glycosylation and associations with other proteins with the plant P transporters may be required prior to the P transporter being able to function. In yeast, the *pho84* gene has been functionally associated with other genes, suggesting a more complicated model for Pi transport (Bun-ya *et al.*, 1992; Bun-ya *et al.*, 1996; Yompakdee *et al.*, 1996; Leggewie *et al.*, 1997). Plant P transporters may not complement the complicated model for Pi transport in these yeast mutants, thereby yielding K_m values higher than those obtained with root

systems (Smith *et al.*, 2003a). Hence, it is important to measure the kinetics of transporters in homologous systems.

2.2.5 Verification of function and kinetics in plant cell suspension cultures

The use of transgenic plant cells as expression systems may overcome the putative post-translational problems of the yeast systems and should provide kinetic information more closely related to the expression of the genes in plants. This plant system involves the production of transgenic cells transformed with the P transporter of interest under the control of a constitutively expressed promoter. Use of tobacco cells transformed with *ARAth;Pht1;1* indicate a K_m of 3.1 μM for this gene (Mitsukawa *et al.*, 1997b), which is indicative of a high-affinity P transporter and considerably lower than the value of 110 μM previously obtained by Muchhal *et al.* (1996) using yeast. Rice cells transformed with *HORvu;Pht1;1* or *HORvu;Pht1;6* yielded K_m's of 8 μM and 320 μM, respectively (Rae *et al.*, 2003) indicating their roles as high affinity and low affinity transporters, respectively. The use of transgenic plant cells to determine P transporter kinetics yields results consistent with those observed in uptake experiments with excised barley roots that exhibited both high affinity of the range 1 – 10 μM and low affinity in the range of 0.2 – 0.5 mM kinetics (Barber, 1972). These high affinity K_m values are also consistent with the low concentrations of Pi found in most soil solutions. This plant-based protocol has the advantage of providing results that may be more reliable than those obtained with yeast mutants. The kinetic values of P transporters provided from yeast systems should be used with care until they are tested in plant-based systems.

2.2.6 Split root and hydroponic experiments

Split-root experiments with potatoes demonstrated that it is the overall P status of the plant that determines the rate of P uptake and not the portion of root system growing in solution lacking P (Cogliatti & Clarkson, 1983). Experiments by Clarkson and Scattergood (1982) in barley and tomato demonstrated that there was an immediate increase in rate of P absorption when plants were moved from hydroponic solutions containing 150 μM P to those with no P. In barley this increase in rate of P absorption continued for several days after the re-application of P, before decreasing to those levels observed prior to P starvation (Clarkson & Scattergood, 1982). The increased rate of P absorption is associated with the increase in transcription of P transporters, as shown by the dramatic increase in transcription of *ARAth;Pht1;1* and *ARAth;Pht1;2* when plants were transferred from 250 μM P to zero P hydroponic solutions (Smith *et al.*, 1997). A similarly high transcription rate was seen for tobacco P transporter genes, *NICta;Pht1;1 – 1;4*, when grown without P. When P was re-supplied transcription levels returned to those levels observed prior to P starvation (Kai *et al.*, 2002). The levels of

transcription can also be reflected in the level of protein as seen in the decreased accumulation of MEDtr;Pht1;1 protein when P deprived *Medicago* plants were re-supplied with P (Chiou *et al.*, 2001).

2.2.7 P transporter kinetics in realistic growth conditions

Much research has used plants grown in hydroponic or sand cultures, with 'low P' supplies of zero and 'high P' concentrations as high as 250 μM. None of these conditions effectively reflect the true growing conditions of plants in soil systems. Uptake in hydroponic growth systems, which are usually aerated, does not involve the formation of depletion zones around actively absorbing roots, so that Pi supply to the epidermal P transporters is maintained. Hydroponic cultures also produce a root architecture that is not identical to that found in soil. Sand culture systems are slightly more realistic, but in these the adsorption of Pi to sand particles does not occur and may result in solution P concentrations atypical of soil. Experiments on potato (Rausch *et al.*, 2001), tomato (Rosewarne *et al.*, 1999), rice (Paszkowski *et al.*, 2002), *Medicago* (Harrison *et al.*, 2002) and *Arabidopsis* (Mudge *et al.*, 2002) have used soil:sand mixes to grow plants for expression studies. These soil:sand systems are likely to deliver data on gene expression that are more closely related to the situation likely to occur in natural conditions. In order to get a realistic view of P acquisition and translocation plants should be grown in conditions that closely mimic the soil environment, where P is usually in low concentrations and soil microflora influence the availability and acquisition of P (Schachtman *et al.*, 1998). This is particularly important in investigations of the potential roles of AM fungi in P uptake. The work described in this thesis utilises a soil:sand system in order to achieve results that are relevant to plants growing in near-natural conditions.

2.2.8 Expression analysis and localisation of P transporters involved with P uptake from AM fungi

The use of soil:sand mixes for detection and analysis of the expression of *LYCes;Pht1;1* (Rosewarne *et al.*, 1999), *MEDtr;Pht1;4* (Harrison *et al.*, 2002), *ORYsa;Pht1;11* (Paszkowski *et al.*, 2002) and *SOLtu;Pht1;3* (Rausch *et al.*, 2001) was necessary because these P transporters are involved in acquisition of P at the peri-arbuscular membrane, delivered by AM fungi. The establishment of AM symbioses can only be realistically achieved in soil-based systems; hydroponic culture is not practicable and, in common with agar-based systems, suffers from the disadvantages outlined above. As previously mentioned AM fungi provide an alternative pathway for P acquisition. At the start of the work discussed in this thesis only *LYCes;Pht1;1* had been identified as showing an expression pattern consistent with the ability to access P via AM fungi. *LYCes;Pht1;1* was shown to be expressed in both roots and shoots of mycorrhizal (*G. intraradices*) and non-mycorrhizal tomato (Rosewarne *et al.*, 1999). The expression studies of *LYCes;Pht1;1* utilised *in-situ* hybridisation protocols (Rosewarne *et al.* 1999). Rosewarne's *et*

al. (1999) results would be enhanced by using localisation expression protocols similar to those used to determine the localisation of *MEDtr;Pht1;4*, *ORYsa;Pht1;11* and *SOLtu;Pht1;3*. The expression studies in MEDtr;Pht1;4 utilised a specific antibody for immuno-localisation (protein detection) and *Medicago* plants transformed with the promoter of *MEDtr;Pht1;4* driving the expression of a reporter gene (GUS). Results using both approaches showed localisation of *MEDtr;Pht1;4* to the peri-arbuscular membrane in *G. intraradices* colonised cortical cells (Harrison *et al.*, 2002). Analysis of *SOLtu;Pht1;3* used *in-situ* hybridisation to detect mRNA and *SOLtu;Pht1;3* promoter-GUS transgenic potato plants to determine localisation to the peri-arbuscular membrane of roots again colonised by *G. intraradices* (Rausch *et al.*, 2001). RT-PCR has been used to show expression of *ORYsa;Pht1;11* in mycorrhizal roots of rice, with expression positively correlated to % colonisation by *G. intraradices* (Paszkowski *et al.*, 2002). These results support the model of AM fungi supplying Pi to the plant via the arbuscule/peri-arbuscular membrane. Of the P transporters associated with P acquisition via AM fungi *LYCes;Pht1;1* had displayed expression in other plant tissue besides colonised cells, but the remaining three P transporters (*MEDtr;Pht1;4*, *ORYsa;Pht1;11* and *SOLtu;Pht1;3*) were only expressed in mycorrhizal roots. Further research has revealed low levels of expression of *SOLtu;Pht1;3* in non-mycorrhizal roots and shoots of potato, while *MEDtr;Pht1;4* is only expressed in mycorrhizal roots (Karandashov *et al.*, 2003). The low levels of expression does not negate the possibility that the main role of these P transporters is in acquiring Pi from AM fungi

While the mycorrhiza-specific or mycorrhizal-related P transporters appear likely to be involved with acquisition of Pi delivered to root cortical cells via AM fungi, the colonisation of plants can also be associated with down-regulation of other P transporters, similar to the down-regulation observed when plants are grown in high Pi conditions (see above). Research on *Medicago truncatula* (Liu *et al.*, 1998b) investigated the expression of the plant P transporters (*MEDtr;Pht1;1* and *MEDtr;Pht1;2*) which had been identified as being expressed in roots colonised by mycorrhizal fungi. There was an increase in transcription and protein levels in epidermal and root hair cells during P starvation, which was opposite to the down regulation of both P transporters during the development of symbiosis with AM fungi (Liu *et al.*; 1998b). This suggests that the plant may not require *MEDtr;Pht1;1* and *MEDtr;Pht1;2* when it is able to obtain Pi from the mycorrhizal fungi. The role of *MEDtr;Pht1;1* is associated with acquiring Pi directly from the soil. The down regulation of root P transporters not involved with Pi acquisition via AM fungi is not uniform across AM fungi. As demonstrated by Burleigh (2001) with expression studies on *MEDtr;Pht1;2* the reduced expression of *MEDtr;Pht1;2* was observed in northern blots and ranged from 60 % reduction to no effect when colonised by *Glomus mosseae* and *Gigaspora rosea* respectively. Colonisation of *Medicago* by other AM fungi produced varying degrees of reduced expression (Burleigh, 2001). Variation in the down-regulation of *MEDtr;Pht1;2* needs to be further examined in order to

determine if the various AM fungi are providing an alternative supply of P and whether the amount of P supplied by the AM fungi is sufficient for the plant to rely solely on that source. Research by Smith *et al.* (2003b & 2004) indicates that P acquired by *Medicago* colonised by *G. intraradices* and *G. caledonium* was provided only via the AM fungi. With 100% of the acquired P being supplied by the AM fungi it would be expected that the *Medicago* plant would not need to expend resources to acquire P directly from the soil, consequently down-regulating the expression of *MEDtr;Pht1;1* and *1;2*. However, experiments to investigate concurrently P uptake via the AM fungi and expression of relevant P transporter genes need to be researched in the future.

Barley has not demonstrated improved P concentration or biomass when colonised by AM fungi, thus suggesting that AM fungi colonisation is not beneficial. The research by Smith *et al.* (2003b & 2004) and Ryan and Angus (2003) indicates that the benefits of AM fungi colonisation should not be dismissed in cereal crops that have shown parasitisation or no plant beneficial improvements. The alternative P pathway via AM fungi may prove to be highly beneficial to cereal crops grown in P deficient soils.

2.3 Aims of the thesis

Research to date highlights the complex nature of the plant-mycorrhizal fungus interaction in Pi acquisition. The process of acquiring Pi depends on the plant species and cultivar, AM fungi colonising the roots and growth conditions. The research covered by this thesis started in March 2000 and investigated the variation in Pi acquisition and colonisation between barley cultivars with three mycorrhizal fungi. P transporters associated with acquiring Pi delivered by the AM fungi in rice, barley, wheat and maize were identified. The colonisation by AM fungi regulating other plant P transporters in barley was also studied. The results from this research adds to the knowledge of Pi acquisition and regulation of P transporters in cereal crops that form symbiotic relationships with AM fungi. Regardless of the complex process of Pi acquisition, the interaction and benefits of mycorrhizal fungi in Pi acquisition should not be ignored if successful advances in Pi acquisition are to be made.

3 Materials and Methods

3.1 Soil conditions and plant propagation

3.1.1 Soil, soil mixes and phosphorus amendments

Two soil types, Millmerran and Ashland, were used for this research. Both contained low levels of plant-available P.

Soil from Millmerran, Queensland, Australia ~ 28°S 151°E (collected from Gaythorne Farm, where no fertilisers had been used) has a pH of 8.5 (H_2O) and bicarbonate extractable phosphorus (P) content of 10 mg kg^{-1} (Colwell, 1963). This soil was used to culture *Glomus intraradices* and *Glomus* sp. WFVAM23 (formerly called *Glomus versiforme*) because both these fungi require neutral to alkaline pH soil for successful colonisation. The Millmerran soil was also used in experiments to investigate P efficiency of different barley cultivars and effects of mycorrhizal colonisation on their growth (Section 3.3).

Soil from Ashland, Withcott, Queensland, Australia ~ 28°S 153°E was collected from uncultivated land on a farm on Ashland Drive. It has a pH of 5.6 (H_20) and bicarbonate extractable P content of 3 mg kg^{-1} (Colwell, 1963) and was used to culture *Scutellospora calospora,* as successful colonisation by this fungus occurs in low pH soils (Dickson *et al.*, 1999).

The analysis of the soils was carried out by Incitec Ltd (Morningside, Queensland, Australia) using methods listed in Appendix 2. Concentrations of organic carbon, nitrate nitrogen, potassium, calcium, magnesium, sodium, chloride, copper, zinc, manganese, iron, boron and sulphur, and electrical conductivity are listed in Appendix 2.

Washed fine quartz sand, supplied by Stone Merchants (Dara, Queensland), was mixed with the soil in the ratio of 1 part soil to 9 parts sand. The soil:sand mix was then sterilised by autoclaving at 121 °C for 90 m twice with an interval of 72 h between autoclavings. Non-draining pots were filled with 2 kg of the soil-sand mix. 200g of soil/root inoculum (Section 3.3) were included in mycorrhizal pots. Pots containing soil from Millmerran had an bicarbonate extractable P content of 1 mg P kg^{-1} soil:sand mix while soil from Ashland had a P content of 0.3 mg P kg^{-1} soil:sand mix. Phosphate in the form of $CaH_4(PO_4)_2.H_2O$ was added to pots following sterilisation to increase the levels of P available to the plants. The $CaH_4(PO_4)_2.H_2O$ was added as a dry fine powder to dry soil:sand and mixed for 10mins. Low P and mycorrhizal pots had 81.3 mg $CaH_4(PO_4)_2.H_2O$ kg^{-1} soil:sand added to give a final P

concentration of 20 mg P kg^{-1} soil:sand. High P pots had 406.5 mg $CaH_4(PO_4)_2.H_2O$ kg^{-1} soil:sand added to give a final P concentration of 100 mg P kg^{-1} soil:sand.

3.1.2 Plants, plant propagation, growth conditions and harvesting

Table 3.1 lists the plant species that were used in the work described in this thesis, together with cultivars and origins.

Table 3.1. Plant species and cultivars used in the work described in this thesis

Plant Species	Cultivar	Source of material
Barley (*Hordeum vulgare* L.)	Golden Promise	Dr. Minesh Patel – CSIRO Plant Industry, Brisbane, Queensland
	Arapiles	Dr. Yongguan Zhu – Adelaide University, Adelaide, South Australia
	Skiff	
	Forrest	
	Franklin	
	Sahara	Prof. Andrew Barr – Adelaide University, Adelaide, South Australia
	Clipper	
Wheat (*Triticum aestivum* L.)	Grebe	Dr. Gangping Zhu - CSIRO Plant Industry, Brisbane, Queensland
Rice (*Oryza sativa* L.)	Jarrah	Yanco Agricultural Institute, New South Wales
Tobacco (*Nicotiana tabacum* L.)	Wisconsin 38	Dr Frank Smith - CSIRO Plant Industry, Brisbane, Queensland
Maize (*Zea mays L.*)	Gold Queen	J.C. & A.T. Searle Pty Ltd, Kilcoy, Queensland
Leek (*Allium porrum* L.)	Vertina	Novartis, Dandonong South, Victoria

Seeds were sterilised by immersion in a bleach solution containing ~5.7% available chlorine for 10 minutes, followed by three washes in sterile H_2O. Seeds were then laid out across the top of a sheet of 3MM paper (30 x30 cm) soaked in 0.5mM $CaSO_4$. The bottom edge of the paper was brought up to the top edge so that the seeds where covered by the paper, allowing the shoots to grow from the open edge and the roots to grow towards the folded edged. The paper and seeds were rolled from one side to the other to form a cylinder and the cylinder was held in place by a rubber band and propped in a beaker (folded edge down) containing 0.5 mM $CaSO_4$. The units were incubated at 4˚C for 24 h, then moved to ~26˚C under growth lights until germination. Seeds usually germinated in five days and were then transplanted into pots.

All plants were grown under conditions conforming to PC2 guidelines (http://www.ogtr.gov.au) in the Controlled Environment Facility at CSIRO, St Lucia, Queensland, Australia. Growth room conditions were a constant temperature of 25°C with a 16 h photoperiod (500 µmol m^2s^{-1} photon flux density) and 65% humidity.

Plants were harvested at times specific to individual experiments and details are given in the relevant chapters. Harvesting involved carefully washing soil away from roots over a 1 mm gauge sieve. Plants were then blotted dry and shoots cut away from roots. Samples were processed to determine fresh and dry weights, concentration of P in samples, extent of mycorrhizal colonisation, and RNA and DNA extractions depending on analysis required, as described in the following sections.

3.2 *Watering*

Pots were watered to weight with distilled water twice per week to maintain soil moisture at 0.1 g g^{-1} dry soil. Pots received 10 mL nutrient solution minus P once per week (Table 3.2).

Table 3.2. Composition of the nutrient solution used in all experiments.

Macro nutrients	**Final concentration**
K_2SO_4	0.4 mM
$MgSO_4.7H_2O$	0.3 mM
$Ca(NO_3)_2$	0.6 mM
Fe EDTA	0.02 mM
$(NH_4)_2SO_4$	0.8 mM
$NaNO_3$	0.4 mM
H_3BO_3 – 2.8 mg L^{-1}	1 mL L^{-1}
$MnCl_2.4H_2O$ – 1.81 mg L^{-1}	1 mL L^{-1}
$ZnSO_4.7H_2O$ – 0.22 mg L^{-1}	1 mL L^{-1}
$CuSO_4.5H_2O$ – 0.08 mg L^{-1}	1 mL L^{-1}
$NaMoO_4.2H_2O$ – 0.025 mg L^{-1}	1 mL L^{-1}

3.3 *Cultures of AM fungi*

Three cultures of AM fungi, were obtained from Prof. S.E. Smith, University of Adelaide, South Australia, Australia.

Glomus sp. WFVAM 23 (formerly called *G. versiforme*). This fungus was obtained as *Glomus versiforme* (Karsten) Berch from Dr P. Bonfante, University of Torino, Italy. It was described as *G. versiforme* in a paper by Gao *et al.* (2001) but it should be noted that the identification of this fungus has been questioned and re-identification and molecular comparison of this fungus with other cultures of *G. versiforme* and *G. intraradices* is currently in progress.

Glomus intraradices Schenck & Smith (DAOM 181602). This fungus was subcultured from an axenic culture on transformed roots obtained from Professor J.A. Fortin, University of Montreal, Canada and subsequently maintained in pot-culture conditions.

Scutellospora calospora (Nicolson & Gerdemann) Walker & Sanders (WUM 12(2)) was originally obtained from Mr Chris Gazey, University of Western Australia, Australia.

Inoculum of the fungi was produced in pot cultures maintained on *Allium porrum* L. cv Vertina (leek) in appropriate soil, section 3.1.1. The pot cultures were grown for at least 6 weeks before the soil, containing root fragments and spores, was used to inoculate experimental plants.

Test plants were transplanted into nurse pots that had been established according to Rosewarne *et al.* (1997).

3.4 *Analysis of mycorrhizal colonisation*

Root samples requiring assessment of mycorrhizal colonisation were cleared and stained by a modification of the method of Phillips and Hayman (1970). Roots were immersed in 10% KOH immediately after harvest and cleared by incubation at 65°C overnight. The roots were then rinsed with water twice and once with 0.1 N HCl, before staining with trypan blue. Trypan blue is a non-vital stain, which stains fungal structures that are both living and dead. The trypan blue stain was prepared as described by Phillips and Hayman (1970) with the omission of phenol. Fungal structures were visualised with bright field microscopy. Assessment of colonisation was done by the line intersect method (McGonigle, 2001) to provide detailed information on the percentage of individual fungal structures present within the roots, as well as total percent colonisation (presence of fungi regardless of structure).

3.5 Plant growth and phosphate analysis

Fresh weights of roots and shoots were recorded at harvest. The roots and shoots were cut into ~4 cm sections and thoroughly mixed before subsampling. Weighed subsamples were then dried at 80°C for ~72 h and dry weights recorded. The dry weights of whole roots or shoots were calculated from the fresh weight: dry weight ratios to correct for weighed subsamples taken for other analyses. Weighed subsamples of the dried material were ashed at 220 °C for ~20 h. Ashed samples were resuspended in 20 mL 0.1N HCl. P concentrations in ashed plant samples were determined by the molybdenum blue method of Watanabe and Olsen (1965), with absorbances read at 650 nm on a GBC 911 UV-Visible Spectrophotometer (GBC Scientific Equipment, Dandenong, Victoria, Australia).

3.6 RNA extraction

3.6.1 Large scale preparations

Subsamples of fresh plant material for extraction of RNA were rapidly frozen in liquid nitrogen immediately after harvest and stored at -80 °C. Total RNA was extracted from plant material through a caesium chloride pad by the method of Chirgwin *et al.* (1979) modified as follows: Plant material that had been frozen was ground to a powder in liquid nitrogen with mortar and pestle. Following centrifugation through the caesium chloride pad the RNA was resuspended in H_2O (treated with 0.1% Diethylpyrocarbonate, DEPC) and immediately precipitated with NaOAc and ethanol to concentrate the RNA. If the RNA pellet was not clear or white, a pheonl:chloroform:isoamlyalcohol extraction was performed and the RNA precipitated again. The precipitated RNA was collected by centrifugation (14000 rpm,15 m, 4 °C) and RNA pellets were air dried on ice before dissolving in H_2O (DEPC) for immediate usage or stored at -80°C in a NaOAc and ethanol precipitation mix.

3.6.2 Small scale preparations

A QIAGEN RNeasy Plant Mini kit #74103, supplied by QIAGEN Pty Ltd, Clifton Hill, Victoria, Australia, was used for small scale RNA preparations. The protocol listed in the handbook was used to extract RNA from samples of ~100 mg plant tissue ground in liquid nitrogen with mortar and pestle. The extraction included the QIAGEN #79254 'on the column' RNase-Free DNase in order to remove any remaining genomic DNA.

3.7 Genomic DNA extraction

Small-scale genomic DNA extractions used the BIO101 FastDNA® Kit (#6540-400) with the FastPrep® Instrument, both supplied by Qbiogene Inc., Carlsbad, California, United States of America. The

FastPrep® System eliminates the need for manual grinding by using a highly energetic mechanical motion with garnet combined with cell lysing reagents. The protocol listed in the handbook was used to extract a maximum of 200 mg of fresh plant tissue with lysing matrix: sphere + garnet + sphere. Genomic DNA was stored at 4˚C.

3.8 Clone analysis

The following section details the techniques used to isolate and manipulate the P transporter genes and corresponding promoters used in the work described in this thesis. Detailed explanations are given in each section.

3.8.1 Vectors

Vectors are generally circular pieces of DNA that are able to utilise the bacterial, yeast, plant or animal cell that it resides in to replicate themselves. Cloning vectors are able to have foreign pieces of DNA inserted into them and maintain their capacity to replicate. This enables a gene of interest to be replicated and studied independently of other genes. The following three vectors were used in the work discussed in this thesis and vector maps and associated components are listed in Appendix 3.

pGemT-easy (#1360) – supplied by Promega Corporation, Annandale, New South Wales, Australia. This plasmid is used to clone PCR products and allows selection of cloned products by transforming *E. coli* strains, followed by growth on LB with ampicillin and blue/white screening. Blue/white screening is achieved by the insertional inactivation of the LacZ α-peptide. The inserted gene of interest disrupts the coding region of the α-peptide, which can then be identified by colour screening on indicator plates. Details of vector and related products can be found in Appendix 3.

pZERO1 (#K2500-01) – supplied by Invitrogen Australia Pty Ltd, Mount Waverly, Victoria, Australia. This plasmid is used to clone PCR products and allows selection of cloned products by transforming *E. coli strains* and growth on LSLB with zeocin selection. Details of vector and related products can be found in Appendix 3.

pWBvec8 – supplied by CSIRO Plant Industry, Canberra, Australian Capital Territory, Australia. This plasmid is used to clone the promoters of the genes of interest so that they control the expression of green fluorescent protein (GFP) or β-glucuronidase (GUS) genes for plant transformation and expression studies. pWBvec8 is transformed into *Agrobacterium* strains and grows in LB with spectinomycin resistance selection. Details of vector and related products can be found in Appendix 3.

3.8.2 Ligation

Ligation reactions involve the joining of any two pieces of DNA. In this research ligations involved the insertion of the gene of interest into a linearised vector, which then created a circularised product that was transformed into bacterial, fungi or plant cells for replication.

All ligations were set up with a 3:1 insert:vector ratio.

Ligations into pGemT-easy followed the protocol supplied by the supplier, with ligations run at 4°C overnight.

Ligations into pZERO1 and pWBvec8 used T4 DNA ligase (#M1801) supplied by Promega Corporation, Annandale, New South Wales, Australia. Ligations followed the supplier's protocol, with ligations run at 4°C overnight.

3.8.3 Transformation of vectors into bacteria.

Transformation is the introduction of DNA into a bacterial, yeast, plant or animal cell. There are two methods for transformation into bacteria and fungi, heat-shock and electroporation. Electroporation was used during the work described in this thesis to introduce cloned genes into bacterial cells. This was achieved by mixing the ligated cloned genes with highly purified *E. coli* cells and subjecting the mix to high electrical pulses, that disrupt the membrane of the *E. coli* cells sufficiently to enable the cloned genes to be introduced. Following electroporation the cells were recovered in 1 mL SOC medium (Appendix 3) for 1 h at 37°C, before being spread onto appropriate antibiotic selection plates (Appendix 3.).

pGemT-easy and pZERO1 vector based plasmids were transformed into *E. coli* DH10B by electroporation in 0.1 cm cuvettes with a Bio-rad Gene Pulser supplied by Bio-rad Laboratories Pty Ltd, North Ryde, New South Wales, Australia. The electroporation conditions outlined in the Bacterial Electrotransformation and Pulse Controller Instruction Manual V2-89 #165-2098 were used. Following recovery *E. coli* DH10B cells were grown at 37°C overnight.

pWBvec8 vector based plasmids were transformed into *Agrobacterium tumefaciens* AGL1 by electroporation in 0.2 cm cuvettes with the Bio-rad Gene Pulser using procedures outlined in the Bacterial Electrotransformation and Pulse Controller Instruction Manual V2-89 #165-2098. *Agrobacterium* cells were grown at 28°C for 36 – 48 h.

3.8.4 Miniprep analysis

Following transformation, individual colonies were selected from plates and used to inoculate 5 mL liquid broth containing the appropriate antibiotic for the vector used. Plasmid DNA was extracted from 1.5 mL of culture by the alkaline lysis method proposed by Birnboim (1983), with variations according Sambrook at al. (1989).

3.8.5 Restriction enzyme digestion and analysis

Restriction enzymes were used to digest the DNA for ligation and clone analysis. Three companies supplied the restriction enzymes used in the work described in this thesis: New England Biolab enzymes supplied by Genesearch Pty Ltd, Arundel, Queensland, Australia; MBI Fermentas enzymes supplied by Progen Industries Ltd, Darra, Queensland, Australia; Promega enzymes supplied by Promega Corporation, Annandale, New South Wales, Australia.

Digests were set up according to manufacturers' instructions and assessed using 1.2% TAE (Appendix 3) gels run at 60 V for approximately 1 h in a submerged horizontal electrophoresis cell (Bio-rad, Regents Park, New South Wales, Australia). Nucleic acid bands were visualised with ethidium bromide and viewed on a UV luminator (excitation at 312 nm; supplied by Sigma, Castle Hill, New South Wales, Australia). The molecular weight markers used were 1kbplus (#10787-018, Gibco Life Technologies), supplied by Invitrogen Life Technologies, Mulgrave, Victoria, Australia.

3.8.6 Transformation of vectors into plants

Rice, barley and tobacco plants were transformed with *Agrobacterium* vectors prepared as described above. The details of the plant transformations are given in the relevant chapters.

3.9 Sequence analysis

ABI PRISM® Big Dye™ Terminator Ready Reaction Cycle Sequencing Kit supplied by Applied Biosystems (Quantum Scientific, Milton, Queensland, Australia) was used to determine the DNA sequences of isolated clones and confirm cloning reactions. Versions 1 – 3 of the sequencing kit updated by Applied Biosystems have been used during the research. Sequencing reactions were set up according to manufacturers recommendations. Purification of the extension products was done with ethanol/sodium acetate precipitation method as outlined in the manufacturers protocols. Sequencing was done by the Australian Genome Research Facility (Brisbane, Queensland, Australia). Sequences

were analysed with programs on the Australian National Genomic Information Service (www.angis.org.au).

3.10 In-situ hybridisation protocol

Barley (*Hordeum vulgare* L. cv Golden Promise), wheat (*Triticum aestivum* cv Grebe), rice (*Oryza sativa* cv Jarrah) and maize (*Zea mays* cv Gold Queen) were grown in mycorrhizal nurse pots (*G. intraradices*, *G.sp. WFVAM23* or *Sc. calospora*) as detailed in chapter 3.1, 3.2 and 3.3. Roots were harvested and immediately cut into 1 cm lengths under fixation buffer (2% formaldehyde and 0.5% glutaraldehyde in 50 mM Na-PIPES buffer pH 7.2). Root segments were incubated in fixation buffer for 2 h at room temperature. Tissue was dehydrated in a graded series of ethanol and ethanol/Histoclear (National Diagnostics, Atlanta, Georgia) and embedded in paraffin wax. Sections (20 µm) of tissue embedded in paraffin wax were sliced using a Biocut 2030 Reichert-Jung (Leica, Australia). Sections were mounted on Superfrost Plus slides (HD Scientific, Brisbane, Australia). Prior to hybridisation, paraffin was removed from sections by immersing the slides in containers with Histoclear, and tissue was rehydrated in a graded series of ethanol and water. Sections were treated with 1 µg mL^{-1} Proteinase K for 30 min at 37°C and 0.25% acetic anhydride in 100 mM ethanolamine buffer pH 8.0 for 10 m at room temperature. Slides were dehydrated again in an ethanol series.

Sense and anti-sense RNA probes were generated by *in vitro* transcription (Promega Riboprobe System, Madison, Wisconsin) incorporating fluorescein-12-UTP (Roche Diagnostics, Basel, Switzerland) as the label. Probes were synthesised from full-length cDNA (sequences in Appendix 4) encoding *HORvu;Pht1;8*, *ORYsa;Pht1;11*, *TRIae;Pht1;myc* and *ZEAma;Pht1;6* and used to screen tissue of appropriate plant species. Probes were hydrolysed to ca. 300 nucleotides by incubating the probe in 60 mM Na_2CO_3, 40 mM $NaCO_3$ (pH 10.2) for 20 m at 60°C followed by the addition of neutralisation buffer (final concentration: 0.2 M NaOAc, 1% acetic acid, pH 6). The probe was precipitated with 1/8 volume LiCl and 3.75 volume ethanol and pelleted by centrifugation at 12000 rpm, 4°C, 20 m. The hydrolysed RNA probe was dissolved in 50 µL H_2O (DEPC) and 1 µL RNasin (Promega, Australia) and mixed with 450 µL hybridisation buffer (50% formamide, 300 mM NaCl, 10 mM Tris-HCl pH 7.5, 1mM EDTA, 5% dextran sulphate, 150 µg mL^{-1} tRNA). Dry sections were hybridised with 1:25 dilution of labelled RNA probe and hybridisation buffer at 47°C overnight. After hybridisation, slides were washed in 2 x SSC (Sambrook *et al.* 1989) for 60 m at 60°C, 1 x SSC for 30 m at 60°C and 0.1 x SSC for 30 m at 60°C. Hybridised probe was detected with anti-fluorescein antibody-alkaline phosphatase (Roche Diagnostics Australia, Castle Hill, New South Wales, Australia) and the substrates nitroblue tetrazolium and 5-bromo-4-chloro-3-indoyl phosphate.

Slides were examined using a Zeiss Axioskop microscope (Carl Zeiss Microscopy, Jena, Germany). Images were collected with an Olympus DP70 Microscope digital camera and supporting computer software (Olympus Australia Pty Ltd, Victoria, Australia).

3.11 Phosphate transporter nomenclature

The Commission for Plant Gene Nomenclature (appointed by the International Society for Plant Molecular Biology) has recently suggested the formal naming of genes (Bucher *et al.*, 2001). Genes are named according to their gene family eg.:

Tomato (*Lycopersicon esculentum*) phosphate transporters confirmed to be in the Pht1 gene family would be named: *LYCes;Pht1;1*, *LYCes;Pht1;2*, *LYCes;Pht1;3* etc. The Pht2 gene family would be named: *LYCes;PHT2;1*, *LYCes;PHT2;2*, *LYCes;PHT2;3* etc.

Appendix 1 contains details of correct names and the common names used in publications and Table 3.3 contains the P transporters most commonly used in this thesis.

Table 3.3: Common names of P transporters and the correct names used in this thesis.

Common name	**Correct name**	**Plant**
PHT1;1, APT2, PHT1, AtPT1	*ARAth;Pht1;1*	*Arabidopsis thaliana*
PHT1;2, APT1, PHT2	*ARAth;Pht1;2*	*Arabidopsis thaliana*
PHT2;1	*ARAth;Pht2;1*	*Aradidopsis thaliana*
HvPT1	*HORvu;Pht1;1*	*Hordeum vulgare*
HvPT2	*HORvu;Pht1;2*	*Hordeum vulgare*
HvPT8	*HORvu;Pht1;8*	*Hordeum vulgare*
LePT1	*LYCes;Pht1;1*	*Lycopersicon esculentum*
MtPT1	*MEDtr;Pht1;1*	*Medicago truncatula*
MtPT2	*MEDtr;Pht1;2*	*Medicago truncatula*
MtPT4	*MEDtr;Pht1;4*	*Medicago truncatula*
OsPT11	*ORYsa;Pht1;11*	*Oryza sativa*
StPT3	*SOLtu;Pht1;3*	*Solanum tuberosum*
TaPTmyc	*TRIae;Pht1;myc*	*Triticum aestivum*
ZmPT6	*ZEAma;Pht1;6*	*Zea mays*

3.12 Statistical analysis

Statistics packages R and Ministat were used for analysis.

R: Copyright 2001, The R Development Core Team Version 1.3.0 (2001-06-22). R is free software and can be distributed under the licence details.

Ministat, FW Smith, CSIRO Plant Industry, Australia.

4 Variation between barley cultivars in P uptake and rate of colonisation.

Introduction

This chapter covers two subjects. The first examines the P efficiency of seven barley cultivars. The second is involved with the rate of colonisation in three barley cultivars by two mycorrhizal fungi. These subjects will be presented separately, all P efficiency sections followed by all rate of colonisation sections.

4.1 Variation between barley cultivars in P uptake and efficiency

The aim of the work described in this chapter was to identify barley cultivars of different P efficiency for use in future experiments. Despite uniform environmental conditions, differences in growth characteristics and nutritional composition of crop species and cultivars have been regularly observed (Lyness, 1936). Plant breeders utilise these genetic differences by crossing cultivars with desired characteristics to produce a single progeny containing both parental characteristics. A characteristic of interest for this research is the ability of a cultivar to maintain productivity when grown on low P soils (agronomic P efficiency). Much research has been done into the variation of P efficiency in cereal crops to determine if there is potential for improvement (Clark, 1991). Scientists define nutrient efficiency differently, depending on the objectives of their research. Many of these definitions are listed in the chapter written by Clark (1991) on mineral nutrient efficiency. I have defined P efficiency (agronomic P efficiency) as the total amount of P accumulated by a plant, so a plant that is highly P efficient is one that accumulates significantly higher amounts of P from low P soils, and low P efficiency is the reverse. The variation of P uptake in several barley cultivars was examined to identify cultivars that may be classified as having high or low P efficiency for the growth environment utilised throughout this research. Having identified cultivars with high or low P efficiency they were then examined to determine if their ability to acquire P interacts with the rate and extent of mycorrhizal colonisation.

Variations in P uptake have been measured in many crops (Clark, 1991). My research focussed on barley. Jensen and Nittler (1971) noticed significant differences in the percentage of necrosis of leaves due to P deficiency in 24 spring barley cultivars. Nielsen and Schjorring (1983) were able to fit a formula to P uptake in barley cultivars that took into account root length (RL), net P influx per unit length of root (I_{max}), affinity of nutrient uptake (K_m) and the minimum concentration of P in solution at which net influx

appears to be zero (c_{min}). This formula, root net influx of P g^{-1} dry matter = $I_{max} \cdot RL \cdot ((c - c_{min})/(c - c_{min} + K_m))$, revealed variations among six barley varieties out of 30 analysed in both field and hydroponic culture. If a variety had high values for RL and I_{max} and low values for K_m and c_{min} then that variety was P efficient – able to accumulate high amounts of P (Nielsen & Schjorring, 1983; Schjorring & Nielsen, 1987). Differences in P uptake (I_{max}) due to diversity in root hair architecture and root exudates between barley cultivars have been reported (Gahoonia & Nielsen, 1997; Gahoonia *et al.*, 2000). Barley cvs Yagan, WI2737, O'Connor and Kaniere were shown to be able to produce higher yields of dry matter when grown at low P (agronomic P efficiency), while cultivars Galleon, Shannon, WI2539 and Skiff are agronomically P inefficient (field studies by R.D. Wheeler pers.comm. cited in Baon *et al.*, 1993a). A different set of barley cultivars used by Zhu *et al.* (2002) were not shown to have any significant difference in the amount of P acquired when grown in low P soils, but when grown in high P soils cultivars Clipper and Skiff were able to acquire larger amounts of P (efficient in high P soil) than cultivars Haruna Nijo, Sahara, Alexis and Galleon (inefficient in high P soil) (Zhu *et al.*, 2002). These differences were also reflected in the specific P uptake (total P (mg) absorbed per g root dry weight), with cultivars Skiff, Clipper and Sloop having higher values for specific P uptake than Sahara (Zhu *et al.*, 2002). The differences in P efficiency reported in cultivar Skiff in low P soil by Wheeler (Baon *et al.*, 1993a) and Zhu *et al.* (2002) may be due to the differences in growth conditions, field versus pots in a glasshouse. Several cultivars examined by Zhu *et al.* (2002 & 2003) were included in my research. The barley cultivars Franklin, Forrest, Clipper, Skiff, Sahara and Arapiles studied in this chapter were chosen from parents of mapping populations used by the Cooperative Research Centre for Molecular Plant Breeding (Adelaide, South Australia, Australia). Cultivar Golden Promise was also included because it is commonly used in transformation studies. This was an important advantage because transgenic plants were to be produced for expression studies (see Chapter 5).

This experiment was designed to determine if there is variation in P efficiency of the chosen barley cultivars under the conditions to be used in later experiments. The results and discussion from this experiment are presented in the following sections.

4.1.1 Materials and Methods: Barley P efficiency

Barley cvs (Arapiles, Clipper, Forrest, Franklin, Golden Promise, Sahara and Skiff) were grown in 1:10 soil:sand mixes using the Millmerran soil (Chapter 3.1.1) with $CaH_4(PO_4)_2$ added to produce total soil P contents of 18, 38, 80 or 118 mg P per pot (P1, P2, P3 and P4 respectively) prepared as outlined in Chapter 3.1.1. The use of sand is to restrict the added P from becoming unavailable by interacting with soil particles. A high proportion of the P added to this low P sorbing soil:sand mix will therefore be available to plants. Following five days germination (Chapter 3.1.2), five plants were sown per pot with three replicate pots per treatment. Each replicate was set out in a block, and pots randomised within the block twice per week. Plants were grown in a glasshouse with natural light for 31 days (August – September 2000). Day length averaged at 11 h 20 m, with day to night temperatures ranging from 23 – 18°C. Pots were watered according to Chapter 3.2. Plants were harvested 32 days after germination.

Fresh and dry weights for shoots and roots, P concentration and specific uptake of P were measured as outlined in Chapter 3.1.2 and 3.5. Specific uptake of P was calculated by Equation 1 (Zhu *et al.*, 2002):

Equation 1: Specific uptake of P = Total P uptake / Root dry weight (mg P g^{-1} root dry weight)

The proportion of acquired P allocated to shoots was calculated by Equation 2:

Equation 2: Percent P allocated to shoots = Amount P in shoot / Total amount P in plant * 100 (%)

The Ministat and R statistics packages were used for analyses of the root/shoot dry weights, P concentration in roots, shoots and whole plants, ratio of total P in shoots to roots and specific uptake of P utilised (Chapter 3.11). The l.s.d. at P = 0.05 was used to separate the means of each treatment. The ANOVA for each set of calculations is presented in Appendix 5.

4.1.2 Results: Barley P efficiency

When grown in low P soil (P1) Sahara produced the largest total biomass and Franklin was ranked the lowest with the smallest biomass, at 1/6th of that observed in Sahara (Table 4.1). Sahara was the most efficient in terms of dry weight production at P1. This was is also reflected in the root and shoot biomass at this P level (Table 4.2). Sahara is an agronomically efficient cultivar, being able to produce large biomass at low P levels. This is supported by the root:shoot ratio that indicates Sahara produced a large amount of roots in low P soil (Table 4.1). The total biomass of each cultivar increased when P was increased from P1 to P2 (Table 4.1). Increasing the concentrations of P in the soil altered the rankings of the barley cultivars between P levels. Franklin remained in the lowest ranking while Forrest (followed by Sahara) was able to produce the largest total biomass when P levels in the soil increased. The differences in total biomass between P levels P2 and P4 were not significantly different, though P4 and P2 biomasses were significantly higher than those observed at P1. Clipper was the only cultivar to increase total biomass when P was increased from P2 to P4. The remaining cultivars had a slight decrease in biomass. At P4 Arapiles (followed by Franklin) became the lowest rank cultivar and Forrest (followed by Sahara) the highest.

Table 4.1: Total biomass dry weight (g/plant) and root:shoot ratio of 7 barley cultivars grown in soil:sand culture. Superscript numbers are the ranking of the values in that P level. P1 = 18 mg P/ pot; P2 = 38 mg P/ pot; P4 = 118 mg P/ pot. Total biomass l.s.d. (P=0.05) P means = 0.140, Cultivar x P means = 0.371. Root:Shoot ratio l.s.d. (P=0.05) P means = 0.310, Cultivar x P means = 0.821.

Cultivar	Total biomass (g)			Root:Shoot Ratio		
	P1	P2	P4	P1	P2	P4
Arapiles	0.301 $_5$	0.793 $_5$	0.547 $_7$	1.556 $_5$	1.394 $_5$	0.929 $_7$
Clipper	0.338 $_3$	0.842 $_4$	0.973 $_4$	1.387 $_6$	1.254 $_6$	1.297 $_4$
Forrest	0.419 $_2$	1.596 $_1$	1.356 $_1$	2.294 $_2$	1.719 $_3$	1.346 $_2$
Franklin	0.141 $_7$	0.600 $_7$	0.569 $_6$	1.304 $_7$	0.991 $_7$	0.976 $_6$
Golden Promise	0.315 $_4$	0.750 $_6$	0.746 $_5$	1.825 $_3$	1.409 $_4$	1.218 $_5$
Sahara	0.858 $_1$	1.258 $_3$	1.167 $_2$	3.551 $_1$	2.691 $_1$	2.512 $_1$
Skiff	0.260 $_6$	1.267 $_2$	0.999 $_3$	1.695 $_4$	2.127 $_2$	1.315 $_3$
P means	0.376	1.015	0.908	1.945	1.655	1.370

As the concentration of P in the soil increased the root:shoot ratio decreased (Table 4.1). Cultivars supporting root development in P1 changed to increased shoot development as the soil P increased. Sahara was consistently ranked the highest for root:shoot ratio at all P levels, while Franklin ranked the lowest at P levels P1 and P2. Arapiles ranked the lowest for root:shoot ratio at P4, followed by Franklin.

Increased production of roots (high root:shoot ratio) in response to low P concentrations is important for P efficiency (see Discussion).

The biomass of roots differed significantly between cultivars (Table 4.2). The variation between cultivars reveals that Sahara was ranked the highest (able to produce the most root biomass) and Franklin the lowest at low P. The root biomass did not increase proportionally with increasing P levels (Table 4.2). Increasing the total soil P from P1 to P2 resulted in a significant increase in root biomass. The difference in root biomass between soil P2 and P4 was not significant. Root biomass decreased slightly in all cultivars with the exception of Clipper when P levels increased from P2 to P4. Sahara and Forrest were ranked in the top two positions for all P levels and Franklin ranked the lowest at P1 and P2 with Arapiles followed by Franklin at P4.

Sahara was ranked the highest for shoot biomass at P1, though the ranking decreased with increasing soil P (Table 4.2). Forrest became the highest ranking at P2 and P4. Franklin maintained the lowest or second lowest ranking regardless of P level. Shoot biomass increased with increasing P levels from P1 to P2, but similar increases were not observed when P was increased from P2 to P4. Analysis of the shoot biomass revealed a cultivar by P interaction, in which each of the cultivars reacted differently when soil P levels were increased (Table 4.2). Forrest was able to increase shoot biomass with increasing soil P from P1 to P2 significantly more than the other cultivars. Forrest was therefore the most responsive to P application.

Table 4.2: Root and shoot dry weight (g/plant) of 7 barley cultivars grown in soil:sand culture. Superscript numbers are the ranking of the values in that P level. P1 = 18 mg P/ pot; P2 = 38 mg P/ pot; P4 = 118 mg P/ pot. Root biomass l.s.d. (P=0.05) P means = 0.121, Cultivar x P means = 0.319. Shoot biomass l.s.d. (P=0.05) P means = 0.032, Cultivar x P means = 0.085.

Cultivar	**Root biomass (g)**			**Shoot biomass (g)**		
	P1	**P2**	**P4**	**P1**	**P2**	**P4**
Arapiles	0.190 $_5$	0.491 $_4$	0.264 $_7$	0.111 $_4$	0.302 $_6$	0.283 $_7$
Clipper	0.195 $_4$	0.469 $_5$	0.554 $_4$	0.143 $_2$	0.373 $_3$	0.419 $_3$
Forrest	0.292 $_2$	1.008 $_1$	0.774 $_2$	0.127 $_3$	0.588 $_1$	0.582 $_1$
Franklin	0.079 $_7$	0.303 $_7$	0.282 $_6$	0.062 $_7$	0.297 $_7$	0.287 $_6$
Golden Promise	0.205 $_3$	0.438 $_6$	0.404 $_5$	0.110 $_5$	0.312 $_5$	0.342 $_4$
Sahara	0.670 $_1$	0.917 $_2$	0.830 $_1$	0.188 $_1$	0.341 $_4$	0.337 $_5$
Skiff	0.164 $_6$	0.872 $_3$	0.578 $_3$	0.096 $_6$	0.395 $_2$	0.421 $_2$
P means	0.256	0.643	0.527	0.120	0.372	0.382

Phosphorus concentrations in roots and shoots varied significantly between cultivars, as did total plant P (Tables 4.3 and 4.4). Forrest and Sahara ranked in the top two positions, accumulating the highest concentrations of P in roots and shoots at all P levels; Franklin and Arapiles had the lowest rankings. The concentration of P increased with increasing P levels for all cultivars, except for Arapiles in which root P concentrations decreased at the highest P level. As seen in the shoot biomass results, there was a cultivar x P interaction arising where cultivars did not increase P concentrations at the same rate with increasing soil P levels in roots, shoots and total plant P uptake. The P concentration in the tissues accumulated from P1 for all cultivars is indicative of P deficiency and P2 and P4 sufficiency of supplied P to all cultivars as recommended by Table 2.1 (Reuter *et al.*, 1997).

Table 4.3: Phosphorus concentrations (mg P g^{-1} DW) in plant tissues of 7 barley cultivars grown in soil:sand culture. P1 = 18 mg P/ pot; P2 = 38 mg P/ pot; P4 = 118 mg P/ pot. Root l.s.d. (P=0.05) Cultivar means = 0.260, P means = 0.170, Cultivar x P means = 0.450. Shoot l.s.d. (P=0.05) Cultivar means = 0.537, P means = 0.351, Cultivar x P means = 0.930.

Cultivar	**Root (mg P g^{-1} DW)**			**Shoot (mg P g^{-1} DW)**		
	P1	**P2**	**P4**	**P1**	**P2**	**P4**
Arapiles	0.368 4	1.004 6	0.889 7	0.252 5	1.483 6	3.223 6
Clipper	0.377 3	1.197 4	1.827 3	0.339 2	1.721 4	4.254 4
Forrest	0.510 2	2.251 1	2.886 1	0.359 1	2.247 2	5.955 2
Franklin	0.259 7	0.754 7	1.102 6	0.139 7	1.227 7	3.142 7
Golden Promise	0.335 5	1.091 5	1.748 5	0.261 4	1.489 5	4.077 5
Sahara	1.026 1	1.688 2	2.615 2	0.339 2	2.248 1	7.266 1
Skiff	0.302 6	1.372 3	1.817 4	0.235 6	1.858 3	4.298 3
P means	0.454	1.337	1.841	0.275	1.754	4.602

The proportion of P allocated to shoots increased with increasing soil P levels (Table 4.4). Cultivar Sahara allocated the highest amount of P to shoots (ranked 1), while Arapiles (ranked 7) allocated the least. The cultivar x phosphate interaction for P concentration in roots, shoots and total plant P and proportion of P allocated to shoots was significant at the 5% level and demonstrated that not all cultivars accumulated P to the same degree. A cultivar x P interaction was found and indicates that not all cultivars allocate P to shoots in a similar manner with increasing soil P levels.

Table 4.4: Total P uptake (mg P g^{-1} DW) and allocation of P to the shoots (%) in 7 barley cultivars grown in soil:sand culture. P1 = 18 mg P/ pot; P2 = 38 mg P/ pot; P4 = 118 mg P/ pot. Total P uptake l.s.d. (P=0.05) Cultivar means = 0.701, P means = 0.459, Cultivar x P means = 1.215. Proportion of P in shoot l.s.d. (P=0.05) Cultivar means = 4.10, P means = 2.68, Cultivar x P means = 7.09.

Cultivar	**Total P uptake (mg P g^{-1} DW)**			**Proportion of P in shoot %**		
	P1	**P2**	**P4**	**P1**	**P2**	**P4**
Arapiles	0.620 4	2.488 6	4.111 7	42.76 4	61.88 1	78.32 1
Clipper	0.716 3	2.918 4	6.080 4	47.56 1	58.96 3	69.95 5
Forrest	0.869 2	4.499 1	8.842 2	41.55 5	49.84 7	67.28 7
Franklin	0.398 7	1.981 7	4.244 6	35.14 6	61.79 2	74.12 2
Golden Promise	0.596 5	2.580 5	5.825 5	44.40 2	57.73 5	69.58 6
Sahara	1.365 1	3.936 2	9.882 1	26.35 7	56.60 6	73.24 3
Skiff	0.537 6	3.230 3	6.115 3	43.78 3	58.02 4	70.69 4
P means	0.729	3.090	6.443	40.22	57.83	71.88

The specific uptake of P increased with increasing P levels for all cultivars and there was no cultivar by phosphate interactions. The rankings for specific P uptake varied between soil P levels (Table 4.5). At P1 Franklin (followed by Arapiles) was ranked the highest and Sahara the lowest. At P2 Arapiles (followed by Franklin) was ranked the highest and Sahara, again, the lowest. At the highest P level, P4, Golden Promise was the highest ranking and Clipper (followed by Sahara) the lowest.

Table 4.5: Specific P uptake in 7 barley cultivars grown in soil:sand culture.
Specific P uptake = Total P in plant (mg) per root dry weight (g). P1 = 18 mg P/ pot; P2 = 38 mg P/ pot; P4 = 118 mg P/ pot. Specific P uptake l.s.d. (P=0.05) Cultivar means = 2.746, P means = 1.798, Cultivar x P means = 4.756.

Cultivar	**Specific P uptake (mg P g^{-1} root DW)**		
	P1	**P2**	**P4**
Arapiles	4.091 $_2$	7.638 $_1$	15.838 $_2$
Clipper	3.864 $_3$	6.287 $_3$	11.393 $_7$
Forrest	3.293 $_5$	4.480 $_6$	13.932 $_4$
Franklin	5.503 $_1$	6.845 $_2$	15.110 $_3$
Golden Promise	3.090 $_6$	5.885 $_4$	16.105 $_1$
Sahara	2.063 $_7$	4.287 $_7$	12.029 $_6$
Skiff	3.432 $_4$	4.490 $_5$	12.283 $_5$
P means	3.619	5.702	13.813

4.1.3 Discussion: Variation between cultivars in P uptake and efficiency

Accumulation of P from low P soil was significantly different between barley cultivars (Table 4.1). This is an important result from this experiment, as a P efficient plant is one that can accumulate higher amounts of P from low P soils (as defined in the Introduction). As the soil P decreases all cultivars increased the proportion of biomass allocated to roots as indicated by increasing root:shoot ratio. This would result in a relative increase in the area of soil accessed. The total biomass decreased with decreasing soil P concentration for all cultivars. In low P soil, Sahara was able to produce double the amount of roots and total biomass than the other cultivars. This suggests that Sahara with a large root system that can access a larger volume of soil is able to deal with P deficiency better than the other cultivars.

Specific P uptake is a measure of the total P accumulated by the plant divided by the root biomass. Franklin had the lowest total plant P concentrations and the lowest root biomass, but the specific P uptake by Franklin was the highest. Conversely Sahara had the highest total plant P concentrations and root biomass, which resulted in the lowest specific P uptake. Franklin will have a higher level of P stress than Sahara and this may be the reason for the relatively higher specific P uptake observed.

Agronomic P efficiency requires the production of large biomass in P deficient conditions. Using this definition and under the growth conditions utilised in this experiment Sahara and Forrest are classified as being P efficient and Franklin and Arapiles as P inefficient. These results confirm those of Zhu *et al.* (2002), who identified barley cv Sahara as P efficient. The P efficient Sahara and P inefficient Franklin cultivars were used in further experiments involving AM fungi colonisation. The use of Sahara and Franklin will identify any differences in colonisation that may be due to the P efficiency of the barley cultivars. Cultivar Golden Promise, which has moderate efficiency, was included as a cultivar that is readily transformed.

In conclusion, barley cvs Sahara and Forrest are P efficient for the growth conditions to be used throughout the experiments reported in this thesis and cultivars Franklin and Arapiles are P inefficient.

4.2 *Rate of colonisation by two mycorrhizal fungi*

Variations in P efficiency of barley have been identified through measurements of the ability of plants to acquire P from the soil directly. As discussed in the literature review an alternative avenue of P acquisition is via mycorrhizal fungi. Just as there is variation among cultivars for P efficiency, the percentage of root length colonised by AM fungi may also vary between cultivars with consequent effects on mycorrhizal P uptake. Baon *et al.* (1993a & b) used the high colonising *G. etunicatum* to look at the effects of colonisation on barley cultivars classified as being high or low P efficient. There was no significant difference in colonisation between the P efficient cultivars Yagan and Galleon and the P inefficient cultivars WI2539 and WI2737 (Baon *et al.*, 1993b). When this experiment was extended to include the P efficient cultivars O'Connor and Kaniere and P inefficient cultivars Shannon and Skiff, significant differences in colonisation with *G. etunicatum* were recorded (8.6% for Kaniere to 28.6% for Shannon) (Baon *et al.*, 1993a). Other variations between cultivars have also been observed. Indigenous mycorrhizal fungi were able to colonise hull-less barley cultivars (1845-10-5, 1853-6-3, 62-25-7-4-36) more than hulled cultivars (DL3, DL70, DL85, Ratna) (Tilak & Murthy, 1987). Conversely colonisation of cultivars Sahara and Clipper with *G. intraradices* was not significantly different (maximum colonisation recorded as 48 % and 49 % respectively), regardless of the variation in P efficiency of these two cultivars (Zhu *et al.*, 2002 & 2003). These observations demonstrate that different cultivars do not always have different degrees of colonisation. Variation may arise as a combination of cultivar and AM fungi as well as inoculum infectivity (see below).

Beside variations between crop species there is also variation between mycorrhizal fungi with respect to colonisation and consequent crop productivity. Inoculum of *Gigaspora margarita* was able to colonise barley (cv Zephyr) grown in field plots to a higher degree than *G. tenuis* (Powell *et al.*, 1980). Varying levels of colonisation have also been observed in barley cultivar Salome colonised by *G. mosseae* (47 %), *G. intraradices* (77 %) and *Gigaspora rosea* (58%) (Vierheilig *et al.*, 2000), and barley (cv Galleon) grown with six mycorrhizal fungi (*G. versiforme* – 1.2%, *G. etunicatum* – 20.5 %, *G. intraradices* – 9.8 %, *G. fasiculatus* – 2.6 %, *G. mosseae* – 0.17 % and G.sp. 'City Beach' – 14.5 %) (Baon *et al.*, 1993b). Field experiments by Clarke and Mosse (1981) identified variation in colonisation of barley cv Royal when inoculated with *G. mosseae* > *G. caledonius* > *G. fasiculatus*. Supplemented P resulted in decreased colonisation of barley by all mycorrhizal fungi (Clarke & Mosse, 1981; Baon *et al.*, 1993b). The extent of colonisation can be dependent upon the AM fungi used and the effectiveness of the inoculum. I utilised mycorrhizal nurse pots (similar to Rosewarne *et al.*, 1997) to ensure colonisation of barley plants studied was rapid and near synchronous.

Mycorrhizal colonisation decreases with increasing P levels in the soil. Colonisation of barley cv Vodka decreased from 97 % to 71 % when soil P levels were increased from 7.9 mg P kg^{-1} to 317.9 mg P kg^{-1} (Plenchette & Morel, 1996). Danish field sites under various fertilisation regimes exhibited reduced colonisation in barley by indigenous fungi with increasing soil P levels (Jensen & Jakobsen, 1980). Barley grown in low P soil with high colonisation had similar shoot P contents to that grown in high P soil with low colonisation (Jensen & Jakobsen, 1980; Baon *et al.*, 1994). These results can be explained in the light of results of Smith *et al.* (2003b & 2004). They showed that in some non-responsive plants (tomato in their experiment) total P uptake into mycorrhizal and non-mycorrhizal plants was the same, even when the mycorrhizal pathway of P uptake made a highly significant contribution.

Temperature can also affect the extent of mycorrhizal colonisation. Inoculum of *G. intraradices* mixed through pots did not colonise barley cv Galleon when grown at 10°C and colonisation at 20°C was higher than colonisation at 15°C (Baon *et al.*, 1994). Spring sown barley cultivars was colonised faster than winter sown barley cultivars grown in fields, reaching a plateau within 15 days and 30 days respectively (Jakobsen & Nielsen, 1983). Differences in colonisation of several field-grown barley cultivars observed by Black and Tinker (1979) over two years of experimentation was attributed to the lower temperature recorded in 1976 compared to 1975. Increased temperature accounted for the increased rate of colonisation measured in barley cv Rupal with *G. mosseae* when two thirteen week experiments were run consecutively from winter into summer (Jakobsen & Andersen, 1982). I have conducted these experiments at ~ 25°C to achieve a high level of colonisation.

This experiment was designed to establish if variation in P efficiency affects mycorrhizal colonisation. The results and discussion from this experiment are discussed in the following sections.

4.2.1 Materials and Methods: Mycorrhizal colonisation of barley cultivars

P1 and P3 pots (details in Section 4.1.1) were prepared for this experiment. 500 g soil:sand was used to prepare *G.sp. WFVAM23* and *G. intraradices* nurse pots for both P1 and P3 soil P levels (Chapter 3.3). Following five days germination (Chapter 3.1.2) barley cvs Franklin, Golden Promise and Sahara were sown, one plant per pot. There were three replicate pots per treatment. Plants were grown in the Controlled Environment Facility at CSIRO, St. Lucia (Chapter 3.1.2) and watered as described in Chapter 3.2. Root samples were collected from harvests at 5, 8, 11, 14, 20 and 32 days after planting (Chapter 3.1.2), and processed for analysis of mycorrhizal colonisation (Chapter 3.4). Statistical analysis of the % colonisation between barley cultivars and P treatments was done with the R statistics package (Chapter 3.11). The l.s.d. at P = 0.05 was used to compare the means of each treatment. The ANOVA is presented in Appendix 5.

4.2.2 Results: Mycorrhizal colonisation of barley cultivars

All barley cultivars were colonised by both G.sp. WFVAM23 and *G. intraradices*, although colonisation was consistently more extensive with G.sp. WFVAM23 (Figure 4.1). As well as hyphal colonisation, both arbuscules and vesicles were observed in roots colonised by *G. intraradices* and G.sp. WFVAM23. There was reduced colonisation of both mycorrhizal fungi when soil P was increased from 18 to 80 mg P per pot for all barley cultivars (Figure 4.2). Increased soil P concentrations reduced colonisation by *G.sp. WFVAM23* significantly more than they did for colonisation by *G. intraradices*.

The percentage colonisation after 32 days was similar in all barley cultivars, but the rate of colonisation during the course of the experiment varied (Figure 4.1). The percentage colonisation by G.sp. WFVAM23 increased with increasing time, reaching a maximum in 11 days when colonising Franklin, but taking 32 days to reach a maximum for Golden Promise and Sahara. Colonisation by *G. intraradices* increased consistently throughout the experiment in all cultivars. A plateau was only reached in Golden Promise after 20 days. There was no clear evidence that colonisation had plateaued in Franklin and Sahara by the end of the experiment.. A difference in P efficiency does not appear to be associated with the final percentage colonisation by either of the mycorrhizal fungi.

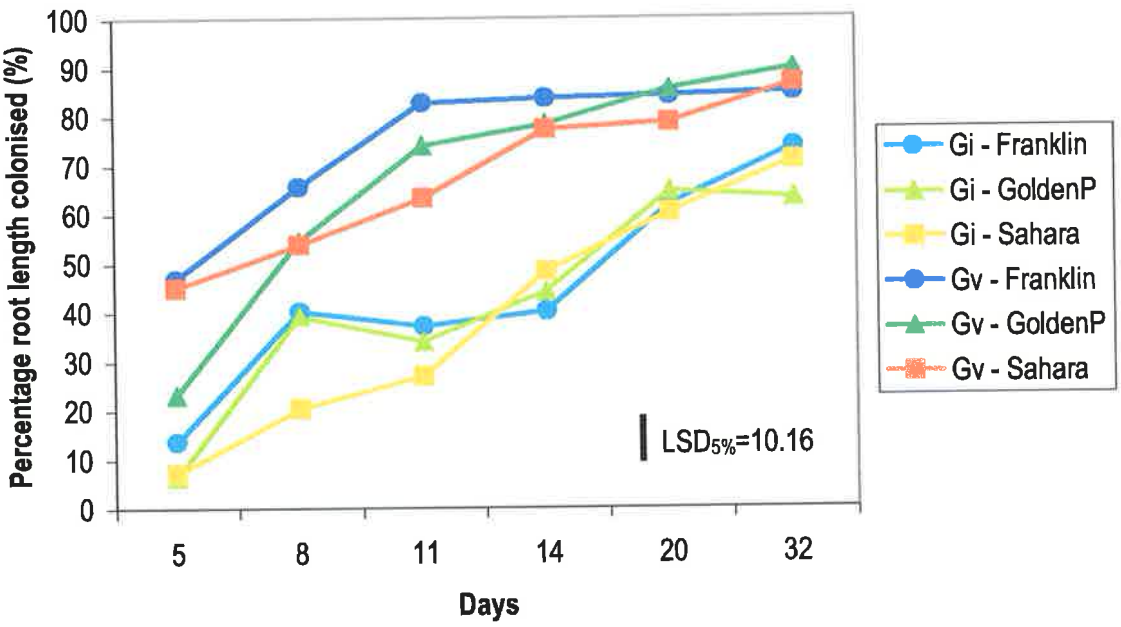


Figure 4.1: Colonisation of barley cvs Franklin, Golden Promise and Sahara by two mycorrhizal fungi grown in low P soil (18 mg P per pot). Gi = *G. intraradices*, Gv = *G.sp. WFVAM23*.

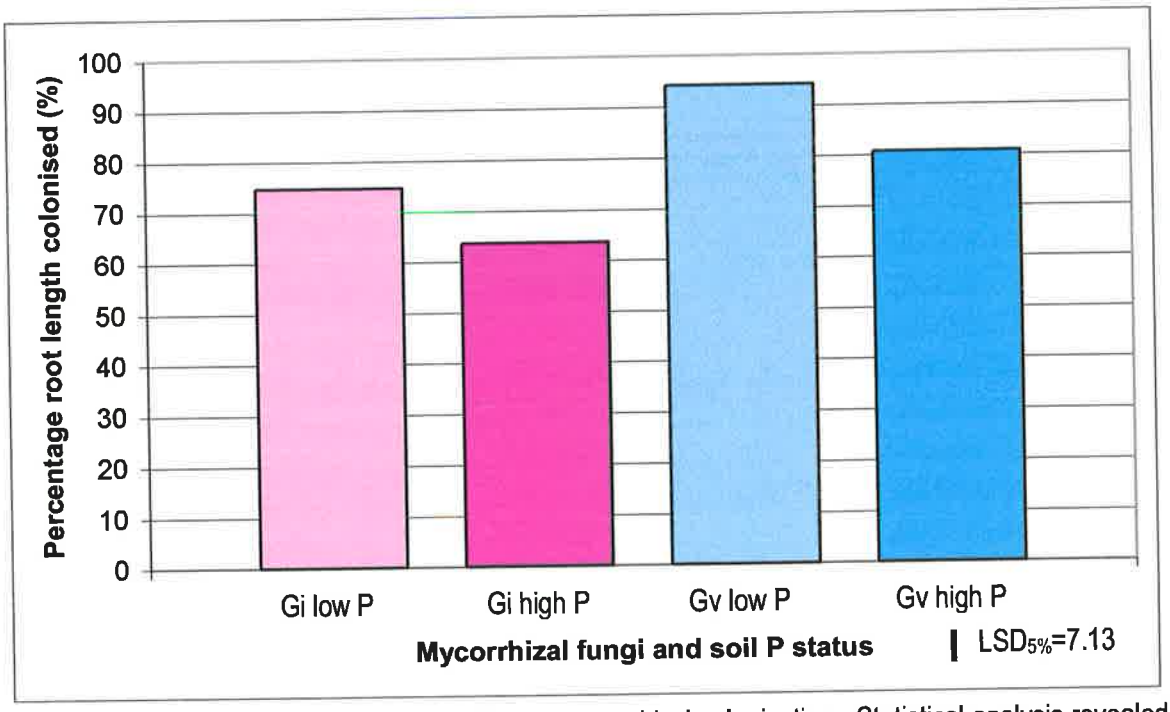


Figure 4.2: The effect of soil P concentration on mycorrhizal colonisation. Statistical analysis revealed a mycorrhizal x phosphate level x day interaction. This histogram demonstrates that the level of colonisation (after 32 days) was higher in barley plants grown in low P soil than those grown in high P soil. *G.sp. WFVAM23* was able to colonise barley plants more than *G. intraradices* regardless of soil P concentrations. Gi = *G. intraradices*, Gv = *G.sp. WFVAM23*, low P = P1 (18 mgP/pot), high P = P3 (80 mgP/pot). The LSD is calculated from the complete set of data for mycorrhiza x phosphate x day interaction.

4.2.3 Discussion: Mycorrhizal colonisation of barley cultivars

All internal mycorrhizal structures, including internal hyphae, arbuscules and vesicles were counted when measuring colonisation in this experiment. Colonisation of cultivars Sahara, Franklin and Golden Promise reached a maximum for both AM fungi after 32 days at values >60% (Figure 4.1). This high level of colonisation was expected in plants grown in nurse pots, in which a high inoculum potential develops, leading to rapid earlier colonisation (between 5 and 45 % in this experiment within five days post planting) (Figure 4.1). Such high levels of colonisation after 32 days of growth have also been recorded when barley cultivar Vodka was grown in *G. intraradices* inoculated pots for 80 days (Plenchette & Morel, 1996). When Delp *et al.* (2000) used *G. intraradices* nurse pots to colonise barley cv Galleon, 55% of roots were colonised 10 days after transplanting, consistent with the rate of colonisation observed in my experiment (Figure 4.1). Nurse pots have the advantage of providing an active mycorrhizal network that can readily colonise plants transplanted into the network. An active mycorrhizal network was also evident with the fast colonisation of field grown spring barley (45 % colonisation after 15 day growth) (Jakobsen & Nielsen, 1983). Vierheilig *et al.* (2000) also observed rapid colonisation when nurse pots of *G. mosseae*, *G. intraradices* and *Gigaspora rosea* colonised barley cv Salome 47 %, 77%, and 58 % respectively, within 12 days (Vierheilig *et al.*, 2000). The significant differences in colonisation between AM fungi observed by Vierheilig *et al.* (2000), Powell *et al.* (1980), Baon *et al.* (1993b) and Clarke and Mosse (1981), were also observed between G.sp. WFVAM23 and *G. intraradices* used in this research. This and the cited research demonstrate that barley can be colonised by AM fungi to a higher level than what has previously been thought. G.sp. WFVAM23 was able to colonise all three barley cultivars tested more extensively than *G. intraradices*.

Soil P concentrations are also important in the symbiotic relationship. The level of P in the soil affected the colonisation by G.sp. WFVAM23 more than *G. intraradices* (Figure 4.2). Reduced colonisation of barley cv Ark Royal as a result of added P was reported for all three mycorrhizal fungi assessed by Clarke and Mosse (1981), *G. intraradices* recorded by Plenchette and Morel (1996) and indigenous fungi reported by Jensen and Jakobsen (1980). While these experiments were not concerned with the P transporters involved with P acquisition, the internal P concentrations may regulate the transcription of genes encoding P transporters as well as colonisation. The level of colonisation is inversely proportional to soil P levels, with shoot P content being similar when plants are grown in high P soils with little mycorrhizal colonisation or in low P soils and highly colonised by mycorrhizal fungi (Jensen & Jakobsen, 1980). Reduced colonisation with increasing soil P concentration was also observed by Baon *et al.* (1993a) for several barley cultivars colonised by *G. etunicatum*, only colonisation in cultivar Kaniere remained unaffected by the increasing soil P level.

The rate of colonisation varied with barley cultivar and mycorrhizal fungi species in this research (Figure 4.1). Variations in the rate of colonisation between AM fungal species was reported by Powell *et al.* (1980) for barley cv Zephyr which was colonised faster by *G. tenuis* than by *Gigaspora margarita*. The colonisation of cultivar Ark Royal varied over time and resulted in different degrees of colonisation depending on AM fungi, with higher colonisation levels achieved by *G. mosseae* than *G. caledonius* and *G. fasciculatus* (Clarke & Mosse, 1981). Regardless of the rate of colonisation in my experiment, all cultivars reached a similar level of colonisation after 32 days independent of AM fungus. There was also no specific pattern for the rate of colonisation with either mycorrhizal fungi that may have been linked to the P efficiency of the cultivar or the root growth. The hypothesis that a cultivar that can effectively acquire P may not benefit from mycorrhizal colonisation and would have a lower level of colonisation is not supported by these results. This was also demonstrated by Zhu *et al.* (2003) and Baon *et al.* (1993b) who showed that there were no differences in colonisation between the P efficient cultivars Sahara, Yagan and Galleon and the P inefficient cultivars Clipper, WI2539 and WI2737. The differences in colonisation observed by Baon *et al.* (1993b) in the P efficient cultivar Kaniere and the P inefficient cultivar Shannon may be specific to the AM fungi or root growth pattern. Relative rates of growth of roots and AM fungi determine the final outcome in terms of percentage root length colonised (Smith & Walker, 1981; Smith *et al.*, 1992).

In conclusion, there was no difference in colonisation between barley cultivars of different P efficiencies or root growth (Sahara, Franklin and Golden Promise). *G.sp. WFVAM23* was able to colonise all cultivars more than *G. intraradices*. With the use of *G. intraradices* and *G.sp. WFVAM23* nurse pots, barley cvs Sahara, Franklin and Golden Promise reach maximum colonisation after 32 days growth. Maximum colonisation ensures the establishment of an active symbiotic relationship, a pre-requisite for the study of P transport in mycorrhizal barley in this thesis.

5 Barley P transporters, wheat mycorrhizal P transporter and maize mycorrhizal P transporter

5.1 Introduction

Mycorrhizal plants are able to acquire P via two pathways (Chapter 2.2). Direct acquisition via the root requires P transporters to be expressed in root epidermal cells, while acquisition via the mycorrhizal fungi requires plant P transporters to be expressed in root cortical cells containing mycorrhizal structures. At the commencement of this research the P transporter *LYCes;Pht1;1* had been reported to be expressed in cortical cells containing arbuscules (presumably at the peri-arbuscular membrane), thereby facilitating P uptake from the mycorrhizal fungi (Rosewarne *et al.*, 1999). This expression pattern was visualised by *in-situ* hybridisation. Another three P transporters involved with P acquisition from mycorrhizal fungi have subsequently been identified by reverse-transcriptase PCR (RT-PCR), by using transgenic plants expressing reporter genes (Rausch *et al.*, 2001), by real-time RT-PCR (Paszkowski *et al.*, 2002), or immuno-localisation (Harrison *et al.*, 2002). These results need to be evaluated in the context of the methods used.

- RT-PCR assesses the expression of a gene, by using gene-specific primers to amplify cDNA from an RNA template. Real-time RT-PCR is a refinement of RT-PCR that provides quantitative information on the initial levels of RNA transcripts in the tissue extract. Real-time RT-PCR was used by Paszkowski *et al.* (2002) to demonstrate the expression of *ORYsa;Pht1;11* in rice roots colonised by *Glomus intraradices*. No expression was detected in non-mycorrhizal roots or roots infected only with root pathogens *Rhizoctonia solani* or *Fusarium moniliforme*.
- Reporter genes such as GUS (β-glucuronidase,(Jefferson, 1987)) and GFP (green fluorescent protein, (Carlson *et al.*, 2001)) are useful in promoter analysis as the expression site of the gene of interest can be visualised in transgenic plants. GUS activity can be visualised in a histochemical assay and GFP can be visualised in living tissue by fluorescence microscopy. GFP has the advantage over GUS in that visualisation is non-destructive. Potato transformed with the promoter of *SORtu;Pht1;3* fused to GUS demonstrated expression of this gene in arbuscule-containing cells of potato colonised with *G. intraradices* (Rausch *et al.*, 2001).
- *In-situ* hybridisation is the annealing of an RNA probe to complementary RNA sequence in fixed tissue, followed by the visualisation of the location of the probe via radioactivity or immunocytochemistry where the gene transcripts accumulate. Rosewarne *et al.* (1999) localised the expression of *LYCes;Pht1;1* with an RNA probe that identified where the gene is transcribed (see above).

- Immuno-localisation is the annealing of a specific antibody to a particular protein in fixed tissue, followed by the visualisation of the location of the probe via radioactivity or immunocytochemistry. Harrison *et al.* (2002) utilised an antibody probe to MEDtr;Pht1;4 that recognised where the gene product was localised. The immuno-localisation of MEDtr;Pht1;4 was to the arbuscules formed by *G. versiforme* in *Medicago*.

The success of RT-PCR, real-time RT-PCR, *in-situ* hybridisation and immuno-localisation is in the specificity of the primers, probes and antibodies to the gene of interest and ensuring that no cross-reaction occurs. RT-PCR, real-time RT-PCR, reporter genes and *in-situ* hybridisation have been used to determine the expression patterns of eight barley P transporters available to my research, with particular emphasis on identifying expression of a barley P transporter at the peri-arbuscular membrane and the effect of mycorrhizal colonisation on expression patterns.

An initial assessment of barley P transporters *HORvu;Pht1;1* and *1;2* by Smith *et al.* (1999) revealed that both genes were down regulated when barley plants were grown in high P conditions. Constructs containing the promoters for both genes linked to the GFP reporter gene were made by Schünmann *et al.* (2003). I was able to utilise these constructs to produce transgenic barley plants in order to assess the expression of these two genes when plants were colonised by mycorrhizal fungi. The expression pattern of all eight barley P transporters was then assessed with RT-PCR in mycorrhizal and non-mycorrhizal roots. Real-time RT-PCR was used to quantitate the level of expression of three of the barley P transporters (*HORvu;Pht1;1*, *1;2* and *1;8*). *In-situ* hybridisation was used to localise the expression of one barley P transporter (*HORvu;Pht1;8*) in mycorrhizal roots.

Having identified a barley P transporter associated with P acquisition from mycorrhizal fungi, the sequence of this gene was used to identify the homologous genes in wheat and maize. Greater than 98% of the publicly available proteins (not only P transporters, all proteins) of maize, wheat, and barley were found to have significant homology in the draft sequence assembly and more than 95% of translated cDNAs were found in the rice draft gene predictions (Goff *et al.*, 2002). Taking advantage of the high degree of synteny between barley and wheat, primers from the barley sequence were used to amplify the homologous gene to *HORvu;Pht1;8* from wheat cDNA by PCR (*TRIae;Pht1;myc*). Similar methods with maize did not yield a result. The homologous maize gene (*ZEAma;Pht1;6*) was identified from a family of P transporter genes cloned by Zhao *et al.* (1999) when the phylogenetic tree of all identified P transporters was constructed. Utilising the sequence information from Zhao *et al.* (1999) the *ZEAma;Pht1;6* was cloned for use in *in-situ* hybridisation experiments. Both the *TRIae;Pht1;myc* and *ZEAma;Pht1;6* clones were used in *in-situ* hybridisation studies to identify the cell types in which these genes are expressed.

Barley and wheat roots colonised by *Sc. calospora* (along with *G. intraradices* and G.sp. WFVAM23) were also included in *in-situ* hybridisation studies to determine if the arbusculate coils, common to *Sc. calospora*, are involved with supplying P to plants. The inclusion of *Sc. calospora* provides an alternative site of P supply via different AM fungi structures that may be part of the indigenous soil microbial community.

This chapter identifies the expression pattern of barley P transporters (*HORvu;Pht1;1* to *1;8*) in roots grown in soil containing low P, high P and mycorrhizal conditions. The isolation of three cereal crop P transporters presumed to be involved with P acquisition via mycorrhizal fungi is outlined and their localisation is explored.

5.2 *Methods and Materials*

5.2.1 Production of transgenic barley Plants

5.2.1.1 Agrobacterium-mediated transformation of barley

Spring barley (*Hordeum vulgare* L. cv Golden Promise) was grown in a plant growth room at 12°C with 16 h/8 h light/ dark periods. Immature embryos were isolated from developing grains to provide material for transformation studies (Patel *et al.*, 2000). Immature embryos, 1.5 – 2.5 mm in length, were isolated from developing barley caryopses. The embryo explants were transformed by co-cultivation with *A. tumefaciens* strain AGL1 harbouring a binary expression plasmid, (Tingay *et al.*, 1997). The binary expression plasmids also carried a selectable marker gene encoding antibiotic resistance to hygromycin. Hygromycin was used for selection of *Agrobacterium*-infected embryos and resistant embryos subsequently used to generate embryonic calli. Plantlets were regenerated from hygromycin-resistant calli after selection for up to 8 weeks, transferred to soil and grown to maturity. Several plantlets regenerated from a single transformed embryonic callus (line) are classified as replicates.

5.2.1.2 Plasmids

Plasmids containing the promoter region of barley P transporters, *HORvu;Pht1;1* or *HORvu;Pht1;2*, controlling expression of green fluorescent protein gene (GFP) were provided by Dr P H D Schünmann (CSIRO Plant Industry, Canberra) (Schünmann *et al.*, 2003). These constructs were transformed into *Agrobacterium* (Chapter 3.8.3).

5.2.1.3 Plant Propagation and growth conditions

Transgenic plants were grown in 1:10 soil:sand mixes using the Millmerran soil with $CaH_4(PO_4)_2$ added to produce a total soil P content of 18 (P1) or 118 (P4) mg P per pot (Chapter 3.1.1) or mycorrhizal nurse pots established with *G. versiforme* and *G.sp. WFVAM23* (Chapter 3.3). *Sc. calospora* nurse pots were established with Ashland soil (Chapter 3.3) Plants were grown and watered as outlined in Chapter 3.1.2 and 3.2.

5.2.2 Reporter gene analysis of transgenic barley

Core samples of roots were taken and washed to remove soil prior to visualisation. GFP fluorescence was observed using a Leica MZ6 dissecting microscope with the GFP PLUS fluorescence module (Leica AG, Heerbrugg, Switzerland). A Bio-rad MRC 600 (Bio-rad Laboratories Pty Ltd, New South Wales, Australia) and Leica TCS SP2 Confocal System on an upright Leica DMRXE microscope was used for Laser Scanning Confocal Microscopy (LSCM).

5.2.3 Plant Propagation and growth conditions for RT-PCR, real-time RT-PCR and *in-situ* hybridisation analysis

Barley cultivar Golden Promise, wheat cultivar Grebe and maize cultivar Gold Queen were grown in the same conditions outlined in section 5.2.1.3.

5.2.4 RT-PCR analysis

RNA was extracted from root core samples as described in Chapter 3.6.2. Approximately 5 μg of total RNA was used as a template for first-strand cDNA synthesis, using a Superscript First Strand cDNA Synthesis Kit (Invitrogen, California, United States of America) according to the manufacturer's instructions. cDNA was produced from a pooled sample of the roots of three plants grown in a single pot for each treatment.

One μL of first strand cDNA was then used for PCR using gene-specific primers for each of the barley P transporters listed. Table 5.2.3-1 provides the details of primers and expected RT-PCR products. All primers were tested with barley gDNA, at various $MgCl_2$ concentrations, prior to RT-PCR analysis. PCR was performed with EXPAND High Fidelity polymerase (Roche Diagnostics Australia, Castle Hill, New South Wales, Australia) according to the manufacturer's instructions. Thermal cycling consisted of an initial denaturation at 94°C for 5 m, followed by 10 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s and extension at 72°C for 60 s, and then an additional 20 cycles during which the extension time was increased by 5 s per cycle, followed by a final extension at 72°C for 7 m. RT-PCR reactions were visualised on agarose gels (Chapter 3.8.5).

Table 5.1. Primer sequences, $MgCl_2$ concentration and expected product size for RT-PCR of barley, wheat and maize P transporters. No $MgCl_2$ concentration optimum was found for *HORvu;Pht1;6*. Sequences of the primers are indicated in gene sequences (Appendix 4). The primers for each gene were designed to areas of low homology between the P transporters listed in Appendix 1, to ensure specificity but this was not tested.

Gene	Sense primer: 5' – 3'	Anti-sense primer: 5' – 3'	Expected product size (bp)	$MgCl_2$ conc.
HORvu;Pht1;1	ggcatcgatgcttaggctgg	cgaatacagagcaccatcag	200	2.5 mM
HORvu;Pht1;2	aactggtgtctaagacatgc	aagcattacattgtcacggc	150	2.5 mM
HORvuPhT1;3	gaccaggcacgaaccgccgc	ggcatatacatgagccggcc	200	2.5 mM
HORvuPhT1;5	tgaatcgcgagaaagcaacac	ctagatgagttctaggcactc	210	4 mM
HORvuPhT1;6	ggcgagaacgacgacgag	ctagtatatctgacgtac	80	-
HORvuPhT1;7	gtagatgatcgtcggtgaatac	cgaccttgatagtttcggttgcag	269	2.5 mM
HORvu;Pht1;8	atcaacagggaggacgcg	cctaagtctaatctcgac	230	4 mM
TRIae;Pht1;myc	caccaccttcatcatacc	cctaagtctaatctcgac	500	2.5 mM
ZEAma;Pht1;6	ctcaagaacaaacacaccacgctc	ctgcttactcgatcacgcatgc	465	3 mM

5.2.5 Real-Time RT-PCR analysis

Samples were prepared as above for RT-PCR analysis and a no-RT control for each sample was included. The first strand cDNA synthesis involved two reactions, one with the addition of the RT-polymerase and one without. The no-RT control is included to diagnose the presence of gDNA.

The Taqman Sequence Detection System 7700 (Applied Biosystems, Victoria, Australia) and corresponding computer programs were used for the design of primers (Table 5.2), real-time RT-PCR reaction and detection of product. The SYBR Green PCR Master Mix (#4309155), Micro-Amp 96-well reaction plate (#N801-0560) and Optical Caps (8 caps/strip, #4323032) were supplied by Applied Biosystems. The PCR reactions were set up according to the SYBR Green PCR Master Mix instructions with the template diluted to 1:10 for gene-of-interest reactions and 1:10000 for ribosomal gene reactions. Thermal cycling consisted of an initial denaturation at 95°C for 10 m, followed by 45 cycles of denaturation at 95°C for 15 s and a combined annealing/extension at 60°C for 1m, with a fluorescence reading detected 30 s into the annealing/extension.

Table 5.2: PCR primers used for real-time RT-PCR for *HORvu;Pht1;1*, *1;2* and *1;8*. Sequences of the primers are indicated in gene sequences (Appendix 4.

Gene	Sense primer: 5' – 3'	Anti-sense primer: 5' – 3'
HORvu;Pht1;1	ggagaacgtcggcgatga	aaatgctgcaaggcgaaaggaa
HORvu;Pht1;2	gacgccattgccccaact	caagccgcagaattaacacaga
HORvu;Pht1;8	ttgggtgattccagggttctt	cacatcacacatggaaatgggt

5.2.6 *In-situ* Hybridisation

Roots were prepared and screened as described in Chapter 3.10.

5.2.7 Pht 1 Family Topology.

A computer program available on http://www.cbs.dtu.dk/services/TMHMM-2.0/ was used to determine the topology of HORvu;Pht1;8, TRIae;Pht1;myc and ZEAma;Pht1;6. The amino acid sequence from each P transporter was input into the program for analysis.

5.3 Results

5.3.1 *HORvu;Pht1;1* and *HORvu;Pht1;2* transgenic barley plants.

Barley embryos transformed with *HORvu;Pht1;1* or *HORvu;Pht1;2* promoters linked to GFP were cultivated and five individual plant lines with *HORvu;Pht1;1* and three lines with *HORvu;Pht1;2* constructs were produced. Plantlets were transferred from tissue culture to soil when roots were well developed. GFP fluorescence was examined after 30 days of growth in soil. Barley roots do not display any autofluorescence, therefore any GFP fluorescence seen is due to the introduced promoter-GFP fused genes. All transgenic plant lines expressing the *HORvu;Pht1;1* promoter-GFP displayed expression in root epidermal cells when grown in low P soil. The expression level of *HORvu;Pht1;1* promoter-GFP was reduced in roots grown in high P soil or in mycorrhizal nurse pots, when compared to non-mycorrhizal roots grown in low P soil. A similar reduction in *HORvu;Pht1;2* promoter-GFP expression in high P and mycorrhizal roots was seen in all transgenic lines and expression was localised to root epidermal cells and vascular tissue (results not shown). One transgenic plant line from each transformation was used for more detailed localisation studies using the confocal microscope (Figure 5.1). Mycorrhizal colonisation exceeded 50% root length for all inoculated plant roots. Mycorrhizal colonisation had the same effect of reducing expression levels of *HORvu;Pht1;1* and *HORvu;Pht1;2* as roots grown in the presence of high P levels (118 mg P kg^{-1} soil). Neither *HORvu;Pht1;1* nor *1;2* was expressed in arbuscule-containing cortical cells. Hence, neither of the proteins encoded by these genes appear to be involved in P transport at the peri-arbuscular membrane, and are unlikely to be involved with P acquisition via mycorrhizal fungi. In order to identify a P transporter expressed at the mycorrhizal interface the expression of all identified barley P transporters was examined by RT-PCR as discussed in the following section.

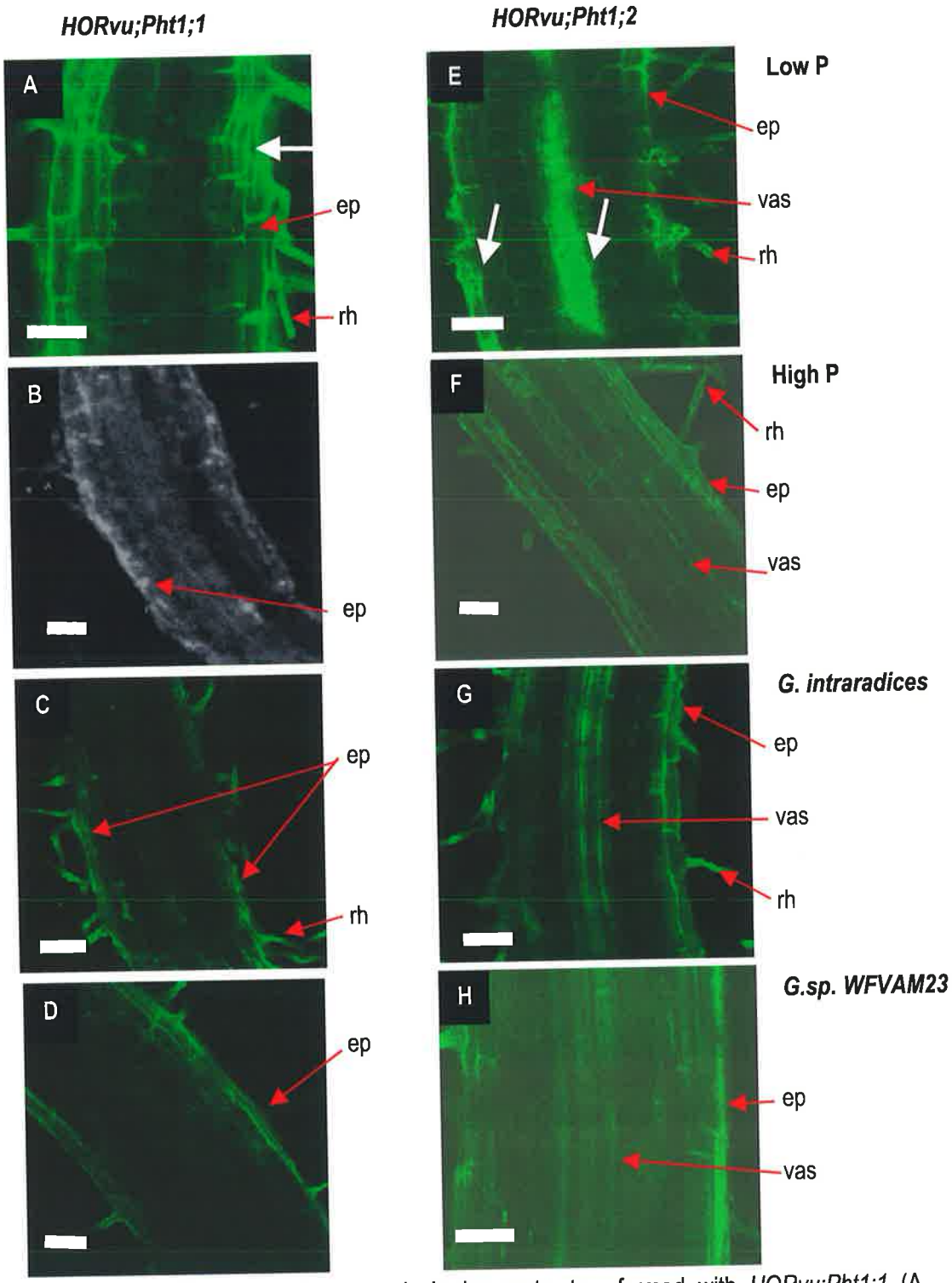


Figure 5.1: GFP images of transgenic barley roots, transformed with *HORvu;Pht1;1* (A – D) or *HORvu;Pht1;2* (E – H) promoters fused to GFP. Transgenic barley plants were grown in low P soil: 20 mg P kg^{-1} soil (A & E), high P soil: 100 mg P kg^{-1} soil (B & F), *G. intraradices* nurse pots (C & G) or *G.sp. WFVAM23* nurse pots (D & H). Fluorescence was brighter in roots grown in low P soil (white arrows) than the other growth conditions. Red arrows identify cell structures, rh = root hairs, ep = epidermis, vas = vascular tissue. Figure B was collect on the Bio-rad MRC 600 Confocal Microscope in black and white; the remaining figures were collected on the Leica TCS SP2 Confocal System in colour. Bars = 84 μM

5.3.2 RT-PCR expression of barley P transporters

Hordeum vulgare cultivar Golden Promise has eight identified genes in the PhT 1 P transporter family. Research by Smith *et al.* (1999) demonstrated via northern analysis that the expression of *HORvu;Pht1;1*, *1;2* and *1;3* genes was reduced when plants were grown in high P hydroponic solutions, compared to low P solutions. Primers specific for each of the 8 barley P transporters were designed by Dr F W Smith (personal communication), except for *HORvuPhT1;3* and *1;4* that display 100% homology throughout the coding region so the primers designed will amplify both genes. These primers were used in RT-PCR analysis of barley roots (cultivar Golden Promise) grown in low P, high P and mycorrhizal nurse pots for > 30 days. All primers excluding those for *HORvuPhT1;6*, successfully amplified products from genomic barley DNA (results not shown), confirming that primers successfully amplified that template. The P concentration and percent mycorrhizal colonisation of the plants were measured for each growth condition (Table 5.3). The concentration of P did not vary in shoots between plants grown in low P soil and mycorrhizal nurse pots, but did increase when grown in high P soils (protocol, Chapter 3.5). In roots the P concentration was slightly elevated in mycorrhizal roots than those grown in low P soil, but not to the extent seen in roots grown in high P soil. Mycorrhizal plants acquired similar P concentrations to those grown in low P soil, suggesting that AM fungi did not increase the supply of P to the barley plant.

Table 5.3: P concentration and extent of colonisation of barley cultivar Golden Promise plants used for real-time-RT-PCR. The same plants were used to measure *HORvu;Pht1;1*, 1;2 and 1;8 expression via real time RT-PCR.

Golden Promise	**P concentration mg P g^{-1} dry weight tissue**		**% colonisation**
	Shoots	**Roots**	
Low P	1.137	0.369	0
High P	3.156	1.197	0
G. intraradices	0.991	0.483	37.5
G.sp. WFVAM23	1.115	0.499	79.5

The results of the RT-PCR are displayed in figure 5.2. No PCR products were amplified in no-RT controls (not shown) indicating that the products amplified were derived from RNA transcripts. Expression of *HORvu;Pht1;1*, *1;2* and *1;3* was reduced considerably when roots were grown in high P soil and slightly reduced when grown in mycorrhizal nurse pots. Barley P transporters *HORvuPhT1;5*, *1;6* and *1;7* were not found to be expressed in root tissue under the conditions used. The expression of *HORvu;Pht1;8* only occurred in roots colonised by mycorrhizal fungi. The RT-PCR protocol restricts the

comparison of expression between samples because it is not sensitive to small variations in starting material, so real-time RT-PCR was used to compare RNA transcript levels of *HORvu;Pht1;1*, *1;2* and *1;8* under different P levels and mycorrhizal colonisation.

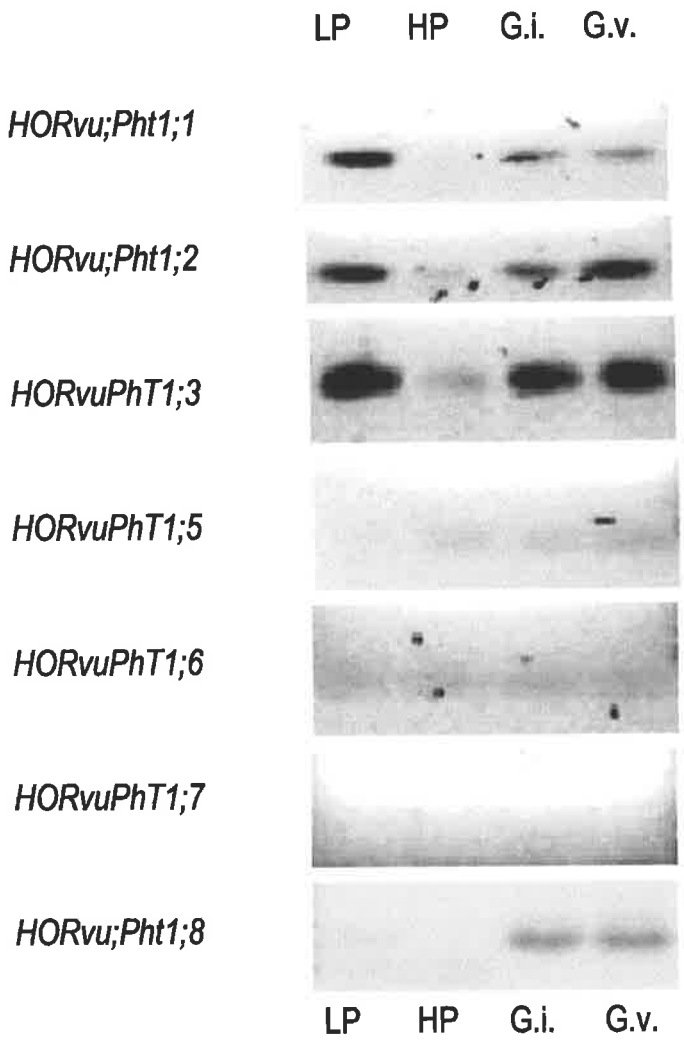


Figure 5.2. RT-PCR analysis of the *Hordeum vulgare Pht1* family of P transporters (cultivar Golden Promise). Expected sizes of products from cDNA are shown in Table 5.2.3-1; all bands amplified are the expected size. LP - Low P soil (18 mg P kg^{-1} soil), HP - high P soil (118 mg P kg^{-1} soil), G.i. - *G. intraradices* nurse pots, G.v. – *G.sp. WFVAM23* nurse pots.

5.3.3 Real-Time RT-PCR of *HORvu;Pht1;1*, *HORvu;Pht1*;2 and *HORvu;Pht1*;8.

Real time RT-PCR is used to quantify the amount of template present in a sample using primers specific to the template. This technique was used to examine the expression patterns of genes encoding barley P transporters *HORvu;Pht1;1*, *1;2* and *1;8*. While exact amounts of template can be calculated, using a standard curve, the most common method of analysis of real-time RT-PCR data is by the comparison of expression between samples relative to one of them. In this experiment I compared the expression of the barley P transporters in barley cultivar Golden Promise when grown in low P soil, high P soil and mycorrhizal nurse pots. I used the expression of each gene in low P soil as the reference value, as demonstrated in figure 5.3. Due to the cost of real-time RT-PCR reactions and insufficient time there are no replicates of the plants used to produce the cDNA. In a real-time RT-PCR reaction duplicates of each reaction are made and these results have been averaged for the presentation of the results in figure 5.3.

The expression of both *HORvu;Pht1;1* and *1;2* was reduced when grown in high P soils and mycorrhizal nurse pots. The reduced expression confirms the results seen in the GFP images (Section 5.3.1) and RT-PCR (Figure 5.2). Colonisation with *G.sp. WFVAM23* resulted in reduced expression levels of *HORvu;Pht1;1* and *1;2* to similar levels to those observed with the supply of high P soil, while *G. intraradices* had a lesser effect on expression levels. The difference in expression levels may reflect the differences in percentage colonisation (Table 5.3.2-1, G.sp WFVAM23 – 79%, *G. intraradices* – 37%). The expression of *HORvu;Pht1;8* was low in non-mycorrhizal roots and very high in mycorrhizal roots, regardless of mycorrhizal fungal species or percent colonisation. This suggests that *HORvu;Pht1;8* may be involved in P acquisition via mycorrhizal fungi. Tissue localisation is discussed in the following section.

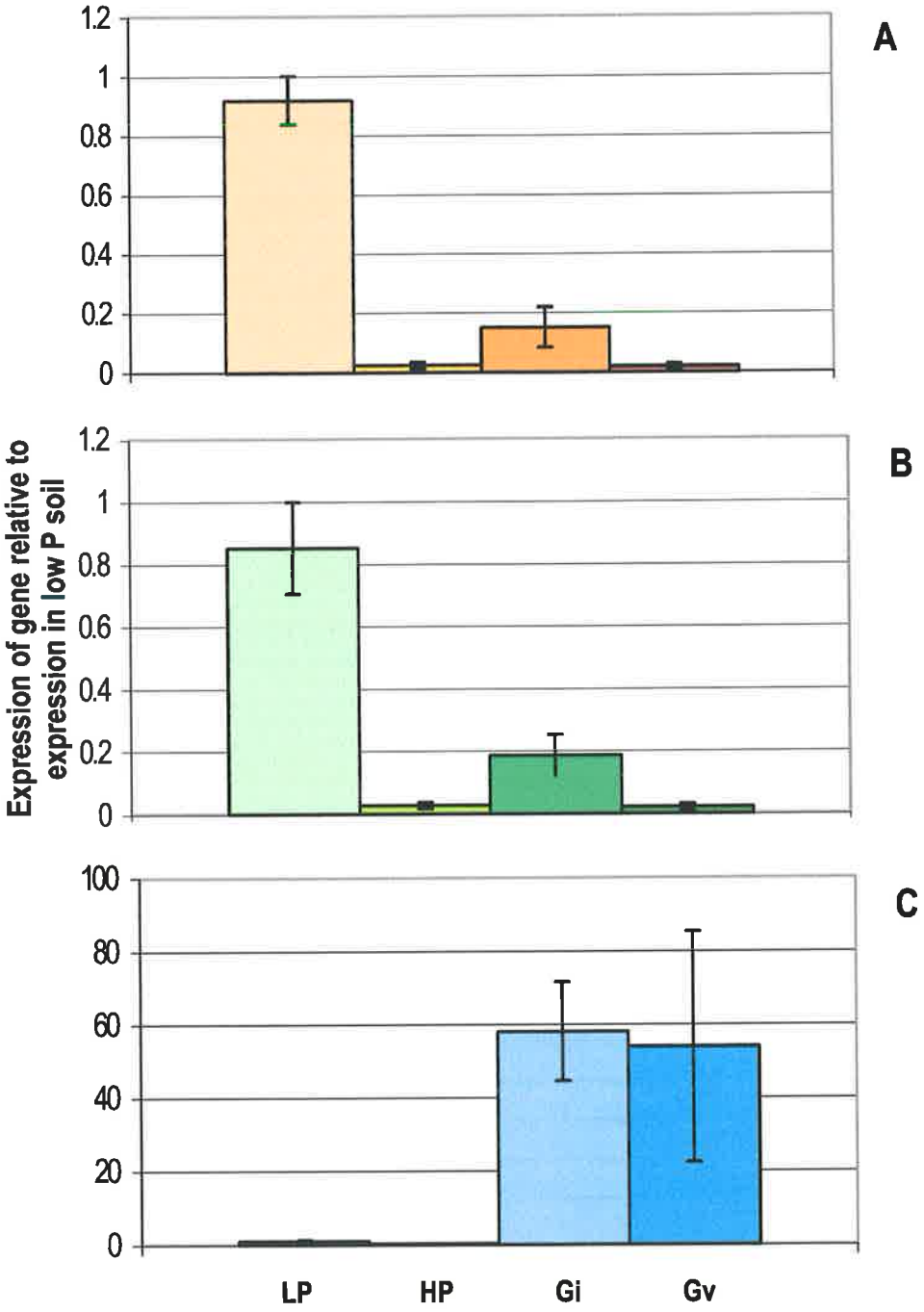


Figure 5.3. Real time RT-PCR results for expression of *HORvu;Pht1;1* (A), *HORvu;Pht1;2* (B) and *HORvu;Pht1;8* (C) in *H. vulgare* cv Golden Promise plants grown in LP - Low P soil (18 mg P kg^{-1} soil), HP - high P soil (118 mg P kg^{-1} soil), Gi - *G. intraradices* nurse pots and Gv – *G.sp. WFVAM23* nurse pots. The expression of each of the genes can only be compared to that gene in each treatment. The expression of different genes within a treatment cannot be compared. The values are means from the duplicate reactions (n = 2) and the bars are confidence intervals.

5.3.4 *In-situ* localisation of *HORvu;Pht1;8*

In-situ hybridisation can be used to localise the expression of a gene (mRNA). In this experiment I used the RNA synthesised from *HORvu;Pht1;8* to localise expression in mycorrhizal barley roots. The anti-sense probe only hybridised to cortical cells containing arbuscules and arbusculate coils (Figure 5.4). Hybridisation to other mycorrhizal or root structures was not observed. The control sense probe showed no hybridisation. From the images it appears that the anti-sense probe is concentrated around the peri-arbuscular membrane. Expression of this gene is likely to occur in the cytoplasm of the cell and transcripts may become concentrated within the highly invaginated membrane around the arbuscule, therefore demonstrating expression closely associated with the arbuscule and arbusculate coils. The probes used did not hybridise to other plant cell tissue, indicating that the probes and experimental design (high temperature hybridisation and high wash stringencies as described in section 3.10) used were specific for the genes being assessed, and giving confidence that the results observed are correct.

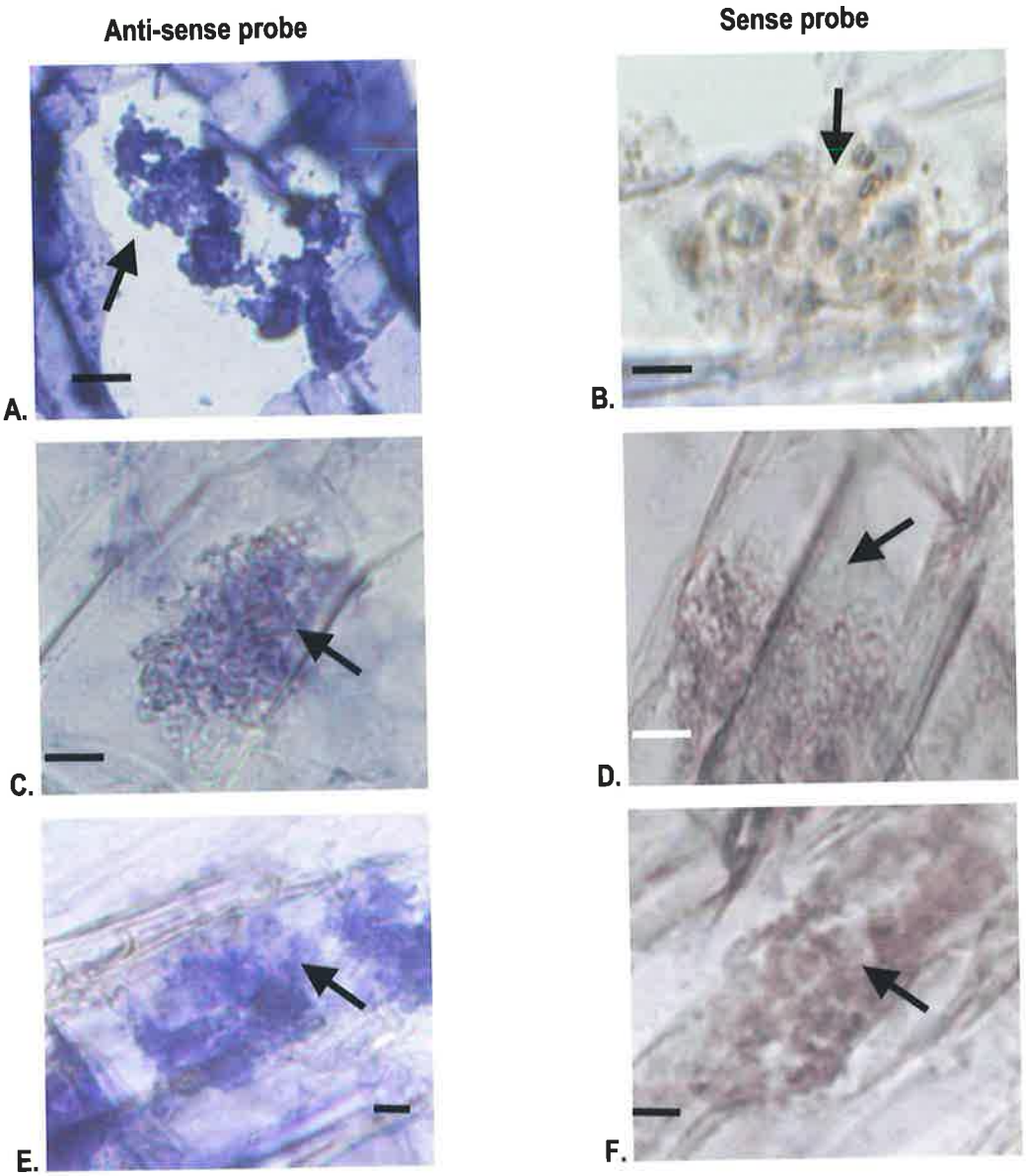


Figure 5.4: Detection of *HORvu;Pht1;8* transcripts by *in-situ* hybridisation on sections of barley roots colonised by *G. intraradices* (A & B), *G.sp. WFVAM23* (C & D) and *Sc. calospora* (E & F). Sections A, C and E have been hybridised with the anti-sense probe, sections B, D and F with the control sense probe. The anti-sense probes hybridised to plant cells containing arbuscules or arbusculate coils (arrowed) and the sense probe showed no hybridisation. Bar = 7.8 µm.

5.3.5 RT-PCR of wheat mycorrhizal P transporter and *In-situ* images

The success in isolating a barley P transporter localised to arbuscule containing cells, and likely to be associated with P acquisition via mycorrhizal fungi, led to the screening of other cereal crops for homologous genes. Barley and wheat have a high level of synteny (as mentioned in the introduction to this chapter) and primers designed to the *HORvu;Pht1;8* gene were used to amplify a homologue from wheat cDNA. This successfully yielded a 1781 bp clone *TRIae;Pht1;myc* (Appendix 4.2.1) with 95% similarity to *HORvu;Pht1;8* at the DNA level and 79% similarity at the amino acid level. An alignment of *TRIae;Pht1;myc* with many full length and partial wheat P transporter sequences on the Genbank database did not yield an identical match. These wheat sequences are listed in Appendix 1. The topology of the wheat gene I have isolated indicates that it is a member of the *PHT1* family of P transporters. Specific primers were designed to *TRIae;Pht1;myc* and used in RT-PCR to determine whether expression primarily occurs in mycorrhizal roots, as was the case for *HORvu;Pht1;8*. The results of this RT-PCR are shown in figure 5.5.

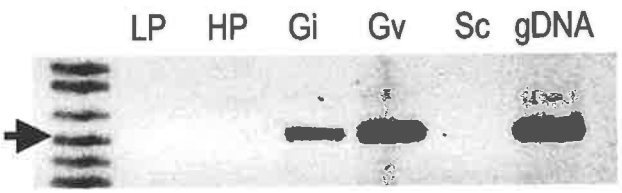


Figure 5.5: RT-PCR analysis of expression of *TRIae;Pht1;myc*. Wheat roots were harvested from 30 day old plants grown in LP - Low P soil (20 mg P kg^{-1} soil), HP - high P soil (100 mg P kg^{-1} soil), Gi - *G. intraradices* nurse pots, Gv – *G.sp.* WFVAM23 nurse pots, Sc – *Sc. calospora* nurse pots and gDNA – genomic DNA. Expected size of product (arrowed) is 500 bp for *TRIae;Pht1;myc* with 1 kb Plus DNA ladder (#10787-018, Gibco Life Technologies, Melbourne, Australia).

TRIae;Pht1;myc was expressed in roots colonised by *G. intraradices* and *G.sp. WFVAM23* (% colonisation > 25%). No measurable expression of the *TRIae;Pht1;myc* gene in LP, HP and *Sc. calospora* samples was observed with RT-PCR. The level of colonisation of wheat roots by *Sc. calospora* was less than 5% and the wheat plants were not as healthy as those plants grown in LP, HP, G.i. or G.v nurse pots. Localisation of *TRIae;Pht1;myc* by *in-situ* hybridisation was carried out to determine if this gene may be involved with P acquisition via mycorrhizal fungi. RNA probes synthesised to *TRIae;Pht1;myc* were used for *in-situ* hybridisation of mycorrhizal wheat genes (Figure 5.6). *TRIae;Pht1;myc* transcripts were localised to wheat cortical cells containing arbuscules or arbusculate coils, in a similar manner to *HORvu;Pht1;8* expression. Despite the apparent lack of

expression of *TRIae;Pht1;myc* in the RT-PCR reactions with *Sc. calospora* colonised roots, *in-situ* hybridisation revealed that transcripts corresponding to this gene were present in cortical cells colonised by this fungal species. No hybridisation was observed with the sections hybridised with the sense probe.

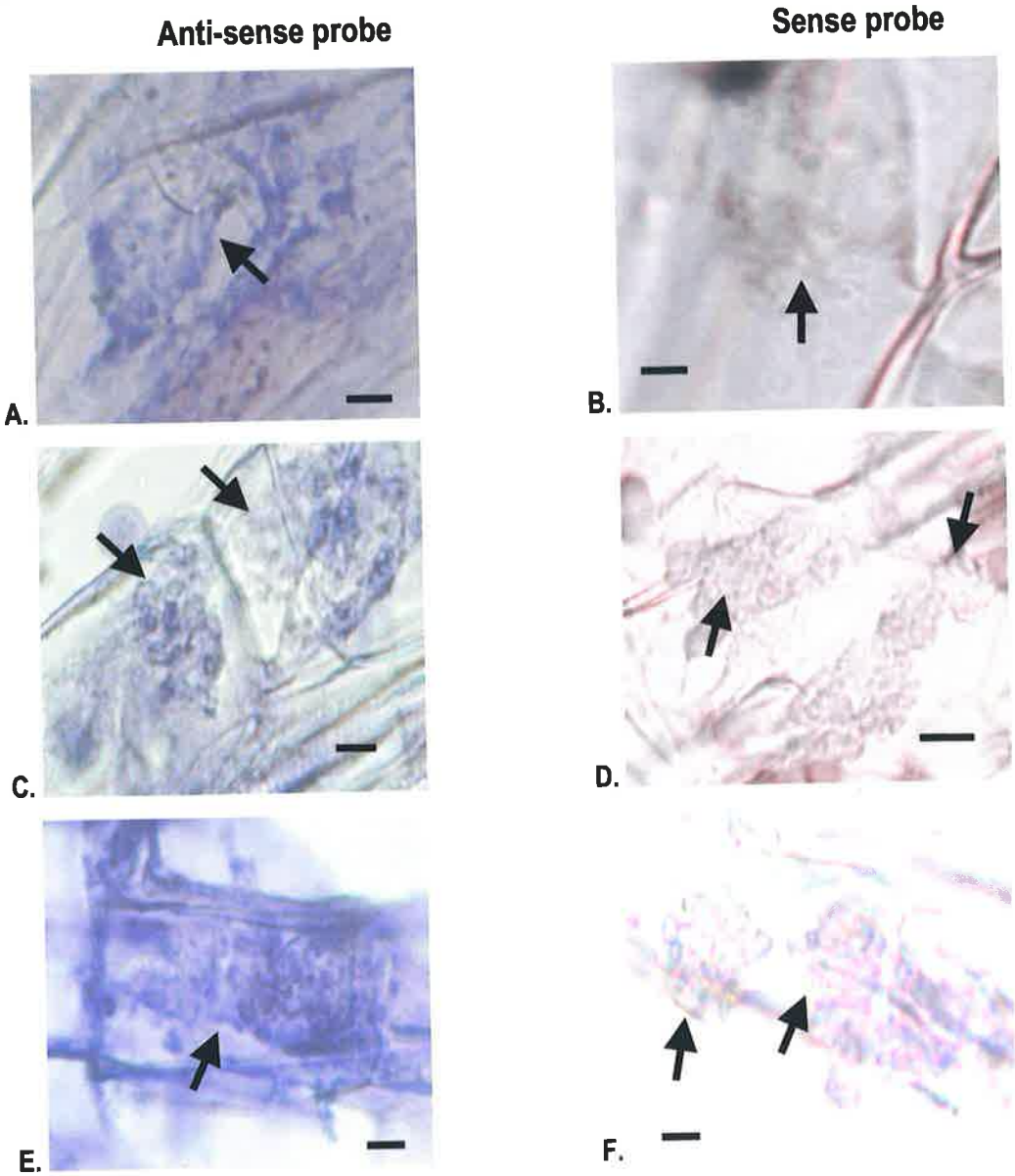
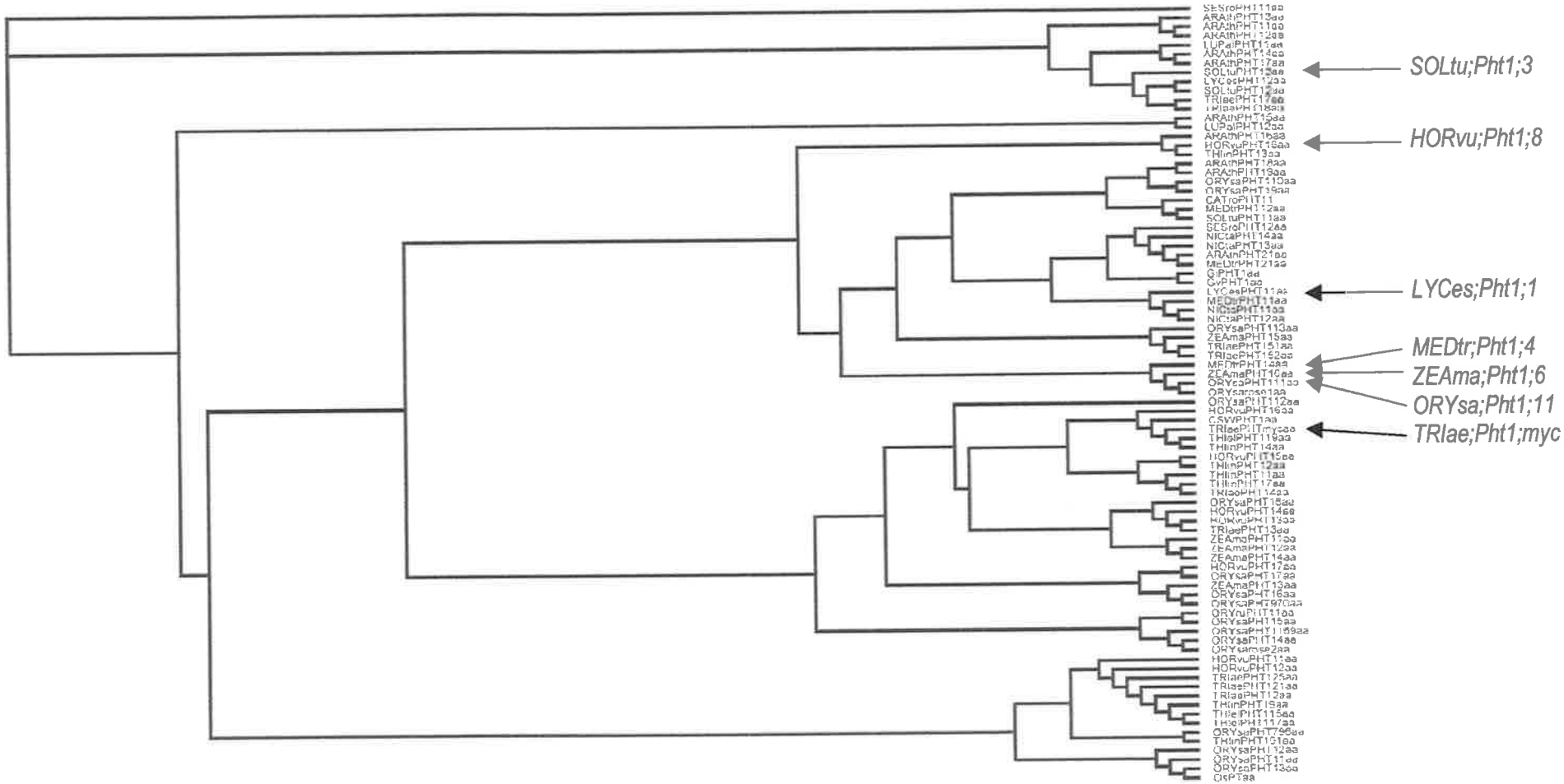


Figure 5.6: Detection of *TRIae;Pht1;myc* transcripts by *in-situ* hybridisation on sections of wheat roots (harvested 30 days after germination) colonised by *G. intraradices* (A & B), *G.sp. WFVAM23* (C & D) and *Sc. calospora* (E & F). Sections A, C and E have been hybridised with the anti-sense probe, sections B, D and F with the control sense probe. The sense probes hybridised to plant cells containing arbuscules/arbusculate coils (arrowed) and the anti-sense probe showed no labelling. Bar = 11.5 µm.

5.3.6 Identifying a maize mycorrhizal P transporter from a phylogenetic tree

The amino acid sequences of all the P transporters tabled in Appendix 1 were processed by the computer program EPROTPARS available through the ANGIS web site (www.angis.org.au). EPROTPARS estimates the phylogenies of genes from protein sequences. The resulting phylogenetic tree (Figure 5.7) nested the maize P transporter, *ZEAma;Pht1;6*, between *MEDtr;Pht1;4* and *ORYsa;Pht1;11*, both of which are expressed in root cells colonised by mycorrhizal fungi (Harrison *et al.*, 2002; Paszkowski *et al.*, 2002). The amino acid sequence of *ZEAma;Pht1;6* is 85% similar to the *MEDtr;Pht1;4* sequence and 90% similar to *ORYsa;Pht1;11* sequence. This suggested that *ZEAma;Pht1;6* may also be involved in P acquisition via mycorrhizal fungi.

Figure 5.7: Phylogenetic tree of all P transporters listed in appendix 1. Prepared by EPROTPARS (www.angis.org.au) based on the amino acid sequences of P transporters. Arrowed P transporters are those transporters expressed in roots colonised by mycorrhizal fungi.



5.3.7 RT-PCR of maize mycorrhizal P transporter and *In-situ* images

In order to confirm that the gene encoding the maize P transporter *ZEAma;Pht1;6* is expressed in roots colonised by mycorrhizal fungi specific primers (Table 5.1) were designed to *ZEAma;Pht1;6* and used in RT-PCR (Figure 5.8). *ZEAma;Pht1;6* was expressed strongly in mycorrhizal roots, and very faint level of expression was observed under low P conditions.

Figure 5.8: RT-PCR analysis of *ZEAma;Pht1;6*. Maize (*Z. mays*) roots were harvested from plants grown in LP - Low P soil (18 mg P/kg soil), HP - high P soil (118 mg P/kg soil), G.i. - *G. intraradices* nurse pots, G.v. – *G.*sp. WFVAM23 nurse pots and gDNA – genomic DNA. Arrowed products were the expected size (Table 5.1), MM - 1 kb Plus DNA ladder (#10787-018, Gibco Life Technologies, Melbourne, Australia).

RNA probes of *ZEAma;Pht1;6* were prepared according to section 3.10 and used for stringent *in-situ* hybridisation analysis of mycorrhizal maize roots (Figure 5.9). The anti-sense probe hybridised to those cells containing arbuscules, as observed with *HORvu;Pht1;8* and *TRIae;Pht1;myc*. No hybridisation was observed in the control sections using the sense probe.

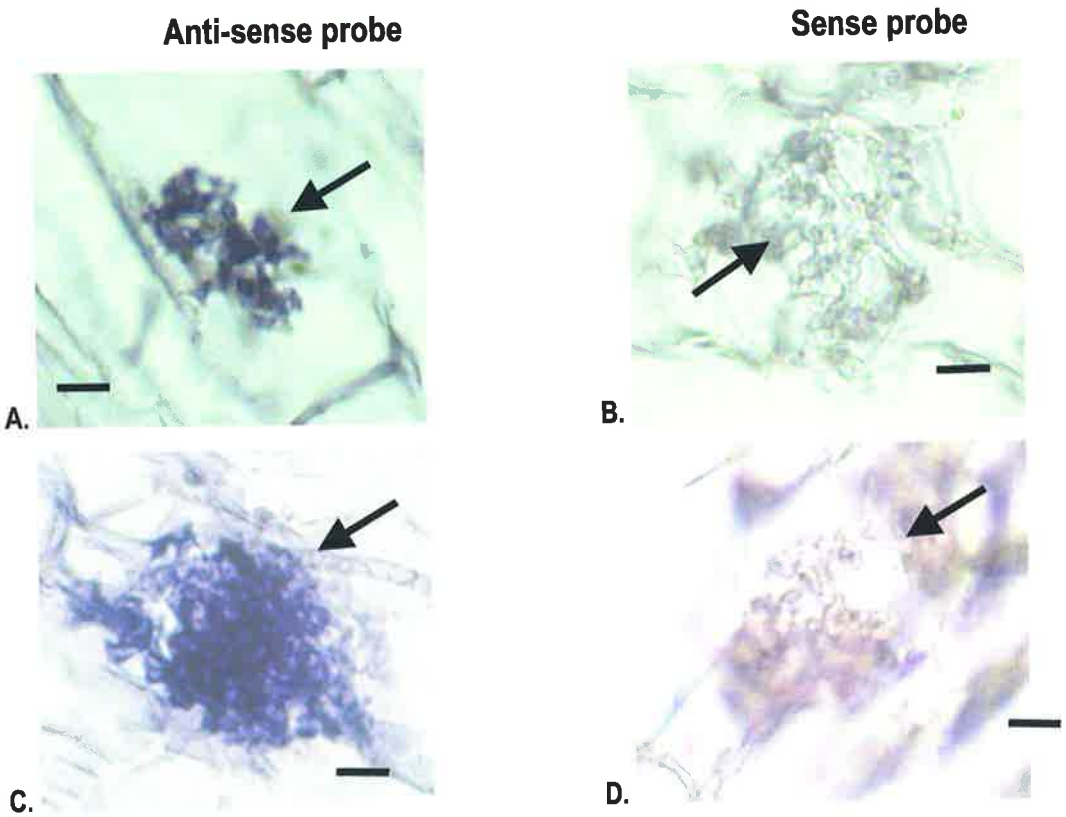


Figure 5.9: Detection of *ZEAma;Pht1;6* transcripts by *in-situ* hybridisation on sections of maize roots colonised by *G. intraradices* (A & B) and *G.sp. WFVAM23* (C & D). Sections A and C have been hybridised with the anti-sense probe, sections B and D with the control sense probe. The anti-sense probes hybridised to plant cells containing arbuscules (arrowed) and the sense probe showed no labelling. Bar = 9 μm.

5.3.8 Pht1 family topology

The amino acid sequence of HORvu;Pht1;8, TRIae;Pht1;myc and ZEAma;Pht1;6 can be processed to determine the 3D topology of the proteins. All three P transporters conform to the Pht1 family topology of 12 hydrophobic membrane-spanning regions divided into two groups of six by a large intracellular hydrophilic charged domain (Figure 5.10). This topology is typical of the members of the major facilitator super family (Marger & Saier, 1993), (Smith *et al.*, 1999).

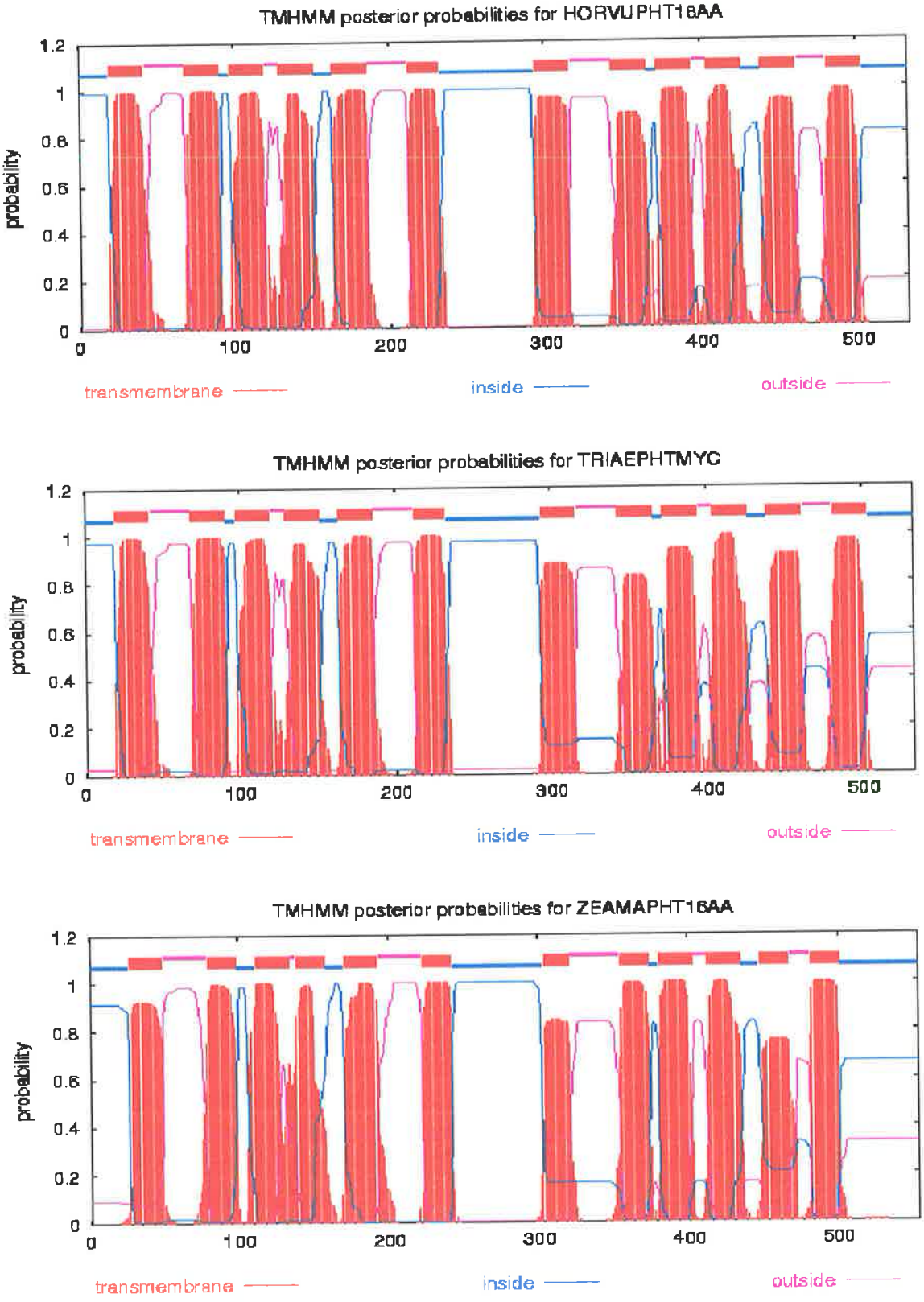


Figure 5.10: Predicted topology of P transporters HORvu;Pht1;8, TRIae;Pht1;myc and ZEAmaPht1;6. Red – Transmembrane, Blue – Intracellular, Pink – Extracellular.

5.4 Discussion

The expression of *HORvu;Pht1;1* in root epidermal cells and *HORvu;Pht1;2* in root epidermal cells and vascular tissue supports the suggestion that these P transporters are involved with Pi acquisition directly from the soil and mobilisation in roots (Smith *et al.*, 1999). The expression of *HORvuPhT1;3* was neither increased nor decreased by mycorrhizal colonisation, compared to expression in low Pi soils, and the expression of *HORvuPhT1;5*, *1;6* and *1;7* was not detected in roots (Figure 5.2). *HORvuPhT1;6* is expressed in phloem of vascular bundles in old and flag leaves and barley ears (Rae *et al.*, 2003). *HORvuPhT1;6* probably functions in remobilisation of stored P from leaves and is the first of the barley Pht 1 family P transporters identified that is not associated with P acquisition from soil. *Arabidopsis* Pht 1 genes, *ARAthPhT1;4*, *1;5* and *1;6*, have also been localised to shoot tissue and pollen grains (Mudge *et al.*, 2002). The publications of Rae *et al.* (2003) and Mudge *et al.* (2002) do not support earlier suggestions that regarded the Pht1 family of P transporters as only being involved with P acquisition from soil and mobilisation within the root system. The genes *HORvuPhT1;5* and *1;7* may be involved in P transport in the shoot or pollen and, if so, it is not surprising that transcripts were not found in the root mRNA tested in my experiments (Figure 5.2).

RT-PCR, real-time RT-PCR and *in-situ* hybridisation of *HORvu;Pht1;8* reveal that this gene is expressed in mycorrhizal colonised root cortical cells (Figures 5.2-5.4). Even though the mycorrhizal plants did not accumulate more P than the low P plants (Table 5.3), the P transporters *HORvu;Pht1;1* and *HORvu;Pht1;2* had been down-regulated and *HORvu;Pht1;8* up-regulated. This suggests that the plant was accessing P via the mycorrhizal fungus rather than via the direct uptake pathway through root epidermal cells. The *in-situ* hybridisations showed that expression of *HORvu;Pht1;8* is localised to cortical cells containing arbuscules and arbusculate coils of *G. intraradices*, *G.*sp. WFVAM23 and *Sc. calospora* (Figure 5.4). The expression patterns that were observed with *HORvu;Pht1;8* were similar to those seen with *ORYsa;Pht1;11* (reported in Chapter 6), *SORtu;Pht1;3*, *LYCes;Pht1;1* and *MEDtr;Pht1;4* P transporters, previously shown to be involved with Pi acquisition via mycorrhizal fungi (Rausch *et al.*, 2001; Rosewarne *et al.*, 1999; Harrison *et al.*, 2002). The expression patterns of *TRIae;Pht1;myc* and *ZEAma;Pht1;6* revealed by RT-PCR and *in-situ* hybridisations are consistent with those observed with *MEDtr;Pht1;4* and *ORYsa;Pht1;11* (Figures 5.5, 5.6, 5.8 and 5.9).

LYCes;Pht1;1, *HORvu;Pht1;8*, *ORYsa;Pht1;11*, *MEDtr;Pht1;4*, *SORtu;Pht1;3*, *TRIae;Pht1;myc* and *ZEAma;Pht1;6* are collectively called mycorrhizal P transporters in the remainder of this thesis. Discussion about the mycorrhizal P transporters sequences and their involvement with the alternative P pathway via AM fungi is detailed in Chapter 7.

Pi acquisition via the soil in barley plants utilises the P transporters *HORvu;Pht1;1* and *1;2*, while Pi acquisition via mycorrhizal fungi utilises *HORvu;Pht1;8*. The expression patterns of these genes identify the pathways of Pi acquisition utilised by the plant, via soil or mycorrhizal fungi, or a combination of both pathways. The main aim of this chapter was to identify P transporters that are involved with Pi acquisition via mycorrhizal fungi. This was achieved with the identification of *HORvu;Pht1;8*, *TRIae;Pht1;myc* and *ZEAma;Pht1;6*. The expression pattern of other barley P transporters (*HORvu;Pht1;1* → *1;7*) together with my RT-PCR results indicates that these P transporters are not involved with Pi acquisition via mycorrhizal fungi.

6 Identification and expression patterns of the PhT1 family of P transporters and a mycorrhizal P transporter in rice

6.1 *Introduction*

April 2002 saw the publication of the draft sequence of the rice genome (*Oryza sativa* L. ssp. *indica*; Yu *et al.*, 2002 and *Oryza sativa* L. ssp. *japonica* cv Nipponbare; Goff *et al.* 2002). The rice genome is approximately 430 Mb in size, 3.7 times larger than the genome of *Arabidopsis thaliana* and 6.7 times smaller than the human genome (Yu *et al.*, 2002). Amongst the cereal crops, the rice genome is the smallest and has a high degree of synteny with other cereals (Yu *et al.*, 2002). The completed rice genome is available to the public via the internet, http://btn.genomics.org.cn/rice (Yu *et al.*, 2002). This database was accessed to screen the rice genomic sequence for P transporters.

There are several rice P transporters identified in this chapter and published. Table 6.1 lists these known rice P transporters. The expression of all of these genes is discussed in this chapter.

Dr.R.Godwin has cloned two rice P transporters from the rice ssp. *indica*, called *OsPT1* (accession number AF493787, submitted April 2002) and *OsPT2* (accession number AF493788, submitted April 2002; Godwin, 2002, sequences in Appendix 4.4). When the sequence of *OsPT1* was Blasted against the GenBank database, http://www.psc.edu/general/software/packages/genbank, there was a 99% match with the partial mRNA sequence of another rice clone from *Oryza sativa* ssp. japonica cv Jingxi 17, thought to be a P transporter pseudogene (accession number AF259980, submitted September 2000; Yu *et al.*, 2000a). The gDNA sequence of *OsPT1* was used to screen the complete rice genome (ssp. *japonica* cv Nipponbare) for other P transporters. The expression of the resulting 11 rice P transporters (named *Rice gene A* → *K*) was then assessed by RT-PCR to determine if any of the genes were up-regulated in rice roots colonised by mycorrhizal fungi.

In October 2002 Paszkowski *et al.* (2002) published a paper identifying 13 rice P transporters from the cv Nipponbare. The expression patterns of these P transporters were assessed by real-time RT-PCR for expression in rice roots colonised by *G. intraradices*. The rice P transporter *ORYsa;Pht1;11* was expressed only in mycorrhizal roots, with the level of expression positively correlated with the degree of colonisation.

Table 6.1: A list of known rice P transporters and references. The names of the genes in this table are those allocated by the authors. Genes in the same row are considered to be identical.

(Yu *et al.*, 2000a)	(Godwin, 2002)	(Paszkowski *et al.*, 2002)	Genes identified by myself.
P transporter pseudogene	*OsPT1*	*ORYsa;Pht1;11*	*Rice gene C*
	OsPT2	*ORYsa;Pht1;4*	*Rice gene H*
		ORYsa;Pht1;1	*Rice gene A*
		ORYsa;Pht1;2	*Rice gene B*
		ORYsa;Pht1;3	*Rice gene D*
		ORYsa;Pht1;5	Rice gene I
		ORYsa;Pht1;6	Rice gene E
		ORYsa;Pht1;7	Rice gene G
		ORYsa;Pht1;8	Rice gene K
		ORYsa;Pht1;9	
		ORYsa;Pht1;10	
		ORYsa;Pht1;12	Rice gene F
		ORYsa;Pht1;13	Rice gene J

The sequences of the 11 rice P transporters identified by myself have been aligned and matched with those published by Paszkowski *et al.* (2002). The *OsPT1/ORYsa;Pht1;11/Rice gene C* gene is of particular interest to myself because the expression of this gene is linked to colonisation by mycorrhizal fungi. The sequence of the *ORYsa;Pht1;11* gene is 100% identical to *OsPT1* previously isolated by Godwin (2002) and the sequence of which I used to screen the rice genome. Although the sequence of *OsPT1* was identified prior to *ORYsa;Pht1;11* it will be referred to as *ORYsa;Pht1;11* in other thesis chapters. In this chapter *OsPT1* will be used to report results found by myself and Godwin, and *ORYsa;Pht1;11* when discussing results by Paszkowski *et al.* (2002) and discussing the gene in general. Prior to the publication of the paper by Paszkowski *et al.* (2002), RT-PCR results with gene specific primers for *OsPT1*, performed by myself, showed expression in rice roots colonised by *G. intraradices* and *G.sp. WFVAM23.* No expression was observed in roots not colonised by mycorrhizal fungi (Figure 6.1). This was consistent with the results subsequently published by Paszkowski *et al.* (2002) in rice roots colonised with *G. intraradices.* I have also performed *in-situ* hybridisations to demonstrate the localisation of expression of *OsPT1* in cortical cells colonised by mycorrhizal fungi. Thus my work proceeded in parallel with and supplemented the work of Paszkowski *et al.* (2002).

This chapter identifies the expression pattern of rice P transporters (identified in *Oryza sativa* ssp. *indica*) in roots subjected to low P soil, high P soil and mycorrhizal conditions. The chapter also presents the results of *in-situ* hybridisation studies aimed at identifying the site of expression of a P transporter presumed to be involved with P acquisition via AM fungi.

6.2 *Methods and Materials*

6.2.1 Screening the completed rice genome.

The rice genome (*Oryza sativa* L. ssp. *indica*) was sequenced by Yu *et al.* (2002) and is available on the web site http://btn.genomics.org.cn/rice. The putative rice P transporter genes identified were initially named rice gene A → rice gene K, correct nomenclature for these genes is clarified in the discussion, and identified from Table 6.1.1 in the Introduction.

6.2.2 Primers designed for RT-PCR

Specific sense and anti-sense primers for 11 of the rice P transporter genes identified from the rice genome were designed with the aid of the Primer3 program (Rozen & Skaletsky, 2000) (Table 6.2). The pair of primers for each rice P transporter gene were checked against the sequence of the other rice P transporter genes, to ensure that primers were specific to only one P transporter, with the computer program Amplify (Engels, 1993). This ensures that a positive result is specific for individual rice P transporters and not a group of known P transporters. Rice P transporter gene D was not a full-length sequence and identifying primers that would not amplify other rice P transporters was not successful. Therefore no RT-PCR reactions were processed for this gene.

Table 6.2: Primers used for RT-PCR of rice P transporters identified from rice genomic sequences. Sequences read 5' → 3'. The size of the PCR product indicated in the table is the correct product for that primer combination, though some PCR reactions yielded other products as well. Rice genes A → K are putative P transporters identified from the rice genome. n.a. = no $MgCl_2$ concentration resulted in the expected product size.

Gene	Sense primer	Anti-sense primer	$MgCl_2$ conc. mM	Length of PCR product, base pairs
Rice A	GTCATGTACGGATTCACCTT	ACCTCCTGCGAGATAACC	2.2	326
Rice B	GTTCGGGTTCCTGTACGC	CCACAAATCCACAACTGTAA	3	351
Rice C	GCTGTCGATCGAGATAGAGG	GCTGTCGATCGAGATAGAGG	2.5	498
Rice E	GATGACCCTCTTCATGCTC	GGCACGAGCATCATGTCA	n.a.	383
Rice F	GAGCATTCGGTTTCCTCTA	ACAAGGAGACATTCCACAAA	3	527
Rice G	CTTCTTCTTCGCCAACTT	TATTTCATCATCCAGCCTCT	2	495
Rice H	GTACGCCTTCACCTTCTTC	TTCGGTACACTACCAGAACC	2.5	493
Rice I	AAACTCCACGACCTTCATC	AATCCTAGTCATGGGCAGTA	3	493
Rice J	AGGACACAGCTGTACCATTT	TTCAGGCTGGATTCAATTAC	3	500
Rice K	TTCATCTGCACGTTCCTC	TCAACACAGCCATAATTGAA	n.a.	470

6.2.3 OsPT1 cDNA

Following the identification that *OsPT1* was only expressed in rice ssp. *japonica* cv Jarrah roots colonised by *G. intraradices* or *G.sp. WFVAM23*, I extracted the cDNA clone of this gene from mycorrhizal colonised rice roots (rice ssp. *japonica* cv Jarrah). Previous attempts by Godwin (2002) to extract the cDNA sequence of *OsPT1* had been unsuccessful because mycorrhizal colonised roots had not been used for the cDNA production in her studies. I produced cDNA from rice cv Jarrah roots colonised by *G. intraradices* as described in Chapter 5.2.3. One µL of first strand cDNA was then used for PCR using gene-specific primers for *OsPT1*. The gene-specific primers for *OsPT1* were 5' atggcggacgcggac 3' for the sense primer and 5' agtacgcacgtacctaca 3' for the anti-sense primer (see Appendix 4 for the position of the primers on the sequence). The PCR reaction was performed with EXPAND High Fidelity polymerase (#1732650 Roche Diagnostics Australia, Castle Hill, NSW) according to the manufacturer's instructions, with a final $MgCl_2$ concentration of 2.5 mM. Thermal cycling consisted of an initial denaturation at 94°C for 5 m, followed by 10 cycles of denaturation at 94°C for 15 s, annealing at 45°C for 30 s and extension at 72°C for 4 m, and then an additional 20 cycles during

which the extension time was increased by 5 s per cycle, followed by a final extension at 72°C for 7 m. The PCR reaction was visualised on an agarose gel according to Chapter 3.8.5. The PCR product was 1668 nucleotides in length and was purified from the agarose gel with the Ultra Clean DNA Purification Kit (#12100-300, Gene Works, South Australia, Australia) according to the manufacturers instructions. Following purification, the PCR product was processed with the QIAGEN A-addition Kit (#231994, QIAGEN, Victoria, Australia) which adds an A residue to a blunt-ended PCR product for easy cloning into pGemT-easy. The A-addition was performed according to the manufacturers instructions resulting in the PCR product then being cloned into pGemT-easy as described in Chapter 3.8. The pGemT-easy-OsPT1cDNA clone was sequenced according to Chapter 3.9 and found not to contain any errors introduced by PCR.

6.2.4 OsPT1 promoter

The genomic clone of *OsPT1* identified by Godwin (2002) consisted of a 3393 nucleotide 5' untranslated region, 1759 nucleotide gene (including one intron) and 390 nucleotide 3' untranslated region (accession number AF493787). Using the gDNA clone of *OsPT1* isolated by Godwin (2002) as a template in a PCR reaction the 5' untranslated region was amplified. Plasmid DNA (750 ng) containing the gDNA clone of *OsPT1* was used as a template in a PCR reaction using EXPAND Long polymerase (#1732650 Roche Diagnostics Australia, Castle Hill, New South Wales, Australia) according to the manufacturers instructions. The sense and anti-sense primers were designed with restriction enzyme sites in order to allow easy cloning into the pWBvec8 vector. The sense primer was 5' gccgccttaattaactcttgattggcttcttagc 3' (site for restriction enzyme PacI is underlined) and the anti-sense primer was 5' gccgaggcgcgccctccgatgatgccgtcgatcgtcc 3' (site for restriction enzyme AscI is underlined). The thermo-cycling conditions were the same as those used in section 6.2.3. The resulting PCR product was 3069 nucleotides in length and was processed as in section 6.2.3, cloned into pGemT-easy and pWBvec8-GFP and transformed into *E. coli* (Chapter 3.8). The pWBvec8-*OsPT1*prom-GFP clones were then transformed into *Agrobacterium tumefaciens* AGL1 as per chapter 3.8.3.

6.2.5 *In-situ* hybridisation of *ORYsa;Pht1;11*

In-situ hybridisations of sense and anti-sense probes of *ORYsa;Pht1;11* were performed according to Chapter 3.10. Rice roots (rice ssp. *japonica* cv Jarrah) were collected and prepared from nurse pots colonised with *G. intraradices*, G.sp. WFVAM23 and *Sc. calospora*.

6.3 *Results*

6.3.1 P transporters identified from the entire rice genome.

The sequence of OsPT1 successfully aligned to 11 different genes in the rice genome (*Rice genes A* → *J*, Table 6.1). There was 100% similarity between *Rice gene C* and *OsPT1*. *Rice gene C* was then referred to as *OsPT1*. The percentage of similarity between *OsPT1* and the other 10 rice clones varied from 95% - 50%. Nine of the rice putative P transporter gDNA genes contained full-length sequences (*Rice genes A, B; E, F, G, H, I, K* and *OsPT1*); the remaining two clones only comprised partial sequences (*Rice genes D* and *J*).

6.3.2 RT-PCR and gDNA PCR of putative rice P transporter genes

RT-PCR reactions were performed only on root tissue from rice plants (ssp. *japonica* cv Jarrah) grown in low and high P soil and nurse pots of *G. intraradices* and *G.sp. WFVAM23*. RT-PCR reactions for putative rice P transporter genes E and K did not yield any amplified products (results not shown). Primers for rice P transporter genes E and K were tested on rice gDNA to ensure that primer design was correct; these PCR reactions did not yield any amplified products (results not shown). The primers for rice P transporter genes E and K need to be redesigned and RT-PCR reactions repeated. RT-PCR reactions for genes F, G and I did not yield any amplified products from rice root cDNA, but did successfully amplify gDNA (results not shown). Successful amplification of gDNA ensured that the primers designed for clones F, G and I were acceptable, but that the genes are not expressed in rice roots grown in low P or high P soils or when colonised by *G. intraradices* or *G.sp. WFVAM23*.

Rice putative P transporter genes A, B, J and H/OsPT2 (Godwin, 2002) were expressed in rice roots grown in low P or high P soils and when colonised by *G. intraradices* or *G.sp. WFVAM23* (Figure 6.1). While putative P transporter genes A, B and H/OsPT2 displayed similar levels of expression in each of the root growth conditions, clone J was expressed more in roots colonised by mycorrhizal fungi than those that were not.

OsPT1 was only amplified from rice roots colonised by *G. intraradices* or *G.sp. WFVAM23* (Figure 6.1). The localisation of this expression was further clarified by *in-situ* hybridisations (Section 6.3.4).

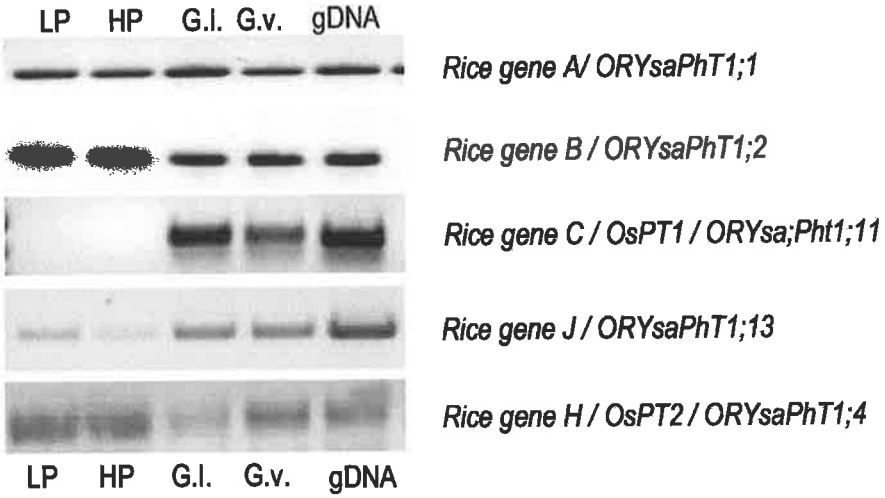


Figure 6.1: RT-PCR results for *rice genes A, B, C, J* and *H*. PCR product sizes are given in Table 6.2-1. LP – low P soil, HP – high P soil, G.I. – colonised by *G. intraradices*, G.V. – colonised by *G.sp. WFVAM23*, gDNA – genomic DNA.

6.3.3 OsPT1 cDNA and promoter

The cDNA (ssp. *japonica* cv Jarrah) of *OsPT1* was successfully cloned from rice roots colonised with *G. intraradices*. The resulting DNA sequence was a 100% match with the gDNA (ssp. *indica*) sequence obtained by Godwin (2002) and the gDNA (ssp. *japonica* cv Nipponbare) sequence obtained by Paszkowski *et al.* (2002). The sequence of OsPT1/ORYsa;Pht1;11 displayed no differences between rice cultivars and subspecies.

The promoter of *OsPT1* was successfully cloned into pGemT-easy and from this into the barley transformation-reporter gene vector, pWBvec8-GFP. The pWBvec8-OsPT1 promoter-GFP clones were transformed into *E. coli* DH10B and cultured, but in spite of several attempts the vector was never successfully transformed into *A. tumefaciens* AGL1. Without a successful *A. tumefaciens* transformation, transgenic rice plants could not be produced for localised expression studies of *OsPT1* via GFP confocal microscopy methods. The production of transgenic rice plants for reporter gene analysis of the expression of OsPT1 would be a useful experiment to complete by future researchers.

6.3.4 *In-situ* hybridisation of *ORYsa;Pht1;11*

RNA probes synthesised to the *ORYsa;Pht1;11* gene were used for *in-situ* hybridisation analysis of mycorrhizal rice roots (Figure 6.2). The anti-sense probe localised to cortical cells containing arbuscules; also confirming specificity of the probe. No hybridisation was observed in the control sections using the sense probe.

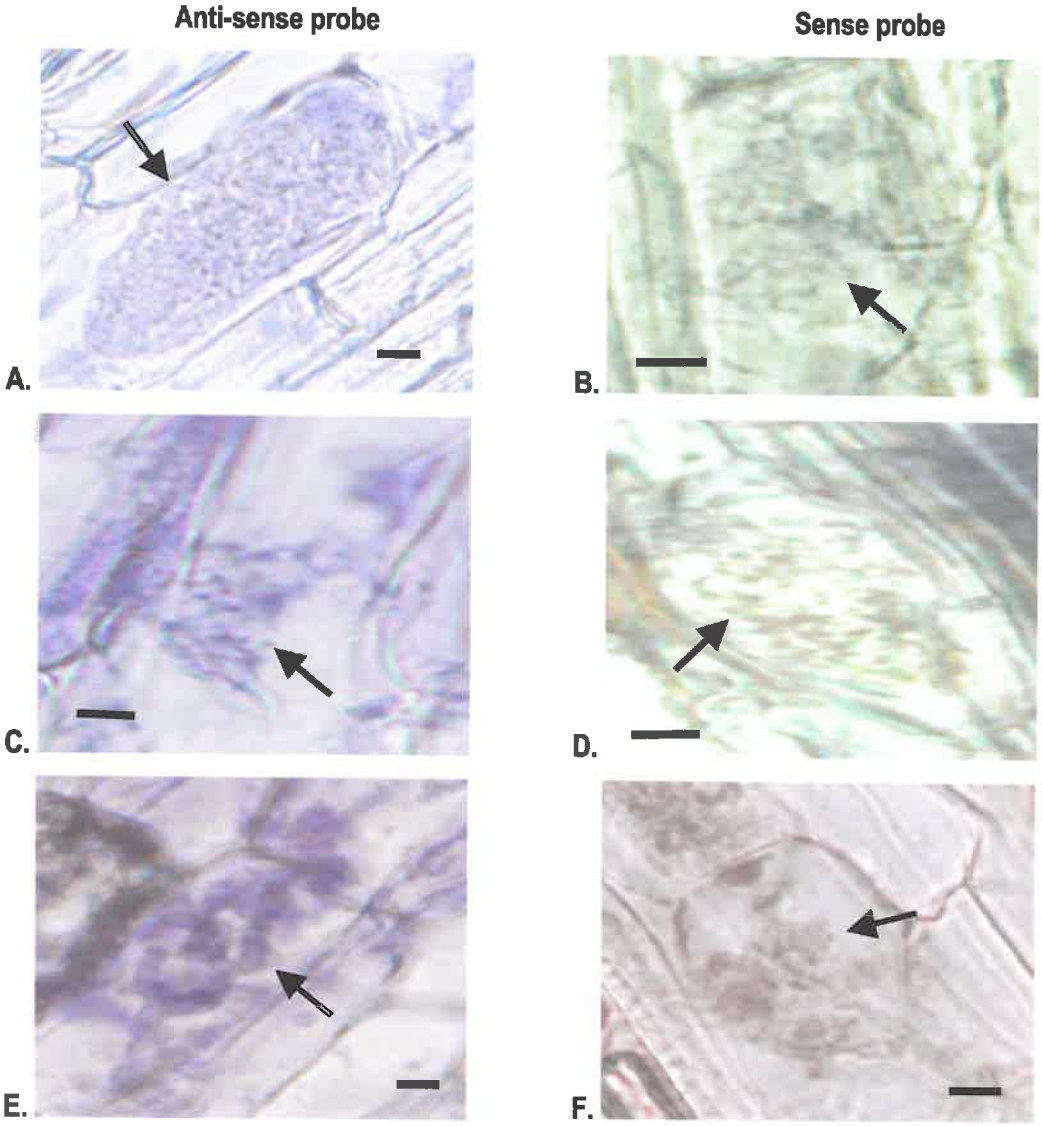


Figure 6.2: Detection of *ORYsa;Pht1;11* transcripts by *in-situ* hybridisation on sections of rice roots colonised by *G. intraradices* (A & B), *G.sp. WFVAM23* (C & D) and *Sc. calospora* (E & F). Sections A, C and E have been hybridised with the anti-sense probe, sections B, D and F with the control sense probe. The anti-sense probes hybridised to plant cells containing arbuscules and arbusculate coils (arrowed) and the sense probes showed no labelling. Bar = 6.7μm.

6.3.5 Pht1 Family Topology

The topology of the OsPT1/ORYsa;Pht1;11 protein can be ascertained from its amino acid sequence. The sequence indicated 11 well-defined hydrophobic membrane-spanning domains (MSD) plus another less well defined MSD (#7) (Figure 6.3). These MSD's are arranged in two groups of 6, separated by a large central loop on the cytoplasmic side of this membrane. Both the N-terminal and C-terminal tails are also orientated toward the cytoplasmic side of the membrane. This topology is typical of the Major Facilitator Superfamily of membrane transporters (Marger & Saier, 1993; Pao *et al.*, 1998). All members of the Pht1 family of P transporters have been shown to have this topology and belong to the Major Facilitator Superfamily (Smith *et al.*, 2003).

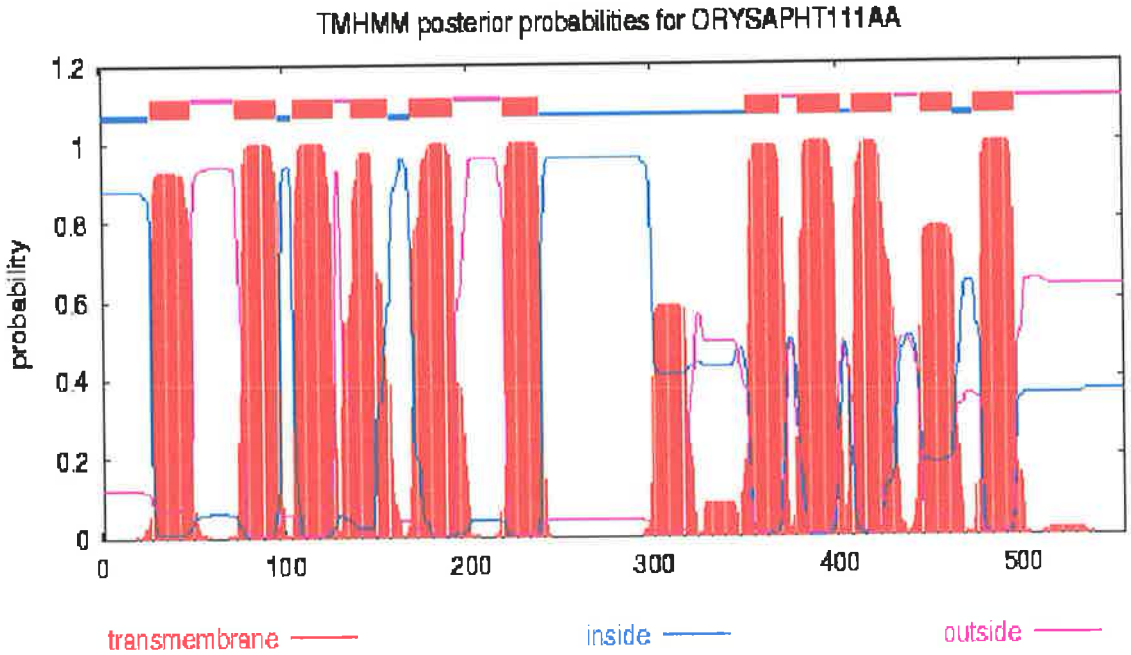


Figure 6.3: Predicted topology of P transporter OsPT1/ORYsa;Pht1;11. Red – Transmembrane, Blue – Intracellular, Pink – Extracellular.

6.4 *Discussion*

The sequencing of complete genomes has allowed researchers to identify whole gene families and study the roles and functions of those genes within the plant. An example of this is the study of nine P transporters identified by Mudge *et al.* (2002) in the *Arabidopsis thaliana* genome. All nine P transporters are of the Pht1 family and it was previously thought that this family of genes were only involved with P acquisition by the roots. Mudge and colleagues demonstrated that this gene family is not only involved with P uptake, but also remobilisation throughout the plant and supplying P to floral organs (Mudge *et al.*, 2002). The studies of gene families give an overall view of how the genes function and interact within the plant. As mentioned in the Introduction, the sequencing of the rice genome will allow gene families to be identified and analysed. This was done by myself and Paszkowski *et al.* (2002) in the identification of rice P transporters from rice ssp. *japonica* cv Jarrah and cv Nipponbare, respectively. Of the 13 full-length sequences identified by Paszkowski *et al.* (2002), 11 sequences (full-length and partial) identified by myself, two full-length sequences identified by Godwin (2002) and the partial sequence identified by Yu *et al.* (2000a), the rice genome may have 13 members of the Pht1 P transporter gene family, significantly more than *Arabidopsis thaliana* which only has 9 members. The RT-PCR and real-time RT-PCR results by myself and Paszkowski *et al.* (2002) reveal that, under the conditions used, all genes are transcribed except *ORYsa;Pht1;12*. *ORYsa;Pht1;12* may be expressed in tissues that have not been studied in these experiments or in developmental stages or under environmental conditions not yet studied. Alternatively, it may be a pseudogene. Pseudogenes are genes with a high degree of similarity to normal genes but they are not transcribed or translated. Further examination of the transcription and translation of this gene within the rice Pht1 family needs to be done before the exact number of functional gene members can be clarified and their roles determined.

The expression of the rice P transporter family has been assessed with RT-PCR (myself) and real-time RT-PCR (Paszkowski *et al.* 2002). Real-time RT-PCR demonstrated that all but clones *ORYsa;Pht1;12* and *1;13* were expressed in rice roots (expression in shoots was not examined). The expression of *ORYsa;Pht1;4, 1;5* and *1;7* was unchanged when rice roots were colonised by *G. intraradices*, while colonisation reduced the expression of *ORYsa;Pht1;1, 1;2, 1;3, 1;6, 1;9* and *1;10* by >25% of that observed in un-colonised roots (Paszkowski *et al.*, 2002). These results confirm and clarify the results with RT-PCR performed by myself for all clones except *ORYsa;Pht1;13*. RT-PCR of *ORYsa;Pht1;13* (*Rice gene J*) resulted in higher levels of expression in rice roots colonised by *G. intraradices* and G.sp. WFVAM23 than non-mycorrhizal rice roots, though no expression was observed in real-time RT-PCR reactions performed by Paszkowski et at (2002) on *G. intraradices* colonised roots. This conflict in

results may be due to use of inappropriate primers by either myself or Paszkowski *et al.* (2002). Sequencing or restriction enzyme digest of the amplified PCR product would confirm that the product is the gene of interest. The primers may cross react with other P transporters – as with *Rice gene D*, or the primers designed do not pick up the desired gene – as with *Rice genes E* and *K*, or different mycorrhizal fungi have different effects on expression. While the RT-PCR results with *ORYsa;Pht1;13* are interesting with respect to enhanced expression in mycorrhizal roots compared to non-mycorrhizal roots, this gene needs to be further examined to confirm this expression pattern. This is discussed further in Chapter 7. Expression of *ORYsa;Pht1;11* only in mycorrhizal roots is confirmed by results from by both RT-PCR and real-time RT-PCR reactions.

The roles of genes *ORYsa;Pht1;1* → *1;10* may be in P acquisition directly from the soil or mobilisation of P through the roots to the shoots. The role of *ORYsa;Pht1;12* may be in shoot mobilisation of P; this would explain the lack of transcripts in root mRNA tested. *ORYsa;Pht1;13* requires further work to clarify expression patterns before its roles can be determined. *ORYsa;Pht1;11* is involved with P acquisition via mycorrhizal fungi.

The gene of particular interest in this chapter is *ORYsa;Pht1;11* that was only expressed in rice roots colonised by mycorrhizal fungi. The *in-situ* hybridisation of *ORYsa;Pht1;11* localises the expression of this gene to rice cortical cells containing arbuscules and arbusculate coils of *G. intraradices*, G.sp. WFVAM 23 and *Sc. calospora*. This expression pattern is consistent with that observed for mycorrhizal P transporters *HORvu;Pht1;8*, *TRIae;Pht1;myc*, *ZEAma;Pht1;6*, *SORtu;Pht1;3*, *LYCes;Pht1;1* and *MEDtr;Pht1;4* (see Chapter 8 for the discussion) (Rausch *et al.*, 2001; Rosewarne *et al.*, 1999; Harrison *et al.*, 2002). The production of plants transformed with the *ORYsa;Pht1;11* promoter linked to GFP would have been another reliable method to confirm the localisation pattern of this gene but unfortunately difficulties were encountered during transformation of this construct into *Agrobacterium* and the time available did not permit this problem to be solved.

As with the other plant species it seems highly likely that rice is able to access P supplied via the mycorrhizal fungi, as well as via root epidermal cells.

7 General discussion

The results from this thesis determined that barley cultivars varied in the way they acquire and utilise P and that colonisation by AM fungi is dependent on several factors including soil P concentrations, AM fungal species and type of inoculum. These results highlight the complex nature of P acquisition and led to the detailed study of the plant-mycorrhizal fungus interactions associated with P acquisition. P transporters (mycorrhizal P transporters) in four plants belonging to the cereal family (barley, wheat, rice and maize) were identified as being involved with acquisition of P via the alternative P pathway provided by the colonisation of these plants by AM fungi. This alternative P pathway is discussed after first analysing the sequences of the mycorrhizal P transporters to determine if there are any regions of homology.

7.1 Mycorrhizal P transporter sequence homology

Analysis of the homology between the mycorrhizal P transporters was carried out in order to determine if there were any areas that are unique to the mycorrhizal P transporters. Unique regions of sequence may reveal motifs that localise the expression of these genes to the peri-arbuscular membrane and not the remaining plasma membrane in colonised cells. The initial step is to align all of the sequences and create a phylogenetic tree to determine if the mycorrhizal P transporters are closely related. The phylogenetic tree displayed in Chapter 5.3.6 contains all of the P transporters reported to date. In the phylogenetic tree the mycorrhizal P transporters are not grouped together. Analysis of the coding sequence alignment of the mycorrhizal P transporters (not shown) does not yield any regions that are unique to the mycorrhizal transporters. This suggests that regions of sequence in the coding region do not control the specific role of the mycorrhizal P transporters in P transport via mycorrhizal fungi. Transcription and initiation of translation of the mycorrhizal P transporters is likely to be controlled by sequence in the 5' untranslated regions.

Analysis of the mycorrhizal P transporters only (Table 7.1 and the phylogenetic tree Figure 7.1) reveals that the lowest percentage of homology is 72% between *HORvu;Pht1;8* and the three P transporters *ZEAma;Pht1;6*, *ORYsa;Pht1;11* and *MEDtr;Pht1;4*. The cereal crop P transporters do not group together (Figure 7.1). The genes from cereals wheat, maize and rice are grouped together, but the barley gene remains quite separate. Further studies on cereal evolution may reveal the reason for the divergence in the phylogenetic tree between barley and the other cereal crops studied.

Table 7.1: Percent homology between the mycorrhizal P transporter amino acid sequences.

	TRIae; Pht1;myc	*ZEAma; Pht1;6*	*ORYsa; Pht1;11*	*MEDtr; Pht1;4*	*SOLtu; Pht1;3*	*LYCes; Pht1;1*	*HORvu; Pht1;8*
TRIae;Pht1;myc	100	75	75	78	87	89	79
ZEAma;Pht1;6		100	90	83	78	76	72
ORYsa;Pht1;11			100	83	78	76	72
MEDtr;Pht1;4				100	79	77	72
SOLtu;Pht1;3					199	91	78
LYCes;Pht1;1						100	77
HORvu;Pht1;8							100

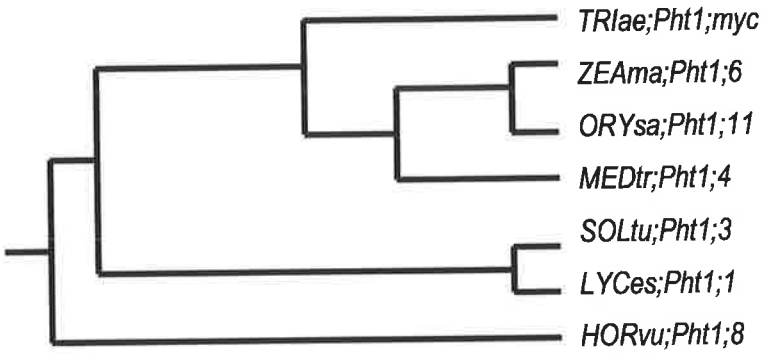


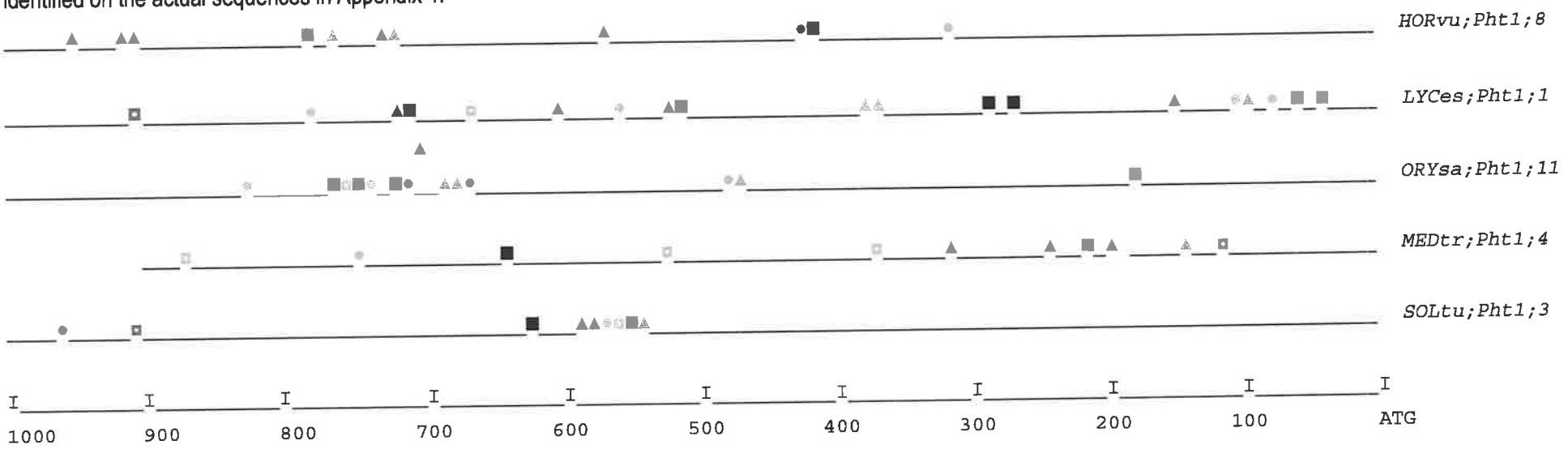
Figure 7.1: Phylogenetic tree of all mycorrhizal P transporters. Prepared by EPROTPARS program (www.angis.org.au) based on the amino acid sequences of the P transporters.

7.2 Putative cis-regulatory elements in mycorrhizal P transporter promoters

Gene regulation is controlled by varied and complex mechanisms (Blancheete & Tompa, 2003). The 5' untranslated region of *SOLtu;Pht1;3* has been analysed for regulatory domains by Rausch *et al.* (2001) and Karandashov *et al.* (2004); and possible domains in *MEDtr;Pht1;4* have been analysed by Harrison *et al.* (2002). Eight putative regulatory motifs have been identified in *SOLtu;Pht1;3*, *MEDtr;Pht1;4*, *LYCesPht1;h* (A.A. Levy, personal communication in Karandashov *et al.* 2004) and *ORYsa;Pht1;11* (Karandashov *et al.* 2004) (Figure 7.2). The promoter region of *HORvu;Pht1;8* contains six of these regulatory domains and *LYCes;Pht1;1* contains seven (Figure 7.2). There is no obvious pattern of these motifs that appears likely to be associated with spatial and temporal expression of these

mycorrhizal P transporters in colonised roots. Gene regulation involves activators, repressors, and positive and negative feedback loops; and more detailed research needs to be done in order to understand the intricate regulatory network that is initiated when plants are colonised by mycorrhizal fungi and P acquired via the alternative pathway provided by AM fungi. The production of transgenic plants, transformed with promoter/reporter gene vectors where the promoter has undergone mutation or deletion of the motif to be analysed, will aid in identifying the role of that motif in localisation and expression of the mycorrhizal P transporters.

Figure 7.2: Illustration of the putative cis-regulatory motifs identified by Rausch *et al.* (2001), Karandashov *et al.* (2004) and Harrison *et al.* (2002) on P transporters described in this thesis. Motifs have been aligned on individual sequences with Vector NTI desktop sequence analysis software (Invitrogen Life Science Software). The 5' untranslated region analysed is ~1000 bp from the start codon (ATG). Motif sequences and icons are listed below the figure. Precise motif location and sequences are identified on the actual sequences in Appendix 4.



■ TGTTATAAGT motif
▲ AAAACTTAG motif
TGCATTCTAT motif
TAACAACTAT
■ CTTCTTGTTCTA motif
TAATATAT motif
● MRR1 - TACATAAATATGTTCTTTAACTTG motif
MRR2 - GCACAAGTAGACCCTTAAACT motif

7.3 *The 'alternative' P acquisition pathway in mycorrhizal plants*

Four mycorrhizal P transporters were identified and characterised in the work described in this thesis. The barley gene *HORvu;Pht1;8* was cloned by Dr Frank Smith (CSIRO Plant Industry, Brisbane), but no expression patterns had been ascertained at the start of my work. Through RT-PCR, real-time RT-PCR and *in-situ* hybridisation experiments it was determined that *HORvu;Pht1;8* is expressed in root cortical cells containing AM fungal structures (arbuscules and arbusculate coils). Similar experimental procedures were also used to identify and determine the expression of *TRIae;Pht1;myc* (wheat), *ORYsa;Pht1,11* (rice) and *ZEAma;Pht1;6* (maize) in respective colonised root cells. The expression of the rice gene *ORYsa;Pht1;11* published by Paszkowski *et al.* (2002) had been found to occur in root samples that had been colonised by *G. intraradices*; I further demonstrated localisation in arbuscule-containing cortical cells via *in-situ* hybridisation experiments. The identification of these four P transporters with expression patterns associated with mycorrhizal colonisation increases both the number of 'mycorrhizal-related' P transporters and the range of plant species in which they have been found. Tomato, potato and *Medicago* genes were published during the course of my work (Rosewarne *et al.*, 1999, *LYCes;Pht1;1*; Rausch *et al.*, 2001, *SORtu;Pht1;3*; Harrison *et al.*, 2002, *MEDtr;Pht1;4)*. The expression patterns of these mycorrhizal P transporters support their potential role in acquisition of P via an alternative pathway for plants that form mycorrhizal associations (Figure 7.3). A second P transporter identified in tomato (*LYCes;Pht1;h*) has also been shown to be induced in plants colonised by AM fungi (A.A. Levy, personal communication, in Karandashov *et al.* 2004). *LYCes;Pht1;h* has not been mentioned prior to this discussion as no publications confirming the expression pattern have appeared in the literature.

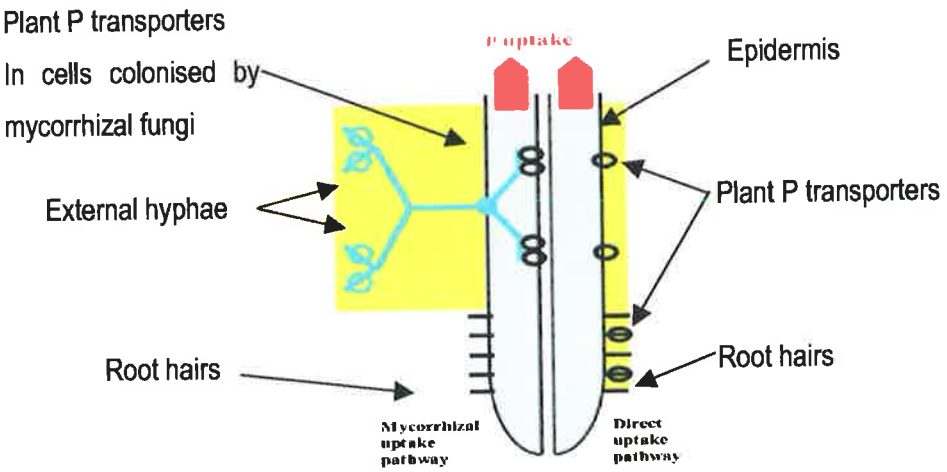


Figure 7.3: Schematic representation of supply of P to plant roots via two alternative pathways. P transporters (black circles) located in the root epidermis and root hairs access P directly from the soil solution in the direct uptake pathway. In the mycorrhizal uptake pathway mycorrhizal P transporters (blue circles) in external hyphae take up P and translocate it to fungus-plant interfaces in the root cortex where plant P transporters (arrowed) access the supply of P via the mycorrhizal fungi. The yellow zones represent the area of accessible P. There is a narrow area of available P along the root in the direct uptake pathway, while the mycorrhizal uptake pathway can access P up to 30 cm away from the root. This figure is modified from Smith *et al.* 2003b.

The expression of the mycorrhizal P transporters is not always limited to cells containing mycorrhizal fungal structures; i.e. they are apparently not completely 'mycorrhiza specific'. The expression of *LYCes;Pht1;1* was detected with *in-situ* hybridisation experiments in the stele and cortex of roots of non-mycorrhizal plants (Rosewarne *et al.*, 1999) and in epidermal cells and root hairs (Daram *et al.*, 1998; Bucher *et al.*, 2001). Low levels of expression of both *SORtu;Pht1;3* (Rausch *et al.*, 2001) and *HORvu;Pht1;8* (this thesis) were detected by real-time RT-PCR in non-mycorrhizal roots. Real-time RT-PCR experiments on *MEDtr;Pht1;4* (Harrison *et al.*, 2002; Karandashov *et al.*, 2003) and *ORYsa;Pht1;11* (Paszkowski *et al.*, 2002) demonstrate that these two genes are the only mycorrhizal P transporters associated with Pi acquisition via mycorrhizal fungi that, to date, have not been observed to be expressed in other plant cells of colonised or non-colonised plants. Expression in cell types other than mycorrhizal colonised cells needs to be examined carefully. *In-situ* hybridisation methods of detecting expression must be performed under very strict stringencies to ensure that expression of groups of P transporters, rather than single genes, are not detected. In order to overcome any problems associated with *in-situ* hybridisation specificity the production of transgenic plants containing promoter-reporter gene fusions and immuno-localisation experiments would be beneficial for future work. While the expression pattern varies between the mycorrhizal P transporters the results strongly suggest that they are involved with P acquisition at the peri-arbuscular membrane, accessing P supplied

by the mycorrhizal fungi. With the up regulation of mycorrhizal P transporters and down regulation of epidermal and root hair P transporters, eg. *HORvu;Pht1;1*, *1;2* and *1;8* expression, it appears that the plant is able to switch from the direct uptake pathway to the mycorrhizal uptake pathway.

Research to date, including that described in this thesis, indicates that the acquisition of P via mycorrhizal fungi does not only involve apparently 'mycorrhizal-specific' P transporters. There is also the redeployment of P transporters from non-mycorrhizal root cells to cells containing mycorrhizal structures when the roots are colonised (Harrison *et al.*, 2002). This supports the theory that a plant colonised by mycorrhizal fungi can change its pathway of P uptake from direct to that via the mycorrhizal fungi. Support for the activity of the 'alternative' P pathway of P acquisition via mycorrhizal fungi in a wide range of mycorrhiza-responsive and un-responsive plants is strengthening. Some plants, such as barley, apparently do not respond to AM colonisation in terms of growth or P uptake (Jensen & Jakobsen, 1980; Baon *et al.*, 1994). The assumption has been that AM fungi make no contribution to P uptake and hence the 'alternative' mycorrhizal pathway is inactive. However, Smith *et al.* (2003 & 2004), Zhu *et al.* (2003) and Schweiger and Jakobsen (1999) were able to demonstrate that the mycorrhizal network can supply up to 100% of the P acquired by flax, *Medicago* and tomato when colonised by *G. intraradices*. Smith *et al.* (2003b) used vials of radioactive P that were only accessible to mycorrhizal fungi hyphae (not plant roots) to determine how much P was being supplied to the plant via the mycorrhizal fungi. Depending on the combination of plant species and mycorrhizal fungal species, the percentage of P supplied by the mycorrhizal fungi varied. For example *G. caledonium* was able to supply flax and Medicago with 100% of the acquired P, but supplied tomato with approximately 75% of the acquired P and *Gi. rosea* only contributed a small proportion of the total P - (Smith *et al.*, 2003b).

Plants that are not acquiring 100% of the P via mycorrhizal fungi must employ both the direct and mycorrhizal P pathways to achieve plant P uptake. P transporters involved with P acquisition in epidermal cells and roots hairs and at fungi-plant interfaces would all be active if the plant was utilising both pathways. P acquisition from mycorrhizal fungi has been localised to cortical cells containing arbuscules and arbusculate coils. The concentration of P in arbuscule containing cells has been measured via cryo-analytcal scanning electron microscopy to be as high as 25 mM, while cells containing no mycorrhizal structures have a concentration of 10 mM P (Ryan *et al.*, 2003). This illustrates that arbuscule containing cells accumulate P. Accumulated P acquired via plant P transporters in the peri-arbuscular membrane requires protons (H^+-ATPase). The presence of H^+-ATPase activity at the peri-arbuscular membrane in colonised cells has been demonstrated and this would provide a source of protons for P transporters (Gianinazzi-Pearson *et al.*, 1991 & 2000). All of

this research and the localisation of several P transporters to colonised cells substantiates the likely importance of the alternative P pathway via mycorrhizal fungi that produce arbuscules. The expression of *HORvu;Pht1;8*, *TRIae;Pht1;myc* and *ORYsa;Pht1;11* in cortical cells colonised by *Sc. calospora* suggests that the arbusculate coils produced by this fungus may also be a site of P transfer (this thesis), though it does not have the same detailed experimental support as for arbuscules. The inclusion of mycorrhizal fungi that form arbusculate coils will be important in future work, if these results are to be used to enhance our understanding of P uptake in field situations that may contain many indigenous mycorrhizal fungi forming different structures in the roots. Understanding the two pathways of P acquisition that work in combination will help determine how soil P and P fertilisers are used by plants and how to take advantage of indigenous mycorrhizal fungi.

Research on P transporters involved with P acquisition via mycorrhizal fungi has revolved around the identification and localisation studies with mycorrhizal P transporters. Paszkowski *et al.* (2002) were able to demonstrate that the expression of the mycorrhizal transporter *ORYsa;Pht1;11* was related to the presence of internal mycorrhizal structures presumed to be involved with delivering P to the plant. Transcripts of *ORYsa;Pht1;11* were only detected when intraradical fungal structures were observed, 25 days post inoculation with 1000 mycorrhizal spores. This expression pattern for *ORYsa;Pht1;11* and all of the other mycorrhizal P transporters needs to be further assessed to determine if the expression is initiated when the root is initially colonised (appressorium formation), or if expression is related to the presence of those mycorrhizal structures that transfer the P into the plant cortical cells such as arbuscules and arbusculate coils. This would determine if the plant immediately identifies the colonisation by the mycorrhizal fungi as being beneficial or whether this recognition occurs at a later stage of development. An alternative avenue of research would involve the use of plant mutants. Plant mutants that are able to inhibit mycorrhizal colonisation at various stages would be useful in examining when the mycorrhizal P transporters are expressed in relation to the stage of colonisation. An example of the usefulness of mutants for assessment of colonisation and mycorrhizal fungi/plant interactions is the tomato mutant *rmc*. When challenged with 8 different mycorrhizal species, three were unable to colonise further than the root epidermal cells, four were able to penetrate past the root epidermis but not into cortical cells and one mycorrhizal species achieved normal colonisation (Gao *et al.* 2001). While mutant plants definitely have a role in the study of colonisation, the research published by Gao *et al.* (2001) emphasises the differences in colonisation depending on the plant and mycorrhizal fungi species that must also be considered.

The coordinate observation of various stages of colonisation by the AM fungi and P transporter expression would also elucidate the timing of the down-regulation of those P transporters involved with

P acquisition via the root/soil interface. The barley P transporters *HORvu;Pht1;1* and *1;2* are involved with P acquisition when the plant is grown in a P deficient environment. When P is supplied via increased fertiliser or via AM fungi the expression of these two genes is significantly reduced. Further investigations, both physiological and molecular, would reveal the processes involved in determining whether one pathway or both pathways are utilised for P acquisition.

The main aim of this thesis was to research the alternative P acquisition pathway via AM fungi. The P transporters involved with P acquisition from AM fungi have already been discussed. The expression pattern of these mycorrhizal P transporters varies depending on the plant species (i.e. whether the P transporter is expressed exclusively at the peri-arbuscular membrane or also expressed at other cellular locations) and the AM fungi colonising the plant. This research has also suggested that there may be more than one mycorrhizal P transporter within a PhT1 family of P transporters. The expression of both *ORYsa;Pht1;11* (Paszkowski *et al.*, 2002) and *ORYsa;Pht1;13* (this thesis) have been shown to be induced in roots colonised by AM fungi, compared with uncolonised roots, section 6.3.2. Similarly, *LYCesPhT1;h* is reported to be induced in AM colonised plants (A.A. Levy, personal communication, in Karandashov *et al.* 2004) in addition to the expression of *LYCes;Pht1;1* in mycorrhizal roots (Rosewarne *et al.*, 1999). The expression patterns of these genes may indicate that in rice and tomato two P transporters are involved with P acquisition via the alternative pathway provided by AM fungi colonisation. Analysis of the two genes suspected to be involved with P acquisition via mycorrhizal fungi in each plant species needs to be done in order to confirm their role in P uptake in the plant. Optimally the assessment of both genes would be conducted in the same tissue to ensure that the same conditions are achieved. The use of real-time RT-PCR, *in-situ* hybridisation and immuno-localisation using different fluorescent labels for identification would be useful to determine the localisation of gene expression relative to the other. Further experiments researching the expression pattern of *LYCes;Pht1;1* and *ORYsa;Pht1;13* would clairify the results of these two genes that are regarded with caution due to the possibility of cross reactions within the Pht 1 gene family. Rice contains a PhT1 gene family that includes 13 genes. Other plant species may contain this number of PhT1 genes and may contain more than one PhT1 gene involved with P acquisition via AM fungi. The presence of more than one mycorrhizal P transporter in a plant species would require further investigation to determine if both transporters are up regulated in the same manner when colonisation occurs or if different expression patterns are observed. Different mycorrhizal P transporters within one plant species may be expressed differently depending on the AM fungi colonising the plant. This would be of particular interest in field applications, where there are many AM fungal species present, if optimal P acquisition is to be achieved.

Given the likelihood that AM symbioses evolved in primitive land plants, before the diversification of present day angiosperm families, it is possible that *Arabidopsis thaliana* (and other non-host plants) contains a PhT1 gene whose expression pattern is potentially influenced by AM fungi, although the non-host status of the plant prevents direct testing of the possible induction of P transporter genes by AM fungi. *Arabidopsis thaliana* is a widely used plant model system in molecular, developmental, physiological and cell biology (Pigliucci, 2002). Nine members of the PhT1 family have been identified in this plant (Mudge *et al.*, 2002). A preliminary attempt was made in this project to analyse the expression of some of these genes by introducing promoter-GFP constructs into the host plant *Nicotiana tabacum.* The assessment of the transgenic tobacco plants was unsuccessful due to masking of GFP fluorescence by autofluorescence of tobacco roots under the conditions used (results not presented). However, the recent identification of 5 *Arabidopsis* ecotypes and 2 mutants that do form mycorrhizal associations (Horii & Ishii, 2003) will now permit further analysis of the expression patterns of the *Arabidopsis* P transporter family. A future experiment could use these *Arabidopsis* accessions to examine the expression patterns of all nine identified *Arabidopsis* P transporters in relation to the effect of mycorrhizal colonisation. If an *Arabidopsis* P transporter was identified as being involved with P acquisition via mycorrhizal fungi, it would stongly suggest that *Arabidopsis* had evolved from once being a host of AM fungi into a non-host plant. This would expand the value of *Arabidopsis* as a model plant for future mycorrhizal research.

The identification of more than one mycorrhizal P transporter per plant species highlights the need for high standards and quality control when researching P transporters to ensure that those results reported are correct for an individual gene and are not from cross-reactions between several P transporters. There is a high degree of similarity between P transporters and primers/probes/antibodies need to be designed in regions that are unique to the P transporter of interest. For example the genes *HORvu;Pht1;1* and *1;2* are identical except for eight amino acids at the 3' end of *HORvu;Pht1;2*. The primers designed for both *HORvu;Pht1;1* and 1;2 for real-time RT-PCR required the anti-sense primer to be developed in the 3' untranslated region of the genes in order to guarantee that the specific gene of interest was being amplified. The specificity of the primers ensured that the different expression patterns seen for these genes are accurate. Nevertheless, the existence of more than one P transporter induced by AM fungi might be expected. Plants evolved alternative pathways to accommodate adaptations to different environmental conditions. Additionally, there is increasing evidence for some degree of specificity in the interactions between different plant and AM fungal species (Helgason et al. 2002) which is likely to involve expression of different genes in signalling and metabolic pathways.

Studies of gene expression will be able to be linked to physiological studies of the quantity of P reaching the plant via the mycorrhizal pathway (i.e. the % contribution of this pathway to total plant uptake) should the project continue. Determining the predominant pathway of P uptake would be useful in field situations when assessing the need to apply fertiliser and the amount required. The identification of P transporters in barley, wheat, maize and rice involved with P acquisition via AM fungi can be used in further research to understand P acquisition of field grown cereals and how they can be used to improve P efficiency when grown in P deficient soils.

APPENDIX 1

PHOSPHATE TRANSPORTERS ISOLATED UP TO June 2003 (plant and AM fungi only).

Those transporters highlighted in yellow in the **Affinity K_m – details** column are classified as high-affinity transporters and those highlighted in blue classified as low affinity transporters (according to the authors).

Those transporters highlighted in the **Sites of Expression** column in pink are classified as being specifically involved with P acquisition via mycorrhizal fungi and those highlighted in purple are classified as being down regulated when P is supplied to the plant as increased P levels or AM colonisation.

References listed in **Affinity K_m – details** and **Sites of Expression** are specific for the results reported in those columns, those references listed in **References** are other researchers involved with identification and reporting of that gene. NA means that no information about affinity or expression was available.

Phosphate transporter names, abbreviated and correct nomenclature (section 3.11)	Plant	Accession number	Affinity K_m – details	Sites of Expression	References
PHT1;1 APT2 PHT1 AtPT1 *ARAth;Pht1;1*	*Arabidopsis thaliana*	AAB17265 - GB	High affinity K_m = 3.1 µM in cultured tobacco cells (Mitsukawa *et al.*, 1997b) Complements yeast strain NS219* when grown on media containing 110 µM P. (Muchhal *et al.*, 1996)	Roots, Hydathodes of cotyledons and leaves, Axillary buds, Peripheral endosperm of germinating seeds. P deficiency increased expression in root hairs and root cap. (Mudge *et al.*, 2002)	(Muchhal *et al.*, 1996) (Smith *et al.*, 1997)

Phosphate transporter names, abbreviated and correct nomenclature (section 3.11)	Plant	Accession number	Affinity K_m – details	Sites of Expression	References
PHT1;2 APT1 PHT2 *ARAth;Pht1;2*	*Arabidopsis thaliana*	BAA24282 - DBJ	Complements yeast strain NS219* when grown on media containing 110 µM P. (Muchhal *et al.*, 1996)	Roots only. P deficiency increased expression in root epidermal cells and root hairs, Near hypocotyl junction expressed in cortical cells, Aged root systems expressed in young lateral roots (Mudge *et al.*, 2002)	(Mitsukawa *et al.*, 1997a) (Smith *et al.*, 1997) (Muchhal *et al.*, 1996)
PHT1;3 PHT3 *ARAth;Pht1;3*	*Arabidopsis thaliana*	BAA24281 - DBJ	NA	Roots only. P deficiency increased expression in stele of the primary roots (pericycle layer) and root hairs Weak expression noted in vascular tissue of young leaves and hydathodes (Mudge *et al.*, 2002)	(Mitsukawa *et al.*, 1997a)
PHT1;4 PHT4 AtPT2 *ARAth;Pht1;4*	*Arabidopsis thaliana*	U62331 - NCBI	NA	Roots, hydathodes, axillary buds cotyledons, senescing anther filaments and abscission zone at the base of siliques. P deficiency increased expression in root epidermis, root tips and cells interior to pericycle. (Mudge *et al.*, 2002)	(Muchhal *et al.*, 1996) (Okumura *et al.*, 1998) (Lu *et al.*, 1997)

Phosphate transporter names, abbreviated and correct nomenclature (section 3.11)	Plant	Accession number	Affinity K_m – details	Sites of Expression	References
PHT1;5 PHT5 *ARAth;Pht1;5*	*Arabidopsis thaliana*	AC003033 - NCBI	NA	Vascular tissue of cotyledons, senescing leaves strongest in the phloem, young floral buds and sepals later in floral development, weak expression in stele of P deficient roots (Mudge *et al.*, 2002)	(Lin *et al.*, 1999a) (Okumura *et al.*, 1998)
PHT1;6 PHT6 *ARAth;Pht1;6*	*Arabidopsis thaliana*	BAA34390 - DBJ	NA	Anthers, tapetum of flowers, dry mature pollen grains (Mudge *et al.*, 2002)	(Okumura *et al.*, 1998)
PHT1;7 *ARAth;Pht1;7*	*Arabidopsis thaliana*	AL138650 - EMBL	NA	Mature pollen grains, P deficient roots (Mudge *et al.*, 2002)	(Obermaier *et al.*, 2000)
PHT1;8 *ARAth;Pht1;8*	*Arabidopsis thaliana*	AC007369 - EMBL	NA	P-deficient roots (Mudge *et al.*, 2002)	(Federspiel *et al.*, 1999)
PHT1;9 *ARAth;Pht1;9*	*Arabidopsis thaliana*	AC015450 - EMBL	NA	P-deficient roots (Mudge *et al.*, 2002)	(Lin *et al.*, 1999b)

Phosphate transporter names, abbreviated and correct nomenclature (section 3.11)	Plant	Accession number	Affinity K_m – details	Sites of Expression	References
PHT2;1 *ARAth;Pht2;1*	*Arabidopsis thaliana*	AB028611 - EMBL	Low affinity Complements yeast strain PAM2† when grown on media containing 394 μM P.	Leaves Chloroplast envelope. *Arabidopsis* PHT2;1 null mutant (pht2;1-1) reveals that PHT2;1 is involved with P allocation, P deficiency responses and translocation of P within leaves.	(Daram *et al.*, 1999) (Versaw & Harrison, 2002)
CrPT1 = PIT1 *CATro;Pht1;1*	*Catharanthus roseus*	AB004809 - GB	Transformed yeast mutant DpU‡ able to grow on low phosphate (55 μM) medium	Roots, stems and young shoots	(Kai *et al.*, 1997)
HvPT1 *HORvu;Pht1;1*	Hordeum vulgare	AF543197	High affinity K_m = 8 μM in barley roots.	Expressed in roots, induced by P deficiency.	(Smith *et al.*, 1999) (Rae *et al.*, 2003)
HvPT2 *HORvu;Pht1;2*	Hordeum vulgare	AY187019	NA	Roots	(Smith *et al.*, 1999)

Phosphate transporter names, abbreviated and correct nomenclature (section 3.11)	Plant	Accession number	Affinity K_m – details	Sites of Expression	References
HvPT3 *HORvu;Pht1;3*	Hordeum vulgare	AY187026	NA	Roots	(Smith *et al.*, 1999)
HvPT4 *HORvu;Pht1;4*	Hordeum vulgare	AY187024	NA	NA	Dr Frank Smith personal communication
HvPT5 *HORvu;Pht1;5*	Hordeum vulgare	AY187021	NA	NA	Dr Frank Smith personal communication
HvPT6 *HORvu;Pht1;6*	Hordeum vulgare	AF543198	Low affinity K_m = 320 µM in barley roots	Expressed in old leaves and flag leaves. Expressed in phloem of vascular bundles in leaves and ears.	(Rae *et al.*, 2003)
HvPT7 *HORvu;Pht1;7*	Hordeum vulgare	AY187022	NA	NA	Dr Frank Smith personal communication

Phosphate transporter names, abbreviated and correct nomenclature (section 3.11)	Plant	Accession number	Affinity K_m – details	Sites of Expression	References
HvPT8 *HORvu;Pht1;8*	Hordeum vulgare	AY187023	NA	Expressed in mycorrhizal roots.	D. Glassop and Dr Frank Smith personal communication
GmPT *GLYma;Pht1;1*	Glycine max	BG791142 - GB	NA	NA	(Shoemaker *et al.*, 1999)
LaPT1 *LUPal;Pht1;1*	Lupinus albus	AF305623 - GB	NA	Strong expression P deficient roots – normal and proteoid Weak expression stems and leaves	(Liu *et al.*, 2001)
LaPT2 *LUPal;Pht1;2*	Lupinus albus	AF305624 - GB	NA	Strong expression in roots – normal and proteoid	(Liu *et al.*, 2001)
LePT1 *LYCes;Pht1;1*	*Lycopersicon esculentum*	AF022873 - GB	High affinity Complements yeast strain PAM971† when grown on media containing 31 µM P.	P deficient roots (rhizodermal and root cap cells) and shoots, stele of root, cortical cells, phloem tissue and cells containing arbuscules	(Daram *et al.*, 1998) (Rosewarne *et al.*, 1999)

Phosphate transporter names, abbreviated and correct nomenclature (section 3.11)	Plant	Accession number	Affinity K_m – details	Sites of Expression	References
LePT2 *LYCes;Pht1;2*	*Lycopersicon esculentum*	AF022874 - GB	NA	Roots	(Liu *et al.*, 1998a)
MtPT1 *MEDtr;Pht1;1*	*Medicago truncatula*	AF000354 - GB	Low affinity Complements yeast strain NS219* when grown on media containing 192 µM P.	Expressed in roots and up regulated when P deficient, down regulated when mycorrhizal. Localised to epidermal cells and root hairs.	(Liu *et al.*, 1998b) (Chiou *et al.*, 2001) (Versaw *et al.*, 2002)
MtPT2 *MEDtr;Pht1;2*	*Medicago truncatula*	AF000355 - GB	NA	Roots and up regulated when P deficient, down regulated when mycorrhizal	(Liu *et al.*, 1998b)
MtPT3 *MEDtr;Pht1;3*	*Medicago truncatula*		NA	NA	(Harrison *et al.*, 2002)

Phosphate transporter names, abbreviated and correct nomenclature (section 3.11)	Plant	Accession number	Affinity K_m – details	Sites of Expression	References
MtPT4 *MEDtr;Pht1;4*	*Medicago truncatula*	AY116210 – cDNA AY116211 - gene	Low affinity Complements yeast strain NS219* when grown on media containing 493 µM P and PAM2† when grown on media containing 668 µM P.	Expressed in mycorrhizal (G. versiforme) roots only with RNA transcripts being detected 14 days post inoculation with increased expression positively correlated to colonisation. No expression detected at day 7. Expression also noted in roots colonised by Gigaspora gigantea. Expression localised to the peri-arbuscular membrane and coordinated with the life of the arbuscule.	(Harrison *et al.*, 2002)
MtPHT2;1 *MEDtr;Pht2;1*	*Medicago truncatula*	AF533081	Low affinity Complements yeast strain PAM2† when grown on media containing 600 µM P.	Expressed in all photosynthetic tissues, dependent upon light (light > dark), development (young > old) and Pi status of the plant (high P slightly > low P). Localised to the chloroplast envelope.	(Zhao *et al.*, 2003)
NtPT1 *NICta;Pht1;1*	*Nicotiana tabacum*	AF156696 - GB	NA	Weak expression in immature leaves, mature leaves, old leaves, stems and roots. All expression increased when P deficient. NtPT1/2 detected together.	(Kai *et al.*, 2002) (Baek *et al.*, 2001)

Phosphate transporter names, abbreviated and correct nomenclature (section 3.11)	Plant	Accession number	Affinity K_m – details	Sites of Expression	References
NtPT2 *NICta;Pht1;2*	*Nicotiana tabacum*	AB042950 - GB	NA	Immature leaves, mature leaves, old leaves, stems and roots. All expression increased when P deficient. NtPT1/2 detected together.	(Kai *et al.*, 2002)
NtPT3 *NICta;Pht1;3*	*Nicotiana tabacum*	AB042951 - GB	NA	No expression when P sufficient. Expressed in old leaves and roots when P deficient. NtPT3/4 detected together.	(Kai *et al.*, 2002)
NtPT4 *NICta;Pht1;4*	*Nicotiana tabacum*	AB042956 - GB	NA	No expression when P sufficient. Expressed in old leaves and roots when P deficient. NtPT3/4 detected together.	(Kai *et al.*, 2002)
OrPT *ORYru;Pht1;1*	Oryza rufipogon	AF337531 - EMBL	NA	NA	(Ming *et al.*, 2001)
OsPT *ORYsa*	*Oryza sativa*	AF239619 - GB	NA	NA	(Yu *et al.*, 2000a)
OsPT1169 *ORYsa;PhT1169*	*Oryza sativa*	AF271893 - EMBL	NA	NA	(Yu *et al.*, 2000b)

Phosphate transporter names, abbreviated and correct nomenclature (section 3.11)	Plant	Accession number	Affinity K_m – details	Sites of Expression	References
OsPT796 *ORYsa;Pht;796*	*Oryza sativa*	AF229169 - GB	NA	NA	(Yu *et al.*, 2000b)
OsPT970 *ORYsa;Pht;970*	*Oryza sativa*	AF335588 - GB	NA	NA	(Ming & Shen, 2001)
OsPT1 *ORYsa;Pht1;1*	*Oryza sativa*	AF536961 - GB	NA	Low expression in roots, transcription reduced when colonised with *G. intraradices*.	(Paszkowski *et al.*, 2002)
OsPT2 *ORYsa;Pht1;2*	*Oryza sativa*	AF536962 - GB	NA	Expressed in roots, transcription reduced when colonised with *G. intraradices*. Transcription reduced with increasing P content.	(Paszkowski *et al.*, 2002)
OsPT3 *ORYsa;Pht1;3*	*Oryza sativa*	AF536963 - GB	NA	Low expression in roots, transcription reduced when colonised with *G. intraradices*.	(Paszkowski *et al.*, 2002)
OsPT4 *ORYsa;Pht1;4*	*Oryza sativa*	AF536964 - GB	NA	Low expression in roots, transcription unaltered when colonised with *G. intraradices*.	(Paszkowski *et al.*, 2002)

Phosphate transporter names, abbreviated and correct nomenclature (section 3.11)	Plant	Accession number	Affinity K_m – details	Sites of Expression	References
OsPT5 *ORYsa;Pht1;5*	*Oryza sativa*	AF536965 - GB	NA	Low expression in roots, transcription unaltered when colonised with *G. intraradices*.	(Paszkowski *et al.*, 2002)
OsPT6 *ORYsa;Pht1;6*	*Oryza sativa*	AF536966 - GB	NA	Expressed in roots, transcription reduced when colonised with *G. intraradices*.	(Paszkowski *et al.*, 2002)
OsPT7 *ORYsa;Pht1;7*	*Oryza sativa*	AF536967 - GB	NA	Very low expression in mycorrhizal or non-mycorrhizal roots.	(Paszkowski *et al.*, 2002)
OsPT8 *ORYsa;Pht1;8*	*Oryza sativa*	AF536968 - GB	NA	Low expression in roots, transcription unaltered when colonised with *G. intraradices*.	(Paszkowski *et al.*, 2002)
OsPT9 *ORYsa;Pht1;9*	*Oryza sativa*	AF536969 - GB	NA	Low expression in roots, transcription reduced when colonised with *G. intraradices*.	(Paszkowski *et al.*, 2002)
OsPT10 *ORYsa;Pht1;10*	*Oryza sativa*	AF536970 - GB	NA	Low expression in roots, transcription reduced when colonised with *G. intraradices*.	(Paszkowski *et al.*, 2002)

Phosphate transporter names, abbreviated and correct nomenclature (section 3.11)	Plant	Accession number	Affinity K_m – details	Sites of Expression	References
OsPT11 OsPT1 *ORYsa;Pht1;11*	*Oryza sativa*	AF536971 – GB (OsPT11) AF493787 – GB (OsPT1)	Low affinity Complements yeast *pho84* mutant when grown on media containing 200 μM P	Expressed in mycorrhizal (*G. intraradices*) roots with RNA transcripts being detected 25 days post inoculation at 2% colonisation with increased expression positively correlated to colonisation. No expression detected at day 15 (0% colonised). No expression when roots infected with other soil microbes. OsPT1 (Godwin, 2002)	(Paszkowski *et al.*, 2002)
OsPT12 *ORYsa;Pht1;12*	*Oryza sativa*	AF536972 - GB	NA	Not detected in roots.	(Paszkowski *et al.*, 2002)
OsPT13 *ORYsa;Pht1;13*	*Oryza sativa*	AF536973 - GB	NA	Not detected in roots.	(Paszkowski *et al.*, 2002)
OsPT2	*Oryza sativa*	AF493788 - GB	NA	NA	(Godwin, 2002)

Phosphate transporter names, abbreviated and correct nomenclature (section 3.11)	Plant	Accession number	Affinity K_m – details	Sites of Expression	References
StPT1 *SOLtu;Pht1;1*	*Solanum tuberosum*	X98890 - GB	Low affinity Complements yeast strain MB192 (*PHO84* mutant) when grown on media containing 280 μM P	Roots, tubers, source leaves, floral organs and P deficient roots	(Leggewie *et al.*, 1997)
StPT2 *SOLtu;Pht1;2*	*Solanum tuberosum*	X98891 - GB	Low affinity Complements yeast strain MB192 (*PHO84* mutant) when grown on media containing 130 μM P	P-deficient roots	(Leggewie *et al.*, 1997)
StPT3 *SOLtu;Pht1;3*	*Solanum tuberosum*	AJ318822 - GB	High affinity Complements yeast strain PAM2† when grown on media containing 64 μM P.	Root sectors where mycorrhizal structures are formed, most prominent in arbuscule containing cells, presumably located in the peri-arbuscular membrane	(Rausch *et al.*, 2001) (Harrison *et al.*, 2002)

Phosphate transporter names, abbreviated and correct nomenclature (section 3.11)	Plant	Accession number	Affinity K_m – details	Sites of Expression	References
CSWPT1	*Triticum aestivum* Chinese spring wheat	Q9XEL6	NA	NA	
TaPT1 *TRIae;Pht1;1*	*Triticum aestivum*	AF110180 – EMBL Q9XEL6	NA	NA	(Li *et al.*, 1999)
TaPT2 *TRIae;Pht1;2*	*Triticum aestivum* Xiaoyan 54	AJ344240	NA	Expression mainly seen in roots, weakly in leaves. All expression dependent on cultivar, P content. Cultivar Jing411 expressed only in –P roots.	(Davies *et al.*, 2002)
TRIae;Pht1;2-1	*Triticum aestivum* (81(85)-5-3-3-3)	AJ344241	NA	NA	(Davies *et al.*, 2002)
TRIae;Pht1;2-5	*Triticum aestivum* (81(85)-5-3-3-3)	AJ344242	NA	NA	(Davies *et al.*, 2002)
TaPT3 *TRIae;Pht1;3*	*Triticum aestivum* Chinese spring wheat	AJ344243	NA	Expression mainly seen in roots, weakly in leaves. All expression dependent on cultivar, P content.	(Davies *et al.*, 2002)

Phosphate transporter names, abbreviated and correct nomenclature (section 3.11)	Plant	Accession number	Affinity K_m – details	Sites of Expression	References
TaPT4 *TRIae;Pht1;4*	*Triticum aestivum* Chinese spring wheat	AJ344244	NA	NA	(Davies *et al.*, 2002)
TaPT5.1 *TRIae;Pht1;5-1*	*Triticum aestivum* (81(85)-5-3-3-3)	AJ344245	NA	Cultivar 81(85)-5-3-3-3 expressed in roots and leaves (not P dependent)	(Davies *et al.*, 2002)
TaPT5.2 *TRIae;Pht1;5-2*	*Triticum aestivum* (81(85)-5-3-3-3)	AJ344246	NA	Cultivar 81(85)-5-3-3-3 expressed in low P roots only	(Davies *et al.*, 2002)
TaPT6 *TRIa;ePht1;6*	*Triticum aestivum* Chinese spring wheat	AJ433247	NA	Expression mainly seen in roots, weakly in leaves. All expression dependent on cultivar, P content. Strong expression –P leaves of 81(85)-5-3-3-3. No expression in *T. elongatum* or *T .intermedium*.	(Davies *et al.*, 2002)
TaPT7 *TRIae;Pht1;7*	*Triticum aestivum* Xiaoyan 54	AJ344248	NA	No expression noted in roots or shoots of hydroponically grown varieties.	(Davies *et al.*, 2002)
TaPT8 *TRIae;Pht1;8*	*Triticum aestivum* Xiaoyan 54	AJ344249	NA	No expression noted in roots or shoots of hydroponically grown varieties.	(Davies *et al.*, 2002)

Phosphate transporter names, abbreviated and correct nomenclature (section 3.11)	Plant	Accession number	Affinity K_m – details	Sites of Expression	References
The-15 *THIel;Pht1;15*	*Thinopyrum elongatum* wheat grass	AJ413955	NA	NA	(Davies *et al.*, 2002)
The-17 *THIel;Pht1;17*	*Thinopyrum elongatum* wheat grass	AJ413956	NA	NA	(Davies *et al.*, 2002)
The-19 *THIel;Pht1;19*	*Thinopyrum elongatum* wheat grass	AJ413957	NA	NA	(Davies *et al.*, 2002)
Thi-1 *THIin;Pht1;1*	*Thinopyrum intermedium* wheat grass	AJ413958	NA	NA	(Davies *et al.*, 2002)
Thi-2 *THIin;Pht1;2*	*Thinopyrum intermedium* wheat grass	AJ413959	NA	NA	(Davies *et al.*, 2002)

Phosphate transporter names, abbreviated and correct nomenclature (section 3.11)	Plant	Accession number	Affinity K_m – details	Sites of Expression	References
Thi-3 *THlin;Pht1;3*	*Thinopyrum intermedium* wheat grass	AJ413960	NA	NA	(Davies *et al.*, 2002)
Thi-4 *THlin;Pht1;4*	*Thinopyrum intermedium* wheat grass	AJ413961	NA	NA	(Davies *et al.*, 2002)
Thi-7 *THlin;Pht1;7*	*Thinopyrum intermedium* wheat grass	AJ413962	NA	NA	(Davies *et al.*, 2002)
Thi-9 *THlin;Pht1;9*	*Thinopyrum intermedium* wheat grass	AJ413963	NA	NA	(Davies *et al.*, 2002)
Thi-91 *THlin;Pht1;91*	*Thinopyrum intermedium* wheat grass	AJ413964	NA	NA	(Davies *et al.*, 2002)

Phosphate transporter names, abbreviated and correct nomenclature (section 3.11)	Plant	Accession number	Affinity K_m – details	Sites of Expression	References
SrPT1 *SESro;Pht1;1*	*Sesbania rostrata*	AJ286743 - GB	NA	NA	(Aono & Oyaizu, 2001)
SrPT2 *SESro;Pht1;2*	*Sesbania rostrata*	AJ286744 - GB	NA	NA	(Aono & Oyaizu, 2001)
ZmPT1 *ZEAma;Pht1;1*	*Zea mays*	Patent WO9958657	NA	NA	(Zhao *et al.*, 1999)
ZmPT2 *ZEAma;Pht1;2*	*Zea mays*	Patent WO9958657	NA	NA	(Zhao *et al.*, 1999)
ZmPT3 *ZEAma;Pht1;3*	*Zea mays*	Patent WO9958657	NA	NA	(Zhao *et al.*, 1999)
ZmPT4 *ZEAma;Pht1;4*	*Zea mays*	Patent WO9958657	NA	NA	(Zhao *et al.*, 1999)
ZmPT5 *ZEAma;Pht1;5*	*Zea mays*	Patent WO9958657	NA	NA	(Zhao *et al.*, 1999)
ZmPT6 *ZEAma;Pht1;6*	*Zea mays*	Patent WO9958657	NA	Mycorrhizal roots.	(Zhao *et al.*, 1999)

Phosphate transporter names, abbreviated and correct nomenclature (section 3.11)	Mycorrhizal fungi name	Accession number	Affinity K_m – details	Sites of Expression	References
GvPT	*Glomus versiforme*	U38650 – GB Q00908	High affinity Complements yeast *pho84* yeast mutant when grown on media containing 18 µM P.	Expressed in external hyphae.	(Harrison & van Buuren, 1995) (Versaw *et al.*, 2002)
GiPT	*Glomus intraradices*	AF359112	NA	Expressed in external hyphae.	(Maldonado-Mendoza *et al.*, 2001) (Versaw *et al.*, 2002)

Databases: GB (Genbank), DBJ (data base), NCBI (National Centre Biotechnology Information), EMBL (European Molecular Biology Laboratory), GenPept

Gene names of *Arabidopsis* clones from http://www.mips.gsf.de/

† PAM2, PM971 – *Saccharomyces cerevisiae* yeast mutant defective in two high-affinity Pi-transporter genes *PH084* and *PH089* (Martinez & Persson, 1998)

‡ DpU - *Saccharomyces cerevisiae* yeast mutant defective in high-affinity Pi-transporter gene *PH084* (Kai *et al.*, 1997)

* NS219 - *Saccharomyces cerevisiae* yeast mutant defective in high-affinity Pi-transporter gene *PH084* (Bun-ya *et al.*, 1991)

Appendix 2: Soil Analysis

[redacted]

SOIL ANALYSIS REPORT

Incitec Ltd
Australian Company Number 010 707 283
Paringa Road, Gibson Island, Murarrie
P.O. Box 140, Morningside, Qld 4170
Tel (07) 3867 9300

NUTRIENT ADVANTAGE

QUALITY MANAGEMENT SYSTEM

Millmerran soil sample

CSIRO TAG

Phone
Nearest Town
Distance to Town
Australian Map Grid Ref . .

Fax
Postcode
Direction to Town
Average Annual Rainfall mm

SOIL SAMPLE AND SITE INFORMATION:

Sampling depth(cm) - Surface
Sampling depth(cm) - Deep
Paddock area ha
Soil type
Slope
Soil profile depth m
Reasons for sampling 1.
2.

Months of Fallow
Age of cultivation yrs
Drainage
Stubble/Trash
Tillage
Irrigation

PRODUCTION INFORMATION:

Main species to be fertilized
Variety
Previous best yield
Yield last year
Vigour of growth
Row/tree spacing(Hort) m X m
Row spacing(Grain/Cotton)
Interrow treatment
Stage in Crop Cycle(Sugar Cane)
Method of fertilizer placement:
1. 2. 3.

yrs mths
Age established -
Root stock(Hort)
Plant population ha
Canopy radius(Hort) m
Grain protein
Legume content
Stock type
Stock number

FERTILIZER HISTORY: Fertilizers | Application Rate | Units | Date Applied Year Month

Most Recent Crop

Previous Crop

Previous Crop

Other Relevant Comments

Form No 804 (Rev 5)

Results of Analysis

PADDOCK NAME	
ORDER NUMBER	155532
PRODUCT	SG GE00/049
SAMPLE BAG NUMBER SURFACE	10403
CORRESPONDING DEEP SOIL BAG No	
DATE OF SAMPLING	
DATE RECEIVED	13/07/00
DATE OF REPORT.	19/07/00

pH(1:5 Water)	8.5
*pH(1:5 CaCl2)	7.7
Organic Carbon %C	1.1
Nitrate Nitrogen mg/kg	9.1
Phosphorus(Colwell) mg/kg	10
Potassium(Amm.Ac.)meq/100g	0.18
Calcium (Amm.Ac.) meq/100g	9.31
Magnesium(Amm.Ac.)meq/100g	8.01
Sodium (Amm Ac.) meq/100g	2.54
Chloride mg/kg	115
Electrical Conduct. dS/m	0.20
Copper (DTPA) mg/kg	0.7
Zinc (DTPA) mg/kg	0.4
Manganese (DTPA) mg/kg	48
Iron (DTPA) mg/kg	28
Boron (Hot CaCl2) mg/kg	1.1
Sulfur (MCP) mg/kg	17

Calculations

Cation Exch. Cap. meq/100g	20.03
Calcium/Magnesium Ratio	1.16
Sodium% of cations(ESP)	12.65

Methods, Calculations outlined overpage.

FOR INTERPRETATION OF THESE RESULTS, PLEASE CONTACT YOUR DEALER:
ACCOUNT
GIBSON ISLAND LAB

OR YOUR INCITEC AREA MANAGER
NARELLE JAGER

NATA

Registered Signatory

The Laboratory [illegible] associated with the sampling process. Samples [illegible] as received after drying at 40°C.

Report # g0041[illegible]

[redacted]

SOIL ANALYSIS REPORT

Incitec Ltd
Australian Company Number 010 707 263
Paringa Road, Gibson Island, Murarrie
P.O. Box 140, Morningside, Qld 4170
Tel: (07) 3867 9300

NUTRIENT ADVANTAGE

Ashland soil sample

Phone
Nearest Town
Distance to Town
Australian Map Grid Ref . .
Fax
Postcode
Direction to Town
Average Annual Rainfall mm

SOIL SAMPLE AND SITE INFORMATION:

Sampling depth(cm)	- Surface	Months of Fallow		
Sampling depth(cm)	- Deep	Age of cultivation		yrs
		Drainage		
Paddock area	ha	Stubble/Trash		
Soil type		Tillage		
Slope		Irrigation		
Soil profile depth	m			
Reasons for sampling	1.			
	2.			

PRODUCTION INFORMATION:

			yrs mths
Main species to be fertilized		Age established	-
Variety		Root stock(Hort)	
Previous best yield		Plant population	ha
Yield last year		Canopy radius(Hort)	m
Vigour of growth		Grain protein	
Row/tree spacing(Hort)	m X m	Legume content	
Row spacing(Grain/Cotton)		Stock type	
Interrow treatment		Stock number	
Stage in Crop Cycle(Sugar Cane)			
Method of fertilizer placement:			
1.	2.	3.	

FERTILIZER HISTORY:	Fertilizers	Application Rate	Units	Date Applied Year Month
Most Recent Crop	[redacted]	[redacted]	[redacted]	[redacted]

Previous Crop

Previous Crop

Other Relevant Comments

Form No 804 (Rev 5)

Results of Analysis

PADDOCK NAME	
ORDER NUMBER	155532
PRODUCT	sg gl00/049
SAMPLE BAG NUMBER SURFACE	10402
CORRESPONDING DEEP SOIL BAG No	
DATE OF SAMPLING	
DATE RECEIVED	13/07/00
DATE OF REPORT	19/07/00

pH(1:5 Water)	5.6
*pH(1:5 CaCl2)	4.0
Organic Carbon %C	0.6
Nitrate Nitrogen mg/kg	<0.2
Phosphorus(Colwell) mg/kg	3
Potassium(Amm.Ac.)meq/100g	0.37
Calcium (Amm.Ac.) meq/100g	0.32
Magnesium(Amm.Ac.)meq/100g	4.36
Aluminium (KCl) meq/100g	2.72
Sodium (Amm Ac.) meq/100g	1.48
Chloride mg/kg	35
Electrical Conduct. dS/m	0.05
Copper (DTPA) mg/kg	0.3
Zinc (DTPA) mg/kg	0.8
Manganese (DTPA) mg/kg	2
Iron (DTPA) mg/kg	67
Boron (Hot CaCl2) mg/kg	0.4
Sulfur (MCP) mg/kg	10

Calculations

Cation Exch. Cap. meq/100g	9.24
Calcium/Magnesium Ratio	0.07
Aluminium Saturation %	29.4
Sodium% of cations(ESP)	15.97

Methods, Calculations outlined overpage.

FOR INTERPRETATION OF THESE RESULTS,
PLEASE CONTACT YOUR DEALER:
BACCOUNT
GIBSON ISLAND LAB

OR YOUR INCITEC AREA MANAGER
NARELLE JAGER

NATA

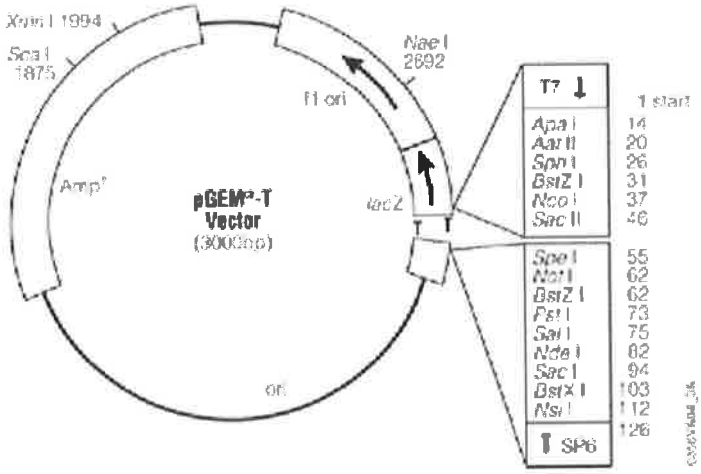
Registered Signatory

The Laboratory is not associated with the sampling process. Samples are analysed as received after drying at 40°C.

Report # g0041776

Appendix 3: Vectors

pGemT-easy (#1360) – supplied by Promega Corporation, Annandale, New South Wales, Australia.



This vector can be transformed into *E. coli* DH10β and grown on LB Ampicillin/XIA plates or LB Ampicillin broth.

LB (Luria-Bertani) broth per L:

To 950 mL distilled H_2O add:

10 g bacto-tryptone

5 g bacto-yeast extract

10 g NaCl

Adjust the pH to 7 with 5 N NaOH, adjust the volume to 1 L with distilled H_2O and sterilise by autoclaving for 20 m at 15 lb $sq.in.^{-1}$ on liquid cycle.

LB plates per L:

Add 15 g bacto-agar prior to autoclaving.

LB Ampicillin/XIA plates:

Prepare LB plates and cool media to 50°C before adding:

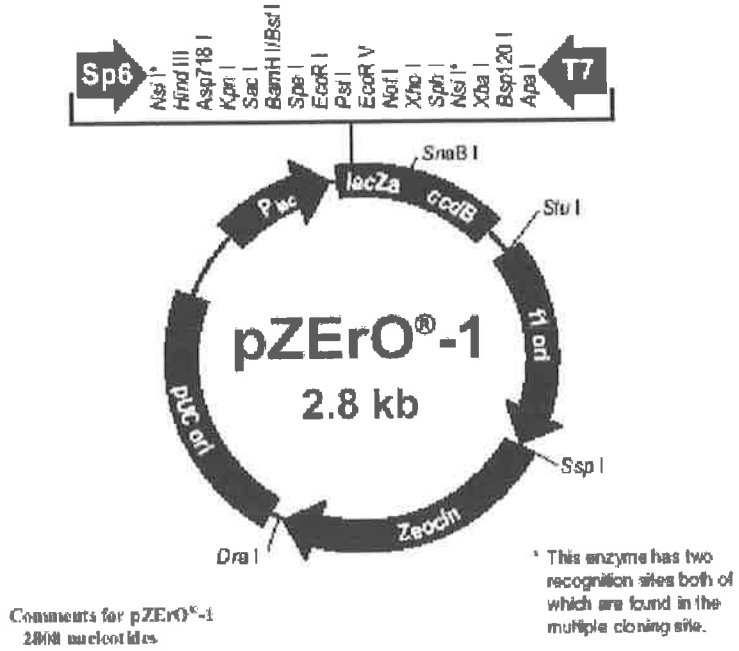
Reagent	method of preparation	final concentration in media
Ampicillin	dissolved in H_2O and filter sterilised	100 μg mL^{-1}
Isopropylthio-β-D-galactoside (IPTG)	dissolved in H_2O and filter sterilised	0.2 mM
5-Bromo-4-chloro-3-indolyl-β-D-galactoside (X-gal)	dissolved in dimethylformamide, no need to filter sterilise	40 μg mL^{-1}

Mix gently and thoroughly before pouring plates in sterile laminar flow.

LB Ampicillin broth:

Prepare LB broth and cool media to 50°C before adding Ampicillin, as above.

pZERO1 (#K2500-01) – supplied by Invitrogen Australia Pty Ltd, Mount Waverly, Victoria, Australia.



pZERO1 can be transformed into *E. coli* DH10β and grown on LSLB Zeocin plates or LSLB Zeocin broth.

LSLB (Low Salt Luria-Bertani) broth per L:

To 950 mL distilled H_2O add:

10 g bacto-tryptone

5 g bacto-yeast extract

5 g NaCl

Adjust the pH to 7 with 5 N NaOH, adjust the volume to 1 L with distilled H_2O and sterilise by autoclaving for 20 min at 15 lb sq.in.$^{-1}$ on liquid cycle.

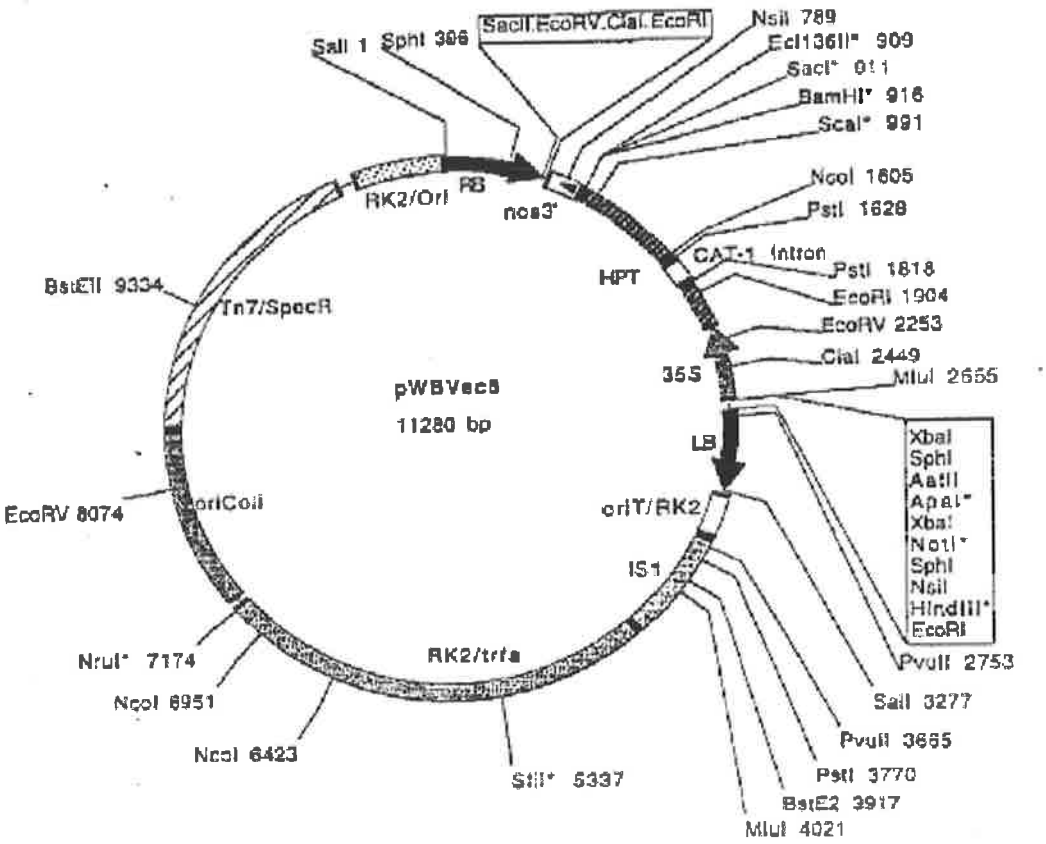
LSLB plates per L:

As for broth, add 15 g bacto-agar prior to autoclaving.

LSLB Zeocin plates/broth:

Prepare LSLB plates and cool media to 50°C before adding Zeocin to a final concentration of 25 μg mL^{-1}. Zeocin (#R250-01) is supplied by Invitrogen Australia Pty Ltd, Mount Waverly, Victoria, Australia.

pWBVec8 – supplied by CSIRO Plant Industry, Canberra, ACT, Australia.



pWBVec8 is transformed into *Agrobacterium tumefaciens* strain AGL1 and grown on YEP/Rifampicillin/Spectinomycin broth or plates.

YEP broth media per L:

To 950 mL distilled H_2O add

10 g Bacto peptone

10 g yeast extract

5 g NaCl

Mix and adjust volume to 1 L, autoclave.

YEP medium per L:

As for broth, add 15 g bacto-agar prior to autoclaving.

YEP Rifampicillin/Spectinomycin:

Prepare YEP and cool media to 50°C before adding:

Reagent	Method of preparation	Final concentration in media
Spectinomycin	dissolved in H_2O and filter sterilised	100 μg mL^{-1}
Rifampicillin	dissolved in Dimethyl Sulfoxide	50 μg mL^{-1}

Other Reagents:

SOC Medium per L:

To 950 mL distilled H_2O add:

20 g bacto-tryptone

5 g bacto-yeast extract

0.5 g NaCl

10 mL 250 mM KCl

Adjust the pH to 7 with 5 N NaOH, adjust the volume to 1 L with distilled H_2O and sterilise by autoclaving for 20 min at 15 lb $sq.in.^{-1}$ on liquid cycle.

Before use allow media to cool and add:

5 mL $MgCl_2$ [2 M, sterilised by autoclaving]

20 mL 1 M Glucose (filter sterilised through a 0.22-μm filter)

TAE 1 x buffer:

0.04 M Tris-acetate

0.001 M Ethylenediaminetetraacetic Acid (EDTA)

Appendix 4: Sequences

The origin of the sequences is from Genbank: accession numbers or patent numbers for each sequence is given in the introduction of that sequence.
Coloured bases highlight the sequence of the primer named underneath.
Promoter regions = plain text
Start codon = ATG
Coding region = **Bold**
Stop codon = **TAA** or **TAG** or **TGA**
3' untranslated region = underlined.

Barley Phosphate Transporters

HvPT1

Accession number = AF543197
HvPT1 total gene = 3202 bp
Promoter = 1403 bp
cDNA = 1563 bp = 521 amino acids
3' untranslated region = 236 bp

```
5'      1  GAGCTCCGAC TACCCCCGCG ATATCAAGCA AGACCCATGG AGGGCAGGCG
       51  GCGGCCTACA AAGTTCCAGG ACCTAACCTA CGGCCGGTGG CGTGGCCTCA
      101  CCTCGTTCAA CGAGTCCGGC GGCGAGGCGT GGGCAGGCAT CGGCGTAGAA
      151  AGCGATGGAT CAACGGGGGC CATCTACAAC TGCCTACGTG GACTGTTTGA
      201  CTCCGCCACG TTGGCACTGA CACTGGGTCC TGTTTGTTAG GTTTTCTGTC
      251  AAAATGCAAT CAACCGACTA TTTAGTGCTT GAATTTTTTA CTGAATAACG
      301  TGTTTTGGGC AAACTTTTTT GAAAACGATT GTTTTCGGCA CTGAGCGACG
      351  CAATTTCGGT GGTTTTTGAC AATTTACTCT AAACATCAGC AGACCAATGC
      401  CTCAGTTACA GACCCAACTC AAAAACACCT CAATTACAGT CAGGCATGTT
      451  GCCAATGGGT TCTTCGTTGT TCTCACACCT GGGTGGGTGT TTATTCGAAA
      501  GCATGTTGAT ATTGCTACGG TCCATGAGGT ATAGGCCAGA GACTCTCGTA
      551  AACTGATAGC ACGTTTACTT GCTGGTTGCT GCATATCGTA CACAATAAAC
      601  AAACACGATG AACTCCAACT TGGTATAAAC TACAGCCGTC ACAACTCATA
      651  CCTCATCCCT AACGAATTGT GGTGCCCATC CGGGAAAATA TCGGTCACTC
      701  TAATGCAATC AATTTTTCGT TACCTTTCTA ACTATGGAAG ATTTGGGACG
      751  TGTAAACAAC ACCATGTCAT GGTCATATAT ACATAAAACT GCCAACTTGC
      801  TAATTAAGTT TCTCAGGCAT ATTCGGATCT TCTCATTATT TTCTCTCGTT
      851  TACAGTTGCT AAGTTTGACA TAGCTACTAG TGGTAGTATA GTCTACTACT
      901  ATTCGAAGCT GTGATCCGCT TGATGTACTT CAGCAGTTCG AGATCCATCT
      951  TATATGTTCA TATATATGTC ATGTATATTC CTAGCATAAC AATTATGTGG
     1001  GTTCCAGTTT TATGTATATG AAACTTCAAA GTCAAATCTC ACATAGACCT
     1051  TATATAAGAA ATCCCTAGCT CAATCCTTGC GATAACTAAA ATGCATCCCA
     1101  ACTTAGCAAG CTGCAGCTTA AATTAAGGGA CGACATTTTA CATTCTTGTG
     1151  TGTCTATTTT ATAAGAGTAT CATGCCAAAA TCTTGCTGTC TTGATAATGT
     1201  ACGGCTGTAC GTACGTGCAA TGCCGTACAT GGAGATTTTA ATATTTACAA
     1251  GTAGCGAGGA AATGTCCCTT TGGCATATCC GCCGAACCTG CATTATGCCT
     1301  ATATATACGT ACTAGAAGAA CGGAACAAAT CACACAACAA GAGAAAACCA
     1351  GAAGAAAGAT AGAAGGGGGC AGGAGTTAAG CTGAGAGATC GCCGGCGACC
     1401  ATGGCGACTG AACAGCTCAA CGTGTTGAAA GCGCTCGATG TTGCCAAGAC
     1451  GCAACTGTAC CATTTCAAGG CCGTGGTGAT CGCCGGCATG GGCTTCTTCA
     1501  CCGACGCCTA CGACCTCTTC TGCATCGCGC TTGTCACCAA GCTGCTGGGG
     1551  CGCATCTACT ACACCGATCC TGCTCTCAAC GAGCCCGGCC ACCTCCCGGC
     1601  AAACGTGTCG GCCGCCGTGA ACGGCGTGGC CCTATGCGGC ACACTTGCCG
     1651  GCCAGCTCTT CTTCGGCTGG CTCGGTGACA AGCTCGGCCG CAAGAGCGTC
     1701  TACGGCTTCA CGCTCATCCT CATGGTCCTC TGCTCCATCG CGTCGGGGCT
     1751  CTCGTTTGGA CACGAGGCCA AGGGCGTAAT GGGGACTCTA TGTTTCTTCC
     1801  GCTTCTGGCT CGGCTTCGGC GTCGGCGGCG ACTACCCTCT GAGCGCCACC
     1851  ATCATGTCCG AGTATGCTAA CAAGAAGACC CGCGGCACCT TTATCGCCGC
     1901  CGTGTTTGCC ATGCAGGGGT TTGGCATCCT ATTTGGTACT ATTGTTACCA
     1951  TCATCGTCTC GTCCGCATTC CGACATGCAT TCCCTGCACC GCCATTCTAC
     2001  ATTGACGCCG CGGCATCCAT TGGTCCGGAG GCCGACTACG TGTGGCGCAT
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2051  CATCGTCATG TTCGGCACCA TCCCGGCCGC CCTGACCTAC TACTGGCGCA
2101  TGAAGATGCC CGAAACTGCA CGGTACACAG CACTCATCGC CGGCAACACG
2151  AAGCAAGCCA CATCAGACAT GTCCAAGGTG CTCAACAAGG AGATCTCAGA
2201  GGAGGCTGGG CAGGGTGAGC GGGCCACTGG TGATACCTGG GGCCTCTTCT
2251  CCAGGCAGTT CATGAAGCGC CATGGGGTGC ACTTGCTAGC GACCACAAGC
2301  ACTTGGTTCC TGCTCGATGT GGCCTTCTAC AGCCAGAACC TGTTCCAGAA
2351  GGACATCTTC ACCAAGATCG GGTGGATCCC GCCGGCCAAG ACCATGAATG
2401  CATTGGAGGA ATTGTACCGC ATCGCCCGTG CCCAAGCGCT CATCGCGCTC
2451  TGCGGCACGG TGCCGGGCTA CTGGTTCACC GTCGCCTTCA TCGACATCAT
2501  CGGGAGGTTC TGGATCCAGC TCATGGGATT CACCATGATG ACCATTTTCA
2551  TGCTTGCAAT CGCGATACCG TATGACTACT TGGTGAAACC AGGGAACCAC
2601  ACCGGCTTCG TCGTGCTTTA TGGGCTCACT TTCTTCTTCG CCAACTTCGG
2651  CCCCAACAGC ACAACCTTCA TCGTACCGGC AGAGATCTTC CCGGCGAGGC
2701  TCCGGTCAAC ATGCCACGGT ATATCGGCCG CAACCGGTAA GGCGGGCGCG
2751  ATCATCGGCG CATTCGGGTT CTTGTATGCG TCGCAGGACC AGAAGAAGCC
2801  TGAGACAGGG TATTCACGGG GAATCGGCAT GCGCAACGCC CTCTTCGTGC
2851  TCGCTGGCAC AAACTTTCTG GGCCTGCTCT TTTCCCTGCT GGTGCCGGAG
2901  TCCAAGGGCA AGTCGCTCGA GGAGCTCTCC AAGGAGAACG TCGGCGATGA
                                    Sense primer → RT-PCR
2951  TGGCATCGAT GCTTAGGCTG GTGCACATCT GGAGACACAG AGTCATGCAC
       Sense primer → real-time RT-PCR
3001  AAGTGTTTCC TTTCTCCTTG CAGCATTTCT TTCCTTCTGT GTGGCCACTT
            ← Anti sense primer real-time RT-PCR
3051  CCTGAATTTG TGGTGTCGCT TACCGTTCGT GTGTTGCTGT TTCTGGCTGG
3101  ATTTTGCCTA AACCCAGCGA GATTGCAATT TTCTGATGGT GCTCTGTATT
                                  ← Anti sense primer RT-PCR
3151  CGTTTGTGAA GAATTGCCAA TAAAATATCC CCTTCATTTG ATTTGATTCC
3201  AA   3′
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HvPT2

Accession number = AY187019
HvPT2 total gene = 3063 bp
Promoter = 1295 bp
cDNA = 1578 bp = 525 amino acids
3′ untranslated region = 190 bp

```
5′      1  GAGCTCAATT AACCCTCACT AAAGGGAGTC GACTCGATCC TTTTTTTAGA
       51  AAATGAGGAT TATCCACCGC CTCTCTTCAT TTTTTTTAAC TGAGTCAAAA
      101  GCTTTGTCTC ATCCATTAAA TAAAAGAGAA TAGAGTTTTT TACATCCCGC
      151  CTCTCTTCAT ATGCAGTCAT AATAACTGCA AGACCTACCA CTGCATATCC
      201  ATTTATCATT GATGTTTTGT TTTCTACTTT TGCTTTCCTT CTTATTCTCG
      251  AGCAACCGGC TAGAGCTTTG CCTTTTCATA CTATAAAACA AGATAATTGT
      301  CCAATTAATA TGAAAGACTG GCCGAAAACC GATATAACGA GCCATACAAA
      351  CGACATCCCC GTGGCCACAC CCCCAATGGC CCGACTACTC TATCACCCAA
      401  GAAATCCTAG TCGACACCCT ACCTAATGAT ACAGAGTCGT CACTCCGGCT
      451  TACAAACATC AGAAACACAC CACACTGGCC TCGAGGCCGC GCTCACCCAA
      501  CAGGTCGACG ACTCAACTAC GCGAATAGAT TCTCAAACCA CACCAAGGTG
      551  ATCTCCGGAG CCGCCACTCC GCTTCCTCAC CAGCCCCGAG GCTACACACC
      601  CGATGATTTG TTGAGAGCAC CACCCAAGCG ACAGAACAAA CATCTTTCAT
      651  TACAAAAGCA ATCTCCGGAG CCGCCGCTCC GGCTTTGACA CTGGCCCCAA
      701  GGAGACACAC ATGCCTAGTC GATAGCGACA CGCCATGAGA ACACGAGCCA
      751  CAAGTGCCTA CCTCTTTCTG CCATACCATG TAGTTATAGA GGAGCGACCA
      801  AATTAACCCC ATCTCTGAAC AAAGGTCACC AAATCCATGT TTGTAGTGAG
      851  AACATAAGAT ACAACTCATC GTATAATCTT GACCTTTTTC TCACAAAGCT
      901  TGCTTGTGTC TGTACAATCT GTTCCTTTGA GGTATATTCC AAGCATAACC
      951  ATGATTTGAC TTCAGTATTT TTATTTCCCA AATTCTATAG TACTGAAATT
     1001  TGAAGGTCAA ACCTGATAAA TTAAAGTTTA GCATTCCTTA ATCCTTCTAA
     1051  GAACCATCCT GATATGTACA ATTCGTTGTA CTTACAAGCG ATGCCGTACA
     1101  TATCATATTT TATACTTGCA ACTAGCAGTG TTAGATTGGC ATACCACAAC
     1151  ATATGTCCTT ACCTGTATGC CTTCACCCTG CATTATGGCT ATATATACGT
     1201  ACCAGATGAG CACCGAATAA AGCACACCAG AAGAGAGCAA AAAAAGAAGA
     1251  AAGATAGAAG GGGGCAGGAG TTAAGCTGAG AGATCGCCGG CGGCCATGGC
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1301  GACTGAACAG CTCAACGTGT TGAAAGCACT CGATGTTGCC AAGACGCAAC
1351  TGTACCATTT CAAGGCCGTG GTGATCGCCG GCATGGGCTT CTTCACCGAC
1401  GCCTACGACC TCTTCTGCAT CGCGCTTGTC ACCAAGCTGC TGGGGCGCAT
1451  CTACTACACC GATCCTGCTC TCAACGAGCC CGGCCACCTC CCGGCAAACG
1501  TGTCGGCCGC CGTGAACGGC GTGGCCCTAT GCGGCACACT TGCCGGCCAG
1551  CTCTTCTTCG GCTGGCTCGG TGACAAGCTC GGCCGCAAGA GCGTCTACGG
1601  CTTCACGCTC ATCCTCATGG TCCTCTGCTC CATCGCGTCG GGGCTCTCGT
1651  TTGGACACGA GGCCAAGGGC GTAATGGGGA CTCTATGTTT CTTCCGCTTC
1701  TGGCTCGGCT TCGGCGTCGG CGGCGACTAC CCTCTGAGCG CCACCATCAT
1751  GTCCGAGTAT GCTAACAAGA AGACCCGCGG CACCTTTATC GCCGCCGTGT
1801  TTGCCATGCA GGGGTTTGGC ATCCTATTTG GTACTATTGT TACCATCATC
1851  GTCTCGTCCG CATTCCGACA TGCATTCCCT GCACCGCCAT TCTACATTGA
1901  CGCCGCGGCA TCCATTGGTC CGGAGGCCGA CTACGTGTGG CGCATCATCG
1951  TCATGTTCGG CACCATCCCG GCCGCCCTGA CCTACTACTG GCGCATGAAG
2001  ATGCCCGAAA CTGCACGGTA CACAGCACTC ATCGCCGGCA ACACGAAGCA
2051  AGCCACATCA GACATGTCCA AGGTGCTCAA CAAGGAGATC TCAGAGGAGG
2101  CTGGGCAGGG TGAGCGGGCC ACTGGTGATA CCTGGGGCCT CTTCTCCAGG
2151  CAGTTCATGA AGCGCCATGG GGTGCACTTG CTAGCGACCA CAAGCACTTG
2201  GTTCCTGCTC GATGTGGCCT TCTACAGCCA GAACCTGTTC CAGAAGGACA
2251  TCTTCACCAA GATCGGGTGG ATCCCGCCGG CCAAGACCAT GAATGCATTG
2301  GAGGAATTGT ACCGCATCGC CCGTGCCCAA GCGCTCATCG CGCTCTGCGG
2351  CACGGTGCCG GGCTACTGGT TCACCGTCGC CTTCATCGAC ATCATCGGGA
2401  GGTTCTGGAT CCAGCTCATG GGATTCACCA TGATGACCAT TTTCATGCTT
2451  GCAATCGCGA TACCGTATGA CTACTTGGTG AAACCAGGGA ACCACACCGG
2501  CTTCrTCGTG CTTTATGGGC TCACTTTCTT CTTCGCCAAC TTCGGCCCCA
2551  ACAGCACAAC CTTCATCGTA CCGGCAGAGA TCTTCCCGGC GAGGCTCCGG
2601  TCAACATGCC ACGGTATATC GGCCGCAACC GGTAAGGCGG GCGCGATCAT
2651  CGGCGCATTC GGGTTCTTGT ATGCGTCGCA GGACCAGAAG AAGCCTGAGA
2701  CAGGGTATTC ACGGGGAATC GGCATGCGCA ACGCCCTCTT CGTGCTCGCT
2751  GGCACAAACT TTCTGGGCCT GCTCTTTTCC CTGCTGGTGC CGGAGTCCAA
2801  GGGCAAGTCG CTCGAGGAGC TCTCCAAGGA GAACGTTGGC GACGATGACG

2851  CCATTGCCCC AACTGGTGTC TAAGACATGC AGGTGTACTT GCACACTCGT
                Sense primer → RT-PCR
      Sense primer → real-time RT-PCR
2901  GCATTATTGG TGTGTTTGAT ATGATTTTCT GTGTTAATTC TGCGGCTTGT
                          ← Anti sense primer real-time RT-PCR
2951  TTTGTTGCTG TAATTACTCT CTGAAATTTA ATTGTTGTAA TGCCGTGACA
                                                ← Anti sense
3001  ATGTAATGCT TAAACTGTAA AAGACAACGA TTCAATTTTG AAAGAACAAA
      primer RT-PCR
3051  AATATCATCT GTA  3'
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HvPT3

Accession number = AY187026
HvPT3 total gene = 6561 bp
Promoter = 4841 bp
cDNA = 1611 bp = 537 amino acids
3' untranslated region = 108 bp

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5'      1  ACTAGTGAAT CAAAGGTTCC TTTAGAACTT GTGTTTTCGG ATGTATGGGG
       51  TCCTGGCCCA ATCTCGGTTG GTAGACAAAA GTATTACGTG AGCTTTATTG
      101  ATGATTTTAG TAAATTTTCT TGGATCTATT TACTCAAAAA TAAGTCTGAT
      151  GTTTTTGAGA TGTTTCATCT GTTTCAACAG CTTGTTGAAC TCCTCTTTAA
      201  TCGCAAGATT TTGTCTATGC AAACCAATTG GGGGTGAGTA CCAAAAGCTT
      251  AACTCCTTCT TTGAGTGCAT TGGTATCTCC ACCATGTTTC CTGCCCTCAT
      301  GCTCATCAAC AGAACGAATC TGCCGAGCGC AAACATTGCC ATATTGTTGA
      351  GGTTGGCTTG TCCCTGCTCG CTCATGCCTC TATGACATTG AAATTTTGGG
      401  ATGAAGCGTT TCTTACAGCG GTCTATCTTA TCAACCGTGT CCCTAGTCGA
      451  GTCATCCACC ACCAAACTCC ACTAGAACGC ATGTTTGATA TTAAACCAAA
      501  CTATAACTTT CTTCACATTT TTGGTTGTGC GGTATGGCCA AATCTACGGC
      551  CTTTCAACAA ACACAAGCTC GAATTCCGTT CCAAACTGTG CGTATTCATA
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 601  GGATACAGCA ATCTCCACAA AGGGTACAAG TGTCTTGATG TTTCCTCTGG
 651  CCGGGTTTAT ATTTCCTGCG ATGTTGTTTT TGATGATCAC ATCTTCCCTT
 701  TCGCCACCTT ACATCCAAAT GCCGGCGCTC AACTCCGCAA GGAGCTCATA
 751  CTTCTTCCGC CCAACCTTCT ACCTTTGTCC GGTCCTTTAC CACGGGGAGG
 801  AGTAGATTTT GATCATATGT CTATATCTCA TAACCCTGGT GCAAGTGTGC
 851  AGGAACATAC GGAAGAAGAA ATCGCCGAAA ACGGCCTTGA TTTTATGCAG
 901  CAACCAGATC ACAGCGGTGC AACAAATCCT GGTGGAGATC CTGATGCTGA
 951  TTCTGGCGCA GAATCTGCCT CGGAGTCACG CGCTGCAACT GCAGCAGACA
1001  GATCCTCCCC GGGATCAGCG CCATCGCCAG GCCGGGCAGG CGGATCCTCT
1051  CCGGGTCTCG CGCCAGCACC AGGTGGGTCG GGCGGGCCCT CGGTAGGTGG
1101  ATCTCCTTCG GCCCCGCGCT AGCACCAGGC AGGACGGACG GGCCACATGC
1151  ACTGTCCCCC GCGTGCCCCT CCCGACACTG GTCACACGCA TGCACCCACT
1201  CCGGAGCCTC CAAGTGGCGG CACTGCGGCT GATCTGCATG GCGGATCTTC
1251  TACGACTGAT GCAACCGATG CTTCTCCCGT GCATCAAACT CGcCTCCATC
1301  AACATCTcTC TCGACCACCG CCGCCACCAC CTGATCGACT CCAAACCAGG
1351  TCTCGTAGTG GCATTATTAA ACCTAAAGTT TATAAAGATG GTTGCGTACG
1401  CTGGGGTTCT TTCTGTTCTA CAGGTGAACC GCAAACTCTG GATGAGGCCC
1451  TTAGTCAGTC ACAATGGAAG GCTGCTATGG ATGAGGAGTA TTCTGCTCTT
1501  ATGGAGAACA ACACATGGCA ACTTGTTCCT CCTGTCAAGG GCAGAAATGT
1551  TATTGGCTGC AAATGGGTCT ATAAAGTTAA AAGGAAGTCT AACGGCACCA
1601  TTGACAGGTA CAAGGCTCGG TTGGTTGCAA AAGGGTTTAA GCAAAGGTAT
1651  GGACTTGACT ATGAGGATAC TTTCAATCAT GTAGTTAAAG TTGCCACTAT
1701  CAGAATTGTT CTTTCAGTAG CAGTATCTAG AAGCTGGTGC ATACGGCAAT
1751  TAGATGTGAA GAACGCGTTT TTGCATGGTG TTCTGGAAGA AGAAGTGTTT
1801  ATGAAGCAAC CTcCTGGATA TGAGAATCCA CAGTTACCAC AACATGTTTG
1851  CAGGCTTGAC AAgGCCTTGT ATGGTCTCAA ACAAGCACCA AGAGCTTGGT
1901  ACTATAGGTT GTCTTCCAAA TTGCAGCATT GGGTTTTATG CCCTCAAAGG
1951  GTGACACTTC ATTGTTCTTT TATCATAGGA AAGGAGTCAC TATTTATATG
2001  CTCATTTATG TTGATGATAT AATTGTCACC AATTCATGTT CCCAGGCTGT
2051  TGAAGCTCTT CTCAAGGATT TGCGCATGGA TTTTGCTCTC AAAGATCTTG
2101  GTGATCTCCA CTACTTCCTT GGCATTGAGG TAAAACATGT GGCAAGTGGC
2151  ATTGTGCTAT CACGGGAGAA ATATGTGCAG GATATACTCC AGAGAGCAGG
2201  AATGAAGAAT TGTAAGCCAT CTCCTACTCC TTTGTCAACT TCTCAAAAAC
2251  TGTCACTTTA TTCTGGGAGG GTACTTGTGC CAGAAGATGC TACCAAGTAC
2301  AGAAGTGTTG TAGGAGCCCT ACAATACTTA ACATTGACTA GGCCATATAT
2351  CTCATACTCA GTGAATAAAG GATGGTAGTT CTTACATGCT CCAACCAGNG
2401  GACACTTTTT GTCACGCCCA AGATGCGACC CTATCCTTAA ATTTGGCACC
2451  GAGAAGCATC ATCGGGGATA GAAGCGCATC TCGTCGTGTC GCAtGAATGG
2501  ATATCGGTTA CAAGTACATG gTACTGAAAG GAAGAGATAT ATAATAGAAT
2551  TGGGCTTACA CTCGCCACAA GCTACATCAG AGTCACATCA GTACATTACA
2601  TAATCATCAA GGGTAAGAGC AGGGTCCGAC TACGGACGAA AACAACCGAG
2651  AAAAGAAGAA CGACGTCCAT CCTTGCTATC CCAGGCTGCC GGTCTGGAAC
2701  CCATCCTAGA TTGATGAAGA AGAAGAAGAA GAAGAAGAAG AAGAAGAAGA
2751  AGAAGAAGAA GCAACTCCAA ATAAACAATC CACGCGCTCG CGTCAAGTAA
2801  CCTTTACATG TACTTGCAAC TGGTGTTGTA GTAATCTGTG AGCCATAGGG
2851  GACTCAGCAA TCTCATTTCC AAAGATATCA AGACTAGCAA AGCTTAATGG
2901  GTGAGGCATG GTTAAGTGGT GAGGTTGCAG CAGCGGCTAA GCACATATTT
2951  GGTGGCTAAA CTTACGAGTA CAAGGAATAA GAGGGGATGA TCTACGCATA
3001  ACGTAGTGAA CTACTAATGA TCAGATGAAT GATCCTGAAC GCCTACCTAC
3051  GTTAGACATA ACCCCACCGT GTCCTCGATC GGAGTAAGAA CTCACGAAAG
3101  AGACAGTCAC GGTTACGCAC ACAGTTGGCA TATTTTAATT AAGTTAACTT
3151  CAAGTTATCT AGAACCAGTG TTAAACAAAG CTTCCACGTT GCCACAATTT
3201  TAGACTATGG TCTAAATACA TGTAGCTAGC GGGTTAGGTT TAGGGACATC
3251  TGGACCCTCA GATTTAGATC GGGTGGTCAA GATGATTAGG TTAGGGAGCC
3301  CAATGGACAA ACCGAAGACG GCTTGCGGTA AAACAGGGTT GATCCGGATA
3351  CAACGGTCAC GACCGTATGT TTCGGGTACC GAGAGGTTTT CGAACTAGGC
3401  TGCGCGTAGG GTCGATGCAC TGTGCAGAGG GGCTAGGCGG AGATTAGAGG
3451  GAAAACGGGC GACCCGGCGA CGATTTTTAA AACACCGACA ACCGTCCGAC
3501  GGTAGACCGA ATACGGTGCC GCTACGGTCG ACCGTTCGGG TACCAGACGG
3551  ACTCCGATCG CGACGAAATT CGACAGGCAG CCTAGCTATA TCTAATTACG
3601  ACCGCATGCC AAGTTTCACC TCGATCAGAG AAAGTTTTAT GCACACTTTT
3651  GAAAACAAGA TTTGACGATG TCGCGGGCGC GTGCGAGTGC GGTCGGGCTC
3701  AGAACGGACA ACGACGAGAA CCGGCAACTA ACAACGGATG CAAGTTTTGA
3751  AAACTGGCGG CAACGGAATG CTGATGCAAT GCAGATGATT CGAATGATGC
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3801  GATGATGATG CGACAAAAGA AAATAGACAC ACGACGAAAA CGGAATAAAG
3851  GGGGGATCTT CTGGAACGTC GGTCTTGGGC TGTCACAACT TTGCAGCTGT
3901  CAAAAGAATA CTTTGGTATC TTCAAGCAAC CAAGGGCCAT GGACTTAAGC
3951  TTGGTAGGTC AGACTCAATG CTAGTCAGTG CCTTCTCTGA TGCAGATTGG
4001  GCAGGATGCC CTGATGACAG GAGATCAACA CGGGCAGGAT GCAGATTGCT
4051  AAGTCTTCTT AGGCAGCAAC TTAGTTTCCT GAAGTGCTCG CAAGCAAGCT
4101  ACTGTATCCA GGTCAAGCAC GGAAGCTGAA TATAAAGCAC TAGCAAATGC
4151  TACCGCTGAA ATCATATGGG TGCAGAATAT GTTGATAGAA TTGGGTGTTT
4201  CACACCCATC ATCAGCATCT CTTTGGTGTG ATAATCTTGG TGCCACGTAC
4251  TTATCTGCTA ATCCTATCTT TCATGTCAGG ACTAACACAT ATCGAGATTG
4301  ACTATCACTT TGTTCGTGAA AGAGTAGCCA GCAAACAATT AAACATCCGG
4351  TTTGTACTCA CTGGAGATCA AGTGACAGAT GGTTTTACTA AACCATTGAC
4401  AGCACAACAA CTAGCTTCAT TTAGACACAA TCTTAACTTA GATAGTTTCG
4451  ATCGAGGAGG AGTGTTGGAA GTTGTAATCT ACGGTATGTA TAAACCGTAT
4501  AGAGATAACT TAGACTTGGA GATAAGTTAG TTTAAACCAT CTATACCGAA
4551  GAGATATGAC TTGAAGATCA ATCCTCGACA TAACAAACTT TGTATATCTT
4601  ATGCTATATA TTAACACGCA TCGCATCGCG TTCGTGCAAG CCATACGGTT
4651  AACCTAGCTT TTCCACGCTG CGGCCGGTCT CCTCCTCCTC GCCCTATTTA
4701  TACGAGCAGT AGGCGGCCCA TTATTTCTGC ACCACAACAC AACAAAGTCT
4751  TCCGGCCGGC GGGCACCGTC GTCTAGCTCT CACACTCGCA GCGTGCCGCG
4801  GCCAAACGTC AGTCCCCTGT GCAGCAACAG CAGCAGCAGC ATGGCGCGGT
4851  CGGAGCAGCA GGGGCTGCAG GTGCTGAGCG CGCTGGACGC GGCCAAGACG
4901  CAGTGGTACC ACTTCACGGC CATCGTCGTC GCCGGCATGG GCTTCTTCAC
4951  CGACGCCTAC GACCTCTTCT GCATCTCCCT CGTCACCAAG CTCCTCGGCC
5001  GCATCTACTA CACCGACCTC TCCAAGCCCG ACCCCGGCTC CCTGCCCCCC
5051  AGCGTCGCCG CCGCCGTCAA CGGCGTCGCC TTCTGCGGCA CCCTCGCCGG
5101  CCAGCTCTTC TTCGGCTGGC TCGGCGACAA GATGGGCCGC AAGAGCGTCT
5151  ACGGCATGAC CCTCCTCCTC ATGGTCATCT GCTCCATCGG CTCGGGCCTC
5201  TCCTTCGCGC ACACACCCAA GAGCGTCATG GCCACGCTCT GCTTCTTCCG
5251  CTTCTGGCTC GGCTTCGGCA TCGGCGGCGA CTACCCGCTC TCGGCCACCA
5301  TCATGTCCGA GTACGCCAAC AAGAAGACCC GCGGCGCATT CATCGCCGCC
5351  GTCTTCGCCA TGCAGGGCTT CGGCATCCTC GCCGGCGGCA TCGTCACCCT
5401  CATCATCTCA TCCGCCTTCC GCGCCGGGTT CCACGAGCCG GCCTACCAGG
5451  ACGACCGCGT CGCGTCCACC GGCACGGAGG CCGACTTCGT GTGGCGCATC
5501  ATCCTCATGC TCGGCGCCCT GCCGGCCCTG CTCACCTACT ACTGGCGGAT
5551  GAAGATGCCC GAGACGGCGC GCTACACCGC CCTCGTCGCC AAGAACGCCA
5601  AGCTGGCCGC CGCCGACATG TCCAAGGTGC TGCAGGTGGA GCTGGAGGAC
5651  GAGACGGAGA AGATGGACGA GATGGTGAGC CGCGGGGCGA ACGACTTCGG
5701  CCTCTTCTCG CCGCAGTTCG CGCGGCGCCA CGGCCTCCAC CTCGTCGGCA
5751  CGGCGACCAC GTGGTTCCTG CTGGACATCG CCTTCTACAG CCAGAACCTG
5801  TTCCAGAAGG ACATCTTCAC GAGCATCAAC TGGATCCCCA AGGCGCGCAC
5851  CATGAGCGCG CTTGACGAGG TGTTCCGCAT CTCCCGCGCG CAGACGCTCA
5901  TCGCGCTCTG CGGCACAGTG CCGGGCTACT GGTTCACGGT CTTCCTCATC
5951  GACGTCGTCG GCCGCTTCGC CATCCAGCTC ATGGGATTCT TCATGATGAC
6001  CGTCTTCATG CTCGGCCTCG CCGTGCCGTA CCACCACTGG ACAACGCCGG
6051  GCAACCAGAT CGGCTTCGTG GTCATGTACG GCTTCACCTT CTTCTTCGCC
6101  AACTTCGGGC CCAACGCAAC CACCTTCGTC GTGCCGGCGG AGATCTTCCC
6151  GGCGAGGCTG CGATCGACGT GCCACGGGAT ATCGGCGGCC GCGGGGAAGG
6201  CCGGAGCCAT GATCGGGGCG TTCGGGTTCC TGTACGCGGC GCAGGACCCG
6251  CACAAGCCGG ACGCCGGGTA CAGGCCCGGG ATCGGGGTGC GCAACTCCCT
6301  CTTCGTGCTC GCCGGGGTCA ACCTGCTGGG GTTCATGTTC ACCTTCCTGG
6351  TGCCGGAGGC CAACGGGAAG TCGCTGGAGG AGATGTCCGG CGAGGCACAG
6401  GACAACGAGA ACGAGGACCA GGCACGAACC GCCGCCGTGC AGCCGTCCAT
                    Sense primer →
6451  GGCCTAGGAC AACTCGTGCG TGCTAGCTAT TGCAGCTGCA GGCTGTTGAG
6501  TTGGTCGAAG ATCCTTAATT TGGTTTTTGT GATACATATA AACGCTTAAA
6551  CTACTACTAG TATGATATGT TTTGCGGGCC ACCCAATCAG GGCCGGCTCA
6601  TATATATACA CAGGCCGGCT CATGTCTATG CC  3'
                  ← Anti sense primer
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HvPT5

Accession number = AY187021
HvPT5 total gene = 3224 bp
Promoter = 1318 bp
cDNA = 1620 bp = 540 amino acids
3′ untranslated region = 286 bp

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5′      1  AAACGTATGC TTTACCAGCA CGGACGTATG CGTGTTATAT AGGGCATAAA
       51  GCCAACCGAA TACAAAGATT ACATGAGATA GAAGTGGTTA GGATTGATCT
      101  TTATCTATAC AAGATCTATC TACAAGATCA ATCCTAATAT TCTATCTCTA
      151  AATTAAACAC ACGCACCCCT AAGATAATTA CAATGTCTAA CAATAATCTT
      201  ATCTTACCTA CTAATAAAGC AAATATTGCT TCCGTCGTAC GTCATTTAAA
      251  TTGTTCTTAA AGTTGGCTAA AATTACCCAC CAATGCCACT CATAGAAAAC
      301  GTTTCAAACA GGAAAATCTC CGGACTGGGC CGGCCCATGT AGGCGTCTCC
      351  TATATTACGC TCTGGGAATT AGAAAAAGAT GCAGCACCCA AATGCGCCCA
      401  TGTGAGTGCC TGTTTTTTTT AGTTTAGTTT TAATTTTTTT TCTTTTCAGT
      451  TCCATTTTGT TTTTCTACTT TAAATAATTT AGAACTTCAA ATAACTTTTC
      501  TTAATTTTAA GAAACTAGGA ATTTCGAACT AAAATATTCA AAAAAAACAT
      551  AATTTTTTTG AGAATTCAAA AAGTACTCAG GAGTTTTGAA AAATGTTTGC
      601  ATATCAAAAA ATGTTTAAAT TTTGGGAAAT TGTCCATAAA ATAAAAAAAA
      651  GTCAATGATT TTAAACAAAA GTCTGTGTAA AAATCTTAAA AACAGTTCGT
      701  GCCTCTGTTT TTAGTCTCGT TTTTTATTTT CATTTTTTGT CCCATTTTCT
      751  AATTTAAATA ATTTAGAACT TTGAAAAACT TTTGCAATTT ATAAAACTTA
      801  GAATTTGAAA TAAAATTTTG AAGAAAACAT AAAATGTTTG TGAGTTTAAA
      851  AAATGCTCGG GATTTTTGTA AAAATATTCG CATATTCAGA AAAATGTTCA
      901  TAATTTTGAG AACAATGTTG CTAAAAATAA TAAAATTCTA TGATTTTGAA
      951  AAACAAGTTT GTGTATTATT TTTTGAGCGC AATTAAAAAA TGTTTGCTAA
     1001  TTCAAAAAAT GTTCATGCAT TTCAAGAAAG GTCTTAAAAT TTAAAAGACA
     1051  TAATATGATC AGCACGATTG GAGATTATAA TTGTTTTTCT TCCGTTGCAA
     1101  CGACACCCTT TTGCTAGTAA AACGAATTAC CAAATCGTGA TCTGTCCGGT
     1151  TTTCCGTAGC TGTATCCGAG CAAGTCTTCC AACTCCATGC CGGACTCGGT
     1201  TTTGTCACCA AATCTATATA AACATTCCGC TCTATCGCCG GGGCACAGCA
     1251  GGTTGGCAGC GACAGATCAA AGCAGCAACA CGAGCTTTGG CACGAGACAT
     1301  CCAACACCCA GCGGCGCGAT GGCGAGCCGG CAGCAGCAGC AGCTGCAGGT
     1351  GCTGAGCGCG CTGGACGGCG CCAAGACGCA GTTGTACCAC TTCAGGGCGG
     1401  TCGTCGTCGC CGGCATGGGC TTCTTCACCG ACGCCTACGA CCTCTTCTGC
     1451  ATCTCGCTCG TCACCAAGCT CCTCGGCCGC ATCTACTACG CCGACCCCTC
     1501  CAGCCCCAAC CCTGGATCGC TGCCGCCCAA CGTGGCCGCG GCCGTCAACG
     1551  GCGTCGCGCT CTGCGGCACC CTCGCCGGCC AGCTCTTCTT CGGCTGGCTC
     1601  GGCGACAGGC TCGGCCGGAA GAGCGTCTAC GGCATGACGC TCATCCTCAT
     1651  GGTCGTCTGC TCCGTCGCCT CCGGGCTCTC GTTCGGGCAC ACCCCGGCCA
     1701  GCGTCATGGC CACGCTCTGT TTCTTCCGCT TCTGGCTCGG CTTCGGCATC
     1751  GGCGGCGACT ACCCTCTGTC CGCCACCATC ATGTCCGAGT ACGCCAACAA
     1801  GAAGACGCGC GGAGCCTTCA TCGCGGCCGT CTTCGCAATG CAGGGCTTCG
     1851  GCATCCTCGC CGGCGGCGTC GTCACGCTAG TCCTCTCCAC GGTTTTCCGT
     1901  AGCGCGTTCC CGGCGCCGGC GTACCAGACC GACGCCGCAG CGTCCACCGT
     1951  GCCGCAgGCC GACTATGTGT GGCGCATCAT CCTCATGCTC GGCGCGCTGC
     2001  CTGCGGCGCT CACGTACTAC TGGCGAACGA AGATGCCGGA GACGGCGCGG
     2051  TACACGGCGC TGGTCGCAAA GAACGCGAAG AAGGCCTCGC TGGACATGTC
     2101  CAAGGTGCTC CAGTCGGAGG TCGAGGCGGA GCCGGAGAAG CTGGACGAGA
     2151  TCATGGCCAG AGGCGAGGAC TACGGCCTCT TGACGTCGCG GTTCGCCAAG
     2201  CGCCACGGCC TCCACCTGCT CGGCACGGCG ACGGCGTGGT TCCTGGTCGA
     2251  CGTCGCGTAC TACAGCCAGA ACCTGTTCCA GAAGGACATC TTCGGCAGCA
     2301  TTGGCTGGAT CCCCAAGGCG CGCACAATGG ACGCGCTCGA GGAGGTGTTC
     2351  CGCATCTCCC GCGCGCAGAC GCTCATCGCG CTCTGCGGCA CCGTGCCGGG
     2401  CTACTGGTTC ACCGTGTTCC TCATCGACGT CATCGGAAGG TTCTGGATCC
     2451  AGCTCGTGGG GTTCGCCATG ATGGCTGTTT TCATGCTCGG CCTCGCGGTG
     2501  CCGTACCACC ACTGGACGAC GCCGGGCAAC CACGTCGGCT TCGTCGTCAT
     2551  GTATGGGCTC ACCTTCTTCT TCGCCAACTT CGGGCCGAAC GCGACGACGT
     2601  TCATCGTGCC GGCCGAGATC TTCCCGGCGC GGCTCCGGTC GACCTGCCAC
     2651  GGCATCTCCG CTGCGGCGGG GAAGGCCGGT GCCATCATCG GGGCGTTCGG
     2701  GTTCCTCTAC GCGGCACAGT CGCCGGACCt GGCGCACGTG GACGCCGGGT
     2751  ACAAGCCTGG GATCGGCGTG CAGAAGGCGT TGTATGTGCT CGCTGCGTGC
     2801  AACCTCCTGG GGTTCTTGGT CACGTTCCTC GTGCCGGAGT CGAAAGGGAA
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```
2851  GTCGCTCGAG GAGATGTCCG GCGAGGCCGA CGCCGAGGAA GGCAACGGCG
2901  CCAATAACGT TCGCCCGTCG GGAGAGCAGC TGGTTTGAAT CGCGAGAAAG
                                                Sense primer →
2951  CAACACGCGC ATACGTGCAT GTTTGCGCAT GCGACGAGTT TTTGTGTCGT
3001  GACTACTGTA GAACTTTGGG TAGGTGTGTA TTACACTACA CTTAATCTTA
3051  GCCATAGCCA AAAGGTCGAC ACTAACGTGT AATTTTTTTA AAAGAGTGCG
3101  TAGAACTCAT CTATATGAAA TATATTTTGG TCTCATTCAC ATTTTTTTAA
3151  AGAGTGCCTA GAACTCATCT AGTGGGATAT AGTTTGATCG AGTCGACTCC
        ← Anti sense primer
3201  CTTTAGTGAG GGTTAATTGA GCTC  3'
```

HvPT6

Accession number = AF543198
HvPT6 total gene = 2876 bp
Promoter = 916 bp
cDNA = 1608 bp = 536 amino acids
3' untranslated region = 352 bp

```
5'     1  ACTAGTTATA CAGGTAGTCT ATTCCCTATG GGATTTTACC ATGATGAttc
      51  GTCAAAATTT ATAGATGATT ATCAATATTG CATTTTTACT GTCGCAACTG
     101  TCCGCTGATG ATTGATTTAT TTGATACTGT TTATGCATAG GTATTATTGG
     151  CGTTATGAAA ACAAATTTAG TTCGGCGACA AGCTGATAGT CGCAAAGGAG
     201  GAATTAAACG AGAGGCTTTA CATACCAAAG CAAGTAATCA TGGGCATTTA
     251  AATCATCTAC GATTCCGGCC GGTGTTTTAT CACCTGCTTG AACTGCTGCA
     301  GCGCATCACT TCTTGCTACC ACCTTAAACT GCTAGCTCTT CAAAAGGAGG
     351  TTTCTAGCAA ACTCTGCTGA CACACATAGC CATCCAAACC ATCTTTGTGC
     401  ACACAAGAAA CATGAGTAGG TCGAGAGAAG CCAAAGGGAT CTTCCAAATT
     451  TCGTCTTTCG AACTAGCAAG TTATGGTGAG TGGAGGCGGT ATCCGAAACT
     501  GAAAAAATGC GGATCAACCA AATCCCACAG TAACTGCCTG CAGGAGTCAC
     551  CTTTTGATGG GATGTCACGT CACCTTATTA CTTGCCTCCA TTTTTTTTGT
     601  TTTTCATGCC TCCTTGATCG TGACAGCCTT CATTTATTTC CTCTTTTCCA
     651  TCCTCACCTT ACCAAGATAC CACAAAATAA ATTATAGTTT TCATTTACTA
     701  GAAAATAAAA AAAATAGTTA GATTTTCCTT TTCACCAACA AAATCGGAAC
     751  AAGATAACTT ATTAGGGTGC GAATATTCCC CTTCAGGAGG AGGAGCCGCA
     801  GTATTTATCT TGTCCAACCC TCGCGGCTCG TCCCACCCTA AAATCTCTGA
     851  TCTTGTCTAT CTCTCAGCAC AACCAACAGC GATCCCCGGC GGCGGCGAGA
     901  GATAAGGCTC GTGGCCATGG CGCGCGAGCA GCTGGAGGTG CTGTCGGCGC
     951  TGGACACGGC CAAGACGCAG TGGTACCACT TCACGGCGAT CGTCATCGCC
    1001  GGCATGGGCT TCTTCACCGA CGCGTATGAT CTCTTCTGCA TCTCGCTCGT
    1051  CACCAAGCTG CTCGGCCGCA TCTACTACTA CCGCGAGGGT GCCGACGCCC
    1101  CCGGCTCGCT GCCGCCCAAC GTCGCCGCCG CCGTCAACGG CGTCGCCTTC
    1151  TGCGGCACGC TCTCGGGCCA GCTCTTCTTC GGCTGGCTCG GCGACCGCAT
    1201  GGGCCGCAAG CGCGTCTACG GCATGACCCT CATGTGCATG GTGCTCTGCT
    1251  CCATCGCCTC GGGCCTCTCC TTCGGCTCCA CCCCCGGCTC CGTCATGGCC
    1301  ACGCTCTGCT TCTTCCGCTT CTGGCTCGGG TTTGGGATCG GCGGCGACTA
    1351  CCCGCTCTCC GCCACCATCA TGTCCGAGTA CGCCAACAAG AAGACGAGGG
    1401  GCGCCTTCAT CGCCGCCGTA TTCGCCATGC AGGGCTTCGG CATCCTCACC
    1451  GGCGGCGTCG TCACGCTCAT CGTGTCCGCC GCGTTCCGCG CCGCCTTCCA
    1501  CGCGCCCGCC TACGAGAAGG GCGCCGTCGC ATCCACGCCC CCGCAGGCCG
    1551  ACTTCGTGTG GCGCTTCATC CTCATGTTCG GCGCCGTCCC GGCCCTGCTC
    1601  ACCTACTACT GGCGGATGAA GATGCCCGAG ACGGCGCGCT ACACGGCGCT
    1651  CGTCGCCAAG AACGCCAAGC AGGCCGCGGC CGACATGTCC AAGGTGCTCC
    1701  AGGTGGAGAT CGCCGCCGAG GACGAAACCA AGGACAACGA CGGGGCCGGC
    1751  GAAGACCGCA ACTCGTTCGG GCTCTTCTCC GGCGAGTTCC TTCGGCGGCA
    1801  CGGGCTCCAC CTCCTCGGCA CGGCCACCTG CTGGTTCCTC CTCGACATCG
    1851  CCTTCTACTC GCAGAACCTG TTCCAGAAGG ACATCTTCAC GGCGATCAAC
    1901  TGGATCCCCA AGGCCAAGAC GATGAGCGCC CTCGAAGAAG TGCACCGCAT
    1951  CGCGCGCGCG CAGACGCTCA TCGCGCTCTG CGGCACGGTG CCGGGCTACT
    2001  GGTTCACCGT GGCCCTCATC GACCGGATCG GGCGGTTCTG GATCCAGCTC
    2051  GGCGGATTCT TCTTCATGGC GGTGTTCATG CTGGGGCTGG CCTTCCCGTA
    2101  CCACCACTGG ACGACCCCGG GCAACCACAT CGGGTTCGTG GTGCTGTACG
    2151  CGCTCACCTT CTTCTTCGCC AACTTCGGGC CAAACTCCAC CACATTCATC
    2201  GTGCCGGCGG AGATCTTCCC GGCCAGGCTC CGGTCGACGT GCCACGGCAT
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2251  CTCCGCCGCC GCCGGGAAGC TGGGCGCCAT CGTGGGGTCG TTCGGGTTCC
2301  TGTACCTGGC GCAGAACCAG GACCCCAGCA AGGTGGACCA CGGGTACAAG
2351  GCCGGCATCG GGGTCAAGAA CTCGCTATTC ATCCTCGCCG CCTGCAACTT
2401  CCTCGGCATG GCCTTCACCT TCTGCGCGCC CGAGTCCAAC GGCATCTCGC
2451  TCGAGGAGCT CTCCGGCGAG AACGACGACG AGGCGCCGGC GCCGGCGACG
                        Sense primer →
2501  CACGCCAGGA CGGTGCCCGT GTGAGACGTC GCCGTACGTC AGATATACTA
                                          ← Anti sense primer
2551  GTATATGCTA CCGGTGGTAT ACTCTGCAGG TTTGGATGGA TGTATGGGTG
2601  TTTTCTTTTT CTTGGTTGGG ACGTTCAACT CAGGAGTGAG TACAACAACA
2651  ATACTACTAT TGTCATGTGT CAGAGTTCTC CATGTTATAA TTAATTAATT
2701  AGAGTTTATG GTCGAGTTAA TTATTATTAG TAATATATAT ACTACGTAGG
2751  TAATACCAGG GACGGAGCTA GCATTCATGC ATAGAGGAGG CAAGTTTGTT
2801  TATTTAAAGG GCAGAATTCA TATGAAGTGA ATTTTTTTTA CTACAATTAC
2851  TACAATCATA ATAGAAGAAC CAATTT  3'
```

HvPT7

Accession number = AY187022
HvPT7 total gene = 3700 bp
Promoter = 1347 bp
cDNA = 1584 bp = 528 amino acids
3' untranslated region = 769 bp

```
5'     1  AAGTTTTTCT TGGATGGTTT TTTATGNAAG GTTTTTAATG AGGCAATAGT
      51  AATGCAGACA TTGTGATATG TCAGTTTCTC CTTATTTTCC CACTGGGTTC
     101  TTGGAAGGAG TTCTTGATGG CATATTGTAT GTATCTCATC ATATTCTTCC
     151  TACATGGTTT TTGGAGGAGA CTAATCAAGT TGATGATGAT GATGGTGATG
     201  ATGATGTTAC AAGAATGATT GAAGCAGAGA TTAGGGAGAG TGTTAGGATT
     251  TAATTAATTA GTTTAATTAA AGGGAATCTC TGCCCGTGGA ACAGATAGGT
     301  AGTTTGGCTC CCGAACGATC ACATCCGAAT GTAACGGATG CGTCATTTTG
     351  ATCGTGGCTG TTGTAACCAT CGTGTCTGTG TGATGCGCCT CTTCAGGAGG
     401  TATATATATG TACATCATCG GTATCAATAA TTATCTATTG TTACTGTTCA
     451  TTAACAATTT TCTGAAAGAA TTGGTAATGA GTTGTTATGT GGTAACAATC
     501  CTCCATGTTA AACTTGCGGT AATAAAGTAT TGGGAAAGTT GTGAATGAGC
     551  CGGGATGTAT TTATACACAT TAACTTTAGA CATGATTTTG CCAAAAAAAT
     601  TACAAGGGGA TTCATGATAT TATAATATTT TTTGGAAAAT AGAAAACCAT
     651  GGTTACAGTA CAAGACAATA AAATGTTACA CATAAAACAT CAGGAAAACC
     701  CGATGGTGTC TGGTACAGAC AAGATGACGA TTGGTAACCT ATAAATATAA
     751  ATTCATAGAA ATGCCTATTT TTACCCTCAA ATGGCACAAC ATGTCACCAT
     801  GAAACTTTTC AAATCAAAGC AACTTTAATC AACTACTAGA AAAAAGCAAG
     851  TTTATCATCA GGGTGTATAC AATTTTATTC GGAATCCTGG TACACTGGTA
     901  GGTTACCCGC GAGCCCCTCT CAGGAGCTAA GACTGGCATG TTTTGGTTCA
     951  TGTTGTGACA AGTTTCATCA TGCGTGGCTA CAAACACAAC AAATATATGT
    1001  ACATTTTGTT TCGATATACT TGCTGCAGAC AAAACAAGAA TATGCCCATC
    1051  CATTATTCCT AGAATATGCT CGCAAAACAA AAGAGCTTCG CTATGCATGG
    1101  GAAACTCTGA GCCCATCCAT GTTTTCCTCT ACAAATTAAG AAGAGAACTG
    1151  TTAAACAGGG AGAAGAAGTT GGGCTCCAAG TAACGAAGGA CCAGGATCCC
    1201  GGAATATTCT CACCTCTCCG TCCGTATATA CATGGCTAAG TGACACCTGT
    1251  GCCTTCTCCC TCAGTTCACT CCCATCTTAA ACCTCAACAA CCTACCATCG
    1301  CGGCTGATCG CACGAGCAAT CTCGCCGCCG GCAGGTCGGA GCTGGCAATG
    1351  GCGGGCGACC AGGTACACGT GCTCGCGGCG CTGGACGGGG CCAAGACGCA
    1401  GTGGTACCAC TTCACGGCCA TCGTCGTCGC CGGCATGGGC TTCTTCACCG
    1451  ACGCCTACGA CCTCTTCTGC ATCTCCCTCG TCACCAAGCT CATCGGCCGC
    1501  ATCTACTACA CCGTCCCGGG CTCTCCCAGC CCAGGCAGCC TCCCGCCGAC
    1551  CGTCTCCGCG GTCGTCAACG GCGTGGCGTT CGTCGGCACG CTCTCAGGCC
    1601  AGCTCTTCTT CGGCTGGCTC GGCGACAAGG TCGGCCGGAA GAGCGTGTAC
    1651  GGCATGACGC TGATGCTGAT GATCATCTGC TCCGTCGCGT CGGGGCTCTC
    1701  GTTCGGCCGC ACGCCCACCA GCGTCATGGC CACGCTCTGC TTCTTCAGAT
    1751  TCTGGCTGGG CTTCGGGATC GGCGGCGACT ACCCGCTCTC CGCCACCATC
    1801  ATGTCCGAGT ACGCCAACAA GCGGACGCGC GGGGCGTTCA TCGCCGCCGT
    1851  CTTCGCGATG CAGGGGTTCG GCATCCTCGC CGGCGGCGGC GTGGCGATCG
    1901  GGATCACGGC GCTGTTCAGG GCCCTCTTCC CGGCGCCGCC GTACGCGGCG
    1951  GACCCGGTGG CATCCACCCC GGACCAGGCG GACTATGTGT GGCGCATCGT
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2001  GCTCATGCTC GGCGCGCTCC CCGCCGCGCT CACCTTCTAC TGGCGGATGA
2051  AGATGCCGGA GACGCCGCGG TACACGGCGC TCATCGCCAA GAACGCCGAG
2101  CGCGCCGCGG CCGACATGTC CAAGGTGCTC AACGTGGAGA TCACCAAGGA
2151  GCAGGCCGGC GACCTGGAGA CCGCGATCTC CATCAAGTCC CACACGTCGC
2201  CGTCGTTCGG CCTCTTCTCC AGGGAGTTCA TGCGGCGTCA CGGGCTCCAT
2251  CTCTTGGGCA CGGCGTCGAC CTGGCTCCTC CTGGACATCG CCTACTACTC
2301  GCAGAACCTG TTCCAGAAGG ACATCTTCAG CGCCATCGGG TGGATCCCTC
2351  CGGCGCCGAC GATGAGCGCG CTGGATGAGC TCTACCACAT CGCGCGCGCC
2401  CAGATCCTGA TCGCGCTGTG CGGCACCGTG CCGGGCTACT GGTTCACCGT
2451  CGCCTTCATC GACTCCGTCG GCCGCTTCAA GATCCAGCTC ATGGGTTTCT
2501  TCATGATGAC GGCATTCATG CTCGGCCTCG CCGGGCCGTA CGACTACTGG
2551  ACGGGCCAGG GCCACCAGGT CGGGTTCGTC GTCATGTACG CGCTGACCTT
2601  CTTCTTCGCC AACTTCGGGC CCAACGCGAC CACCTTCATC GTCCCCGCCG
2651  AGATCTACCC GGcCAGGTTC CGCGCGACGT GCCACGGGAT ATCGGCCGCG
2701  TCGGGGAAGG TGGGCGCCAT CATCGGCTCC TTCGGGTTCT TGTACCTGGC
2751  CCAAAGCCCC GACCCGGCCA AGACAGCCCA TGGATACCAC CCCGGCATCG
2801  GCGTGCGCTA CTCCCTCTTC GTGCTCGCCT TGTGTAGCTT GCTGGGGTTC
2851  ATGCTCACGT TCCTCGTTCC CGAGCCCAAG GGCAAGTCGC TGGAGGAGAT
2901  GTCGCGCGAG ACCGAGCCCG ATCATTGCTA ACTAGTCTAC TCGTTGCTGC
2951  ATGAAGATCC GCTAGTCTAC TTGTAGATGA TCGTCGGTGA ATACTGCATT
                              Sense primer →
3001  AAGTTTTGGC AATTAGGGGG CGACGATATG ATGAAATAAT CCTCAGAATT
3051  GTTTCTCGAA GTAGATCAAG AAGGTTCTTC ATTTGAAAAT TCCTTTCATT
3101  TGAATGTGTA TCTTAGCGAG TTTATGTCTC AAAACGCTCG TTATGTGTCA
3151  CAAGACTACA CTTATGAGGA TACGATTTGA GCACATCAAG GATAAAACGA
3200  ACATGCAAAC CGCGTGATCT GCAACCGAAA CTATCAAGGT CGGGCCGGCA
                          ← Anti sense primer
3251  AAATCTAGGG ACATGTGCAA AACTAAAACA TGAATCCTAT TTCAATAAAA
3301  AAACTAACGA TTAGTTTTTT TTAACACGGT ACAGAAACAA ACGCTGATAC
3351  ATACACATAC ACTCACTCCT ATGAACGCAA ACACGCATAC TCTACCCTAT
3401  GAACATCTCC GTGAGACTTA GACGGCATAG CATCTTAAGA TTTACGAAGT
3451  CACCATAGAA TCATAGATGC CTCCTCGTCG ATGAAAACGT CTCCTCCCAT
3501  TGAATGTGCA TCGTCAAAAA TCCTAAAATA ACTCCAGGAA TTATGCGAGC
3551  ACCGGGATTT GAAACCTGTT GGGCTGCGGA TACCACATTT TATTTAACCA
3601  TCCAACCACA GGTTGGTTCG CACTAACGAT CAGTTATAAT GCATATATAT
3651  CTCACATAAT TGNTTATAGT GNATACATCT CGCAATTGGT TTTTATAATG
      3'
```

HvPT8

Accession number = AY187023
HvPT8 total gene = 3163 bp
Promoter = 1371 bp
cDNA = 1602 bp = 534 amino acids
3' untranslated region = 190 bp
Putative cis-regulating motifs (referenced in section 7.2) have the motif aligned under the sequence with the percentage of similarity.

```
5'     1  GCGTTAAGAG CAACACGGTT TTCTCTATAT TCTGTCGCAA ATGGTAATAT
      51  GGCATAGCCA CGCCAGTCGG TATGAATTCA GGAAAAATGT TTGAAAAACG
     101  AATATTTTTT ATATTTGTAA CATTTTTGAA AAGCAAGGAC ATTTTTTGAA
     151  ATTAAAGAAC AATTTTGGAA CACAAACTTG GTTTAATAAT TGAATAATAT
     201  TTAAATATTT CGAACATTTT TTAAAGTACC AAACATTATT TGAAAATATC
     251  AAACATTTTT TAAAACCACA AATTTAAGGA AATCGCACAC CTTTTTTTAG
     301  TTATGAATAT TTTTAAAAAA CGGAAACTAT TTTGAAATTT AGAGAAAAAA
     351  TAGAAACTAA AATTATTTTC CAAATTTAAA GAACAGTTTT TGAACATGAG
     401  CTTATTTGAA AAAAGATAAA ATAACCTTAG AACAAAGAAA AAAAGAAACG
                                AAAACTTAG-Motif 78%
     451  GAAAACAAAA ACATAAAAAA AAGCAAAAAC ATAAAAAGGA AAAAGAAAAA
                 AAA ACTTAG-Motif 78%AAAAC TTAG-Motif 78%
     501  CAGATAAAAA AAAGGTTCAG GAACCTACCA GAAGGTTCCC AAAACTGGTG
     551  GACTGGGCCA GCCCAAGTAT CGCCTGGGGA GCCTCGACTA CTTGTTACAA
                                                  CTT CTTGTTCTA-Motif 75%
```

601 AATACGTCAA ATAAGATATT CCGGATTTAG AGTCTGTTTT TCTTTTAAAA
TAATATAT-Motif 88% AAA
651 AATCAGCTAA AATATGTTAT TACTTTAAAA AAATACTGAA GAAAACCAGG
ACTTAG-Motif 78%
TAATATAT-Motif 88%
701 TTAGAAATCG GCTGACAACG CATGTAAAAC CGGTCAACCC CATAAGCACG
751 AATCACAAAG CAATAAATGG ACGGTAGCAG ATTTGACCGA AAGCTAAAAA
801 AAACAATTGC CGACAAATAG CAAACCGATT ATTGACACAA TAATGCTACT
851 CCTACAAAGA CTTATAAAGG GTTACTTAAC CTTTCAAACT AGTTCTCCCC
AAAA CTTAG-Motif 78%
901 CCTCCTCCGA TTTTCAGTGG AGGTGGGGGT CCTCATCTTC AATTACCAAT
951 CATAATTATA CACATGAGTT TATACGTAAA ATTGTAGACG TAGCATTACT
TACATAAATA TGTTCTTTAA CTTG-MRR1 Motif 63%
TGTT TATAAGT-Motif 82%
1001 CTCATTCACA CCTGCACCCA ACGACACAAT CATTCCCGTG CGTATATTGC
1051 GTATTGCACC GGCCCTGCGA TGCCCACCGG CGCCTTGGAG TTTCCACCCC
1101 CTTTGTTCCT TCTCCCCGCT TTCAGCAAGT TCATTGTCTG GTACATGCAC
T GCATTCTAT-Motif 70%
1151 ACGCTACAAG ATAACCACAT GCAGCTATAC CATCCTCGTA TCTTGCTATT
1201 GTTTCCATTT GGCACATCTA AACAAACCAA AGCAATAAGC CGGCTTACAT
1251 AACCCCCTTG CTCGCCTATG CTTACTTGGC TCCCATTCCC TGTAAACACA
1301 CGATCGAGGA CCGTCTTGAA TCTTGCTTTG CACCGGCCAA GAAGTGCGGA
1351 CGGGCAGACG TACGTCCGGC GATGGCACGG CAGCAGCTGC AGGTGCTTCA
1401 CGCGCTGGAC GTGGCCAGGA CACAGAGGTA CCACGCGTGG GCGGTGGTGA
1451 TCGCCGGCAT GGGCTTCTTC GCCGACGCGT ACGACATCTT CTGCATCACC
1501 CTGGTCACCA AGCTCCTGGG ACGCATCTAT TACCACGTCC CTGGCCAACC
1551 AGACCCCGGA ATGCTCCCCC GGCGGATCGA GGCGGCCATC AACGGCGTCA
1601 CCTTCTGCGG CATGATCGTG GGGCAGCTCT TGTTTGGCTG GCTCGGCGAC
1651 AAGGTCGGCC GGAAGATGTT CTACGGCAAG ACCATCATGC TCATGATCAT
1701 GGGCTCCTTT CTCTCGGGCT TGTCATTCGG GAACACGGCC GACGGCGTTA
1751 TGGCCACGCT GTGCTTCTTC CGGTTCTGGC TCGGCGTCGG TATCGGCGGA
1801 GACTATCCGC TCTCCGCGAC CATCATTTCC GAGTACTCTA ACAAGAGATC
1851 GCGCGGGAGC CTCATCGCGG CCGTGTTTGC CATGGAAGGG TTTGGCATTC
1901 TTGCAGGCTG CATTGTCACC TTGGTCGTGT CGGCCACGTT CCAGGCCCGC
1951 TTCAACCCGC CGGCGTATGA GGAAGACCCC ATGGCCTCGG TCCCGCCGCA
2001 GGCTGACTAC GTGTGGCGCA TCATCCTCAT GGTGGGTGCC ATCCCAGCCG
2051 TCTTCACCTA CCGCTGGAGG GTGATGATGC CGGAGACGGC GCGCTATACG
2101 GCGCTGGTGG CCCGCGACGC CGAGAAGGCC GCGCGCGACA TGTCCAAGGT
2151 GCTCAAGGTG GAATTCACCG GCGAGCAGGA CAAGATCGAG AGCTTCACCA
2201 GGGACAGGGA CTACGGCGTC TTCTCCCGCC GTTTCGCCCG CCGCCATGGC
2251 TGGCATCTCG TCGGCGCCGT TGCGTCCTGG TTCGTGCTCG ACATCGTCTT
2301 CTACTCCCAG ATCATTCTCC AGGAGGAGAT ATTCAGGGAC GTCAAGTGGA
2351 TCCCCGAGGC ACGCACCATG AGCGCGCTCG AGGAAGCGTA CCGCGTCGCC
2401 CGTGGACAGG CGATCATCGC GCTCTGCGGC ACACTACCTG GCTACTGGTT
2451 CACCGTCGCC TTTGTGGATG TCGTCGGGCG GAAGGCCATC CAGTTCCTCG
2501 GGTTCACCAT GATGAAGGGT CTCATGCTCG TCGTCGCCGC CTTCTACCAC
2551 CACCTGACGC AGCCTGGCCG GCGAATATGG CTGGTGGTCA TGTACGCCTT
2601 CACCTTCTTC TTTGCCAACT TTGGGCCCAA CAGCACCACC TTCATCATAC
2651 CGGCCGAGAT TTTTCCGGCA CACGTCCGGA CGACCTGCCA TGGGATATCA
2701 TCGGCGGCAG GCAAGGTAGG CGCCATTGTC GGGACGTTTG GCTTCCTGTA
2751 CGCCTCGCAG AGGGCGGACG GCAGCAACGA GGTGAAAAGT GGGTACCCGT
2801 CGGGCATCGG CGTGCGTGCC TCACTGTTCG TGCTGGCCGC GTGCAATGTG
2851 TTGGGCATAA TTTTCACCTG TCTCCTGCCT GAGCCGAATG GGAGGTCGCT
2901 GGAGGAGGTG TCCGGCGAGC CCATCAACAG GGAGGACGCG GATTTGGGTG
Sense primer → RT-PCR
2951 ATTCCAGGGT TCTTCCCTTG TAGAACCTGT TTGAACGTAG GCTGTGCGCA
Sense primer → real-time RT-PCR
3001 TCACTTGATG AAAAAGAACC CATTTCCATG TGTGATGTGC TGGGAACCCG
← Anti sense primer real-time RT-PCR
3051 GGATTAGATG CAGTCCAAGG ATCCCTACAT GACGTTTGGC AACTATTGCG
3101 GAGGAGAAAT GTGAATTATA AGAGGAAGGA ATTGTCGAGA TTAGACTTAG
← Anti sense primer RT-PCR
3151 GATTTCACAT TTT 3'

Wheat Phosphate Transporter

TRIae;Pht1;myc Accession # AJ830009
TRIaePHT1;myc total gene = 1781 bp
cDNA = 1602 bp = 534 amino acids
3′ untranslated region = 179 bp

```
5′      1  ATGGCACGGC AGCAGCTGCA GGTGCTTCAC GCGCTGGACG TGGCGAGGAC
       51  GCAGAGGTAC CACGCGTGGG CGGTGGTGAT CGCCGGCATG GGCTTCTTCG
      101  CCGACGCCTA CGACATCTTC TGCATCACCC TAGTCACCAA GCTCCTCGGA
      151  CGCATCTATT ACCAAGTCCC GGGCCAACGA GAGCCCGGGA TGCTCCCCCG
      201  GCGGATCGAG GCGGCCATCA ACGGCGTCAC CTTCTGCGGC ATGATCGTGG
      251  GCCAGCTCTT GTTTGGCTGG CTCGGCGACA AGGTCGGCCG GAAGATGTTC
      301  TACGGCAAGA CCATCATGCT CATGATCATG GGCTCCTTCC TCTCCGGCTT
      351  GTCGTTCGGG AACACGGCCG ACGGCGTCAT GGCCACGCTA TGCTTCTTCA
      401  GGTTCTGGCT CGGCGTCGGC ATCGGCGGAG ACTATCCGCT CTCCGCGACC
      451  ATCATTTCCG AGTACTCCAA CAAGAGGTCG CGCGGGAGCC TCATCGCGGC
      501  CGTGTTTGCC ATGGAAGGGT TTGGCATCCT TGCAGGTTGC ATTGTCACCT
      551  TGGTCGTGTC GGCCACGTTC CAGGCGCGCT TCAACCCGCC GGCGTATGAC
      601  GAAGACCACA TGGCCTCGGT CCCGCCGCAG GCTGACTACG TGTGGCGCAT
      651  CATCCTCATG GTGGGTGCCA TCCCAGCCGT CTTCACCTAT CGCTGGAGGG
      701  TGATGATGCC GGAGACGGCG CGCTATACGG CGCTGGTCGC GCGCGACGCC
      751  GAGAAGGCCG CGCGCGACAT GTCCAAGGTG CTCAAGGTGG AACTCAGCGG
      801  CGAGCAGGAC AAGATCGAGA GCTTCACCAG GGACAGGGAC TACGGCGTCT
      851  TCTCCCGCCG TTTCGCCCGC CGCCACGGCT GGCATCTCGT CGGCGCCGTT
      901  GCATCCTGGT TCGTGCTCGA CATCGTCTTC TACTCCCAGA TCATTCTCCA
      951  GGAGGAGATC TTCAGGGACA TCAAGTGGAT CCCCGAGGCA AACAGCATGA
     1001  GCGCGCTCGA GGAAGCGTAC CGCGTCGCCC GTGCACAGGC GATTATCGCG
     1051  CTATGCGGCA CACTACCTGG CTACTGGTTC ACCATCGCCT TTGTGGACGT
     1101  CGTCGGGCGG AAGGCCATCC AGTTCCTCGG GTTCACCATG ATGAAGGGAC
     1151  TCATGCTCGT CGTGGCCGGC TTCTACCACC AACTGACGCA GCCTGGCCGG
     1201  CGAATCTGGC TGGTGGTCAT GTACGCCTTC ACCTTCTTCT TTGCCAACTT
     1251  TGGGCCCAAC AGCACCACCT TCATCATACC GGCCGAGGTT TTTCCGGCGC
                          Sense primer →
     1301  ACGTCCGGAC GACCTGCCAC GGGATATCAT CAGCGGCAGG CAAGGTCGGT
     1351  GCCATTGTCG GGACGTTTGG CTTCCTGTAC GCCTCGCAGA GGGCGGACGG
     1401  CAGCAATGAG AGGGAGACCG GGTACCCGTC GGGCATCGGC GTGCGTGCCT
     1451  CACTGTTCGT GCTGGCCGCG TGCAATGTGT TGGGAATAAT TTTCACCTGT
     1501  CTCCTGCCTG AGCCGAACGG GAGGTCGCTG GAGGAGGTGT CCGGCGAGCC
     1551  CATCAACGGG GAGGACGCAG ATCTGGGTGA TTCCAAGGTT CTTCCCTTGT
     1601  AGAACCTGCC TGAACGTAGG CTGTGCGCAC CACTCGATGA AAAAGAAGCT
     1651  ATTTGAATGT GTGTTGTGCT GGGAACCCGG GATTAGATGC ACTCCAAGGA
     1701  CCCTACGTAC ACGGCGTTCG GCAATCATCG CGGAGGGGAA ATGTGAATTA
     1751  AGAGGAAGGA ATTGTCGAGA TTAGACTTAG G  3′
                          ← Anti sense primer
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Maize Phosphate Transporter

ZEAma;Pht1;6

Patent number = WO9958657. Accession # AJ830010
ZEAma;Pht1;6 total gene = 1726 bp
Promoter = 49 bp
cDNA = 1665 bp = 555 amino acids
3′ untranslated region = 12 bp

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5′      1  GACACCTGCC TTACATTGCC GGCGCGCTTG CGTGACTGGG CGCGGCGAGA
       51  TGGCGGCGCC GGGCGGGTCG AACCTGGCGG TGCTGGACGC GCTGGACTCG
      101  GCGCGCACCC AGATGTACCA CATGAAGGCC ATAGTCATCG CCGGCATGGG
      151  CTTCTTCACC GACGCCTACG ACCTCTTCTG CATCTCCACC GTGTCCAAGC
      201  TGCTCGGCCG CATCTACTAC CCGGACGACA ACCTGTACAT AGACAAGCCC
      251  AAGCCCGGCA CTCTGCCCGT GTCCGTCAAC AACATGGTGA CAGGCGTCGC
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 301  GCTCGTCGGC ACGCTCATGG GCCAGCTCGT CTTCGGCTAC TTCGGCGACA
 351  AGCTCGGGCG GAAGCGCGTG TACGGCATCA CGCTCGTGCT GATGGCCGCC
 401  TGCGCCATCG GCTCGGGCCT CTCGTTCGGC AGCTCGGCGC ACGCCGTCAT
 451  CGGCACGCTC TGCTTCTTCC GCTTCTGGCT CGGCTTCGGC ATCGGCGGGG
 501  ACTACCCGCT GTCCGCGACC ATCATGTCCG AGTACTCCAA CAAGAAGACG
 551  CGGGGCGCGT TCATCGCCGC GGTGTTCGCG ATGCAGGGCG TCGGCATCAT
 601  CTTCGCGGGG CTCGTGTCCA TGATCGTCTC GGGCATCCTC CTGCACTACC
 651  ACCCGGCGCC GGCGTGGAAG GAGAACCACG ACCGGTCGTG GCAGGACCAG
 701  ATGCCGGCGG CGGACTACAT GTGGCGCATC GTCCTGATGA TCGGCGCGTT
 751  CCCGGCGCTG GCCACGTTCT ACTGGCGGAT GAAGATGCCC GAGACGGCAA
 801  GGTACACCGC GCTCATCGAG GGCAACGCCA AGCAGGCGGC CAACGACATG
 851  CAGAAGGTGA TGGACGTCGA GATCCAGGCC GAGCAGGACA AGCTCGCGAG
 901  GTACAAGGCG GCGAACGACT ACCCGCTGCT GTCGAGGGAG TTCGCCCGGC
 951  GCCACGGCCT GCACCTCATC GGCACGGCCA CCACGTGGTT CCTTCTCGAC
1001  ATAGCCTTCT ACAGCCAGAA CCTGACCCAG AAAGACATCT TCCCGGCGAT
1051  CAAACTGACG AGCCCCGTCG ACGACATAAA CGCCCTCAAG GAGGTGTTCG
1101  AGATTTCCAA GGCCATGTTC CTCGTCGCAC TCCTCGGCAC CTTCCCCGGC
1151  TACTGGGTCA CCGTCGCGCT CATCGACAAA ATGGGCAGGT ACCTGATCCA
1201  GCTCATCGGT TTCTTCATGA TGTCTGTCTT CATGCTACTG ATGGGCGTCA
1251  TGTACAACGA CCTCAAGAAC AAACACACCA CGCTCTTCGC CCTCTTCTAC
                  Sense primer →
1301  GCGCTCACCT TCTTCTTCGC CAACTTCGGC CCCAACAGCA CCACCTTCGT
1351  GCTGCCGGCC GAGCTATTCC CGACGCGCGT GCGCTCCACC TGCCACGCCA
1401  TCAGCGCCGC GTCAGGCAAG GCCGGCGCCA TCGTCGCGGC CTTCGGGGTG
1451  CAGAGCCTCA CGCTCAAGGG AGACGTGGGC CACATCAAGA AGGCGCTCAT
1501  CATCCTCTCC GTCACCAACA TCCTCGGCTT CTTCTTCACC TTCCTCGTCC
1551  CGGAGACCAT GGGCCGCTCG CTCGAGGAGA TCTCCGGGGA GGACGGCAAC
1601  GTCGAAAACG GTCCCGGTGC TCCCGCCGGC GTGGCCATGG GCGTCGCGGA
1651  CGTGAGCAAG GATGACAAGA TGCCTGTTTC CAGTACTGAG TGGCAGAGCT
1701  CCATGCATGC GTGATCGAGT AAGCAG  3'
          ← Anti sense primer
```

Rice Phosphate Transporters

OsPT1, Rice C, ORYsa;Pht1;11

The sequence of OsPT1 (personal communication Godwin) was used to screen the rice genome. There was a 100% match with a gene designated *rice C*. Upon publication of a paper by Paszkowski (2003)it was revealed that all three genes have 100% similarity. The name *ORYsa;Pht1;11* will be used when referring to any of these genes.

Accession number = AF493787 (*OsPT1*), AF536971 (*ORYsa;Pht1;11*)
ORYsaPhT1;1 total gene = 5451 bp
Promoter = 3393 bp
cDNA = 1668 bp = 556 amino acids
3' untranslated region = 390 bp
Putative cis-regulating motifs (referenced in section 7.2) have the motif aligned under the sequence with the percentage of similarity.

```
5'     1  AAGCGAGGTG CCTCTGGGGC CAAATGCTCG ATCTACCGGA GCGGCGGAGT
      51  CATTTTCTAG GATCCGCAAG CGGCGTCGAG GAAAACCGTG ACCATACTGA
     101  CCAACCACGC AAGACATCGC TGGCGCTCGG CCACTCCGCC CCTCCACCAT
     151  TTCTCCTTGC TGAAGCTGCT GCTTCTCCCC ATGCCTATCA TCTCCGGTCT
     201  ACGCCCCCTC TTCAAGCTTC TCCGCGCCAC TCTCCTTGAT TCTTGCATAT
     251  CCTTCTCGCA CAATTGATTT TCTGGACAAG TTTGGTACAT CGATTGATTT
     301  GAGAACACAT CTAGTAGCGA GAAATTCTCT TGATTGGCTT CTTAGCAACA
          Sense primer → with Pac1 at 5' GCCGCCTTAATTAA
     351  TGCATTACTA GAGTATTAGT CAGGTAGGCA GCTTAATTTG CTGGCTAAAT
     401  AGTACCAACG AAGGCCCACA ACACCAGCAA GTGGATGTTG GCATTATCGT
     451  ATTAGACCGG AATAAGTCTG TTGTTCCTCC CTCATTTGTT GCTCCCGATT
     501  AAATCGTCTT CCTCAACCAC AAAACCAGTT ATAACGTACT ACCCATCTCC
```

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 551  GAAACTCGGT GCAAATAAAC TCCTTCAGTG ATTTAAGTAG CGGTTTCTGC
 601  TGACGTGGCA AGTTGACCAC GTTGACTCGC TGAGTCAGTA GGTGGGTCCC
 651  ACATGTTAGC GATTTCTTCC TCATCTCATC TCCTTCCTCA CCTATCCTCT
 701  CTCTCTCCCC CCCCCCCTCT CCCTCTCTCT CTCTCTCTCT TCTCTTCCCT
 751  CTTCCTCCTG TCTCACCTTC CAGCAGCAGT AGAGCCCGGG ATGGTGGTGG
 801  TGGTTGTGAG CTGAAGCCGA AGAAGGCAGC TAAGTAACCG AGGAAGCCCG
 851  ATGGGAGGAG GCGACTAGTG AGAAGGGCCC CGTCCCCCCA AGGGTGATGG
 901  ATGTGGAGAA GTTTGGATGG AGTAGGAAGA GGCCCCAAGC GACGACGAGA
 951  GCTTAGCGAG GCTGAGGCGG CTAGAAACAT GAGGCCAAAT CTAGTCATTG
1001  TCGACATGCT GTCAAGGAGC GTGCACGTGG TGGTGCTCGA GGTAGCGGCA
1051  GGCGGCACTC GAGGCTGCAA CTTCATGGAT GTCGCAGTCC ACGTCGTATG
1101  CGCTCGTGGC CTCCTCCGGC CCGGCCCCTT GCCCCCTCCC AAGATGGTGT
1151  CGTAGCGGCA AACGGCCGGA AGGAAGGGCT CGATGTTGGC GGCTCCTCTC
1201  CCGAGTGGGG TTGGGGTTCG CGAGGGTGCT CACCAGCTAG CACCGTCTTC
1251  ACCTGGCTCC GGGGATGCGT GCACCTAACA CTACAGACTA ATAGGGGAGT
1301  GATGGGAGAG GGTGGAGCTT TGGTGTCGTC ATCGATGTCT TCTCGTCTTC
1351  AGCTGTCGTT GCTAGTGAAG ATCTTCCAAG TTCCAAGTCT CACTTGGCAC
1401  ATCCCTACCG GTCACGTCCT CCTTCCCTGA ATGCTCCGAG GTGGCCACCA
1451  TCCTCGGCTC CGGGTCACCA CCACCATCGC CCTGTCGGGG CCATCTCGCC
1501  ACTACCGCTA CCCTCCCGAG CTTTACTGTT GTTGGAAGGT GAGAGACGAA
1551  GAAGAGGGAA GAGAGAAAAG AGAGAGGTGG AAGGGGGAGG GGGAGGTGAG
1601  GAGGAAGATG AGACAAGGAA ATCACCAATA TGTGAATCCC ACATGCTAAT
1651  ATAGTAAGTC AACTGCAGTA AACCTGGCAT CTCAGGAGAT ACCGCTTCCC
1701  AAATTCTCGA AGTAGTTGAT TTACATCGGT TTTTGAAGTT AGGGAAGGCA
1751  TTATACCCGG TTTTGCGGCT GAGGGAGCGA TTCATTCAGG AGCAATAGAC
1801  GAGGGATGCC AAATAGACTT GTTCCTATTA GACCGTAAGA TTATTGATGG
1851  GACGAAAGGC CTTGCATAGA TAGTGATATC TGAGCCCATG CAGGGGCTAT
1901  CACCAATGCA GTGTTGCTAG AAAGTGCAGT ATCACTACTA GCAAAATAAA
1951  AAGTTTGTCT GCTAAGAGAA AAAAAAGGGA CCCATTTAAC ACAATTCTAG
2001  CTCTAGATAA GTGGGACTGT ACAAATTCTT CATTTTTTTT AGCGAAGTTA
2051  GAAGTTAGTA GAGTTGCTCC ACAAAATTGT ACTATGGGGG TGGAGATGGG
2101  TTTTTGCTAC TTCACCGCTC CACTCCATCC AAAAAAGCTA ACTAACTTTT
2151  AATTTTTCAT GTATTTACCG AATATGCCAC TGAACTACCC ATTCATACCC
2201  TCTCTCATCC CGTTCTTCCT CCCAACTAGG CGGCTGGGTG GCGACCGGCC
2251  GCTGGGACTG GTGGCGGCGA GCGGGTTGCA CAGCTGCTAG GCGGCTTGGT
2301  GGTGTGCGAG GGCAGCAAGG GAGAGGGCGG TGGCACGCGG GCTGAGCATC
2351  GATCGGCAGG GTCAGCAGGA CAACGTGTGG GTGACGGGGT GTGGGATGAG
2401  CGACAACGGT GTCGGTTGGT TGGGTGGCAT GCATAGGCCG TAACCAGTCG
2451  TGGGGACCAN CGGCTATTGG GTGTCTTGGT GGCGCACAAG CGCAACAGGG
2501  CGGCGCACGT GTGGGCAGCA GTGCACAGGC TGAGCGGTGA CGCGCAACGG
2551  CGGTGGTGGC AAGCCGCGGA CGCCAACCAA GTATTCGATG AAATGAGATG
                                      T GCATTCTAT-Motif 70%
2601  GGATGTGGTA GCGTGGAGCT AACCAAATCT AGCCAAACAT TTTTACAACT
                                                T GTTTATAAGT-Motif 73%
                                                  TAACAACT
2651  ACCTTCAGCC TCTATAAGAG CTAGCTTCTA TTAGAGTTGG AGTTTAAAAT
      AT-Motif 80%           TGCAATTCT AT-Motif 70%
        CTTCTTGT TCTA-Motif 67%                    TGTTTATAAG
                                                     AAAAC
                                                   TACATAAA
2701  TAGGAGTTGA AATTTGGAGG CTTAACAAAT GGGGCCGAAA TATCTGCTTT
      T-Motif 73%              TAATATAT-Motif 75%
      TTAG-Motif 78%                          TAA TATAT-Motif 75%
      TATGTTCTTT AACTTG-MRR1 Motif 54%   TACATAAA TATGTTCTTT
2751  TAGGCAGACG GCGTAGCGGT AAATCGATGA TGTAGACAGT TGGCCGCAAC
      TAACTTG-MRR1 Motif 54%
2801  TGCAAGGAAA CAACACTCTG TCACACAGGT TCCGCCGTAT GGTAGGAATC
2851  GCCAATCATG ATTATCTGCC GAATATCCTA CCAAAAACGA CGCATTTCCG
2901  TCCTAGTTAA CTTCGATGCT GCTGCGCTGC ATCCTCCAGG TAAAGTAAAA
                                    TGC ATTCTAT-Motif 70%
                                                      TAAT
2951  TCTTGCAAGG GACAAAACAC ACAGGCTCTT CTCCCATATC CATCAGCTGC
      ATAT-Motif 75%
3001  CCAATTGACG CATCACTGAG TTGAATTACA CGCGCGCAAG GGCAACGCAG
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3051  CATGATTGAT TTCGTCATTC TCCTCTCGAG ACAAAGAATC CGATCTCATC
3101  TCCGCACGAG GGACGGCCAA CTGCTTCCAC CCTTCACAAT GCCGCCTAGA
3151  CGCCTAGTGT ACGCGCCGAA TATGCTGCCA AACCAACACG GGACAATCTC
3201  CCGCGCTTGG CGACAGCTTC TCCTCGGTGC AGACGCCCTC TCGTTCATCG
                                                CTTC TTGTTCTA-Motif 67%
3251  ACCTAGCTTG AGGGTGAAAT CCCAGCTATA AGATCGGGCA AGGCAGCGAG
3301  CAGTTTGGTG ATCAGAGGTA GCTAGCTAGC CTCGTAGCAG TGTTCCCCGG
3351  CGGCGAGAGC GGCAGGAGAG GACGATCGAC GGCATCATCG GAGATGGCGG
      ← Anti-sense primer with AscI site at 3' GGCGCGCCTCGGC
3401  ACGCGGACGG GGGCTCGAAC CTGGCGGTGC TGGACGCGCT GGACTCGGCG
3451  CGCACGCAGA TGTACCACAT GAAGGCGATC GTGATCGCCG GCATGGGCTT
3501  CTTCACCGAC GCCTACGACC TGTTCTGCAT CTCCACGGTG TCCAAGCTGC
3551  TCGGCCGTCT CTACTACCAA CCCGATGGCT TGACGGACAG TAAGCCAGGC
3601  GCTCTGTCCA AGACCGCCAA CAACATGGTC ATCGGCGTCG CGCTCGTCGG
3651  CACGCTCATG GGCCAGCTTG TCTTCGGCTA CTTCGGCGAC AAGCTCGGCC
3701  GGAAGCGCGT TTACGGCGTC ACCCTCATCC TGATGGCCGC CTGCGCCATC
3751  GGGTTCGGCC TGTCGTTCGG CAGCTCGCGC AAGGCAGTCA TCGGCACGCT
3801  GTGCTTCTTC CGCTTCTGGC TCGGCTTCGG CATCGGCGGG GACTACCCGC
3851  TGTCGGCCAC CATCATGTCC GAGTACTCAA ACAAGAAGAC GCGCGGCGCG
3901  TTCATCGCCG CGGTGTTCGC CATGCAGGGC GTCGGCATCA TCTTCGCGGG
3951  GCTCGTGTCC ATGATCGTCT CTAGCATCTT CCTCACCTAC AACAAGGCGC
4001  CGTCGTACAA GGGGAACCAT GACCTCTCGA GGCAGATGCC CGCGGCTGAC
4051  TACGTGTGGC GCATCGTCCT GATGATCGGC GCGTTCCCGG CGTTGGCGAC
4101  CTTCTACTGG CGGATGAAGA TGCCGGAGAC GGCGAGGTAC ACGGCGATCA
4151  TCGATGGCAA CGCGAAGCAG GCGGCGAACG ACATGCAGAA GGTGCTGTCG
4201  ATCGAGATAG AGGCCGAGCA GGAGAAGCTG GCCAAGTTCA ACGCGGCCAA
      Sense primer → RT-PCR
4251  CAACTACCCG CTCCTGTCGA TGGAGTTCGC CCGGCGCCAC GGCCTGCACC
4301  TCATCGGCAC GACGACCACG TGGTTCCTCC TTGACATCGC CTTCTACAGC
4351  CAGAACCTGA CCCAGAAGGA CATCTTCCCA GCTATGGGCC TGATCAGCGG
4401  CGCTGCCGAA GTCAACGCTC TCACGGAGAT GTTCCAGATA TCCAAGGCCT
4451  CGTTCCTCGT CGCTCTCCTC GGCACCTTCC CCGGCTACTG GGTCACCGTC
4501  GCTCTCATCG ACAAGATGGG CAGGTACGTA CGAACCGTAT AAACATGGAC
4551  ACTTGATGCA AATGCAATCG ATGCGAACAT ACACGAAATG AATGAATTCA
4601  TGGTCACATA TGCAGGTACA TGATCCAGCT GATCGGTTTC TTCATGATGT
4651  CCATGTTCAT GCTGGCGATG GGCATCCTGT ACGACTACCT CAAAACCCAT
4701  CACTTCCTGT TCGGGCTCCT GTACGCGCTC ACTTTCTTCT TCGCCAACTT
4751  CGGGCCGAAC AGCACCACCT TCGTGCTGCC GGCCGAGCTG TTCCCGACGC
4801  GCGTGCGCTC CACCTGCCAC GCCATCAGTG CCGCGGCGGG CAAGGCCGGC
4851  GCCATCGTCG CGGCCTTCGG CATTCAGAAG CTCACGTACA ACTCTCAAGT
4901  CAAAAGCATC AAGAAGGCGC TCATCATCCT CTCCATCACC AACATGCTCG
4951  GCTTCTTCTT CACGTTCCTC GTCCCGGAGA CCATGGGTCG GTCGCTCGAG
5001  GAGATCTCCG GCGAGGACGG CAACACCGGC GCCGGTGGCG GCGGCGCCCC
5051  TGCCGCTGCC AATGCCGGCG TTGGCGTGAG CGCTTCGGAT GTGAGCAGGG
5101  ACGAGAAGTT CCCTGCTTCA AGCACCGAAT GGCAGACATC CATGCACGCA
5151  TGATACGCTC ATCTGGGATA TGCATACCTA CACAATACCA GTACGTATAC
5201  CTACGCAATA ATAGTACTAT ATTGATATAT CTGTATTATG AGAGTGGAAA
5251  TGGACCAAAA TAATGGCAAT AACTTGAATT GCCAGATGCT AGCTTGGGAA
5301  TTTGATATAC AAGTATATAT CTCCATTACC ATGTTAGAGT AATATATATG
5351  TTTGAGTGTG TGCACTCATG CAACAATAGT ATATATCGTG ACCATTCACA
5401  ATGTTGAGAA CTACTAGCTA GCAAGAAACT GAGAATGAGA GGGACAATGC
5451  AACTATGCTC CTTTTACTAC TTCGTATTGG TGTATGCGTA TTGCAAGCAG
5501  CTAATCAATC ATTCTGTTAG TTCTTTCTCT ATATGACGAG CTC  3'
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OsPT2

Accession number = AF493788
ORYsaPhT1;2 total gene = 2510 bp
Promoter = 781 bp
cDNA = 1617 bp = 539 amino acids
3' untranslated region = 112 bp

```
5'     1  ACTAGTCTCC CTCCTCGTCA TACTTCAGCA ACCACAAGAT TTCTTTTCTG
```

```
  51  AACTTTTTAC TAATGAACAT TCAGAAATTT CTGTGCAATA TTATCTCATG
 101  ACCTGAACCA AACGATGCTT GAGCCACGAA ATAGTAGAGG AGACAAAGAT
 151  ATAGTTTCGT CAATTCGAGA AGTTTGTCCG GATACTACGG ATGATAGCGG
 201  CAGATTTGGA CTGGTTCCAT GAAAGTTGTA CAGTAAGGTG CGAATCTTGA
 251  GTTGCAGAGA TGCACCTGGA TCCGGCTATC TAGCTTCACG AGAATCCCAT
 301  CTCTACTCTC CTAAATTGCC CACGAAACTG AATTTATGTA GGGATTTTTA
 351  GCGAAATTCA GACATTTTTC ACGGGGATGG GTCGGGGATT GTTGACTGAT
 401  AAAGCTGGAT TTGAAGAAAC AACAAAATTT TGATATATGA TACCTTGAAT
 451  AAACGAGGAG TTTCTGAAGT AGTGGCATGG TCTGTTCCAG ATGTCTCTCT
 501  GAACTTCCGT TTCAGTTTCA GTGGACCATA TTGTTGGTGA ACTGAAACGA
 551  ATATTATCTT CTCGTAGCCA CGTGCATTCT GTAGATTTTC TTTTGCTCAG
 601  TTCGACACAT AGACATCTGA GGCTAATTAG CTCTGTTAAT CGCGCGGTTT
 651  GTGTAATTCT CACAAATAAT TAGTTTCTCG TTCATTGCAA ATTGCAGCGA
 701  GATTTTGTCG AAATAATAAA CTTGGTGTTC AGTTATTCTC TGCAAAAAAT
 751  TGCATATTGC AGAGTAGCTG AGATTGGCGC CATGGCCGGC GAGCTCAAGG
 801  TGCTGAACGC GCTCGACTCG GCGAAGACGC AGTGGTACCA TTTCACGGCG
 851  ATCGTGATCG CCGGCATGGG GTTCTTCACC GACGCCTACG ACCTCTTCTC
 901  CATCTCCCTC GTCACCAAGC TGCTCGGCCG CATCTACTAC TTCAACCCGG
 951  CGTCCAAGAG CCCTGGCTCC CTCCCGCCCA ACGTCTCCGC CGCCGTCAAT
1001  GGCGTCGCCT TCTGCGGCAC CCTCGCCGGC CAGCTCTTCT TCGGTTGGCT
1051  CGGCGACAAG ATGGGGCGCA AGAAGGTGTA CGGCATGACG CTCATGCTCA
1101  TGGTCATCTG CTGCCTCGCT TCCGGCCTCT CGTTCGGGTC GTCGGCGAAA
1151  GGCGTCATGG CCACGCTCTG CTTCTTCCGC TTCTGGCTCG GCTTCGGCAT
1201  CGGCGGCGAC TACCCGCTCT CGGCGACCAT CATGTCGGAG TACGCTAATA
1251  AGCGCACCCG TGGAGCGTTC ATCGCCGCCG TGTTCGCCAT GCAGGGCTTC
1301  GGCAACCTCA CCGGCGGCAT CGTGGCCATC ATCGTGTCCG CCGCGTTCAA
1351  GTCGCGGTTC GACGCGCCGG CGTACAGGGA CGACCGGACC GGCTCCACCG
1401  TGCCGCAGGC CGACTACGCG TGGCGCATCG TGCTCATGTT CGGCGCCATC
1451  CCGGCGCTGC TCACCTACTA CTGGCGGATG AAGATGCCGG AGACGGCGCG
1501  CTACACCGCG CTGGTCGCCA AGAACGCGAA GCAGGCCGCC GCGGACATGA
1551  CGCAGGTGCT CAACGTCGAG ATCGTGGAGG AGCAGGAGAA GGCTGACGAG
1601  GTCGCGCGGC GCGAGCAGTT CGGGCTCTTC TCCCGCCAGT TTTTGAGACG
1651  CCATGGGCGC CACCTGCTGG GCACGACGGT GTGCTGGTTC GTGCTGGACA
1701  TCGCCTTCTA CTCGTCGAAC CTGTTCCAGA AGGACATCTA CACGGCGGTG
1751  CAGTGGCTGC CCAAGGCGGA CACCATGAGC GCCCTGGAGG AGATGTTCAA
1801  GATCTCCCGG GCACAGACGC TCGTGGCGCT GTGCGGCACC ATCCCGGGCT
1851  ACTGGTTCAC CGTCTTCTTC ATCGACATCA TCGGCCGCTT CGTCATCCAG
1901  CTCGGCGGCT TCTTCTTCAT GACGGCGTTC ATGCTCGGCC TCGCCGTGCC
1951  GTACCACCAC TGGACGACGC CGGGGAACCA CATCGGCTTC GTGGTCATGT
2001  ACGCCTTCAC CTTCTTCTTC GCCAACTTCG GGCCCAACTC CACGACCTTC
2051  ATCGTGCCGG CGGAGATCTT CCCGGCGAGG CTGCGTTCCA CCTGCCACGG
2101  CATCTCGGCG GCGGCGGGGA AGGCCGGCGC CATCGTCGGG TCGTTCGGGT
2151  TCCTGTACGC GGCGCAGAGC ACGGACGCGA GCAAGACGGA CGCCGGCTAC
2201  CCGCCGGGCA TCGGCGTGCG CAACTCGCTC TTCTTCCTCG CCGGATGCAA
2251  CGTCATCGGG TTCTTCTTCA CGTTCCTGGT GCCGGAGTCG AAGGGGAAGT
2301  CGCTGGAGGA GCTCTCCGGC GAGAACGAGG ACGATGACGA TGTGCCGGAA
2351  GCGCCCGCGA CGGCCGATCA CCGGACTGCG CCGGCGCCGC CAGCTTGATA
2401  CCCCGCGGCA AAACCCAAAT GGTCAATCAT CAGTGTTTTG TTGTAATATA
2451  TGTGCAATGG ATGATTATTC TGGTTCTGCT AGTGTACCAA ACAAAATTAC
2501  AAATACTAGT  3'
```

Rice A, ORYsaPhT1;1

The contig (5095) containing the rice A sequence has a total length = 12280 bp

RiceA total gene = 4248 bp
Promoter = 2406 bp
cDNA = 1587 bp = 529 amino acids
3' untranslated region = 255 bp

```
5'     1  CAGTTTCTAA ATTCTTCATT TTCTGGATTG TACTTCTTCT TCAAATATTG
      51  AGAAAAGCTG GACTCGGCTG GTGATAACAT GTAGATGTTG GAAAAATAAC
     101  TTTTTCCATC TAGGGTGTAT TTAGTTCACG CTAAAATTGA AAGTTCGGTT
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151 GAAATTGGAA CGATGCGACG AAAAAGTTGA AAGTTTGTGT GTAGAAAAGT
201 TTTAATGGGA TGGAAAAGTT GAAAGTTTGA AAAAAAATTA CAACTAAACA
251 TGGATCAAGT TCATTTTTTT TTATATTCTA TAACAATAGT TTCTTATAAT
301 ATGAAAAGTA GTAGCTTTGC TATTAGACCT TTGCTATTAG TAGTAGCTTG
351 TTTGGCGCCA TCCTCTTGTT GAGCAATCAT TCTTCAGAAT TCATCTGAAG
401 TAGCGAATGG CAACAAAGGG GTAAATCGTT TTGTTTGTTT AGCTCGAAGG
451 AGTAAACTTT GATGTAACCA CCCATACTCA GGATGGGTGT AGGCAGTGCA
501 GATGGCCTAA CGCATTCCGA CGGCAACGGA GTTCTGGCGA AGGAGAGGGC
551 TCCGTGACGA GGACGAGGAA GGAGCCATCA GAGGAGGAAG TCGGGGTGGC
601 CAAGACCGAG GTGGAGCAAG GAGGGATGTG CCCCTGGCAG CGGCGAAAGG
651 CAGAAGTTCG GAGTAGGTCG GCACAGGCAG GAGGAGGTTG GGGAAGTCCG
701 GCGAAAGTAT CGGCCGTGGA GCTATACGCC GGAGAAGGGA AGGTGGCCAT
751 GGGGCCACCA TTAGCAAGTG GGTATGGCTG GTCAGATTTG GCTACGTTTG
801 CCTGGAGCAA CAGCAACCAA CAAGGACACA ACGAGGTGAA GAGTTATCCT
851 CGAGGCGTAA AAGGTGAAGA TGGCAATTCG CGGGGAGCTA TAGCTAGCCT
901 TCTCCAGTAC TTCTGTAGTT CTGTCCATAT CCTGTTCTGC GTCACGAGCC
951 ATGCAAACAA AACACACACA TCTCTGCGTC ATTCGCGGGG AGTAGTTTCG
1001 CTCGCTCGCT AGGGCATGCA TGCTTGTCAA GTTGCTCGCA AGAGTTCATC
1051 GTCAAGAAGC TTCGCTGGAA ATTTCGTCGA CCTCTTCTCC GATTGTTGTA
1101 GCGAAAGGGA CATGGTTTGT TCTCTAGCTG AAGTTTCAGC CCCATGTCAA
1151 TTCCATCACC AAAACGAGAT AGCGACAAGC CAGATCGTAT TAGCTAGGCA
1201 TGTGCCATGT GGAAAACCAA GAAACAAGAT AATTTAAGAC GGGAAGGCCC
1251 AAGTGTGTAC TGCAACTACT GTTGCGAGCT GTGTGTCCAC CCTACCGAAG
1301 GACGCCTATG AACTGGACTT CATTGAGCCA GTAGTGCAGC CAGCCCATGC
1351 TCTGCAAAAT CCTACCCTCG CATACTTCAA CGGCGATGAC TTCCATATCA
1401 AAGAAAGAAT GTTCAGTCAG AACTACTCCG GCTAACAAGT ACAGTAGTAA
1451 TAACCAATTG AACAAACACG GCATTCAGCT GCTGATTTCC AAGCCTATCC
1501 ATATACTCCC TCCGTTTTTT AATAGATGAC GCCGTTGACT TTTTCTTACA
1551 TGTTTGACCA TTCGTCTTAT TCAAAAATTT TATGCAATTG TATAAGATAT
1601 AAATCACACT TAAAGTATTA TGACTGATAT AACAACTCAT AATAAAATAA
1651 ATTATAATTA CGTAAAATTT TTGAATAAGA CGAATGGTCA AACATGTGAG
1701 AAAAAATCAA CGGCATCATC TATTAAAAAA CGGAGTTAGT ACTTGGTTCT
1751 TGCTCAGCTT GTCGCTGTCT TGTTTCAACC TGCTAAGAGC ACCCGCAATG
1801 GTAAAGTAAT GTGCTATCTA TAAAACATGT ACACCTCAGC AATAGACTCG
1851 ATTAATAGTA AACCACTTCA ATGGTATGTC TACATTGGTA TCTATAGCTC
1901 TCTCATGCAT TGTCTCGTTT TTCTCTATAG ACTATCTCTA AGTTAGTAGA
1951 TAGCTTTGCT CTTTCTCTTC ATTTAATATA TTCCAAGTAG GAAAATATGC
2001 TGACATGGAT CTCTTGTAGA GAGCCTATAG ATAATCATTG TGGGTGCCCT
2051 AATGAGAAGC GATTGTGTTA CGTACTACAC AGTACAGACT ACAGACTACA
2101 AAGTATCCAT CTCAACATGC TTCGTCAGTA ATTGATGAAG ATGTGCCGGT
2151 TAATTACTGC TCCAAACGCG GGAAGAACGG AGACCAAAAG ACTTCCAGTT
2201 TTGCAGTCCG CCCCCTCTAC TGTGCCCCTG CCCTGCTTTT GATAAGAACG
2251 GCAACCATTA GAGTTGACTA GTTTGAGCAT TCAACACTGG ACCTGCGACT
2301 GCGAGTAGTA TATATCTGCG TCCAGTAGAG AACAGTCAGT TCAGCGGCAA
2351 AGCAAAAGAA AAAGTTAGAT CAGAGGAGAG CTCAGAGCTC AAAGAGTTGG
2401 GAAGCCATGG **CGGGAGGGCA GCTCAACGTG CTGAGCACGC TCGACCAGGC**
2451 GAAGACGCAA TGGTACCACT TCATGGCGAT CGTCATCGCC GGCATGGGCT
2501 TCTTCACCGA CGCCTACGAC CTCTTCTGCA TTTCCCTCGT CACCAAGCTG
2551 CTCGGCCGCA TCTACTACAC CGACGATTCC AAGGACACCC CCGGCGCGCT
2601 CCCGCCCAAC GTGTCGGCCG CCGTCACCGG CGTCGCGCTC TGCGGCACGC
2651 TCGCCGGCCA GCTTTTCTTC GGATGGCTCG GCGACAAGCT CGGACGCAAG
2701 AGCGTGTATG GTTTCACGCT GATTCTGATG GTCGTGTGCT CGGTCGCGTC
2751 CGGGCTCTCG TTCGGGAGCT CGGCCAAGGG CGTCGTGTCG ACGCTCTGCT
2801 TCTTCCGGTT CTGGCTCGGC TTCGGCATCG GCGGCGACTA CCCGCTCAGC
2851 GCCACCATCA TGTCGGAGTA CGCGAACAAG AGGACGCGCG GGGCCTTCAT
2901 CGCCGCCGTG TTCGCCATGC AGGGGTTCGG GATCCTCTTC GGCGCCATCG
2951 TCGCGCTCGC GGTGTCGGCG GGGTTCCGGC ACGCGTACCC GGCGCCGTCC
3001 TACTCCGACA ACCACGCCGC GTCGCTCGTC CCGCAGGCCG ACTACGTGTG
3051 GCGCATCATC CTCATGTTCG GCACCGTCCC GGCGGCGCTC ACCTACTACT
3101 GGCGGATGAA GATGCCCGAG ACGGCGCGGT ACACGGCGCT CATCGCCCGC
3151 AACGCGAAGC AGGCGGCGGC CGACATGTCC AAGGTGCTGC ACACCCAGAT
3201 TGAGGAGAGC GCGGACCGCG CCGAGACGGT GGCCGTCGGC GGCGAGAGCT
3251 GGGGCCTCTT CTCGCGCCAG TTCCTGCGCC GCCACGGCCT CCACCTCCTC
3301 GCCACCACCA GCACGTGGTT CCTCCTCGAC ATCGCCTTCT ACAGCCAGAA

```
3351  CCTGTTCCAG AAGGACATCT TCAGCAAGGT CGGGTGGATC CCGCCGGCGA
3401  AGACCATGAA CGCGCTCGAG GAGCTCTACC GCATCGCCCG CGCCCAGGCG
3451  CTCATCGCGC TCTGCGGCAC CATCCCGGGC TACTGGTTCA CCGTCGCATT
3501  CATCGAGATC ATGGGCAGGT TCTGGATCCA GATCATGGGC TTCGCCATGA
3551  TGACGGCGTT CATGCTCGGC CTCGCCATCC CGTACCACCA CTGGACGACG
3601  CCGGGGCACC ACACCGGCTT CATCGTCATG TACGGATTCA CCTTCTTCTT
                                  Sense primer → RT-PCR
3651  CGCGAACTTC GGGCCAAACA GCACCACCTT CATCGTGCCG GCGGAGATAT
3701  ACCCGGCGCG GCTCCGGTCG ACGTGCCACG GCATCTCCGC CGCCGCCGGG
3751  AAGGCCGGCG CCATCATCGG AGCGTTCGGA TTCCTGTACG CGGCGCAGGA
3801  CCAGCACAAG CCCGAGCCTG GGTACCCCAG GGGGATCGGC ATCAAGAACG
3851  CGCTCTTCGT GCTCGCCGGC ACAAACTTCC TCGGGACGAT CATGACGCTG
3901  CTCGTGCCGG AGTCCAAGGG CATGTCGCTC GAGGTTATCT CGCAGGAGGT
                                  ← Anti-sense primer RT-PCR
3951  CGCCGACGGC GACGACGAGG AGGCGGCCTA CCCGAAGTAA TTTGACCGCG
4001  CGCGTGATCA CGCAGGGAGT GGTTGCCGCT AACCATTGGT GTCATCTCTT
4051  TTCCCAACTG TAACAACTCT AGTCGTCGCT TCCGTACGAG TGGTAGTTTT
4101  TTCTTTTTCT TGGATAAGTT TGTAGAAATT TCAATTAGTG ACTAGTTTGT
4151  AGTATATGTG AGTGAGATGT GTGTATATGT TCTTGAAGAA TTGGTGAACT
4201  TTTCCTGGAT TTGAAAGAAC CGTGTAGTTT GAAAAAAGAA TGCAATGGAT
      3'
```

Rice B, ORYsaPhT1;2

The contig (5095) containing the rice B sequence has a total length = 12280 bp

RiceB total gene = 2938 bp
Promoter = 1200 bp
cDNA = 1587 bp = 529 amino acids
3' untranslated region = 151 bp

```
5'      1  TACCATTTTT TATAGAGTTG TCAAATATGT GTATCTATTT AGTTTCTTAC
       51  CAAATTTTGA TAAATACATA AGAAATCGTA CCAAAATTTA GTAATATTGT
      101  CAAACTACCA AAATTTTAAA TTTATCTTGG CTACAATCTG AACAGCCCTT
      151  CTTTAAGTTT GACTAAGTTT AAAGAAAAAT ATAGTACTAT TTTAAACACA
      201  AAACAAACAT ATTATGAAAA TATAAAATGT ATTCAATGTT AAACTAGTTA
      251  AGTATTTTAG ATGTTGCTAA TCTTTTTATA AATTTGATCA AACTTATCAA
      301  AGTTTAACTA GAGAAAAGGC TAAACGAATT ATAATATGAA ATCGGAAACT
      351  GATGGTGTAG GACTTAACCA AATGATCACT GCTCAAGACC AAGACCCTAT
      401  TTGCATGCAT AGCTTTGTCA CTGCCCAGCC GATCCTGACA CCGTAGAATT
      451  CTAAACAGTG GGAGGCCATA AGCTGATATG CATAGATAGT TGAAAGTTAA
      501  TTTCACATTC CACGATCCTG CAAATAGAAT AACATTTGAC TTCTGTACGG
      551  GCATTTTAAA GACAATATAT GTCACTATCT GCTTGCAATC TACAGTATGT
      601  ACGGTACATG CAATGCAGTA GAAAGAAAAT CTTTTTTCTG CAACCATGCC
      651  CTGTCTGTAT CCTGTCCGGA CTGCGCTGCG GCTATATATA CAGCACCATG
      701  GCAACAGAGA TGAGGAAACT AACAGAGCAC ACAGCGATCC CCTCTGCTCT
      751  CTCTCCGGAG TCAACCCATC CTCAATCTTC CCAAGGTACT CAACCCTCTC
      801  TCCTGCATCT GAGTTCCATG TTAGCAGAGA TGCTAGATTA ATTAGTGTTG
      851  CGGTTTAGCA TATTCAGGAA ACGTTCTACT GAATATTTGT TGAATCATAT
      901  GCGTAATATT ATTACCCGCC AATAAGATTA GTTGTGCAAA CATGTGCTTC
      951  TACTATTTAG TACAGTACGC ATTAAAAATG GACACACCCC TATCTATTGA
     1001  AGTTTCAATT ACTGAGCGGT AAATCATGAA TTCTCAATCT CAACAGTGCG
     1051  AAACCTGAAA TTTCTCTGTT CAATTAACAT TCAACGATCT AGTCAGATAT
     1101  AAATGGAGAT GCCTAGGTGA AGAATTTTCT GTTCTTTTGT GATATGTTGT
     1151  CTGTACTAAG CAAAGCTTAT AACTTTGCAG CTTGAGGAAA AAAGATCATC
     1201  ATGGCGGGAT CGCAGCTCAA CGTTTTGGTC AAGCTCGACC AGGCCAAGAC
     1251  GCAATGGTAC CATTTCATGG CGATCGTCAT CGCCGGGATG GGGTTCTTCA
     1301  CAGACGCCTA CGACCTCTTT TGCATCGCGC TGGTCACCAA GCTGTTGGGT
     1351  CGCCTTTACT ACACCGACAT CACCAAGCCC AACCCAGGGA CACTCCCGCC
     1401  CAACGTGTCG TCCGCCGTCA CTGGCGTCGC GCTCTGCGGC ACGCTTGCCG
     1451  GCCAGCTCTT CTTCGGATGG CTCGGCGACA AGCTCGGCCG CAAGAGCGTC
     1501  TATGGATTCA CGCTCATCCT CATGGTCGTG TGCTCCATCG CCTCCGGTCT
     1551  CTCGTTTGGC CACACGCCCA AGAGCGTGAT TGCCACGCTC TGCTTCTTCC
     1601  GCTTCTGGCT CGGCTTCGGC ATCGGCGGCG ACTACCCGCT CAGCGCCACC
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1651  ATCATGTCGG AGTACGCGAG CAAGAAGACC CGCGGGGCCT TCATCGCCGC
1701  CGTGTTCGCC ATGCAGGGGT TCGGGATCCT CTTCGGCGCC ATCGTCGCGC
1751  TCGTCGTCTC GGCCGGCTTC CGTCACGCGT ACCCGGCGCC GTCGTACGCC
1801  CAGAACCCCG CCGCGTCGCT CGCGCCGCAG GCTGACTACA CGTGGCGGCT
1851  CATCCTCATG TTCGGCACCA TCCCGGCTGG GCTCACCTAC TACTGGCGCA
1901  TGAAAATGCC CGAGACGGCG CGGTACACGG CGCTCGTCGC CCGCAACGCC
1951  AAGCAGGCGG CGGCTGACAT GTCCAAGGTG CTCCACGCCG AGATCGAGGA
2001  GCGGCCGGAG GTGGTCGAGA GCCAGGTGGT CGCCGGGGAG ACCTGGGGCC
2051  TCTTCTCACG GCAGTTCATG AAGCGCCACG GGATGCACCT CCTGGCGACC
2101  ACCAGCACGT GGTTCCTGCT CGACATCGCC TTCTACAGCC AGAACCTGTT
2151  CCAGAAGGAC ATCTTCAGCA AGGTCGGGTG GATACCGCCG GCGAAGACCA
2201  TGAACGCGCT CGAGGAACTC TACCGCATCT CCCGCGCCCA GGCACTCATC
2251  GCACTCTGCG GAACCATCCC GGGCTACTGG TTCACCGTCG CCTTCATCGA
2301  CATCGTCGGC AGGTTCTGGA TCCAGATCAT GGGCTTCTTC ATGATGACCG
2351  TGTTCATGCT CGCGCTCGGC GTGCCCTACG ACCACTGGAC GCACCCGGCG
2401  CACCACACCG GCTTCGTCGT GCTCTACGCA CTCACCTTCT TCTTCGCCAA
2451  CTTCGGGCCC AACAGCACCA CCTTCATCGT GCCGGCTGAG ATCTTCCCGG
2501  CGAGGCTCCG GTCGACGTGC CACGGCATCT CCGCCGCGTC CGGCAAGGCC
2551  GGCGCGATCA TCGGCGCGTT CGGGTTCCTG TACGCTGCGC AGGACCAGCA
                          Sense primer → RT-PCR
2601  CAATCCCGAC GCGGGATACT CCCGCGGCAT CGGCATCCGG AACGCGCTCT
2651  TCGTGCTCGC CGGCACAAAC TTCCTCGGTA TGCTCATGAC GCTGCTGGTG
2701  CCGGAGTCCA AGGGCTTGTC GCTGGAGGAG ATGTCCAAGG ACAACGTCGT
2751  CGACGAGACC GCCCAAGAAG CGATCGCCCA AGCGTGATGT CATAAACATG
2801  CCGTCTCGAC GTGAGTGACT GAAAAAAATG TATGCTTTAT TACTCTATTG
2851  GTGTGATTAC TTAATCTAGT TTTGTATACT TTTGTAGTGT CTCTCCTTTT
2901  ACAGTTGTGG ATTTGTGGGG TTTCTCTTTC TTTCTTTT  3'
      ← Anti-sense primer RT-PCR
```

Rice D, ORYsaPhT1;3

The contig (24795) containing the rice D sequence has a total length = 4083 bp

RiceD total gene = 3483 bp
Promoter = 2511 bp
partial cDNA = 972 bp = 324 amino acids

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5'      1  TGTTTTATTG TGTTCCTCAG CTATGATTCT ATATGTACTT CTTTTCATGT
       51  AACGTTTAGC TGTGTATATT TATTTGTGGA TATAGAGATT GGATTTTTAT
      101  CCATTATCTT AAAAAAGAGC TACCATACTG TGAAAATAAT GGCTGAAATC
      151  ACACAAATTG CTTAAGTACA TCAGGACCTA GTACGTGCTA TCCAAAGCCA
      201  GACCGTTTTG CTGCATTCCA TGGACCATGC ATGATTAACC TCTGGCTCTA
      251  TGTCAAAAGT TCTGGGCACA AAAGAGTCAA AACTTCCAAT GTATATTTTA
      301  CAAACACCAA ACAAGACAGA TACAAATTAT CCCCATATTT TATTACAGGG
      351  TCCAGTAGGA TGACATGGAG TACAAATTAG TTGGTCGCTT ACCACACGGG
      401  TAATTAAAGC AGGTAGGTGA AAATGAAATC ATGAAACAAT CGATACTTAG
      451  CTTATTTAAC ATCATGCGAT GCATGCTGAT GCTGAATACT CCAATGTTCA
      501  AGCTTGCCCT CTATCAGTCT CCAAATCATG TAGGTTCAAT TGTAACGGTC
      551  CTTAATTAAT TCATCAGTCA TCACTGCCCA AATTCATAAA TATTTTGATA
      601  AAAAAACTCC CAAATTTACA CCAGGGCAAT ATTTAATTTT GGAGATCCAA
      651  GCTGTCATAT TAGCATAAGA AAGATACCTA GGAATCTGAC TTGGCAATGT
      701  GCAGACACGG GTGACTTGGA AGACCAGGCA AAGCATCTCT GCTTCTTGGC
      751  ATCAAATGAT GCTCCATCGT GGTCAATTAC TCTATCCATT AATGAAGGAG
      801  AGAGATGGGA TGAAAATATG AAATAGGCTA AAATAGTTTA GTTAAGCGGT
      851  TGTACCATCA AATATATATG TGTTCAACTT GCAACAACTC ATTAATGTTA
      901  GTACATTGAG ACAGACGCCG TGTTTAGTTC CAAAATAATT CTTTAAACTT
      951  CTAACTTTTT CATCAAATTA AAACTTTCCT ACACACAAAC TTTTAACTTT
     1001  TCCGTCACAT CGTTTCAATT TCAATCAAAC TTCCAATTTT AGTGTAAACT
     1051  AATCACATCC AGAGAGATGT GTACATTCAG TTCAGATTGC TCTGAACTTG
     1101  GGAGTATGCT TCAGACCAAG ATTAGCATGG ATTATCACCA CACGATGTCA
     1151  CACCAAATAG GAACTGAAGT TAGATTCAGC AGATACAAAA TTAACTACTA
     1201  CTTCTGGAGT TAAATTTAAA AGTTGGAGCT CTACCAAACG TACCCAAAGC
     1251  ATATTGTAGA ATTTTTTTTT TTTGACAATC CTTAATTAGT TAAGAGTTCT
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1301  TCAGAGATAA AGGTACCTTG TAGCGTTAAT CAGTAGTGCT TGCTAATAAC
1351  TCTGAAGAAC CTAAGACAAA ACTATATTTT TGGAGGGAAA GAAACTAATT
1401  TCGCGATAGC TTTAGCTAGT GTGTCATCCA CACTCCTAAG AATTATATCC
1451  TACATATGCT ACTTGGAGAT ACTAAGCAGA GCCGCTGCAA GCTTCAAGCT
1501  TCAAGACCAG AAGATCAAGC TTGGATGTCT TGGATCACAT TCACTATGAC
1551  TTGGAATGCA AAACACTTAC AAGAAATGAA AAAGAAAGAT GCTCCCTTTC
1601  ACAAAATTGA TAAAACTTTA TTTTCTTGTG AGTGTGCAAG TAGTGCTGGG
1651  AACTAACTTG TTAAGGTACA TGTAACACGG CATTGGGAAT AGTTGACTAG
1701  GATTAAACAA CATCCAAATG GAGGAGACTT CTCGAGATCA GGTATATTCC
1751  TAGGATCAAA CCATAGTACA AATAACCAAA TACTACAATT TTAGATTATT
1801  TGGGCAGTTA GGAATAATAA TTTTCAGTGT CCTTTGCAAA ACCTTAGCTC
1851  CAAGCTCATT AACATAATCG TCTAACCATA TAAATGTTTT TAATTTGAAG
1901  AAATTCAATA TTATCATTTA CAATTTAGAT CATTTGGACA GTTAATTCCT
1951  AACATGAAAG GGGTTAAAAA ATATATTTTA TTCCAAAAAA CACAACACAA
2001  ATGTAGACAC TCATAACGCG TATACTCAAC GCACACATGG ATATCCCTCT
2051  AAAAGGATGG ACCGGCAAAT CATGAAATTG ACGTGTCACC ATGGGCGTCA
2101  CACTGTCAAT AGGTATATAG TCTACCACTG AAAAATTTAA TAGCCATAAA
2151  TACGATTACA TATATCAAGT CTATAACTTG AACCTAGATG GGTTAGTTCC
2201  ACCATATATA ACGAATCTAA CCAATTGAGC TACTCTATCA TTTGGAAGTT
2251  TTTAATTTGA AGAAATTTAA AATGTTCTTT CGGAAATAGA ATTTTAAATC
2301  GAGCCTATCT CTCGTTGACT CAGATCACTC CAATCATCAT GTCCCTAAGC
2351  CGGGAAGCAG CATTAACCAA TAAAATCTTA AGCAAGTGCA TCTAGCTCAC
2401  ACGTACTAGT ACATTAATTC TCTAGGGTTA TATATATTTG CACTGCAGCT
2451  AGTTGCCTTG CTAGCTCGGC GAGGAGCAAG ACGAACAACG AGGCTACGTA
2501  CGCCGGCGAC AATGGCGGAC GGGCAGCTCA AGGTGCTGAC GACGCTGGAC
2551  CATGCGAGGA CGCAGTGGTA CCATTTCATG GCGATCGTGA TCGCCGGAAT
2601  GGGCTTCTTC ACCGACGCGT ACGACCTCTT CTGCATCTCC CTCGTCTCCA
2651  AGCTGCTCGG CCGCATCTAC TACACCGACA TCGCCAGCGA CACCCCCGGC
2701  AGCCTGCCGC CCAACGTGTC GGCGGCGGTG AACGGCGTCG CGCTGTGCGG
2751  CACGCTCGCG GGGCAGCTCT TCTTCGGGTG GCTCGGCGAC AAGCTCGGGC
2801  GGAAGAGCGT GTACGGCTTC ACGCTCGTGC TCATGGTGGT GTGCTCCGTC
2851  GCGTCGGGCC TCTCGTTCGG GCGCACGGCG AAGGGCGTCG TCGCCACGCT
2901  CTGCTTCTTC CGCTTCTGGC TCGGCTTCGG CATCGGCGGC GACTACCCGC
2951  TGTCGGCGAC GATCATGTCG GAGTACGCCA ACAAGAGGAC GCGCGGGGCG
3001  TTCATCGCCG CCGTGTTCGC CATGCAGGGG TTCGGCATCC TGTTCGGCGC
3051  CATCGTGGCG CTCGTCGTGT CGGCCGGGTT CCGGAACGCG TACCCGGCGC
3101  CGTCGTACGC CGACGGCCGC GCGGCGTCGC TGGTGCCCGA GGCCGACTAC
3151  GTGTGGCGGA TCATCCTCAT GTTCGGCACC GTCCCGGCGG CGCTCACCTA
3201  CTACTGGCGC ATGAAGATGC CGGAGACGGC GAGGTACACC GCGCTCATCG
3251  CGCGCAACGC CAAGCAGGCC GCCGCCGACA TGTCCAAGGT GCTCGACACG
3301  GAGATCCAGG AGGACGCGGA CCGCGCCGAG GCGGTCGCCG CCGGCGGCGC
3351  CGGCAACGAG TGGGGGCTCT TCTCGCGTCA CTTCGTGCGG CGGCACGGGG
3401  TGCACCTGGT GGCGACGACG AGCACGTGGT TCCTGCTCGA CATCGCGTTC
3451  TACAGCCAGA ACCTGTTCCA GAAGGACATC TTC  3'
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Rice E, ORYsaPhT1;6

The contig (19209) containing the rice E sequence has a total length = 5406 bp

RiceE total gene = 3899 bp
Promoter = 2345 bp
cDNA = 1521 bp = 507 amino acids
3' untranslated region = 34 bp

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5'     1  GGAACGAAAG CATCTCCAAT CTCTCATTGC TGACCTCTTT AAATTAACCC
      51  CCTTAATTTG ACCACCCTAC GTACCAGCTT TCATTTCTTA ATTTAATTCT
     101  CTGGCTTCTG TATTATATTG ACAGTGCTCC TATATATATG TACTTTAGCG
     151  TGGTTAGTTA TATATTATAA CAGCACGTAA TCACTGCTCA GCATGTACTT
     201  AATTTGTCCC TTGGTAGAAT GATCAGCCGA TGCTTTTGCA CGATCAGTTC
     251  ACTGTGCCAT ATAGGGGCTC CCATGCATCA TGAACAAATT AACATGACCT
     301  TCGGTAAGGC GGTACTCTCG AAAGTCGCTA TTGGGGTTGC ACGAGGGATG
     351  AGTTCATCTT CTTCTCTCTT ATTCCTCCTC CTCATCAACC TGTTTCTATA
     401  GGATGACTAG GAGTGTGGGT AGCTAGTAGC TTGCAGTTAT GGTAGGACAG
     451  AGTGTCTATG TCCCGCTGTA GAGCTGTGGT GACAGGCCAC ACATAACAGT
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 501  GATAGGCTCG GCGTTAGTTC TTTTCTCAAG TTGGTGGTTG GGAGTCGTTA
 551  GTTTCATGCT GGTGAGTTTT TCTTTTCTTT CCTGATTGTA GCCTCCTAGA
 601  GTTGTAATAT TGTATATTTT TTCATGCTAT ATTAATATGA AGCCTCGCAG
 651  CTATCTCATA TGGTTTGTTC AAAAAAATAA CACGATCAAT TGTTGTTTGT
 701  TGTGTAGTGG TCTCTCCGGT GTCACTATCG GTGATTTGAC AAAGACGCTC
 751  CCAATGTTGG ATGGTGTAAA GATAGTCATG TCTCCAGTCT TTGCACATAT
 801  ATATAATACA TAGAGTCAAA GTTTAAGATG AAAGGTAACC TTAATAAGCG
 851  ATTGAATGCT CTATGTGATG GAAATGTGCA TGACGTCTAT AATCCTTTCT
 901  AAGACTCTAA GAGTGAGTTA CAAACCGCCT TTGCTACCTG TACGTTGATT
 951  GAGTTTTAGA AAAATCGTAG TTGATTGATC AATCATCAAT CTGTTGTTGA
1001  TCTAAGGGCT ATAGCTCTAT CTTGTACCTC GAGCCAAGAG GGAGACATGA
1051  TAAGGAGAGG GATATGTCAG AGAGGACTCA CGACAACGTC CTGGTGGAGA
1101  TGGGCGGGCG GTGGGTGGGG GTGAGATCAT GTGGTTAGGT TGTAAGGGTG
1151  ACAGATCTAG CAGCGTGAGC CACCCCGAGA TGAAGGAGAA AATACATATG
1201  GATTTGTTGA TGAAGAGGGT GATGGCAGTG ATGTGGAGGG AAAGAGGACA
1251  GGGATAGGAA AGGCTTGTCC AACCTCGCCG GTGTCAGCGC TGGCAGTGCA
1301  AAGAGGAATG CCATCACACG CGAGTCAAAG TTGGGACTTA GAACGTTGGT
1351  GGCACAAGAG AAGGGGATCA GGAGAGGGGG AGGCTATGTT AAAATTAGAT
1401  TGTTACACAA ATAACAATGT AATCTGAGCT GTTTAATAAA CTATATATAA
1451  AACATCAGGC AACTAATGAA TGGTCAAGGC CGTTATCAAT TGGTCTGGAT
1501  GGTAACTCCC TGCCTTCGAT GCAGGACGGA GTGTAGCGGG GGCCCCTGTC
1551  CGACCATCCC TTGCGATAAG AGTTTTTTCC TTTCTCAGTA ACTTATATTT
1601  CCTGTATTTG TGTTTTTTGT TTCTTTTTTT CCTTGACCCC TAGCAACGGC
1651  CCTCTGACCG TTTGCGTTGT GTAACCAAAC TCTGTTGTCT TCTTTTAATA
1701  TATTGACGTG CAATCATTTA GCGCGTTCGC GAGAAAAAAA TGGTTTGGAT
1751  GGTTAAATCT TACTAGAAAC CAGAACGAGA TAGGCTACGC AATTAGCAAT
1801  GGATGGTTGG CATTAGGTCA TCTAAGGTCA AACCGATGGA GATACATTTG
1851  CTGCATAAAT AGCAACTAGC GTAATATGTG ATGTGATGTA CTTCCTACGT
1901  CTTATAATAA GTTTATTTTT TAGCTACTTA TATTTGTCTA GAAATAAGTT
1951  AATTTTTAGA ATAATTATTT GTATCGGAGT TTGTGAAAGT AAAAAGTAAT
2001  TGTATTTGGA GTACATAAAG TGAGAAAGTA TTGAGATTTG ATAAAGTAGA
2051  GGTATTTTAG TTTTTTTTAT TGGTACGTGT GAGATTAGTG AAAAATAAAT
2101  TTATTTGGGA AGGGATGTAG TACCTAGCTA CTCTTGTACC AGCTAGATTA
2151  AGTATAAACC AAAAACAACG GGAGTAGTAG TAGTAAGAAT GTAAAACGGT
2201  TCCAACATAA CGCAACAACG GAACGGTTGG ATATTCGGTT GGGAGAAGAA
2251  GGGAGATTCC GTTTGAGTAG GTTATAAATA GCAAGGAATA CATACGTAAG
2301  TTCCAAATCA TCACAAAGCA AAGCAAAGCA AGCAATTAAG CTGGCATGGG
2351  CGGCGGCGGC GGGGAGCAGC AGCAGCTTGA GGTGCTCCAC GCCCTGGACG
2401  TGGCCAAGAC GCAATGGTAC CATTTCACGG CCATCGTGGT GGCCGGAATG
2451  GGGTTCTTCA CCGACGCCTA TGACCTCTTC TGCATCTCCC TCGTCACCAA
2501  GCTGCTGGGC CGCATCTACT ACCGCGTCGA CGGGTCCCCG TCCCCCGGCA
2551  CGCTCCCCCC GCACGTCTCC GCCTCCGTCA ACGGCGTGGC CTTCGTGGGC
2601  ACGCTCTCAG GGCAACTCTT CTTCGGCTGG CTGGGCGACA AGCTCGGCCG
2651  TAAGCGCGTC TATGGCATCA CCCTCATGCT CATGGTGCTC TGCTCCCTCG
2701  CCTCCGCGCT CTCCTTTGGC CACACCCCGA CCTCCGTCAT GGCCACCCTC
2751  TGCTTCTTCC GCTTCTGGCT CGGCTTCGGC ATCGGCGGCG ACTACCCGCT
2801  CTCCGCCACC ATCATGTCCG AGTACGCCAA CAAGAAGACG CGTGGCGCCT
2851  TCATCGCCGC CGTCTTCGCG ATGCAGGGCT TCGGCATCAT CACCGGCGGC
2901  CTCGTCGCCA TCCTCGTCTC CGCCTCCTTC AGGGCCGCCT TCCCGGCGCC
2951  TCCCTACGGC GAGGACCCCG TGGCCTCCAC GCCGCCGCAG GCCGACTTCG
3001  TGTGGAGGAT CATACTCATG CTGGGCGCGC TGCCGGCGGC GCTCACCTAC
3051  TACTGGCGCA CCAAGATGCC CGAGACGGCG CGCTACACGG CGCTCGTGGC
3101  CAACAACGCC AAGCAGGCCG CGGCCGACAT GTCCAAGGTG CTGCAGGTGG
3151  TGGAGATGCG TAATATTGGT AATAATGGTG GCAGCAGGAG GCCGTTCGGG
3201  CTGTTCTCCG GCGAGTTTGT CCGGCGGCAC GGGCTGCACC TGGTGGGCAC
3251  GTCGGCGACG TGGTTGCTGC TGGACATTGC GTTCTACAGC CAGAACCTGT
3301  TCCAGAAGGA CATATTCAGC GCGGTGGGGT GGATCCCCAA GGCGGCGACG
3351  ATGAGCGCGC TGGAGGAGCT GTTCCGCATC GCGCGGGCGC AGACGCTGAT
3401  CGCGCTGTGC GGGACGGTGC CCGGCTACTG GTTCACCGTC GCGCTCATCG
3451  ACGTGGTGGG CCGTTTCAAG ATCCAGGCCG TTGGCTTCGC CGGGATGACC
3501  CTCTTCATGC TCGCCCTCGC CCTGCCGTAC CACCACTGGA CGGCGCCGGG
      Sense primer → RT-PCR
3551  GAAGCCATGC CTGGCAACCA GGTCGGCTTC GTCTTCCTCT ACGGCCTCAC
3601  CTTCTTCTTC GCCAACTTCG GGCCGAACGC GACGACGTTC ATCGTACCGG
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3651  CCGAGATCTT CCCGGCGCGT CTCCGGTCAA CCTGCCACGG CATCTCCGCC
3701  GCGTCCGGCA AGGCCGGCGC GATCATCGGA GCATTCGGTT TCCTCTACGC
3751  GGCGCAGCCA CAGGACAAGG CGCATGTCGA CGCCGGCTAC AAACCTGGGA
3801  TTGGCGTGCG GAACGCGCTC TTCGCTGCTC GCCGGGTGCA ACCTCGTTGG
3851  GTTCCTCATG ACATGATGCT CGTGCCGGAA TCGAAAGGGA AGTCGCTGGA
      3'        ← Anti-sense primr RT-PCR
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Rice F, ORYsaPhT1;12

The contig (17657) containing the rice F sequence has a total length = 12280 bp.

```
RiceF total gene = 4871 bp
Promoter = 2901 bp
cDNA = 1626 bp = 542 amino acids
3' untranslated region = 344 bp

5'     1  TAAAACAACT CATAACAAAA TAAATATAAT TACGTAAATT TTTTGAATAA
      51  GACGAATGGT TAAACATGTA AGAAAAAACC AACGGCATCA TTTATTAAAA
     101  AACGGAGGGA GTATATATCT CGTGGTCTTG TTCATGTTGG TCAATGAAGG
     151  ACTATAATAT ACTCTCTCCG NTTTTTTAAT AGATGACACC GTTGACTTTT
     201  TTAATATGTT TGACCATTCG TCTTATTCAA TAAATTTACG TAATTATAAT
     251  TTATTTTGTT ATGAGTTATT TTATCACTCA TATTTAACCA TTCGTCTTAT
     301  TCAAAAAATT TACGTAATTA TAATTTATTT TGTTATAAGT TGTTTTATCA
     351  CTCATAGTAC TTTAAGTGTG ATTTATATCT TATACATTTG CATAAAATTT
     401  TTGAATAAGA CGAATGGTCA AACATGTGAG AAAAAGTCAA TGGTGTCATC
     451  TATTAAAAAC GAAGGTAGTA ACATGGAAAC GGTGAATTTT CCATACTACT
     501  GATACTACTT TCTCTCGTTC GAATACTACT TTGTTTTCCC GTATAAAGTT
     551  TATTGACCGA AATACGGAAT CCATGAAAAG ATAAAGATGC TCTTATTATC
     601  TGATGCTACT GTTAACTCTC TACTTCTTGA GAACAGTAAA CCCTCCCTCT
     651  TTCAACTAGT CAGCAAATTA ACCAAAGCCG GTGTCTATAC CAGTCAGTCC
     701  CAAAAAAAGT CAAACCCTAG TTATGAACTT AGGCACGTAT ATGTCCGGAT
     751  TCGTAGTTAG GATTGGACTT TTTTTAGGGC TGGGGTAGTC TGGTAAAAAC
     801  CGATTCCCCA ACAGGTAAAA AGAAGTGGTC CTCGGAAGTT GCCAAACCGA
     851  ACCCACCGAT CGAATCGAGG CGTGTGTGCC GCGTGCGCGC CCGTACAGTA
     901  CGAGGAGAGC GGGACGCGGC CGGGTTCGCG ACGGCGCACA CGCAGGCTGG
     951  GCCGGTGATG GGCTGCGTGG GAGGTGTGCT GCTGATGCAT GGGCCAATGC
    1001  GCGAGATAGC TCGGTCGCGT GGTATGTGTC GGGCCTCGGT TCCCACGGGC
    1051  TTCGCACGCA GGCCGGATAC AGAAACAGAT CGAATCTCGG AACAAAAACA
    1101  GAGAGCCACA TATCACTATA TCAGATGTCA CGGTGGATTT GCCGCCGCGC
    1151  AGATCGGGAT AGCTGGCAGG CATTCGTACA CCCGGATTTC TGCTTTGATA
    1201  TTCTGTTATC ACAGTATATC CGAGGGCACC GTATCTGGTG CAAACCAGGG
    1251  TTGCTGTGCA GCCTTGCAAA TTTTCAATTA AGACCATAGA ATACCCATCC
    1301  GATGGCTAGG TATAGAGGTG GGTTACTTTT GCACTTAACC GCCCACACTC
    1351  ATCTATGCTA ATCTGTTTTT TATGCAAATC CCCCCTAATC ACAGCATCCG
    1401  TCCGCCCGCT CTTCCCGCGT CGTTTCGTTT CTGCAGCACA CGCGATTCGC
    1451  CGCCGCCCGC TCGAGTCCCC GCCGCCCGCC AGCGACTCGC CGGCGCCCCT
    1501  TCGCCACGCC ACCACCGCCA TGTTGCAAAC CCTCCCGTTG TGAGGTCACG
    1551  GGGTAGACCG TGCCGCTGCC GCCATGGAGT CTGGCACGCG CGACGTTTCG
    1601  GATTCCAACG CGATAGACCT TGTGCAGGAC CAGCGGGCGG GTGCCGTTGA
    1651  TCCTGTCGTC GCTATTGATC CCGTCTCCGT TGAGGCCGCC GCCATATATC
    1701  ATGCCGTCAT TATCGTGGAC GCGGGCCAAA CAAGGTGTGA ACTATGAACC
    1751  GTGGAAGTGT ACTTGCTGGA AGTTTATTCT GAACTTGTAT CCCTATACTT
    1801  GATGTATTCC TGAATGAGAC ATGCTTATTT TCGTATTGTG AACATTTTAA
    1851  TCTTAACCTG TATGCTCTTG TTATTGGTAG ATGCTGCCAA AATTTAGTAA
    1901  AAAGGGATAC ATGCTACCAA AATCTGAACT GCATCAAGAG TTTCTTATCT
    1951  GAGACATGTT TATCATCAAT ATATGACAAT ACATGAAACT GAACCTGTAT
    2001  CAAACCTATC AAGCTGTTGT GTTCTAGCAC ATGTGCAGAA AATGCAGAAC
    2051  AAGTAGTTTG ACAGTTTGAC ATATTACAAA ATCCGTGCTG GTTTCAGTTC
    2101  ATCTGTCATC CGAATGCATG AAGCTGAGCA TATATTCAGT TTGACAGATT
    2151  GACACAGAAA ACAGCTGGTA GTTCATTCAG GCTATTATTC AGTTACACAC
    2201  CCATAGGTTC CACTGCCACC ACAAGCAAGA AAAATGTTTT TTTATGGTTC
    2251  CACTTGACTT GACTCAATCT CATTATCAAA TGACAGAACA CAATTCCATT
    2301  GCATATTTGC AGTCACAATC TTTCAGAGTT TCAGATTACA AAATTAGTAC
    2351  ATCAGTGTCA ATCTCAGAAC ACGATTCCAT TGCATATCAG CTCGGCGGGC
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2401  CAGCGACCGC GTCGAGCCGG CTGGGCGGGG ACGCGGTCGC GGCGGCGAGC
2451  GGCGACGGCG GGAGGGCGGA GGACGGCGGC CACATGAAGC AGGGGGGATT
2501  TGCATAAAAA ACAGATTAGC AGAGATGAGT GCGGGCGGTT AAGTGCAAAA
2551  GTAGCCTACC TCTGTCCGTA GCCATCGGAT GAGCATCATG TGGTCTTGAT
2601  TGAAAGTTTG CAAGGTTGCA CAGCAACTCT GGTTTGCACG AGATACGGTG
2651  CCGTATCCGA GGGGGTGCTA GTGTCTGCCA ATGGCGTTGC ATACGTATCT
2701  CGTTTGTATA ACGGAATCGG GCTCTTTGCA CGGCACACGG TTCATCTCGT
2751  ACTCGAACTC CGATCTGTTA TAACCATCGC GTTGGATCGT AGCAGCACAC
2801  GGTTCATCTC GTACTCGAAC TCCTGATCTG TTATAACCAT CGCGTTGGAT
2851  CGTAGCAGCA GCCGCCGACC CAAACGCAAA CGCAAACGCA AACGCGACGC
2901  CATGGGAAGG CAGGACCAGC AGCTGCAGGT GCTGAACGCG CTCGACGCGG
2951  CCAAGACGCA ATGGTACCAC TTCACGGCGA TCATCGTCGC CGGCATGGGG
3001  TTCTTCACCG ATGCCTACGA CCTCTTCTGC ATCTCGCTCG TCACCAAGCT
3051  TCTCGGCCGC ATCTACTACA CCGACCCCGC CAGCCCCACC CCCGGCTCGC
3101  TGCCGCCCAA CATCGCCGCC GCGGTGAATG GCGTCGCGCT CTGCGGCACC
3151  CTCTCCGGCC AGCTCTTCTT CGGATGGCTC GGCGACAAGC TCGGCCGCAA
3201  GAGCGTCTAC GGGATGACGC TGCTGCTCAT GGTGATTTGC TCCATCGCCT
3251  CAGGGGCTCT CCTTCTCGCA CACGCCGACG AGCGTCATGG CCACGCTCTG
3301  CTTCTTCCGC TTCTGGCTCG GCTTCGGCAT CGGCGGTGAC TACCCGCTGA
3351  GCGCCACCAT CATGTCCGAG TACGCCAACA AGAAGACCCG CGGCGCGTTC
3401  ATCGCCGCCG TCTTCGCCAT GCAGGGGTTC GGCATCCTCG CCGGCGGCGT
3451  TGTCACGCTC GCCATGTCCG CGGGGTTCCA GGCCGCGTTC CCGGCCCCAG
3501  CGTACGAGGT CAATGCCGCT GCGTCCACCG TGCCGCAGGC CGACTACGTG
3551  TGGCGCATCA TCCTGATGCT CGGTGCGCTG CCGGCCATAC TGACGTACTA
3601  CTGGCGGATG AAGATGCCGG AGACGGCGCG GTACACGGCG CTCGTCGCCA
3651  AGGACGCGAA GCAGGCGTCG TCGGACATGG CCAAGGTGCT GCAGGTGGAA
3701  ATCGAGGTGG AGGAGGAGAA GCTCCAGGAC ATCACGAGGG GCAGGGACTA
3751  CGGCCTCTTC TCGGCGCGGT TCGCCAAGCG CCATGGCGCG CACCTCCTGG
3801  GCACGGCGGC GACGTGGTTC CTCGTGACGT CGCGTACTAC AGCCAGAACC
3851  TGTTCCAGAA GGACATCTTC ACCAGCATCC ACTGGATCCC CAAGGCGCGC
3901  ACCATGAGCG CGCTCGAGGA GGTGTTCCGC ATCTCCCGCG CGCAGACGCT
3951  CATCGCGCTC TGCGGCACCG TGCCGGGCTA CTGGTTCACC GTCTTCCTCA
4001  TCGACATCAT CGGCCGCTTC AAGATCCAGC TCCTCGGCTT CGCCGGGATG
4051  ACGGCGTTCA TGCTCGGCCT CTCCATCCCG TACCACCACT GGACCATGCC
4101  TGGCAACCAG GTCATCTTCG TCTTCCTCTA CGGCTTCACC TTCTTCTTCG
4151  CCAACTTCGG GCCGAACGCG ACGACGTTCA TCGTACCGGC CGAGATCTTC
4201  CCGGCGCGTC TCCGGTCAAC CTGCCACGGC ATCTCCGCCG CGTCCGGCAA
4251  GGCCGGCGCG ATCATCGGAG CATTCGGTTT CCTCTACGCG GCGCAGCCAC
                          Sense primer → RT-PCR
4301  AGGACAAGGC GCATGTCGAC GCCGGCTACA AACCTGGGAT TGGCGTGCGG
4351  AACGCGCTCT TCGTGCTCGC CGGGTGCAAC CTCGTTGGGT TCCTCATGAC
4401  ATGGATGCTC GTGCCGGAAT CGAAAGGGAA GTCGCTGGAG GAGATGTCCG
4451  GCGAGGCCGA CGACGAGGAA GCTTCTGCCA ACGGCGGTGC CATCGCCGTC
4501  AACTCGTCCG GAGTTGAGAT GGTGTAATCC TTCAGGACGC AACGAGATGA
4551  CGAACACTTG CATGCGAAGC TCGTACTTGT AGCGTGATAG GAAATGTTAT
4601  ACTTATATTT ATTAGATCGT ACTCCTACTA GTAACTATCA TAACTATGTT
4651  AGTACTTGCT TTTTAGGTAC AGGAGTTCTC TTTGTACCTC AAGTTGATCC
4701  CTAATTTTCG TAGAACTTAA TTAATTCATG GCAAGAAGTT GCTCATTACT
4751  CATTAATAGA AGCTATTCTA AACTTTTGTG GAATGTCTCC TTGTTATTTG
                          ← Anti-sense primer RT-PCR
4801  CATGTTACTT AAACAATTAT AAAAAAAATA GAAAAAAATT AAATAGATAG
4851  ATTACGATAT ATCATTACAC A  3'
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Rice G, ORYsaPhT1;7

The contig (2059) containing the rice G sequence has a total length = 17252 bp

RiceG total gene = 4801 bp
Promoter = 2852 bp
cDNA = 1581 bp = 527 amino acids
3' untranslated region = 368 bp

```
5'      1  TCTAGAAAGA TTAAAGAAAC AGATGACACA AGGAATCAGT CTTATCCCGT
       51  CCCTGGTTGC AACAAGGACA ACCACAATGG CTAATAAGCA TAGTGTTAGG
      101  CTGGCTGTTG TCTTCGGTCT GATATCATCT GAGATTGGAT CCCCTATTCT
      151  TGTCAAGAAG AATGTGCGTA TATGCAACCA CTGTCATCAT GCATTGAAGT
      201  TGATATCAAG ATATTCAGGA CGGAGGATTG TTGTCGGTGA CTCAAAGATC
      251  TATCATGAAT TCTCTGATGG CTCTTGTTGC TGTGGTGACT ACTGGTGAAT
      301  GGATATCACT GTTTCAGTAT TTGACAATGT TCTTCACACC TTCGGCAACA
      351  CCAATGGAAC GCCTGAATGA ATAGGTGTTG TGGCAAAATA TCATCATAAA
      401  GTCACAGCTT AGATGAACCA TCGAGTATGA GTCCAAATGC TACAGCTGTA
      451  TCAATTTTCG GGGGGCACAT AGACGAGAAA TCAATTCCCA GGTACTGGTA
      501  CAATGTTACT CCGGTAGCAG CGGCTCAAAG TGAAAGGGTG AATATGCACA
      551  CTGGACAGGA ACCTCCTCTT AATACTGTAA CACATTGATA TGATGTACAA
      601  AGTCCATTCT TTTCGAGTGA AGATTCTGAA TCACATAGCT CCATGTACCA
      651  CACATCATTC AATAATGAAA TTTAACAGAA AACTACTTTC ATTTGCAATT
      701  CTTAACAAAA CAGAATTTAG AGATTTAGAA GAACATTTGC TTGACACAAG
      751  GCCAAGTTTT ACTCGTTTTC ATACAAACTT CAGAACAAAA TTAAGCATAT
      801  GAAACGCATC GAAACGGCAT GTAGCCGCAC TTCAGCTATG CTCCGCCCCG
      851  GCGCTTGGCC TCCTCGGCCT CGACCATGCG GCGGGACGCC TCGGCGAGCC
      901  GGTCGAGCGC CTGCTGCACG GCGTCGCGGC CGCCGATGAG GAGCCGCGCC
      951  ACCACCTCCG GGTCGCGCTC GCCGCGGGCC TCCTCGAACT CCCGCCGCGC
     1001  GTTGGCGCGC AGCACCTCGC GCCACGGGAC GCCGCGGTCG TCGGGCCACT
     1051  CGAACAGCCG CGTGACCCGG AGGATGTCCC GGTACAGCCC CAGCGCCTCC
     1101  CGCCGCGAGC TCGTCAGCCG GCGGCGCGCC TCGGCGTCCG CCGCGTCTCC
     1151  GTCGAGCGCC CCGGAAGAGG AGGAGGGGGA CTTCTTGGCG AGGTGGCGGT
     1201  CGAGCAGCTC GTCGATGGTG TCGGGCCCCT CGTGGATGGA TCGGACCGGC
     1251  GGCCGCCGCC TCAACACGGC CACGCCGCGG AGGAGGGAGG AGGAGGCGCG
     1301  TGCTGCGACG GCCATGGCTG GGTTATTCTT CCCCTCGTCC CGTCTATTTA
     1351  CCGTTGCGTT GCTCCTGAGT TGGGTATTTG GGTGAGATCC CCAATTCTTG
     1401  ACCTGGTTGG GTCGGTATCC AGCCGATGGG CACCGTGCCA CCGTGCTACT
     1451  AGTATGTGGC AAACCGGAGA CTGGATTTAC CAAGAAATTG CGTTGATTTA
     1501  CAAAGAAACC ACACCATAAT TCTCATCGGT TTCATCATCC CATAGTACTA
     1551  CTACAATTTA TTTATTTGCA ACAACGGCGA ACCAAGATCA CAATGGAATA
     1601  CGTTTTACAT GTCCTACTTG TAACACCGAA CGCTACTACG ATGTTGATGT
     1651  ACAAGGTTTC ATCTCATCTG TACGGTGCGG TAGCCGGAGG ATTGGCCACG
     1701  GTGACGGCCT TGGTCTTGAT CCGGTACCTC GGCCGGCGAG GGATGACCTG
     1751  GCTGGGGCCC ATCCCGAACT GGCCGGTGCC GTCCATGGCC GGCGGCCCGT
     1801  CGTCGTAGAC GTAGCCGAGG AGGCGGCCGC AGGCGTCGCA GCTGATGCGG
     1851  GTGCGCTTCC GCTGGATCCC CCAGTAGTTG AGCGTCTCGA AGAAGGGGCG
     1901  GATCTTGTCC TCCGGCGCGA ACCGCAGCCG CGACTCGTCC ACCCACGAGA
     1951  AGGAGAGCGT CCCCTTGTTG CCGGCCTCGA AGTAGACCCC CTCCGGGTAT
     2001  AGCTGCGCCG CCGTCAGGTT CAGGTCCGCC CCGCACTCCG CGCACCGGTA
     2051  CGTCGCCGCC GCCGCCGATG AGGACGCCAT CCCGCCGCGC CGCGCCGCGC
     2101  CACCCGAGCA CGGAGATCTT CTGCGTGGCC GCAACGATGG ATCTGCGTAG
     2151  TTCAGTGTAA TTTTGTCCAA TTTAGGGACG ATGATTTCTA GGGAGGACAC
     2201  GGCACCGGAA GCGAAGCCGC GTTGGACTGG AATTTCTTGC TACGACCATG
     2251  AGAGGGTTCT TCTATGGTGA GAAGTCAAAG CCAAGACGCC ATGTTTTTTC
     2301  GAGTTTCGCA ATGGTTTCAC GACGGAATAC GGTGCGGCCC ATTCAGGCCC
     2351  AGTTTGTTTT GGATCGCCCG GCCCATTAGC CGTTGCTTCC TCTCTCTCCG
     2401  TTCCGTGTTC TACGAGATTT GTCTCAACAA TCAATCCGAA TTTTGGAAGC
     2451  AGAGTTGTTA CGAATTGTAT CGGCAAACAC ATATCATGTG TATCATGTGA
     2501  TCATCAGAGT ATATACATAA CAAGTAACAA AATCTGCAGG TTTGCACGTC
     2551  TCGTGTGTAG TACGGCGATA AGCTAATGGG ATATGGATCC AAAACACGCA
     2601  GAGCCATGCG ATTGCGATGC GAGCCCGTCA AAACTTGTTG CTGGAAAGGA
     2651  GGGAGAAGGC GTTGCATTCT CCCGAGAAAA ATGAAGGATA TGACCTCGGA
     2701  ATATTCTCGC GTCACCCGCG TATACATAGC AACCAACCAC CTGTTCCATC
     2751  TCTCTGTAGC TCACTCCCTC GCCGCCATTT ACGAGGCAGG AAGGTGTTTG
     2801  TGTGTGAGAG AGAGAGAGAG CCTTTGACCG CCGGAGCAGC AGCGTCACCG
     2851  CCATGGCGGG CGATCAGATG CACGTGCTCT CCGCGCTGGA CAGCGCCAAG
     2901  ACGCAGTGGT ACCACTTCAC CGCCATCGTC ATCGCCGGCA TGGGCTTCTT
     2951  CACCGACGCC TACGACCTCT TCTGCATCTC CCTCGTCACC AAGCTCATCG
     3001  GCCGCGTCTA CTACACCGCC GACGGCGCGT CCAAGCCGGG CAGCCTGCCG
     3051  CCCAACGTCT CGGCGGCCGT GAACGGCGTC GCCTTCGTCG GCACGCTCAC
     3101  GGGGCAGCTC TTCTTCGGGT GGCTCGGCGA CAGGGTCGGC CGGAAGAGCG
     3151  TCTACGGCAT GACGCTGCTC TTGATGATCA TCTGCTCCGT CGCGTCGGGG
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3201  CTGTCGTTCG GGGACACGCC GACGAGCGTC ATGGCCACGC TCTGCTTCTT
3251  CCGCTTCTGG CTCGGCTTCG GCATCGGCGG CGACTACCCG CTCAGCGCCA
3301  CCATCATGTC GGAGTACGCG AACAAGCGGA CGCGCGGGGC GTTCATCGCC
3351  GCCGTGTTCG CGATGCAGGG GTTCGGGATC CTCGCCGGCG GCGCGGTGGC
3401  GATCGGGATC ACCGCGATCT TCAGGAGCCG GTTCCCCGCG CCGCCGTTCG
3451  CCGCCGACCC GGCGGCGTCC ACCCCGCCCC AGGCCGACTA CGTGTGGCGG
3501  CTCATCCTCA TGTTCGGCGC GCTTCCCGCG GCGCTCACCT TCTACTGGCG
3551  GATGAGGATG CCGGAGACGG CGCGGTACAC CGCCATCGTC GCCAAGAACG
3601  CGGAGCGCGC CGCGGCCGAC ATGTCCAAGG TGCTCCAGGT GAAGATCACG
3651  GCGGAGCAGG CGGAGATGGC CTCGCCGGTG GACAAGCCCT TCACCAGCAA
3701  GCCCTTCGGC CTCTTCTCCG GCGAGTTCGC GCGGCGCCAC GGGTTCCACC
3751  TCCTGGGCAC GACGTCGACG TGGCTCCTCC TGGACATCGC CTACTACTCC
3801  CAGAACCTGT TCCAGAAGGA CATCTTCAGC GCCATCGGGT GGATCCCGGA
3851  GGCGAAGACG ATGAGCGCGC TGGACGAGCT GTACCACATC GCGCGCGCGC
3901  AGACGCTGAT CGCGCTGTGC GGGACGGTGC CGGGCTACTG GTTCACGGTG
3951  GCGCTGATCG ACGTGGTCGG GCGGTTCAAG ATCCAGGCGG CGGGGTTCTT
4001  CATGATGACG GCGTTCATGC TGGCGCTGGC GGTGCCGTAC GACCACTGGA
4051  CGGCGGCGGG GAACCAGATC GGGTTCGTGG TGCTGTACGC GCTCACCTTC
4101  TTCTTCGCCA ACTTCGGGCC GAACGCGACG ACGTTCATCG TGCCGGCGGA
      Sense primer → RT-PCR
4151  GATATACCCG GCGAGGCTGC GCGCGACGTG CCACGGGATA TCGGCGGCGT
4201  CGGGGAAGGT GGGCGCGATC GTCGGGTCTT TCGGGTTCCT GTACCTGGCG
4251  CAGAGCCCCG TCCCGGCCAA GGCGGCGGCG CACGGCTACC CGCCGGGCAT
4301  CGGCGTCCGC AACTCGCTCT TCGCGCTCGC CGGCTGCAGC TTGCTCGGCT
4351  TCCTCCTCAC CTTCCTTGTG CCGGAGCCCA AGGGCAAGTC GCTCGAGGAG
4401  ATGTCACGGG AGAACGAGGT CGGCCAGCCG TGATCCACC  CGTTAATTCC
4451  ACCGCCGTCC GTCTGCATGC AAGATCCATG CGTATGCGTG GTTAGTCCAC
4501  TAGAGATTTT TGTTCTCTTT TTTCTAGAAT CCATTGGAAT GCATATGTTC
4551  TTTTTTTTTT CTAGAATCCA TTAGAGGCTG GATGATGAAA TAATGGCCGC
                            ← Anti-sense primer RT-PCR
4601  CAATTAATTG TTGACGACAA TGTAGTTTAG CATTAGGTGA GTTTTTCATA
4651  TAATGAAACT ATCATTAGAG TTCATGCTGA TTCTGTTTCG GCACGAGGGA
4701  TCCTCGCGTC GTTCCTTTTT TTCTGTTGAT TGTGATGATC AAGAGCGATC
4751  TCTCCTCCAA AAAACAAAAC AGAATGCATC TCTCTATAAT CAAAAGGAAA
4801  A  3′
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Rice H, ORYsaPhT1;4

The contig (1494) containing the rice H sequence has a total length = 19078 bp

RiceH total gene = 5050 bp
Promoter = 3056 bp
cDNA = 1617 bp = 539 amino acids
3′ untranslated region = 377 bp

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5′      1  AAATTTGGTA GAATTGTTCA GTGGATTCGG TTATGGAAAT AAACTAATTG
       51  GTAGATGGAA ATGGATATGA CTGAATTTAG TATATCCCCT AAATAGGTAT
      101  GATTAATTAG TTGTCTTTTC TTATTAGGAA ATCTTATCTC GTGTGTAACT
      151  TGCACTTTTA CAAACCCGAG GTTATAAATA TATACATCTA GGGTTTTTTA
      201  ATCTATCTCT CTAGATCAAT ATAATTACTT TCAACGCATC AACACATTTT
      251  AATTCTGGTG AGTTCTTAGT TTCGAGCGAG GCTGCATCAT TTCATCAGGC
      301  ATGGAAGTAA GTTCCACCAA CTTCAATTGT ATTGGCTTAG GTAGGACTTT
      351  CTAGGTTCGT TCCGATATTC TAATTAGTTG CTATAATCGT TGCATATACT
      401  AGCTTTGGCT AAAGCTGAGT CGTTTCAATC TTTTGGTCTG ATCTTATATA
      451  TCCATCACGC TTGTATAGAT CTCTCAACTA AATTGTTTTT CTTATTATCT
      501  ACAATTTTAT AGTGTCTTGG TTAGGTCCGA TCTATTAGAT TGCTGCCAAT
      551  AAGTTTAATT CTACTAAGTC GATAGGGTTT TATGCAACAT TCATAAGAAT
      601  TCTAGCCAAT TAGTTATCTT GCTATAATTT CTGGGTGTTA CATCGGCTCA
      651  TGTTTAATTG AGTATATTCA CTAGTTAGAT CACTTTTTAT TAACTTAAAT
      701  CTTATACTGT CTTGATTTGG TCCGATCTAC CAAGGTTATT TTTAATGTGA
      751  AACGATTTAT TACTGATTAT ACATTGCTTG GTTCTAGCCG ATCGAAGCAT
      801  AAGTTCTTAT ATTAATCTAT TCAAGTTTAC ATTGGAACCA TAGCCATTGA
      851  TTGAAACGCT GTTGACATCG GCTGGCTACG CTATAACACA TCGACCTATT
      901  AGCCGATCAG CTCCTTAATC TTATACGTTC TATTGTTAGT TGTAGAATTA
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951 AACTAATTGA CATGTCCTGA ACTCGGATTT TAGGACCTAC ATTAGAGCTA
1001 AACAGATCTG TCAGGTTCTG GTGTGTTGTA CAAATTTTGA GGCAACAGGC
1051 TTTTTATCGA TTTTAATAAG TGGTGTAAAT CATCGATATC GCTATCTGAG
1101 TGAAGCCATT CTTGTCTTTA CAGTGCAGTG TCATAAGAAA ATCACCAGTC
1151 TTCAAACAAA TAGGCGTACC CACTTAACTT AATTAAACAA ATATAATTGT
1201 CCAATAAAAT GAGAACGTAG TTGAGTTTTA CTTCTACACA CCAGTTGCAA
1251 ATAGAATATT TTTTTAATGA ATATCAACCC GACCTCTATA TTTCGCATTG
1301 ATATGCACAA ACAAAAATCC CCGCTCTTAA AAAATGATAC AAGAAATGGT
1351 ACCAAAAGTC ATGTTCTCTT AAAAAAAAAA AGAAATGTCA CCCAGTTTGA
1401 TACTCACTTT GTCCCAAAAT ATGTAAGTGT TACAGGTTTT GGAGAACTTA
1451 TCCCATCGGA ACACACTTTG TTTACATTTT ATCCGATCCT ACCCCATGCA
1501 CCCTTACGCT TTCCAATTAC TCGTCGAATT GATAATACTC TCTCTCATTC
1551 TTGATCTCTC TAAAAGACCA TGAATCCCTT GAATACGGAG ATAGGTAACC
1601 TTGTCTTGTT TTGACTTGAC AAATATTGAT TCCTCGAGCA TCTAAATGGC
1651 ACATTGAGGA CATGACTATT TGTCTATCTG GCCCCACAGT AGAAGAATCC
1701 TGAATATAAA CAAATGATAC AAAACAATAA AAAGTCAATT CAACATCACT
1751 GGTAGTAGAT GGATAACATA GATAACACGT ACGCTGCCCA AATTTCGAAG
1801 ATATTAGTAC GTGATTTGTT TGACTAATCT TATTCTTATA GGAGTATTGC
1851 ATTAGGGAGA AACAAATCGA AGCGATGACG TGGCTCTTTC TAGCTAGTTT
1901 TGTCATTTGC CTCCCTATAA ATACCATCCA TGTGTACCTT GCCATCGGTT
1951 CTTCAGAGTT ACAGTGCTAA CGGCCTGCAG CAGAGTGCAG CGACTCCCCT
2001 GAAGAAACTG GTATATTAAT ATCAGGTGTG TATATATTTC ACATTTTATT
2051 CTAGTACTAC TATTAATGAC ATGTCTATAT ATGTCAATTT TAAGTATATA
2101 CATGTAATGG GAAATTAAAA TTTTCATATA TTCACAAGTT TCTTGCTCAT
2151 GGAACATGCG TCAAGGCAGG ATGTTGTGTA GGGGTGTTAA TTACTGATTG
2201 GTCATTAGTT GCCCTCATGA ATCCATGAAA AAGTTCTTCA TAAAGTCATC
2251 ACAAGAAGAG ACCTTTTGTG CCCTCTTTAC GGCATGCTAA GGTCACGAAC
2301 AGTTCAACAA AAGCAACCAC AAGATTTCTT TTCTGAAAAC TAATGAACAT
2351 TCAGAAATTT CTGTGCAATT TATCTCATGA CCTAACCAGA CGATGCTTGA
2401 GCCACGAAAT AGAAGAGACA AAGATAGTTT CGTCAATTCG AGAAGTTTGT
2451 CCGGACACTA CTGATGATAG CGGCAGATTT GGACTGATTC CATGAAAGTT
2501 GTACAGTAAG GTGCGAATCT TGAGTTGCAG AGATGCACCT GGATCCGGCT
2551 ATCTAGCTTC ACGAGAATCC CATCTCTGCT CTCCTAAATT GACCACGAAA
2601 CTGAATTTAT GTAGAGATTT TTCTCGAAAT TCAGACATTT TTCACTGGGA
2651 TGGATCGGGG ATTGTTGGCT GATAAAGCTG GATTTGAAGA AACAACAAAA
2701 TTTTGATATA TGATACCTTG AATAAACGAG GAGTTTCTGA AGTAGTGGCA
2751 TGGTCTGTTC CAGATGTCTC TCTGAACTTC CGTTTCAGTT TCAGTGGACC
2801 TTATTGTTGG TGAACTGAAA CGAATATTAT CTTCTCGTAG CCACGTGCAT
2851 TCTGTAGATT TTCTTTTGCT CAGTTCGACA CGCATATACA TCTGAGGCTA
2901 ATTAGCTCAA TTAATCGCGC GGTTTGTGTA ATTCTCCCAA ATAATTAGTT
2951 TCTCGTTCAT TGCAAATTGC AGCGAGATTT TGTCGAAATA ATAAACTTGG
3001 TGTTCAGTTA TTCTCTGCAA AAAATTGCAT ATTGCAGAGT AGCTGAGATT
3051 GGCGCCATG**G CCGGCGAGCT CAAGGTGCTG AACGCGCTCG ACTCGGCGAA**
3101 GACGCAGTGG TACCATTTCA CGGCGATCGT GATCGCCGGC ATGGGGTTCT
3151 TCACCGACGC CTACGACCTC TTCTCCATCT CCCTCGTCAC CAAGCTGCTC
3201 GGCCGCATCT ACTACTTCAA CCCGGCGTCC AAGAGCCCCG GCTCTCTCCC
3251 GCCCAACGTC TCCGCCGCCG TCAATGGCGT CGCCTTCTGC GGCACCCTCG
3301 CCGGCCAGCT CTTCTTCGGC TGGCTCGGCG ACAAGATGGG GCGCAAGAAG
3351 GTGTACGGGA TGACGCTCAT GCTCATGGTC ATCTGCTGCC TCGCCTCCGG
3401 CCTCTCGTTC GGGTCGTCGG CGAAAGGCGT CATGGCCACG CTCTGCTTCT
3451 TCCGCTTCTG GCTCGGCTTC GGCATCGGCG GCGACTACCC GCTCTCGGCG
3501 ACCATCATGT CGGAGTATGC TAACAAGCGT ACCCGTGGCG CGTTCATCGC
3551 CGCCGTGTTC GCCATGCAGG GGTTCGGCAA CCTCACCGGC GGCATCGTGG
3601 CCATCATCGT GTCCGCCGCG TTCAAGGCGA GGTTCGACGC GCCGGCGTAC
3651 AGGGACGACC GGGCCGGCTC CACCGTGCCG CAGGCCGACT ACGCGTGGCG
3701 CATCGTGCTC ATGTTCGGCG CCATCCCGGC GCTGCTCACC TACTACTGGC
3751 GGATGAAGAT GCCGGAGACG GCGCGCTACA CCGCGCTGGT GGCCAAGAAC
3801 GCGAAGCAGG CCGCCGCCGA CATGACGCAG GTGCTCAACG TCGAGATCGT
3851 GGAGGAGCAG GAGAAGGCCG ACGAGGTCGC GCGGCGCGAG CAGTTCGGGC
3901 TCTTCTCCCG CCAGTTCTTG AGACGCCATG GGCGCCACCT GCTGGGCACG
3951 ACGGTGTGCT GGTTCGTGCT GGACATCGCC TTCTACTCGT CGAACCTGTT
4001 CCAGAAGGAC ATCTACACGG CGGTGCAGTG GCTGCCCAAG GCGGACACCA
4051 TGAGCGCCCT GGAGGAGATG TTCAAGATCT CCCGGGCACA GACGCTCGTG
4101 GCGCTGTGCG GCACCATCCC GGGCTACTGG TTCACCGTCT TCTTCATCGA

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4151  CATCATCGGC CGCTTCGTCA TCCAACTCGG CGGCTTCTTC TTCATGACGG
4201  CGTTCATGCT CGGCCTCGCC GTGCCGTACC ACCACTGGAC GACGCCGGGG
4251  AACCACATCG GCTTCGTGGT CATGTACGCC TTCACCTTCT TCTTCGCCAA
                                  Sense primer → RT-PCR
4301  CTTCGGGCCC AACTCCACGA CCTTCATCGT GCCGGCGGAG ATCTTCCCGG
4351  CGAGGCTGCG TTCCACCTGC CACGGCATCT CGGCGGCGGC GGGGAAGGCC
4401  GGCGCCATCG TCGGGTCGTT CGGGTTCCTG TACGCGGCGC AGAGCACGGA
4451  CGCGAGCAAG ACGGACGCCG GCTACCCGCC GGGCATCGGC GTGCGCAACT
4501  CGCTCTTCTT CCTCGCCGGA TGCAACGTCA TCGGCTTCTT CTTCACGTTC
4551  CTGGTGCCGG AGTCGAAGGG GAAGTCGCTG GAGGAGCTCT CCGGCGAGAA
4601  CGAGGACGAT GACGATGTGC CGGAGGCGCC CTCGACGGCC GATCACCGGA
4651  CTGCGCCGGC GCCGCCAGCT TGATACCCCG CGGCAAAACC CAAATGGTCA
4701  ATCATCAGCG TTTTGTTGTA ATATATGTGC AATGGATGAT TATTCTGGTT
4751  CTGGTAGTGT ACCGAACAAA ATTACAAATA CTAGTCGTCA ACCCGTGCGA
      ← Anti-sense primer RT-PCR
4801  GTGATATTAT AAATGACACT TAGATTATGT ATTAAATATA TTTTCTAAAA
4851  TTATTGTGGC TTAAATTTTG TAAAAAAGAA TATTGCGGCT TAGATTGCAT
4901  TAGAATAACA ATAACATCGC CCACAATTCA CTTAGAGCCC CTTTGATTTG
4951  GAAGAAAAAC AAAGGAATAT TGGATGGTTT TAATCCTATA GGAAAATTTG
5001  CTACGAAGGC ATTTGAAACA AAGGATTAAA TCTTATCCTA TCCTTTGGAA
      3'
```

Rice I, ORYsaPhT1;5

The contig (1361) containing the rice I sequence has a total length = 19355 bp

RiceI total gene = 7050 bp
Promoter = 5027 bp
cDNA = 1650 bp = 550 amino acids
3' untranslated region = 373 bp

```
5'      1  CCGGCGGCGA AACACGGGAG ATCGAGGCGT TCAAGGAAGA AATGAAGCAG
       51  CTGTTCAAGA TGAGCGATCT GGGAGAGCTC ACTTTTTACA TGGGCATCGA
      101  GGTGCACCAG AGCACGGCCG CAATCACTCT GCATCAGGGT GGTTACGCGC
      151  GCCAGCTGCT GACCAAGACT AGGATGGATG GGTGCAATGC CTGCACCACT
      201  CCCATGGAGA CCCGCCTACA ACTGAGCAGA TCAAGCTCAG CCTCGCCGGA
      251  GGACACCACT GAATATCACA GCATCGTTGG AAGGTTGTGT TACCTGGTTC
      301  ACATGCGCCC TGACATAGCT TTTGCAGTTG GGTATGTGAG CAGGTTCATG
      351  GAGACCCCCA CCAGTGAGCA CATGGCGGCT ATGAAACGAA TTTTGAGGTA
      401  CGTCCCCAGC ACTCTCGACT TCGGTTGCTA CAACAGAAGT GTCGGGCCGG
      451  CGACATTGAC GGGTTTTAGC GACTCTAACA TGGCTGGAGA CGTTGACACC
      501  CAGAAGAGCA CCACGGGAGT CCTCTTCTTC CTTGGCACAA ATCCGGTGAG
      551  TTGGCAATCT CAGAAGCAAA AAATTGTGGC ATAGGCTACG ACAGCAACCT
      601  GCCAAGACGT GTGGTTGAGC CAGCTGCTGG GAGAGCTCAG CGGATTCAAG
      651  CCGGGTGCCT TCAAACTCAA GGTTGACAAC AAGTCAGCCA TTGCCCTCAG
      701  TAAGAACCCT GTGTTTCATG AACGAAGCAA GCACATCTCT ACCAGATATC
      751  ACTACATTCA CTCATGCGTG GAAGACAGAT GTTGATTCCA TTAGTACTGA
      801  AGGGTAAATT GCAGATATTC TGACCAAGGC TCTTGGGCGC GTGAGGTTCC
      851  AGGAACTCCG CACTCAGATC GGGGTCATCG ATATCAAGAA TCAGCGCAAA
      901  ATTTAGGGAG AGAAATGTTG GCAAATAAAC CAGCGCCGGT CATCGCCGGT
      951  CATCCTCTGC TTCTGCAGTT CTTGTTCTTC TTCGGTCAAC TGTAGCACGT
     1001  CCTGTTTATT TTCGATTTCT TCAGTTTCGA CATTTAAGCA GAGCTGGCCA
     1051  GTCCGCCATG CGCGTTGCGT TGTATGCCTG AGACGTGCGA GTTCACGTCG
     1101  GGGCGTGGGT GTGGTGGAGT CATAGCATAG TATGCGCTCT GCGCTTATTC
     1151  GTGTGGGATG GCACACGATC ACGCGACCGT GGAGCGTTTA GTCTGGCCTA
     1201  TGGGCCACTT CTATGTTTTT TCAATAAAAG GGAAAGTAAA CGGAAAAGCA
     1251  GCAAGCAAGT TAGCTGCGCC AAAATCTTCG TGTGTTCGTG TTGTTTCTAT
     1301  TCTCGCGTGT GTTCAAGAAT TCTTGTTGTT CTGGGACACT GGGATCAAGG
     1351  ATCGTCGGTA ACAATTGTAT CCTACTTATC CAGATCGTCC GTAACAAGTG
     1401  GCATGTTCGC ATTAGCTGCA ATACGAACAA GTGTCATACC TACCTTCACT
     1451  AGACGTTTAT GTTTTATTGC CTCCTGAAGA ATCCGAATGG AAAAAAGGAG
     1501  TGACAAGTCA ACCAAAAATC TCTACGTGAG GTGTGCGATT TTTCAACGTG
     1551  GATGGCATTG AGCCATTGAC GGCCCAATTT TGCACGCAAA TGGGAAAACC
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1601  AGATCAAAAG GAGACACATG TACGGTGTTG TTGCATGGTT AAGAAGGAAT
1651  ATTTTGACTA ATTGGGTCAT AGTGCTCTTC TGAGCACTGT ATATATTGGA
1701  AGACAAAGCA GGCAAATGAT ATGTACCATA TCAACCATAA AGATTCCCTC
1751  GATGTGCATG CAGCACCTAA CTGCAATTTA GACATGATAG AAAGAAGCTG
1801  AATAAACTAT TTCCTGGTAT GAATCTTTTA CAGTACTAAA TAAGACGGCA
1851  GCTTCTTTCT ATCAGGTGTA AATTGCAGTT TGTGTGGTCC TGTTTCTTAA
1901  TTGTTAAACC CGTTCGGTCC ATTTCGTAGT TATGTTTTGT TAGATAATGG
1951  TATTGCTTTG GATCATGTCT TCTTTTTTTT TTTTAACGTA TAGAGCTAGC
2001  CCACACTTGG ACACTCCACA TACACCCATG AGCATGCCTC CTTAGCGTAG
2051  ACTGAAAGAG ATAGAGTCTA ACGTAAATAA ATTCAAAACC TCACTGAATT
2101  TCTGATAAAA AAAAGTATAC TTCCATGAAT GTAACTGTAG GCGTGCACCA
2151  GGATTTGGAT CCTTTCCCTT TTTTAACCAA AGAATATACG AGAGTAAATT
2201  TTATAACACT ACATATATAT ATATTCATTA AATTATCACA AAACAATAGA
2251  TTTAAGATGA TGTATCACAA TAATACAGAT TTAACACCAA ATTTATTACA
2301  AATTAAACTA CAATTTAAGG TGGAGCATCA TAAAACTACA GATTTAATAA
2351  CACAATTATC ACAAAACTAA TAGCGTCAAT TTAATTACAA AAGTAGAACG
2401  TTTATAACTT AATCATAGCA GTAGTACTAA GAATTTAAAC CTCAAAATCT
2451  GTAGTTTTGT AATAATTTTA TTATTAAATC GATAGTTTTA AGATACTTAG
2501  CCTTAAACTT CTAGTTTTGT GACAAATTTG TTATTAAATT TGTAATTCTG
2551  TGATACACCA CCTTAAAACT ATAGTTTTGT GATAATTTGC TCCAAATAGT
2601  GTAAAATTTA CTCAATATAG TTAATTTGTT GTTCTAGTTC ATCCAACATC
2651  GTTTATACTC CCTGTATTCA CCTGATATTA TTTATCTTCA CATTTTGATC
2701  ATATCTTTTT TACCAATAAA ATTATAAATA TTTTAGAATA TACCATATTA
2751  TGAAAATGTT TTGCATCGTA AATATAAACA GACAATTTAA CTTTCATTGA
2801  TTATAGATTA ACCAAGTAAC CATTTAAAAG TTATAGATAG TAGGAATTGA
2851  AACATTAATA ATCAATGCTA GCGATTATTC GCATTCTTAT TTTACTCCCT
2901  CCATCCTATA ATATAATGCG CGCACGCATT TCAAGATTTA ACTTTTAAAA
2951  CATTTGACCA ACACTTAGTA TAATATGAAA TTAATTTTAT TTATCAAAAA
3001  TTATATCATT AGATTGTGAT TTGAATTTAC TTTCGTATGG TTATAATTTT
3051  GTTGCTACAA ACCTTACAGT ATATGAGAAA TTATAAGTTA AAGATTAGTT
3101  TTAGATACTA TACTAATTTT GACCGTGCCT TATATTATAG GATAGAGTGG
3151  GTATCTAATA AATTGCCGTT GTGTTTGGAT GTGAAATTAC CCGACCTTTT
3201  AACTAATTTG ATATTTTAGG AAAAATCCAC ATAAGGTGAG ATGGAAATAT
3251  CCCGTTAACT CCATATTATT TTTACCAAAA ATATTAATTA AACTAACAAG
3301  CTTTGATCGA GCTGGGTGTT TGGTACATTT GAGTACTTAT TATATTTTTA
3351  TCGTTTTGAA AGTACGTTGG CATGTTTGCT GTCTTCCGTT GATTCATGCA
3401  AGATGCCAAG AAAACAAAAT ATTTTCTTAT ATTTAATTTT AGTCGTCGTC
3451  AAGCTCAGGT GTAGAATATC GTGATATGTT GGACCTGTTA GATGTGTCCA
3501  AATCCACGCA GATGGAAGTT TGGAAACACA GTTTCATGGG TCATTCCAGA
3551  AGTTCAAACA ACCAGCCAGC TAGTCCCTCC ATCCTCAAAT ATAAAATAGT
3601  ATCCCTCTCT ATTCGTATTT TAATGTAAGA CGTCGTTAGT CTTTTCACTA
3651  ACATTTGATC ATTTGTCTTT TTCAAAAAAA TTATGTAATT ATTATTTATT
3701  TTATTGTGAT TTAATTTATC ATCAAATGTT TTTAAGCATG ACATAAATAT
3751  TTTTATATTT AAAAAAAATT TGAAATGGTT AAAAAGTCAA CAATATCATA
3801  ATACAAAAGG AGTACCTTTT AAAATTTGAG AGGTCAGTTT TTTTTTAGAA
3851  CTCATACCCA CAAACTTAAA CATTTATGAG GTACTTTTAT TATTGTCCTC
3901  TCCCCCTTAT TCACTGGACA GGAACATTCC ACAGTTGATG GTTTCCTTCA
3951  ATTAGGGGCA TATCGTCCAA AAAACTATAA TTGTTTTGAT TTTCCTTCGT
4001  CTTAATCTCT ATAAGAAAAT AGAAATTTTT ATTTTTTTTG GTGGATCCAT
4051  ATAGTATTAT ATATAGGTGA TATATACATA AGCTTCTCGT GCGCACACAC
4101  ACTAACATAA AAATATCATC GAAATTTTCT TAAAAAAATA TATACATGTG
4151  CCTCCTATAG TAATAGAGAA TGAAAACACC TATATAGAAA GTTTCATCTT
4201  CAAATTCATC ATTTAAAGAG GAAAAAAACA AAATTCTTGT AGTTAATATT
4251  CATAAAACTC AACTTTTTTT CTCTTTTTTG CAGGTATAAT ATAATGAACT
4301  TACAGTTGAG ACATACATAT GAATAATACT ATTGAAAATA TTTTTATAAA
4351  CTTTTCTAAA ATTTTCACTC GAAGTTTGTG CACCCAACAT ATGTTCTCTT
4401  ATATAACATA CCTGGTATGA ATAAGCAACT ACATTACAAT AGAACAACAC
4451  AAGGCAAGAT TAATATATAT ACCCAATGAA ATATTCCCTC TGTTTTCTTT
4501  AATTTAACAT TGGAAAGTAT AAAATAGAAC TAACCAACGT CAGAAAGTAA
4551  AAATAGAGGA AACATATAAA CCTAAAGATC AGCATTTAAA ATACTTTTCG
4601  TGTATTTTCC TCCAATTTAC AAGATAATTC ACATAACAGT ACAACCTACT
4651  ATTTAGTGGA GAAAATAGTT AATAATGACC TATATGATCC AGAGGTTCAA
4701  ACTGTATATG TTCTAGACCA TTCACAACCT GTGCCAAAAT ACCAATGAGA
4751  TTATGACATG CATGCATATG CATTGCCCTA TAGTATGATG GTAACCATGC
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4801  ATACCAGCCA TAACAAATTA AACTTGTCCA TGCATGAACA AACCAGAATG
4851  TTCCAGGATT TATTCTCTAG CTAATACCTG TCACTTACAC CAAATTCCTT
4901  GCACTATATA TATAGCTACA ATAATTCATG CGTGCATGCC ATTGCCATTG
4951  ACCATATGCA AGACTAGTGC CATAACAATT TCACAATAAC TTAGAGTATT
5001  ATATCAAAGA TAGTCGCCGG CGCCGCCATG GCTCAGGATC GCAAGGTGCT
5051  CGACGCGCTG GACACGGCGA AGACGCAGTG GTACCACTTC ACGGCGGTGG
5101  TGATCGCCGG CATGGGGTTC TTCACCGACG CCTACGACCT CTTCTCCATC
5151  TCCCTCGTCA CCAAGCTGCT CGGCCGCATC TACTACTTCA ACCCGGCGTC
5201  CAAGAGCCCC GGCTCCCTCC CGCCCAACGT CTCCGCCGCC GTCAATGGCG
5251  TCGCCTTCTG CGGCACCCTC GCCGGCCAGC TCTTCTTCGG CTGGCTCGGC
5301  GACAAGATGG GGCGCAAGAA GGTGTACGGG ATGACGCTCA TGCTCATGGT
5351  CATCTGCTGC CTCGCCTCCG GCCTCTCGTT CGGGTCGTCG GCGAAAGGCG
5401  TCATGGCCAC GCTCTGCTTC TTCCGCTTCT GGCTTGGGTT CGGCATCGGT
5451  GGCGACTACC CGCTCTCGGC GACCATCATG TCGGAGTATG CTAACAAGCG
5501  TACCCGTGGC GCGTTCATCG CCGCCGTGTT CGCCATGCAG GGCTTCGGCA
5551  ACCTCACCGG CGGCATCGTG GCCATCATCG TGTCCGCCGC GTTCAAGTCG
5601  CGGTTCGACG CGCCGGCGTA CAGGGACGAC CGGGCCGGCT CCACCGTGCC
5651  GCAGGCTGAC TACGCGTGGC GCATCGTGCT CATGTTCGGC GCCATCCCGG
5701  CGCTGCTCAC CTACTACTGG CGGATGAAGA TGCCGGAGAC GGCGCGCTAC
5751  ACCGCGCTGG TCGCCAAGAA CGACAAGAAG GCAGCCGCCG ACATGGCGCG
5801  CGTTCTCAAC GTCGAGCTCG TCGACGAGCA GGAGAAGGCA GCGGCGGCGA
5851  CGGCGGCGGC TGCGGAGGAG GAGGCAGCAC GGCGCGAGCA GTACGGGCTC
5901  TTCTCCCGGG AATTCGCACG GCGCCATGGC CACCACCTGC TGGGCACGAC
5951  GGTGTGCTGG TTCGTGCTGG ACATCGCCTA CTACTCGCAG AACCTGTTCC
6001  AGAAGGACAT CTACACGGCG GTGCAGTGGC TGCCCAAGGC GGACACCATG
6051  AGCGCCCTGG AGGAGATGTT CAAGATCTCC CGGGCACAGA CGCTCGTGGC
6101  GCTGTGCGGC ACCATCCCGG GCTACTGGTT CACCGTCCTC TTCATCGACA
6151  TCGTCGGCCG CTTCGCCATC CAGCTCGGCG GCTTCTTCCT CATGACGGCG
6201  TTCATGCTCG GCCTCGCCGT GCCGTACCAC CACTGGACGA CGCCGGGGAA
6251  CCACGTCGGC TTCGTGGTCA TGTATGCCTT CACCTTCTTC TTCGCCAACT
6301  TCGGGCCAAA CTCCACGACC TTCATCGTGC CGGCGGAGAT CTTCCCGGCG
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Sense primer → RT-PCR

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6351  AGGCTGCGTT CCACCTGCCA CGGCATCTCG TCGGCGGCCG GGAAGATGGG
6401  CGCCATCGTC GGGTCGTTCG GGTTCTTGTA CGCCGCGCAG AGCACCGACC
6451  CGAGCAAGAC GGACGCCGGC TACCCGCCGG GCATCGGCGT GCGCAACTCG
6501  CTGTTCCTGC TCGCCGGATG CAACGTCGTC GGCTTCTTGT TCACGTTCTT
6551  GGTGCCAGAG TCGAAGGGGA AGTCGCTGGA GGAGCTCTCC GGCGAGAACG
6601  AAATGGAGGC TGAGCCGGCG GCAGCAACTA ACTCCTACAG GCAGACCGTC
6651  CCTGACAGCG GACAGTCCGA GTAAATAAAC ATAAATTACC GCAATTACTG
6701  TATCTGATCT TGTATACTCT CACTAGTTGC ATTCTGCCAT TCTTGCCTCA
6751  GGCCGTATAA TAATAATCTC GAAATCTTGC TACTGCCCAT GACTAGGATT
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← Anti-sense primer RT-PCR

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6801  CTGGGAACCA GCAGATTTTC ACCATGTTGG TACAGCTTCT CTGGATCTCT
6851  TCTATGGTCT GCAAGACCAC CTGGAATTCT CACATGGCAC CTTGGTGCCC
6901  TCTGAGTCTC ATTTCACAGA TTGGCAAATG AGATAAACTT TAAACGCTCC
6951  AGAGCAATTG ATACTGGCAA ATGAGATAAT GTTCCTTGAG CGGCATTGCA
7001  TCCACGTCTT TTTCACCCTG ATGCTTATAA CTCTGAGGAG CATGGATGTT
      3'
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Rice J, ORYsaPhT1;13

The contig (285) containing the rice J sequence has a total length = 28353 bp

RiceJ total gene = 4499 bp
Promoter = 3838 bp
partial cDNA = 661 bp = 220.3 amino acids

```
5'      1  ATAAATCCAC GAGTTACTAA GTGCTCCTTC ACTTTAAAAG CATCGTCGAA
       51  TTCGCAGGCT ATTTAATTCT TAGAGTCAGC ACATGGACAA ATTATCTTCC
      101  TCATATTGTT CTTCTCAGCG TATGCCTTGG CAATACCAAT AAATTTAAAA
      151  TATGGAGCTC TTCCTTCAAA ATTTTGCATG CATAAATCCA TCACAAATTG
      201  AGGTCTAAAA GCTTAATAAA TTGCATACCC AGTGTGGAAA TGAGTAGATC
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 251  TAAGCACCGT AGAAAAATCC CCCGAAGATT TGAAGTTTAA AACCTCCCCC
 301  CTATATTTAA GTTACTTTAG GAGAGAGAAA CTTCGGGGAG AATGAACAGG
 351  ACTCGGGGAG GAAGAAGACT ATATATATAG GGGCATCTTT GCAGGCGGGC
 401  CATATAAGAG GCACATGCAA AAATCGATGT TGCCCAGGCG CACAACGTCT
 451  GCCTTCTTCG TAGGCGCCGC TTATATGGTC TGCCTGCAAA GATCGATCTT
 501  TGCAGGCGGA CCGCCCCCTC CACCCCGGGT ACATTTTGTG CCGGTAGGGT
 551  TTTGGCTCCG AATTACATGT CCGCCTGCGA AAATGGAACC CCTACGACGC
 601  CGAAAATGAG TTTTCTAGCA GTGCCCTCCG CCTCCACCTC GCATCTTCTA
 651  TGGGCGAAGG AACCGAGCAG CTCTATGCCT CGATGGGCTG CTGCCACTGC
 701  ACCATTGGCC AGCTCTGTCC GAGCCATTGC CGACGTCGCG AGGCCCCTCC
 751  GGTCGCTCGC CCTCTCTTTG GCTGGCTGTT GAATAAACAA TGGCAGATAA
 801  AGGGATAGAG AAAAATAGAG AGGAAAAAGA GCGAGAAGGC TAACTATCGC
 851  TGCCAGCGAT CGTTTTTTTT TCTTTTTTTT TCTTTTCCTC TTTTAGTGAG
 901  TTTATACACC TAAAGTTTAC ACATCTAAAA TTTATCCACC TAAAGTTTGT
 951  GAAGCTAATG TTTATAAGTC AAAAGTTTAT ATACCCAATT AAAATTCAAA
1001  TTCGGATTCA AATATTTTAT ATTAAAATAT TTCTATACAT AAAGTTTATG
1051  TGTGCAAAGT ATACGATATA AAGTCTATGC ACATGAATGA GAGTATTAGA
1101  TTTTTTTCCT AATTTTCTTT TACTATTTTT TTTGAAATTT ATGATGTAAT
1151  AGAAAAGTAA AAAAGAGAGA TGAAAGATGA GTATTAGGGG GGAGGGACGG
1201  GTGATCGCTA GGAGGAGAGG GAAGCGATCA CCCGCCCACT AGCAACCCCC
1251  CAAAAAGAGA GCAGAGAAAC ATAAAAATGC CCCGACATGT GGAGTCCACA
1301  TGGATATATC CCATGCTAAC TCAGCCGGGT TGATTATAGC CAACTCGCCA
1351  CGTTAGCTGA AACCGAGCAC AATACCACCC TAGTAACTCA GGTATTCCAA
1401  TAATTTGTGC ATAAGTAGAG AGAGAGGACG AGGAACGAAA GGGAGGAAGT
1451  GGGCCAACGT ATTGGCCTTG GCCCAAGAAG AATATATTGA GTTAGGTTTT
1501  TTCACGATTT TTAGAGGGAA AAATTGAATG AGTTGGATCA ATGAAGTCAA
1551  TCAGAAGGGA GAAGGGGAAA ATAAGTGGGA AAGGATCGAA AGGGCTCCGA
1601  CTCGACAAGA ATGAACAATG TTGATGAAAA ATATTTTCGC TAATGATTTT
1651  CCCAAATTTG TGGAGTATGT TACTCTAAGC TTGTGTATAT ATAAGCTATT
1701  TTGTAGGTAA TCGAAGAAAA GTAATTTAAA TATAGTGAGA AGAACAGAAG
1751  GTGAAGGAGG CCATTTATAG AAATAGCATA CTAGGACTGT TAAATAGTTA
1801  TCTGTATATT ATCTTTGGAT ACCAAAGATG CGGCCTACTA GTCACTGCCC
1851  ATTAATTCTT TTCAAGTTTG TTGAATACAT AAGCACATAA TGATTTAATT
1901  TATTCCCTAA ATAAATCATG ACATTGCAGA TGTAAACTAG TATAATCATA
1951  TCATACGATT TACACACGTA AACTAGACAG TAAAGCATGA ACAGGTCGAA
2001  TATGCGAAAC ATGTTGAATG CATACCGAGG GTTTTGAAAA ACTGGCTGCT
2051  CAGTAGGAAG AACTCATGGT ACAGTGCGTT GATGACGGCG CGCAGCACGT
2101  GAGTGAAGAG GAAGACGAGC TGTTGTGGAT GAACAGGGAG TAGTCACGCA
2151  AACCGCTTCC TAAAAACCTT AATGCCACCA TCTCTTGGTG CAGGACGTCG
2201  AACACGAAGG TTCTGGAAAC CTACTCTCCC GATCGCCGGT GCATGCCCGC
2251  GAGCGGGATA AAGTAGACTA CGAGTGATGG CGCCGTACAG AGGAGCAGGT
2301  AACCCTAGAT TGATTTCGCA TTTTGCATGG AGGCAGTGAC TCTACTTATA
2351  TAGAGATATC AGGATGCTTG ATCAGGGCGC CTGCACGATC TCCACTACAG
2401  GTAACCGAAC CGAATAAGTC GCTCGTAACT TATCCAGACT CCACGCCGTT
2451  TCACGCACCG GATTATTCAG AGCGTTTCCA AAAAACAAAT CTGAATTTTC
2501  TGCAGCAAAA CAAAACTGCA AAAGGAACCA ATTTTATCAG ACCATTCGAC
2551  GCGTACGTCG TGCACGTGCC CCAGCATGTC CGGCCAGGCG AGGCGAGCGA
2601  GCGTGCGTCT GTGTTCCTCC TGTTCTCTCC ACCTCACATG CCTCAAGTGG
2651  CTAGGAGAGC ATCCTCCCTT TTAAGGAGGT ACTAAACTAC CCATACATGT
2701  TATCCCATGA GGTGTGGTTT TGTGATTTTT CAAAGAATTA ATCTTTGATG
2751  AAGTTGTCGA TAAAGTTTTC TGTGTACAAG TTTGTTCATA ATTGTTTGAA
2801  TTATATATTA TGAAAAGCTA TTAAAAGTAT AACTATATGG ATATATTCTC
2851  ATGAAAACAT TATTGATACC ATTTTTGCAA ATGAATTATG AAACCTTAAA
2901  ATGTTTGTAC ATCCATACAT CCTAAGACCA GATCTATCTG TAAACGGAGC
2951  AGTGTTTTCT GAACTCGCTA GCTCTTACGG AATCTGTATC TTCATTAGAG
3001  AATATTACAC CCAGCAATTC ATCGGGCACG TCAGATGCCA GGATGATTGA
3051  AACAGACATC TTTTACTTTC TTGTTCCTGC TTGCCGTCCG GGACTTCAAG
3101  TGGACGACTT TGATCGAATA AATTGCAACC AGCAAGTTCC AGCTAACTTC
3151  TGATCTGAAG AACCAAGCAC GTACACCACT AGTATTTTCC AGTCTACATT
3201  TAATTTGCAC TACCTAGTAG CTATCAGTTG TTACACTAAA TTCAAGATTT
3251  CTACAGATCC AAGTATATCT GATACAATAA TAAGAATATT CATTCAGTTC
3301  ACCATATGCT AGTGTCTTGT TGCACAAGTA CAGACACATG TTGACAATAA
3351  ACCATTTGTG ACTCCCAGAT GGCTGTCAGG TTCAGCAGAT TGTAGAAATG
3401  AAATTCATAA AGTTCAGGGA ACTATCTTAA AAAAAGTGCA GCAAGTGTTT
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3451  CAAGGAAAAA CATTAAAATC TAACTATAGT ATAATTGTAA TATAATTCCA
3501  CTATAACTAT AATATAAGTT GTATATAAGT ATTAAAATAA TACCTAAAAT
3551  AATATATGCA ACTTTATATC TAACTTATAT AGAATTTACG ATGTAGTTAC
3601  AGTGCAGTTA CACTACAACT GTTGTACTAT AGTTACATTT GAAAGTTTTT
3651  TTTGCCGAAA AAAACTTGTG ATAGTTTTTC AGCAATTCCA TTCAGAAATC
3701  GATTAGATGG AAAGCATAAA ACCTTTGATA TGACAGAGAC AGTTTAATTA
3751  TGCTTGGAAG CTTATATAAC TTAGCAACTT ACAATAGCAA GGACAGGATC
3801  GAAGAAATTA AGGAAGCAAG TCGGATCAGA AAGCAACAAT GGCCGGCAAC
3851  CAGCAGCTGC GGGTGCTGCA CGCCCTGGAC ATTGCAAGGA CACAGCTGTA
                                    Sense primer → RT-PCR
3901  CCATTTCATC GCGATCGTGA TCGCCGGCAT GGGCTTCTTC ACCGACGCCT
3951  ACGACCTCTT CTCCATCTCC CTCGTCGCCG ACCTCCTCGG CCACGTCTAC
4001  TACCACGGCG AGCTCCCCCG GAACATCCAC GCCGCCGTCA CCGGCATCGC
4051  GCTTTGTGGC ACCGTCCCCG GACAGCTCGT GTTCGGCTGG CTCGGTGACA
4101  AGATGGGCCG GAAGCGTGTC TATGGAATCA CCCTCCTCCT CATGGTCGCC
4151  TCCTCTCTCG CCTCCGGACT CTCCTTCAGT AAGCGCGAGG GGAAGAACGT
4201  TATCGCTGTG CTTTGCTTCT TCCGGTTTTG GCTTGGTGTT AGCATCGGTG
4251  GCGATTACCC GCTCTCCGCC ACCATCATGT CAGAGTACGC CAACAAGAGG
4301  ACTCGTGGTG CCTTCATTGC TGCTGTTTTT GCTATGCAAG TAAGTAGAAA
4351  CTATGTTTTT CTTATTGTAA TTGAATCCAG CCTGAACATG AATGATGTTT
                    ← Anti-sense primer RT-PCR
4401  CTGCATATGA CCCACACCAT CCATTCTTTA GTTTTTAAAC ATAACATTTC
4451  GGAAATTATT AATGGACAAA GTTTCAAAAG CTTGACAAAA TCTTTTAAA 3′
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Rice K, ORYsaPhT1;8

The contig (24425) containing the rice K sequence has a total length = 4097 bp

RiceK total gene = 2351 bp
Promoter = 384 bp
cDNA = 1626 bp = 542 amino acids
3′ untranslated region = 341 bp

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5′      1  CTAAAAGCTT TTAGGGTGAA CTGATTGAAA TAAATCATAC ATACAATAAA
       51  TACATTATAT ATCGCATATT AATAAGTCAG CGTGCGAAGA GCTTCTGGGA
      101  TGAACCGATC GGTGACCCGA GCGCGTGCGT TCGGGCACCT ATCAAACCCT
      151  TGTACACAAG CGCATGACCC CGCGTGAGAT TCGTGCCGTT TTTCTTCCCT
      201  CTCCATATTA CAGTGACAAC CAAGCAGGCG TGCCAAATCC ACTGGACACG
      251  GCCAAGTCGG CTACCATTCC TGCGCCTACA TATACCGCGG CATCTCCCGC
      301  GCCATTGCTG TGCCCAGAGA GCTCGACACA AATACAGGGG GACTCGTCTT
      351  CTTCCCCGAG CTTTGCGAGC AGAGTCGTTC AGCCATGGCG CGGCAGGAGC
      401  AGCAGCAGCA CCTACAGGTG CTGAGCGCGC TGGACGCGGC GAAGACGCAG
      451  TGGTACCACT TCACGGCGAT CGTCGTCGCC GGCATGGGCT TCTTCACCGA
      501  CGCCTACGAC CTCTTCTGCA TCTCCCTCGT CACCAAGCTG CTCGGCCGCA
      551  TCTACTACAC CGACCTCGCC AAGGAGAACC CCGGCAGCCT GCCGCCCAAC
      601  GTCGCCGCGG CGGTGAACGG CGTCGCGTTC TGCGGCACGC TCGCGGGCCA
      651  GCTCTTCTTC GGGTGGCTCG GCGACAAGCT CGGCCGGAAG AGCGTGTACG
      701  GGATGACGCT GCTGATGATG GTCATCTGCT CCATCGCGTC GGGGCTCTCG
      751  TTCTCGCACA CGCCCACCAA CGTCATGGCG ACGCTCTGCT TCTTCCGGTT
      801  CTGGCTCGGC TTCGGCATCG GCGGCGACTA CCCGCTGTCG GCGACGATCA
      851  TGTCGGAGTA CGCCAACAAG AAGACCCGCG GCGCGTTCAT CGCCGCCGTG
      901  TTCGCGATGC AGGGGTTCGG CATCCTCGCC GGCGGCATCG TCACCCTCAT
      951  CATCTCCTCC GCGTTCCGCG CCGGGTTCCC GGCGCCGGCG TACCAGGACG
     1001  ACCGCGCGGG CTCCACCGTC CGCCAGGCCG ACTACGTGTG GCGGATCATC
     1051  CTCATGCTCG GCGCCATGCC GGCGCTGCTC ACCTACTACT GGCGGATGAA
     1101  GATGCCGGAG ACGGCGCGCT ACACCGCCCT CGTCGCCAAG AACGCCAAGC
     1151  AGGCCGCCGC CGACATGTCC AAGGTGCTCC AGGTCGAGAT CCAGGAGGAG
     1201  CAGGACAAGC TGGAGCAGAT GGTGACCCGG AACAGCAGCA GCTTCGGCCT
     1251  CTTCTCCCGC CAGTTCGCGC GCCGCCACGG CCTCCACCTC GTCGGCACCG
     1301  CCACGACATG GTTCCTCCTC GACATCGCCT TCTACAGCCA GAACCTGTTC
     1351  CAGAAGGACA TCTTCACCAG CATCAACTGG ATCCCCAAGG CCAAGACCAT
     1401  GTCGGCGCTG GAGGAGGTGT TCCGCATCGC GCGCGCCCAG ACGCTCATCG
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1451  CGCTGTGCGG CACCGTCCCG GGCTACTGGT TCACCGTCTT CCTCATCGAC
1501  ATCGTCGGCC GCTTCGCCAT CCAGCTGCTA GGGTTTTTCA TGATGACCGT
1551  GTTCATGCTC GGCCTCGCCG TGCCGTACCA CCACTGGACG ACGAAGGGGA
1601  ACCACATCGG CTTCGTCGTC ATGTACGCCT TCACCTTCTT CTTCGCCAAC
1651  TTCGGCCCCA ACTCCACCAC CTTCATCGTG CCGGCGGAGA TCTTCCCGGC
1701  GAGGCTGCGT TCCACCTGCC ACGGCATCTC GGCGGCGGCG GGGAAGGCCG
1751  GCGCCATCAT CGGATCGTTC GGGTTCCTGT ACGCGGCGCA GGACCCGCAC
1801  AAGCCCGACG CCGGGTACAA ACCCGGGATC GGGGTGAGGA ACTCGCTGTT
1851  CGTGCTCGCC GGATGCAACC TGCTCGGGTT CATCTGCACG TTCCTCGTGC
                                    Sense primer → RT-PCR
1901  CGGAGTCGAA GGGGAAGTCG CTGGAGGAGA TGTCCGGCGA GGCGGAGGAC
1951  GACGACGACG AGGTGGCCGC CGCCGGCGGT GGCGCCGCCG TGCGGCCGCA
2001  GACGGCGTAG TGTATGACT  GCACGTGAAT ATAGTGTAGG TTTTACTTAA
2051  TTTACTTACT GTTATTATTA TTATACTCCT ACTTGTGTTT GTCTATGTGA
2101  AATTGGGAAT CATGAACCCA TGATCATGTT TTGTTAGGTT AAGAAGGCAA
2151  AAGAAATGTG TGTTAAATAC TTCAATTATG TAAACTCTGT TTTTAAGTAT
2201  TTGGCCACTT GAGGAATAAT TCTTGCAGAC CAGCAATTTG GCACGAATAC
2251  ATTTTATAAT TGAACTACCA CTCTACCAGA GTAGTACACT ACTAATTTGC
2301  CTTAGAGAGG ACAATGAGAT GTCTAAATTT TCAATTATGG CTGTGTTGAG
                                    ← Anti-sense primer RT-PCR
2351  T  3'
```

Tomato Phosphate Transporter

LYCes;Pht1;1

The cDNA sequence of Lycopersicon esculentum (LePT1) was submitted to the Genbank database by Daram *et al.* (1998). The promoter region was cloned by myself and has not been added to the database.
LYCes;Pht1;1 total gene = 3269 bp
Promoter = 1356 bp
CDNA = 1620 bp = 540 amino acids
Putative cis-regulating motifs (referenced in section 7.2) have the motif aligned under the sequence with the percentage of similarity.

```
5'      1  GGCAAGCTTA CTATAGGGCA CGCGTGGTCG ACGGCCCTGG CTGGTACTAA
           LePT1promHindIII →
       51  CCTGTCTTCT AGATTTATGG ATCACATGTA AATGTTAGGC TGTTTCCTTT
      101  ATATTTTTTT GAAAAGTCTT CACTTTTTTT TCTAAGAAAA AATAAATAAA
      151  TACTATCTTT TAATATTTAT ATAGCTCTGC ATGACTTCAT AGTATGGACT
      201  ATATATAAGT AAAACAGGAG TTTACTTGTT TTCTTTTAAT TATTGGTCTT
      251  TGGTAGCAAC TTTAAACCTA ATATTTACTA ATATCAACCA CTCCCATATG
      301  TTAAAAAAAT AATATCACTT GTTTATACTT TTTAAGTAAA TTATGTGTCT
      351  TTGTGATGCA ATCATGCCAC AAATTTTTTG AATGAAACTG TCTCTCTATT
                                        LePT1promA →
      401  TACTATTTAC TACAAGGTAG AGGCAAGGCT GCGTTGATGA TTTCGGAATT
                 LePT1promA1 →
      451  GAGTTATGAT TGAACGACTA GGTCCTTATC ATCTTTTTAG ACTTTAGCTT
           ← LePT1promX
                    GCACAAGTA GACCCTTAAA CT-MRR2 Motif 62%
      501  TTGAGATTAC GTTCGGTTTG TGATGATATC ATCAGTCAGA ATCTCAAGGA
      551  ATCTGTGTAT CTCTATCTGG AAATAACTGT TGTTAGTAGT AAGCACTGTA
                                                   TGCAATTCT
      601  TAGCTGATGT GATTTTAAAC CACTTTTTTT CTCAATTTTG ACTATTTAAA
           AT-Motif 70%                                      AAA
      651  GCTGAGGTTG TTTATTGATT TCACATATAC ACTATAGTTT TATAATACTA
           ACTTAG-Motif 78%
                   TG TTTATAAGT-Motif 73%
      701  TGTCACAACT TTAGGTTACC AGCTTTGAAA AGTAGCACAC ATGCTTAGCA
                       LePT1promq →
             TAACAACT AT-Motif 80%
```

```
 751  TGTGTCATAT TAAAACTAAT AAAATAATAG ACAGAAAACA AGACTTTTTG
                  AAAACTTAG-Motif 78%
 801  TGGCCCCCCT TGCTTTGTCT TTATCAAGAA GTCAATTTTC TACCCCCCTA
            T GCATTCTAT-Motif 70%
 851  CAGGACAACT TACAAATTTA TTAGTATTAA TTATTCAGAT AAGGTTTGAT
                                                 LePT1promB →
          AAAACT TAG-Motif 78%
                    TGTTTA TAAGT-Motif 73%
 901  TAATGCAATT CCCAAATATC TTCTTATTTT TGTAATGTTA TTTGAGTTTA
                 ← LePT1promY
 951  ACTTGTGTGT ACTGACAGTG ACAATGTAAA GCAATAGTAA AAAGTGGGAT
1001  TAGTAACCTT GAACAATAAG TATGTTACTT GTTACAACAG GTTAAAATAT
                                                TAATATAT-Motif 88%
                                                  TAATAT
1051  ATGTTCTTGT GAACCAAGTA TACTAATGTT ATAAGTGATG TAAGTTAAGT
      AT-Motif 88%                 TGTTT ATAAGT-Motif 72%    T
1101  CCTTATAATT TACATAACCT CTTGTTTGAT CAGATTTGAT TTATGCAATC
      GTTTATAAGT-Motif 73%
1151  CTAAACTACC TTTTTGGTTT GTTCTGTTAT TTGAGTTTAA CTTGTGTATA
1201  CTGATAGTGT AACGCGGGAG TAGTAACCTT GAACACTAAG TATGTTCCTT
                            LePT1promC →
                                        AAAACTTAG-Motif 78%
1251  GTTATAACAG GCTAAAGTTA TATATTCTTG TAAAGAATGT TTACTATGTT
                    TGCATTC TAT-Motif 70%                TGCA
                        TAA TATAT-Motif 88%
1301  AGTTATGTAA GTTGAATTGT TATAATTTAC ACCCTCTTGT TCTGTTTTTT
                                      ← LePT1promZ
      ATTCTAT-Motif 70%TGTT TATAAGT-Motif 73%
                                        CTTCTTGTT CTA-Motif 84%
1351  GCAGGAAGTT TAGTCATGGC GAACGATTTG CAAGTGCTAA ATGCACTAGA
      ← LePT1promBamHI cctaggcggc
1401  TGTCGCGAAG ACACAACTGT ATCACTTCAC AGCGATTGTG ATTGCTGGCA
1451  TGGGTTTTTT TACTGATGCT TATGACCTTT TCTGCATTTC TATGGTCACT
1501  AAATTGCTTG GTCGTCTTTA CTACCATCAT GACGGTGCAT TGAAACCTGG
1551  CTCTCTGCCC CCTAATGTTT CAGCAGCTGT TAATGGAGTC GCCTTCTGTG
1601  GCACCCTTGC TGGACAGTTG TTCTTCGGGT GGCTTGGAGA TAAAATGGGA
1651  AGGAAGAAAG TCTATGGAAT GACCCTTATG ATTATGGTCA TTTGTTCAAT
1701  TGCCTCGGGG CTTTCATTTG GCCATACACC AAAAGGTGTT ATGACTACGC
                  ← GWTOMATO389
1751  TTTGTTTCTT CAGATTCTGG CTAGCATTTG GCATTCGTGG TGATTATCCCC
      IPCRTOMATO1 →          ← GWTOMATO452
1801  TTTCTGCCAC CATCATGTCT GAGTATGCTA ACAAAAAGAC CCGTGGAGCG
1851  TTCATTGCTG CTGTGTTTGC TATGCAAGGT TTCGGAATTC TGGCTGGTGG
1901  AATGGTGGCA ATCATTGTTT CTGCAGCATT CAAGGGCGCA TTCCCTGCAC
1951  CAGCATATGA GGTTGATGCT ATTGGTTCAA CAGTCCCTCA GGCTGATTTC
2001  GTGTGGCGTA TAATTCTCAT GTTTGGTGCA ATCCCTGCTG GACTTACTTA
2051  TTACTGGCGT ATGAAGATGC CTGAAACTGC CCGTTACACT GCCTTGGTCG
2101  CCAAGAACTT GAAACAGGCA GCTAACGACA TGTCCAAGGT GTTGCAAGTC
2151  GAAATTGAAG CAGAGCCAGA GAAAGTTACA GCTATTTCTG AAGCAAAAGG
2201  AGCCAATGAC TTTGGTTTGT TCACTAAGGA GTTCCTCCGT CGCCATGGAC
2251  TTCACTTGCT TGGAACTGCT AGCACATGGT TCTTGTTGGA CATTGCTTTC
2301  TACAGTCAAA ACCTTTTCCA GAAGGACATT TTCAGTGCAA TTGGATGGAT
2351  TCCACCAGCA CAAACCATGA ACGCGTTGGA AGAAGTTTAC AAGATTGCAA
2401  GGGCACAAAC ACTTATTGCT CTTTGTAGTA CTGTTCCTGG TTACTGGTTC
2451  ACAGTTGCAT TCATCGATAA GATTGGTCGA TTTGCAATTC AGTTGATGGG
2501  ATTCTTCTTC ATGACAGTCT TCATGTTTGC CTTAGCCATT CCATACCATC
2551  ACTGGACTCT CAAGGATCAC AGAATTGGCT TCGTGGTCAT GTACTCATTC
2601  ACCTTTTTCT TCGCCAATTT TGGTCCAAAC GCCACAACAT TCGTCGTCCC
2651  TGCTGAGATT TTCCCAGCCA GGCTTAGGTC CACATGCCAT GGAATATCAG
2701  CAGCAGCAGG TAAAGCAGGA GCTATGGTTG GTGCATTTGG ATTCTTATAC
2751  GCTGCTCAGC CCACGGATCC AACAAAGACT GACGCCGGTT ACCCTCCTGG
2801  CCATTGGTGT GAGGAACTCG TTGATCGTCC TTGGTTGTGT AACTTCCTCG
2851  GTATGCTGTT CACATTCTTG GTTCCAGAAT CCAATGGGAA GTCATTGGAA
```

```
2901  GATTTGTCGA GGGAAAACGA AGGGGAAGAG GAAACTGTAG CTGAAATAAG
2951  AGCAACAAGT GGAAGGACAG TTCCTGTGTG AGTTTTAGAC AAGTTATCAG
3001  TTAGTATACA CTACAATGCA GTTTGAGTTA ATTTGTGGTA TTTGGGATTA
3051  GAAAGAGATT GTTTGTTGGT TTGTTATAAG AAGATGGAAT AAGCTCTTAT
3101  CTTTTTGTTT GTTTGTTTGG GTAATTAAAC ATTATTACCT TACTTCTGCA
3151  AATCTCAGAA ATTCTGAGAT TATATAAAGT AACCAAAGGA GGTTCTTTGG
3201  TTGTCTATCT CTTTTTATAA AACATTTCTT GACTCTAAAA AAAAAAAAAA
3251  AAAACTCGAG ACTAGTTCA 3'
```

sGFP – synthetic Green fluorescent protein

```
   1  TCTAGAATGG TGAGCAAGGG CGAGGAGCTG TTCACCGGGG TGGTGCCCAT
  51  CTGGTCGAGC TGGACGGCGA CGTAAACGGC CACAAGTTCA GCGTGTCCGG
 101  CGAGGGCGAG GGCGATGCCA CCTACGGCAA GCTGACCCTG AAGTTCATCT
                                  Sense primer → GFPFOR133
 151  GCACCACCGG CAAGCTGCCC GTGCCCTGGC CCACCCTCGT GACCACCTTC
 201  ACCTACGGCG TGCAGTGCTT CAGCCGCTAC CCCGACCACA TGAAGCAGCA
 251  CGACTTCTTC AAGTCCGCCA TGCCCGAAGG CTACGTCCAG GAGCGCACCA
 301  TCTTCTTCAA GGACGACGGC AACTACAAGA CCCGCGCCGA GGTGAAGTTC
 351  GAGGGCGACA CCCTGGTGAA CCGCATCGAG CTGAAGGGCA TCGACTTCAA
 401  GGAGGACGGC AACATCCTGG GGCACAAGCT GGAGTACAAC TACAACAGCC
 451  ACAACGTCTA TATCATGGCC GACAAGCAGA AGAACGGCAT CAAGGTGAAC
 501  TTCAAGATCC GCCACAACAT CGAGGACGGC AGCGTGCAGC TCGCCGACCA
      Antisense primer ← GFPREV511
 551  CTACCAGCAG AACACCCCCA TCGGCGACGG CCCCGTGCTG CTGCCCGACA
 601  ACCACTACCT GAGCACCCAG TCCGCCCTGA GCAAAGACCC CAACGAGAAG
 651  CGCGATCACA TGGTCCTGCT GGAGTTCGTG ACCGCCGCCG GGATCACTCT
 701  CGGCATGGAC GAGCTGTACA AGTAAGATAT C
```

Appendix 5: ANOVA's for results presented in Chapter 4.

Abbreviations: Df = degrees of freedom; Sum Sq = Sum squared; Mean Sq = Mean Sum Squared

Part 1: Results from experiments analysing P efficiency between seven barley cultivars.

ANOVA results for g Root Dry weight per plant.

Trait	Df	Sum Sq	Mean Sq	F value	LSD (P=0.05)	LSD (P=0.01)
Rep	2	0.51154	0.25577	6.840		
Cultivar	6	2.43565	0.40594	10.856	0.184	0.247
Phosphate	2	1.65075	0.82537	22.072	0.121	0.161
Cultivar x Phosphate	12	0.54994	0.04583	1.226	0.319	0.427
Residuals	39	1.45839	0.03739			

ANOVA results for g Shoot Dry weight per plant.

Trait	Df	Sum Sq	Mean Sq	F value	LSD (P=0.05)	LSD (P=0.01)
Rep	2	0.0644	0.0322	11.998		
Cultivar	6	0.2815	0.0469	17.496	0.049	0.066
Phosphate	2	0.9274	0.4636	172.914	0.032	0.043
Cultivar x Phosphate	12	0.1289	0.0107	4.006	0.085	0.114
Residuals	39	0.1046	0.0027			

ANOVA results for concentration of P mg in roots per plant.

Trait	Df	Sum Sq	Mean Sq	F value	LSD (P=0.05)	LSD (P=0.01)
Rep	2	0.7605	0.3803	5.118		
Cultivar	6	11.4060	1.9010	25.585	0.260	0.348
Phosphate	2	20.7007	10.3504	139.305	0.170	0.228
Cultivar x Phosphate	12	3.7874	0.3156	4.248	0.450	0.602
Residuals	39	2.9720	0.0743			

ANOVA results for concentration of P mg in shoots per plant.

Trait	Df	Sum Sq	Mean Sq	F value	LSD (P=0.05)	LSD (P=0.01)
Rep	2	2.1864	1.0932	3.442		
Cultivar	6	22.2248	3.7041	11.664	0.537	0.718
Phosphate	2	203.184	101.5919	319.896	0.351	0.470
Cultivar x Phosphate	12	21.0074	1.7506	5.512	0.930	1.244
Residuals	39	12.7031	0.3176			

ANOVA results for Total P mg per plant.

Trait	Df	Sum Sq	Mean Sq	F value	LSD (P=0.05)	LSD (P=0.01)
Rep	2	5.4672	2.7336	5.041		
Cultivar	6	64.3303	10.7217	19.773	0.701	0.939
Phosphate	2	346.2865	173.1432	319.305	0.459	0.615
Cultivar x Phosphate	12	36.6860	3.0572	5.638	1.215	1.626
Residuals	40	21.6900	0.5423			

ANOVA results for proportion of P in shoot per plant.

Trait	Df	Sum Sq	Mean Sq	F value	LSD (P=0.05)	LSD (P=0.01)
Rep	2	39.6626	19.8313	1.073		
Phosphate	2	538.7776	89.7963	4.856	4.095	5.481
Cultivar	6	10571.3870	5285.6935	285.865	2.681	3.588
Cultivar x Phosphate	12	928.5291	77.3774	4.185	7.092	9.494
Residuals	38	739.6080	18.4902			

ANOVA results for specific uptake of P in root per plant.

Trait	Df	Sum Sq	Mean Sq	F value	LSD (P=0.05)	LSD (P=0.01)
Rep	2	77.5223	38.7612	4.662		
Phosphate	2	77.9059	12.9843	1.562	2.746	3.676
Cultivar	6	1218.2314	609.1157	73.260	1.798	2.406
Cultivar x Phosphate	12	40.6106	3.3842	0.407	4.756	6.366
Residuals	38	324.2620	8.3144			

Part 2: Results from experiments analysing the rate of colonisation of three barley cultivars by two AM fungi.

Trait	Df	Sum Sq	Mean Sq	F value
Cultivar	2	1758	879	7.3795
Mycorrhiza	1	41829	41829	351.0912
Phosphate	1	3962	3962	33.2553
Day	5	70316	14063	118.0405
Rep	2	468	234	1.9629
Cultivar:Mycorrhiza	2	198	99	0.8311
Cultivar:Phosphate	2	31	16	0.1311
Cultivar:Day	10	3130	313	2.6276
Mycorrhiza:Phosphate	1	2068	2068	17.3619
Mycorrhiza:Day	5	3404	681	5.715
Phosphate:Day	5	1067	213	1.7914
Cultivar:Mycorrhiza:Phosphate	2	974	487	4.0895
Cultivar:Mycorrhiza:Day	10	1871	187	1.5707
Cultivar:Phosphate:Day	10	1272	127	1.0675
Mycorrhiza:Phosphate:Day	5	3249	650	5.4542
Cultivar:Mycorrhiza:Phosphate:Day	10	775	78	0.6505
Residuals	141	16799	119	

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