Phosphate uptake process

Introduction

Whilst soil moisture and nitrogen (N) are major limitations to agricultural production systems in the SAT, phosphorus (P) deficiency also limits crop growth on many soils. The cost and availability of phosphatic fertilizers to the majority of farmers in the region restrict their use. Attention has, therefore, turned to making more efficient use of the soil phosphate reserves by seeking crop genotypes and management systems that result in more effective uptake and utilization of soil-P. A number of promising strategies are being explored, many of which are presented in this Workshop. To be effectively developed, all of them require an under- standing of the mechanisms of phosphate uptake and utilization by crop plants. Use of molecular tools by nutritional physiologists in recent years has consider- ably enhanced the understanding of these mechanisms and provided new opportunities for manipulating nutrient uptake and utilization. Key genes involved in the process have been identified and information on their role and regulation is accumulating. This paper provides a summary of the phosphate uptake process and highlights some of the important molecular mechanisms involved.

The external phosphate concentration

Plant roots acquire their phosphate from the external soil solution where it is in equilibrium with phosphate sorbed onto soil minerals and colloids. These sorption reactions maintain low concentrations of phosphate in soil solution whilst buffering the amount of phosphate in solution. The movement of phosphate ions to the sites through which it is taken up into root cells occurs by diffusion. This is a relatively slow process and, in P-deficient soils, results

in the concentration of phosphate in solution being depleted around plant roots. Thus, many of the strategies for improving phosphate uptake are aimed at reducing this depletion zone and increasing the solution phosphate concentration immediately adjacent to the sites of phosphate uptake in the roots.

Extension of roots into undepleted regions of soil provides the root tip with external P concentrations similar to those in the bulk soil solution. Further back along the root axis extension of root hairs from epidermal cells in many plant species considerably increases the volume of soil explored for phosphate. Still further back, the soil volume explored by some species growing in low phosphate soils may be enhanced by the presence of hyphae of mycorrhizal fungi which can extend several centimeters from the root surface. A cone of soil in which the concentration of phosphate in solution is depleted thus develops back from the root tip. Within this zone the equilibrium of the phosphate sorption will have shifted towards release of sorbed phosphate ions into solution. Distance to the uptake sites within the root and any barriers to phosphate diffusion determine whether the plant can access these ions.

The root apoplasm

The walls of root epidermal and cortical cells and the associated intercellular spaces make up the apoplasm. In young roots, these walls are composed of inter- laced fibres that form an open latticework (Peterson and Cholewa, 1998). Soil solution can therefore, move radially towards the central stellar region of the root through the pores in this latticework and the intercellular spaces. The suberised Casparian band around the tangential walls of

endodermal cells prevents radial movement into the central stele of nutrients in the soil solution. The band also restricts nutrients within the stele from leaking out into the apoplasm. Older areas of some roots have another layer of suberised cells in the outer layers of cortical cells that form the exodermis. This layer further restricts apoplastic movement of external soil solution in these regions of the root. In slower growing roots, such as those on plants subjected to stress, the exodermis may be formed closer to the tip than in rapidly growing roots (Perumalla and Peterson, 1986). Movement of solutes through the apoplasm also appears to be restricted near the meristematic region close to the root tip where the microfibrils of the cell walls appear densely packed (Peterson and Cholewa, 1998).

The interlacing fibres of cell walls in the apoplasm serve to filter soil solution. They also increase the path length over which phosphate ions must diffuse to the underlying uptake sites on the plasmalemma. The presence of carboxyl groups associated with the pectic polysaccharides of the cell wall fibres results in an overall negative charge. Anions such as phos- phate are repelled by this charge and restricted to the larger pores within the apoplasm. Mucilages, ex- creted into cell walls and surrounding many roots, carry negatively charged hydroxyl groups which can further modify the flow of anions. These, and other root excretions, provide substrates for rhizosphere micro-organisms that can influence nutrient concentrations close to the uptake sites. The net effect is that movement of phosphate may be impeded within the apoplast, further modifying the concentration of phosphate at the outer surface of the plasmalemma, particularly in cells in the inner cortex. Even in soils well supplied with phosphate this

concentration is likely to be less than 2 micro molar. In the P-deficient soils of the SAT, the concentration will be much lower than this.

Uptake of phosphate into the symplasm

The plasmalemma of root epidermal and cortical cells provides the boundary between the apoplasm and the symplasm. Once inside the symplasm, nutrient ions in the cytoplasm can move radially through to the stele via plasmodesmata connections without encountering further membrane barriers (Clarkson, 1993). Trans- port of ions across the semipermeable plasmalemma is, therefore, a critical step that mediates and regulates the uptake of nutrients into the plant. The physiology and kinetics of transport of nutrients across the plas- malemma has been known for a long time. Epstein and colleagues (Epstein and Hagen, 1952; Epstein, 1953) conducted classical experiments over 40 years ago that showed that ion uptake by plant roots could be described by first order kinetics in a similar manner to many enzyme reactions. They also showed that, for the major nutrients studied, the process could be described by two phases – a high-affinity system operating at low external nutrient concentrations and a low-affinity system operating at higher external concentrations. An implication arising from these experiments was that uptake through the plasmalemma was mediated by proteins embedded in this membrane. However, isolation and identification of the specific proteins involved proved to be very difficult until nutritional physiologists began to apply molecular techniques to the study of the mechanisms of ion transport in plants. With the aid of this new technology over the past 8 years, many of the specific proteins involved in transport of a number of nutrient ions in plants have been characterized, the

genes encoding these proteins identified, and the complex regulatory systems involved have begun to be untangled. Genes encoding the phosphate transporter proteins responsible for influx of phosphate into the cells of roots and some other tissues have been isolated, and the roles of some of these have been defined.

Uptake of phosphate into the root symplasm involves transport from concentrations less than 2 micro molar in the surrounding apoplasm across the membrane to the cytoplasm where phosphate concentrations are maintained in the mill molar range. This, together with the net negative charge on the inside of the plasmalemma, necessitates that strong electro-chemical gradients need to be overcome for successful transfer of phosphate anions into root cells. Trans- port of phosphate across the plasmalemma, therefore, requires a high-affinity, energy driven transport mechanism. The genes encoding such transporters have been isolated from a number of plant species during the past 4 years and the sequence and topology of the encoded transporter proteins inferred from the DNA sequences.

Identification of plant phosphate transporters

An Expressed Sequence Tag from an *Arabidopsis* clone containing similarities to the sequences of genes encoding phosphate transporters that had been isolated from yeast and fungi led to the isolation of the first reported genes encoding plant phosphate transporters (Muchhal et al., 1996: Smith et al., 1997a). These genes were isolated from *Arabidopsis* . They now form part of the rapidly growing *Pht1* family of plant phosphate transporters which includes members isolated from tomato (Daram et al., 1998; Liu et al., 1998a), potato (Leggewie et al., 1997), *Catharanthus* (Kai et al., 1997),

Medicago (Liu et al., 1998b), barley (Smith et al., 1999) and additional genes from *Arabidopsis* (Mitsukawa et al., 1997a). Eight different members of this family of phosphate transporters have been isolated from the barley genome to date (Smith et al., 1999). A member of a second family of phosphate transporters, *Pht2* , that has similarities to the quite different family of phosphate transporters represented by some mammalian Na+/phosphate cotransporters has recently been isolated from *Arabidopsis* (Daram et al., 1999). This transporter, which functions as an H+/H2PO4− cotransporter in plants, is primarily expressed in *Arabidopsis* shoot tissues. It appears to be involved in the internal cycling of phosphorus within the plant.