

## From Fate to Faith and Soil to Cell: Estimation of Phosphate (Pi) Uptake Rate, Pi-Uptake Kinetics and Relative Growth Rate are Important Parameters to Scavenge Pi by *Brassica* Cultivars under P-Starved Environment

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As a rule, ion uptake by plant cells and roots has features of saturation kinetics. This is in accordance with the assumption of control, as for example by the number of binding sites of ions (carriers, permeases), or the capacity of the proton efflux pumps, in the plasma membrane and tonoplast. Solute transport across membranes is carrier mediated transport. Protein macromolecules integrated into the membrane matrix seems to be the carriers. The carrier-mediated process is subject to kinetics assuming that the number of carriers (binding sites) in the membranes is limited. Kinetics of ion transport through a membrane is considered equivalent to relationship between an enzyme and its substrate. To obtain plants of different P status, two genetically diverse *Brassica* cultivars (P-tolerant 'Con-1' and P-sensitive 'Gold Rush') were grown for several weeks in nutrient solution culture media. P-uptake kinetics of the roots with intact plants in short-term experiments by monitoring P depletion in culture media revealed that P-tolerant 'Con-1' cultivar had favorable characteristics for P-uptake because of high  $I_{max}$  or  $V_{max}$  and low  $K_m$  or  $\frac{1}{2} I_{max}$  value than P-sensitive 'Gold Rush' cultivar. By plotting relative growth rate (RGR) and internal P-concentration (PNC) among P-tolerant (group I; Brown Raya, Con-1, Rainbow, Dunkled and Peela Raya) and P-sensitive (group II; Toria, Sultan Raya, B.S.A, Toria Selection and Gold Rush) cultivars revealed that group I cultivars showed large metabolic fraction and small structural fraction than group II cultivars which provided basis for P-stress tolerance.

**Key words:** *Brassica*, Membrane binding sites, Carrier-mediated transport,  $I_{max}$  or  $V_{max}$ ,  $K_m$  Pi-uptake rate, RGR

### 1 INTRODUCTION

Phosphorus (P) is an essential inorganic nutrient for all living organisms. It is required as a structural component in nucleic acids and phospholipids, as an element in intermediates in carbon metabolism, and to allow (in) activation of a wide range of enzymes. After nitrogen (N), P is quantitatively the most important inorganic nutrient for plant growth, and often limits primary productivity in natural systems as well as cropping systems, unless supplied as fertilizer (Vance et al., 2003). P is a non-renewable resource, unlike N, which can be assimilated from  $N_2$  into  $NH_3$  by free-living and symbiotic  $N_2$ -fixing microorganisms, or converted into  $NH_3$ ,  $NO_3^-$  or urea industrially. Moreover, global P reserves are rapidly being depleted; depending on the assumed scenario, current P reserves will be halved (relative to the reserves at the turn of the twentieth century) by 2040 or, more likely, by 2060 (Steen, 1998; Lambers et al., 2006). Whilst our global P reserves are being depleted, P levels in many agricultural

soils are building up, because 80–90% of P applied as fertilizer is sorbed by soil particles, rendering it unavailable for plants that lack specific adaptation to access sorbed P (Gerke et al., 1994; Jones, 1998). With decreasing global P reserves, P-fertilizer prices are bound to increase. There is an urgent need to develop crops that are more efficient at acquiring inorganic P (Pi) from soil and/or at using P more efficiently. Equally, it is becoming increasingly important to use crops that reduce the off-site effects of P fertilization, thus reducing the risks of pollution of streams and rivers. Unlike nitrate, which readily moves in soil towards the roots via both mass flow and diffusion, phosphate (Pi) is highly immobile. Mass flow typically delivers as little as 1–5% of a plant's P demand, and the amount intercepted by growing roots is only half of that (Lambers et al., 1998; 2006). The rest of all required Pi must reach the root surface via diffusion; diffusion coefficients for phosphate in soil are typically very low compared with those for other nutrients:  $0.3\text{--}3.3 \times 10^{-13} \text{ m}^2 \text{ s}^{-1}$  (Clarkson, 1981). Diffusion is particularly slow in dry soil (Bhadoria et al., 2004). Increasing Pi delivery to roots via mass flow can be achieved by enhanced transpiration rates, but this cannot have a major effect, and would be at the expense of a plant's water-use efficiency. Root interception of Pi can be increased by

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root proliferation, increased frequency and length of root hairs, a modified root architecture that enhances allocation to shallow soil horizons, and mycorrhizal symbioses. Diffusion of Pi towards the root can be increased by increasing the moisture content of dry soil, or by increasing the Pi concentrations in the soil solution through release of Pi from complexed, sorbed or organic forms of P.

The rate at which an ion species is absorbed is dependent on its concentration in the nutrient medium. This relationship is not linear but follows an asymptotic curve. Epstein and Hagen (1952) have linked the process of carrier mediated transport of an ion across a membrane to the enzyme mediated catalysis of a substrate. Michaelis-Menten kinetics have therefore been applied to the ion uptake process. The ion being absorbed has been compared with the substrate and the carrier with the enzyme. The active uptake of nutrients by plants follows the saturation kinetics of Michaelis-Menten, described by the parameters  $V_{max}$ ,  $K_m$  and  $C_{min}$ . The maximal uptake rate or influx ( $V_{max}$  or  $I_{max}$ ) is obtained when all the available carrier sites are loaded, that is, the maximal transport rate. During the uptake process of a nutrient, only the net uptake of ions is determined, resultant of the inflow and outflow of ions at root surface.  $K_m$  is the Michaelis-Menten constant, equal to the substrate ion concentration that gives half of the maximal transport rate; the lower this constant, the higher the affinity between the carrier sites and ions.  $C_{min}$  is the concentration at which net uptake of ions ceases; it is the lowest concentration at which roots can extract ions from the soil solution.  $C_{min}$  concentrations differ considerably among plant species (Marschner, 1995). Michaelis-Menten equation as modified by Nielson and Barber (1978) describes net influx,  $I_n$ , of a nutrient into plant roots as a function of external concentration of this nutrient. Michaelis-Menten kinetics is used as part of mechanistic model to stimulate nutrient transfer from soil into plants, information is needed on ion uptake kinetics of plants under conditions of varying nutrient supply.

Roots are able to alter the uptake kinetics in response to low P availability by increasing  $V_{max}$  (Nielsen & Barber, 1978; Schenk & Barber, 1980; Machado et al., 2004). However, studies with wild species showed that a lower P uptake rate might be a characteristic of plant adaptation to infertile soils, since P diffusion from the soil to root surface would be the main restriction in such environment (Chapin III, 1983). Working with contrasting rice inbred lines for P efficiency, Furlani (1988) found lower values of  $V_{max}$  and  $K_m$  for a P-efficient genotype during a kinetics trial under low available P concentration. In sorghum, P-efficient inbred lines under low P developed larger root systems, lower P uptake rates and transported more P from older to younger leaves (Furlani et al., 1984). In maize, Ciarelli et al. (1998) observed inverse relation between P-uptake rate and length of root system among single and double hybrids, and among inbred lines and their progenies. Differences in root morphology and physiology among cultivars are related to different P accumulation by plants in nutrient solution and/or soil. Genotypes with larger and longer root systems

presented also higher dry matter yield of shoot and root. Significant differences in root surface area of field grown maize genotypes were positively correlated with shoot and root dry matter yield at flowering stage (Schenk and Barber, 1980). Similar correlation between root length and dry matter yield of shoot and root was also observed among maize inbred lines and their  $F_1$  hybrids grown in nutrient solution (Furlani et al., 1984; Ciarelli et al., 1998). These results are evidences of the effect of root morphology and P-uptake kinetic characteristics on shoot and root growth and on dry matter yield of plants, grown either in soil or nutrient solution, and that such parameters are inheritable characters, passive of being selected and improved through a plant breeding program. In earlier studies (Akhtar et al., 2006ab; Akhtar et al., 2007), the available genetic pool of *Brassica* cultivars was evaluated under low external P concentration and observed differences among them in relation to grain, shoot and root dry matter yield, and P-concentration and accumulation in plant parts.

The objective of the present work was to determine the kinetics of P uptake and relative growth rate of *Brassica* cultivars, contrasting by yield and P-acquisition, utilization and accumulation characteristics. A more comprehensive understanding of physiological basis of P-uptake and growth rate can lead us to the formulation of strategies aimed at developing better P-efficient cultivars suited for sustainable cropping with less fertilizer inputs. These approaches can also lead to more sustainable cropping systems with less off-site risks of eutrophication of streams and rivers.

## 2 MATERIALS AND METHODS

### 2.1 Relative Growth Rate (RGR) Trial of Genetically Diverse *Brassica* Cultivars

#### 2.1.1 Plant Material and Culture Conditions

Different cultivars tested were; 'B.S.A.', 'Brown Raya', 'Con-1', 'Dunkled', 'Peela Raya', 'Rainbow', 'Gold Rush', 'Toria', 'Toria Selection' and 'Sultan Raya'. Seeds were germinated in polyethylene lined iron trays containing pre-washed riverbed sand and irrigated with distilled water for seed germination and seedling establishment. Seven day old uniform sized seedlings were transplanted in foam plugged holes in thermopal sheets floating on continuously aerated 200-L half strength modified Johnson's solution (Johnson et al., 1957) in 2 polyethylene lined tubs (1 x 1 x 0.3 m) on day zero after transplanting (DAT=0). Plants were maintained in each tub by randomly repeating ten plants of each cultivar in equally spaced numbered holes. The composition of the solution was; [in mM]:  $KNO_3$  [2],  $NH_4NO_3$  [1],  $Ca(NO_3)_2 \cdot 4H_2O$  [2],  $MgSO_4 \cdot 7H_2O$  [0.5],  $K_2SO_4$  [0.5] and [in  $\mu M$ ]: Fe(III)-EDTA (Ferric Dihydrogen Ethylene Diamine Tetra-acetic Acid) [50],  $H_3BO_3$  [25],  $MnSO_4 \cdot H_2O$  [2],  $ZnSO_4 \cdot 7H_2O$  [2],  $CuSO_4 \cdot 5H_2O$  [0.5], KCl [50],  $H_2MoO_4$  [0.5]. The solutions were modified to maintain deficient (20  $\mu M$ ) and adequate (200  $\mu M$ ) P

levels using  $\text{NH}_4\text{H}_2\text{PO}_4$ . The pH of the solution was monitored daily and maintained at  $5.5 \pm 0.5$ . The solution was renewed every 5 days to maintain nutrient concentrations being exhausted because of plant uptake.

### 2.1.2 Biomass Assay and Relative Growth Rate (RGR)

Various growth related parameters [absolute growth rate (AGR), P-stress factor (PSF), P-utilization efficiency (PUE), specific absorption rate (SAR), specific utilization rate (SUR)] have been reported earlier in detail (Akhtar et al., 2007). In relative growth rate (RGR) trial, on 25 DAT, four plants of each cultivar were harvested while remaining six plants of each cultivar were harvested on 31 DAT. Plants of each harvest were immediately washed in distilled water and blotted dry using filter paper sheets. After separating the plants into roots, shoots and stems, samples were immediately put in craft paper bags and air dried in the laboratory for twelve hours before oven drying. Samples were dried at  $70^\circ\text{C}$  for 48 hours in a forced air oven and then weighed. RGR was calculated by assuming growth in exponential terms as following:

$$\text{RGR} ([\text{g g}^{-1} \text{ day}^{-1}]) = \frac{\text{Log } M_2 - \text{Log } M_1}{T_2 - T_1}$$

Where ' $M_2$ ' refers to biomass recorded at time ' $T_2$ ' (second harvest) and ' $M_1$ ' refers to biomass recorded at time ' $T_1$ ' (first harvest).

### 2.2 Depletion Trials and P-Uptake Kinetics ( $V_{\max}$ or $I_{\max}$ and $K_m$ or $\frac{1}{2} I_{\max}$ ) Protocol

Kinetic parameters of P uptake were evaluated by using two cultivars which varied in PUE for biomass synthesis under adequate as well as deficient conditions. Non-efficient: 'Gold Rush' and efficient: 'Con-1' cultivar in terms of PUE. Detailed experimental protocol is described in earlier study (Akhtar et al., 2007). however, brief summary is described below.

The experimental design consisted of randomized complete blocks with five replications. Seven-day-old, uniform-sized seedlings were transferred to 3.0 L plastic pots in continuously aerated nutrient solution. Plants were grown in solutions up to 28 days of age under optimum nutrition and only deionized water was frequently added to the pots, readjusting the initial volume. When plants were 28-day-old, before running the kinetics experiment, an additional trial was carried out with the objective of defining the adequate P-concentration for P-depletion to occur within 24 hours. Three P-concentrations (0.14, 0.20 and 0.26  $\text{mmol L}^{-1}$  using  $\text{KH}_2\text{PO}_4$ ), two contrasting *Brassica* cultivars (P-tolerant 'Brown Raya' and P-sensitive 'B.S.A.'), with 3 replications were used in this test. The P-concentration defined for the short term kinetics experiment was 0.20  $\text{mmol L}^{-1}$ . Considering that *Brassica* plants at this age already had relatively extensive root systems and were grown under P-stress pressure, P was rapidly depleted from solution in less than 24 hours. The 28-day-old plants of cultivars (P-tolerant 'Con-1' and P-sensitive 'Gold Rush') were transferred to other 3.0-L containers with fresh solutions of the same composition, but without P. Plants remained 24 hours in the P-free solution, after which they

were suspended from solution, so that P was added to the pots at the concentration of 0.20  $\text{mmol P L}^{-1}$ . Solutions were carefully agitated and a 10 mL aliquot sample was taken from each pot for the initial P-concentration determination. Plants were put into nutrient solutions and P depletion from solutions was followed by taking 10 mL aliquot samples from each pot at 90 minute intervals. The solution level in each pot was readjusted to its initial volume with deionized water before each sample was taken. During 10 hours and 30 minutes seven samples were taken at 90 minute intervals and a last one 24 hours after the beginning of the test. The aliquot samples were stored in small, capped glass vials under refrigeration, until P analyses were performed using UV-Spectrophotometer. Kinetic parameters were estimated by plotting the quantity of P in the solution (Q) against time (t) graphically using a linear regression or a potential or exponential regression, choosing the one that best fit the data according to criteria proposed by Marschner (1995).  $V_{\max}$  is calculated from the linear equation and  $K_m$  from both equations.  $C_{\min}$  was estimated from the equation adjusted to the data for the last interval of P-depletion from solution (Akhtar et al., 2007). Michaelis-Menton kinetics have therefore been applied to the ion uptake process.

$$\text{Uptake rate (I)} (\mu\text{mol g}^{-1} \text{ h}^{-1}) = \frac{I_{\max} \cdot C_r}{K_M + C_r}$$

I = influx (rate of uptake)

$I_{\max}$  = maximal influx (maximal rate of uptake)

( $\approx$  density x activity of ion carriers)

$K_M = C_r$  at  $\frac{1}{2} V_{\max}$  ( $\mu\text{mol L}^{-1}$ ) (Michaelis constant)

( $\approx$  affinity of ion carriers)

$C_r$  = concentration of ion in solution at the root surface

When  $I = \frac{1}{2} I_{\max}$ . The value is characteristic for a particular ion crossing the specific membrane. The concentration ( $C_r$ ) required for  $\frac{1}{2} I_{\max}$  equals the Michaelis Menten constant.

### 2.3 Statistical Analysis

Data were subjected to statistical analyses according to standard procedures (Steel & Torrie, 1980) using 'MSTAT-C' program and the methods described by Gomez and Gomez (1984). Treatment means were separated using Duncan's Multiple Range Test (DMRT). The values for  $V_{\max}$ ,  $K_m$ , and  $C_{\min}$  were determined for each replication and submitted to analyses of variance, comparisons of means using the DMRT ( $P \leq 0.05$ ).

## 3 RESULTS AND DISCUSSION

### 3.1 Phosphorus Facts, Pi Transport by Diffusion and Active Uptake

Inorganic phosphate (Pi), the fully oxidized and assimilated form of P, plays a pivotal structural and regulatory role in general metabolism and at the nexus of photosynthesis and carbon allocation. Pi is extremely insoluble in most soils because it forms Ca-salts or is complexed by constituents such as Fe or Al oxides or

fixed into organic forms that render Pi largely inaccessible to plants. The reactions controlling the amounts of Pi in solution include dissolution–precipitation of P-bearing minerals, adsorption–desorption of Pi on soil surfaces, and the hydrolysis of organic matter (Hinsinger, 2001). It can be difficult to distinguish adsorbed and precipitated forms of P, but retention or ‘fixation’ of P by soil components is greatest in the presence of Fe and Al-hydroxylated surfaces (from Fe and Al oxides and clay minerals) and, at higher pH, calcium carbonate (Matar et al., 1992). Along with the types and amounts of clay and metal oxides, P availability is also controlled by soil solution pH, ionic strength, concentrations of P and metals (Fe, Al and Ca) and the presence of competing anions, including organic acids (Hinsinger, 2001). There is great disparity in distribution of Pi between plant cells (mM) and soil solution ( $\mu\text{M}$ ) because of its strong reactions with soil components. Pi is principally supplied to plant roots by diffusion rather than mass flow and diffusion of Pi in soils is slow ( $10^{-12}$  to  $10^{-15}$   $\text{m}^2 \text{S}^{-1}$ ; Rausch & Bucher, 2002), hence, P is one of the most dilute and inaccessible macronutrients in the soil. In case of root interception, as roots push through the soil, they contact nutrients and can take them up. Most likely this mechanism can just enhances diffusion and reduces diffusion co-efficient. As outlined in fig. 1, Pi diffuses from higher concentration to lower concentration following the Fick’s law of diffusion.

The diffusion of Pi through soil is the slowest of all macronutrients. Hence, the supply of Pi at the root surface is limited by diffusion. Plants can increase the availability of Pi in a number of ways. Under Pi stress, plant roots decrease in diameter so that, given the same amount of root mass, they increase their absorptive surface area relative to root volume. The absorptive capacity of roots can be estimated by determining uptake rates per unit length or mass of root. Fig. 1A,B,C indicates the moment of Pi from soil particles to the plant roots via diffusion, as mass flow typically delivers as little as 1–5% of a plant’s P demand, and the amount intercepted by growing roots is only half of that (Lambers et al., 1998; 2006).

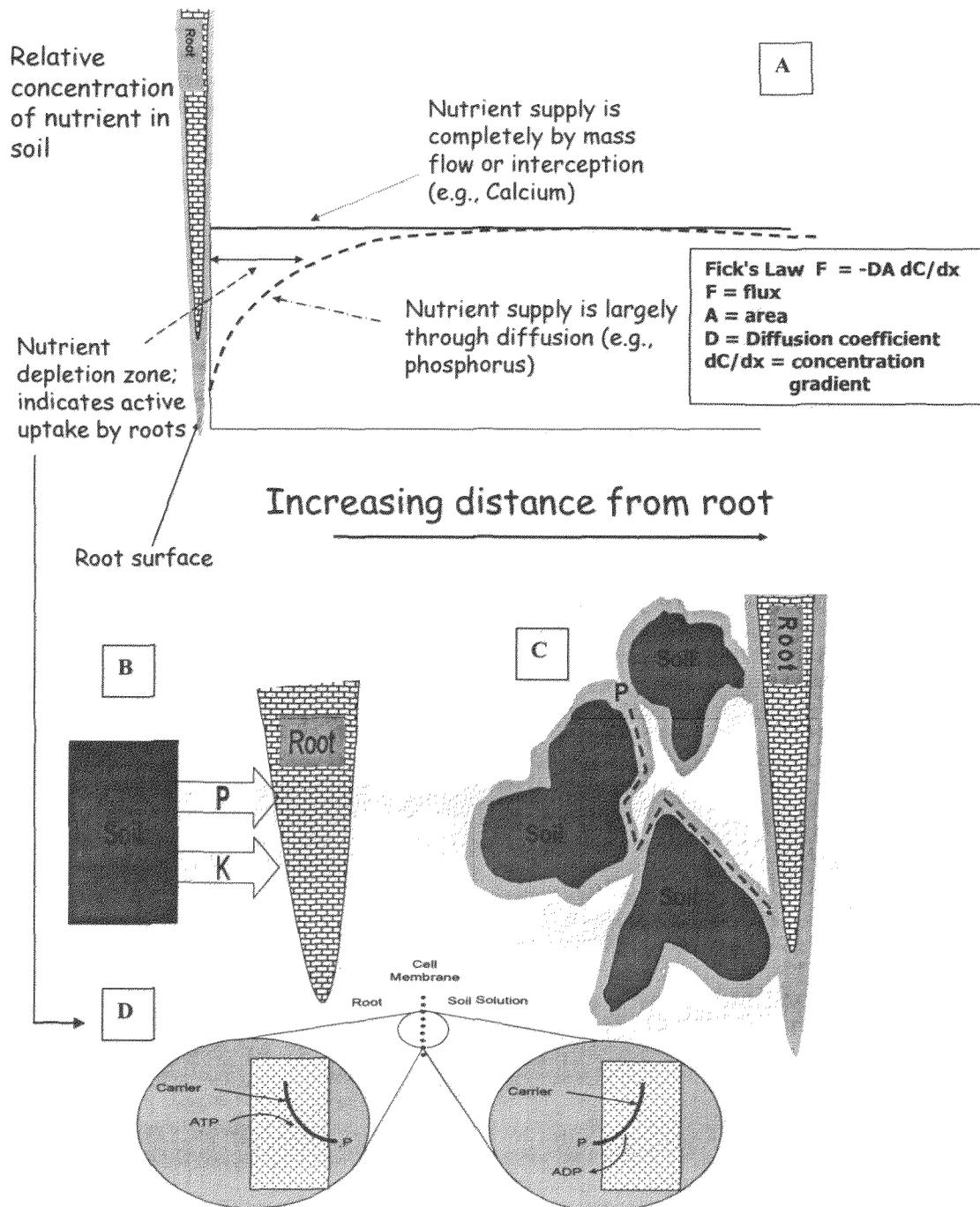
Main site of selectivity in the uptake is the plasma membrane. Active transport in either direction takes place through membranes because active transport is always directional. Another main barrier to diffusion is tonoplast (vacuolar membrane). Vacuole comprises approximately 90% of cell volume and in the main compartment for ion accumulation. Compartmentation by biomembranes is a general prerequisite for living systems. Solute transport into organelles is regulated by membranes and the capability of biomembranes for solute transport and its regulation is closely related to their chemical composition and molecular structure. There is inverse relationship between membrane permeation and the diameter of the uncharged molecule and the rates at which they permeate membranes. Fluid membrane model denotes protein matrix and 2 phospholipid layers with the hydrophilic, charged head regions (amino acids, phosphate, and carboxyl) oriented toward the

membrane surfaces. Different types of phosphate transporters are involved in the uptake of P through membranes. As outlined in Fig. 1D, membrane transport against the gradient potential energy ‘uphill’ linked to energy-consuming mechanism ( a pump in the membrane). The main driving force of membrane transport of ions is  $\text{H}^+\text{ATPase}$ .

### 3.2 Hyperbolic Saturation Curve: Michaelis Menten Kinetics and Influx Rate of two *Brassica* Cultivars

Cultivars may vary in their effectiveness in acquiring P through different levels of expression of transport proteins in roots ( $V_{\text{max}}$  or  $I_{\text{max}}$ ), through differences in the affinity ( $K_m$ ) for P, or through variation in  $C_{\text{min}}$  (minimum P-concentration in the growth medium at which net uptake occurs into roots) for phosphate. Uptake kinetics describes the relationship between nutrient influx into roots and concentration of that nutrient in the external solution at the root surface. Differences among cultivars were observed for the maximal P-uptake rate ( $V_{\text{max}}$ ) and Michaelis-Menten constant ( $K_m$ ) (Akhtar et al., 2007). P-efficient ‘Con-1’ presented high  $V_{\text{max}}$  ( $31.19 \mu\text{mol g}^{-1} \text{h}^{-1}$ ) low  $K_m$  and  $C_{\text{min}}$  [ $(K_m=12.92 \mu\text{mol g}^{-1} \text{h}^{-1}; C_{\text{min}}=0.87 \mu\text{mol g}^{-1} \text{h}^{-1})$ ] than P-sensitive ‘Gold Rush’ [ $V_{\text{max}}=28.25 \mu\text{mol g}^{-1} \text{h}^{-1}; K_m=20.56 \mu\text{mol g}^{-1} \text{h}^{-1}$  and  $C_{\text{min}}=1.12 \mu\text{mol g}^{-1} \text{h}^{-1}$ , respectively], (Akhtar et al., 2007). Results obtained with ‘Con-1’ indicated that this cultivar had favorable characteristics for P-uptake because of high  $V_{\text{max}}$  and low  $K_m$  and  $C_{\text{min}}$  values. Some authors have suggested that plants which are efficient for nutrient uptake should have a high  $V_{\text{max}}$ , which means having high uptake rates under high external concentrations, and low  $K_m$  and  $C_{\text{min}}$ , which means being able to take up the nutrient from dilute solutions (Schenk and Barber, 1980). In the present study ‘Con-1’ satisfied this criterion by exhibiting high  $V_{\text{max}}$  and low  $K_m$  and  $C_{\text{min}}$  value, meaning higher affinity of the carrier binding sites for P ions. On the other hand, ‘Gold Rush’ exhibiting the highest  $C_{\text{min}}$  value, which makes it unacceptable choice under P-stress environment.

The depletion data obtained was transformed into rate of P-uptake (influx) per unit root length. From these influxes, the values of  $I_{\text{max}}$  and  $K_m$  were obtained by means of the Hanes plot. The Hanes plot was chosen because the results are least biased by the variability of the original values as suggested by Jungk et al. (1990). From this plot,  $I_{\text{max}}$  can be obtained from the slope of the line and  $K_m$  as the intercept on the negative x-axis. The relationship between uptake rate ( $I$ ) and ion concentration is presented in Fig. 2. Cultivar Con-1 has high  $I_{\text{max}}$  and low  $K_m$  values than cultivar Gold Rush, evidencing that this cultivar can adapt well to both P-deficient and P-sufficient conditions. The nutrient uptake kinetic parameters  $V_{\text{max}}$ ,  $K_m$  and  $C_{\text{min}}$  are important plant attributes to be considered when looking for efficient genome, evaluating and selecting cultivars for higher efficiency of P-uptake under or low or high external P-concentrations.

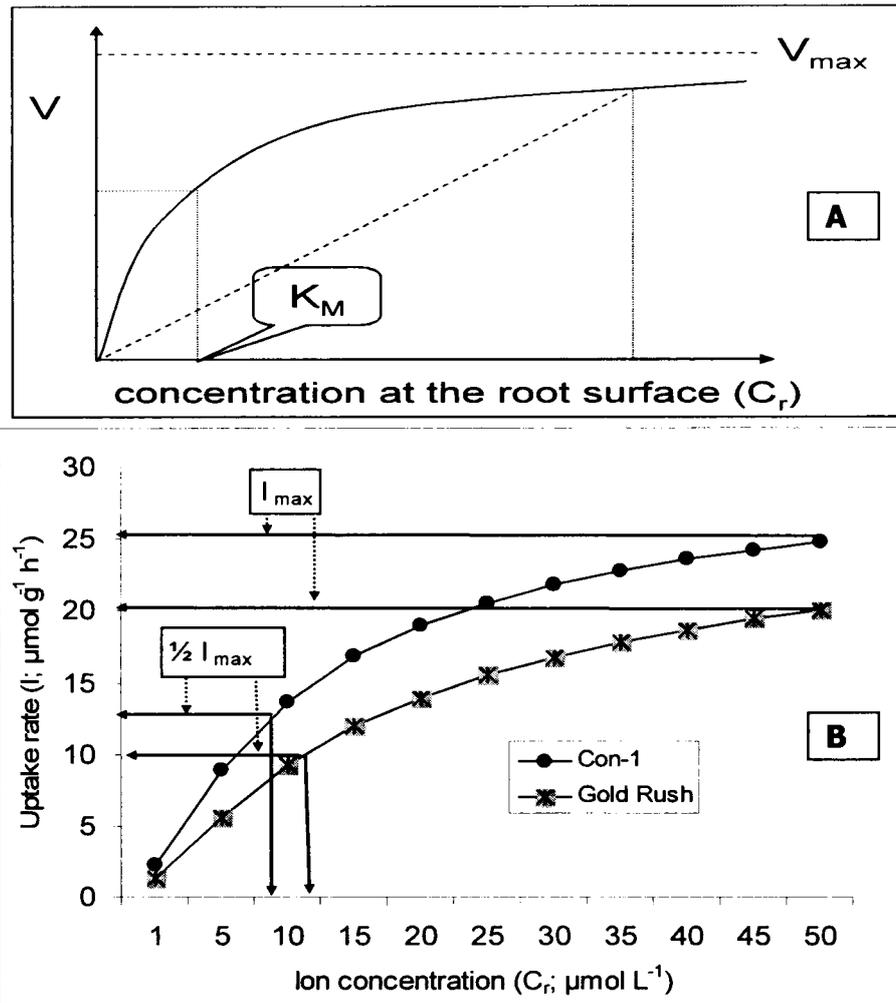


**Fig. 1** Movement of P through the soil solution in response to a concentration gradient; i.e. diffusion 'P will diffuse from higher concentration to lower concentration' via active uptake (biochemical energy used to transport ions into the cell against the energy gradient). Nutrient depletion zone in the root vicinity indicates active uptake by roots.

**3.3 Relative Growth Rate (RGR) of Cultivars**

Fig. 3 represents the plot between relative growth rate (RGR) and internal P-concentration (PNC) among P-tolerant (group I; Brown Raya, Con-1, Rainbow, Dunkled and Peela Raya) and P-sensitive (group II; Toria, Sultan Raya, B.S.A, Toria Selection and Gold Rush) cultivars.

Group I cultivars showed large metabolic fraction and small structural fraction (Fig. 3A) than group II cultivars (Fig. 3B) which provided basis for P- stress tolerance. Efficient cultivars adapt well under P-stress by increasing nutrient uptake per unit root biomass, decreasing internal nutrient requirement and decreasing nutrient losses per



**Michaelis Menten equation of ion uptake ('hyperbolic saturation curve'):**

$$\text{Uptake rate (I)} (\mu\text{mol g}^{-1} \text{h}^{-1}) = \frac{V_{\max} \cdot C_r}{K_M + C_r}$$

$V_{\max}$  = uptake rate at high  $C_r$   
( $\approx$  density  $\times$  activity of ion carriers)

$K_M = C_r$  at  $1/2 V_{\max}$  ( $\mu\text{mol L}^{-1}$ ) (Michaelis constant)  
( $\approx$  affinity of ion carriers)

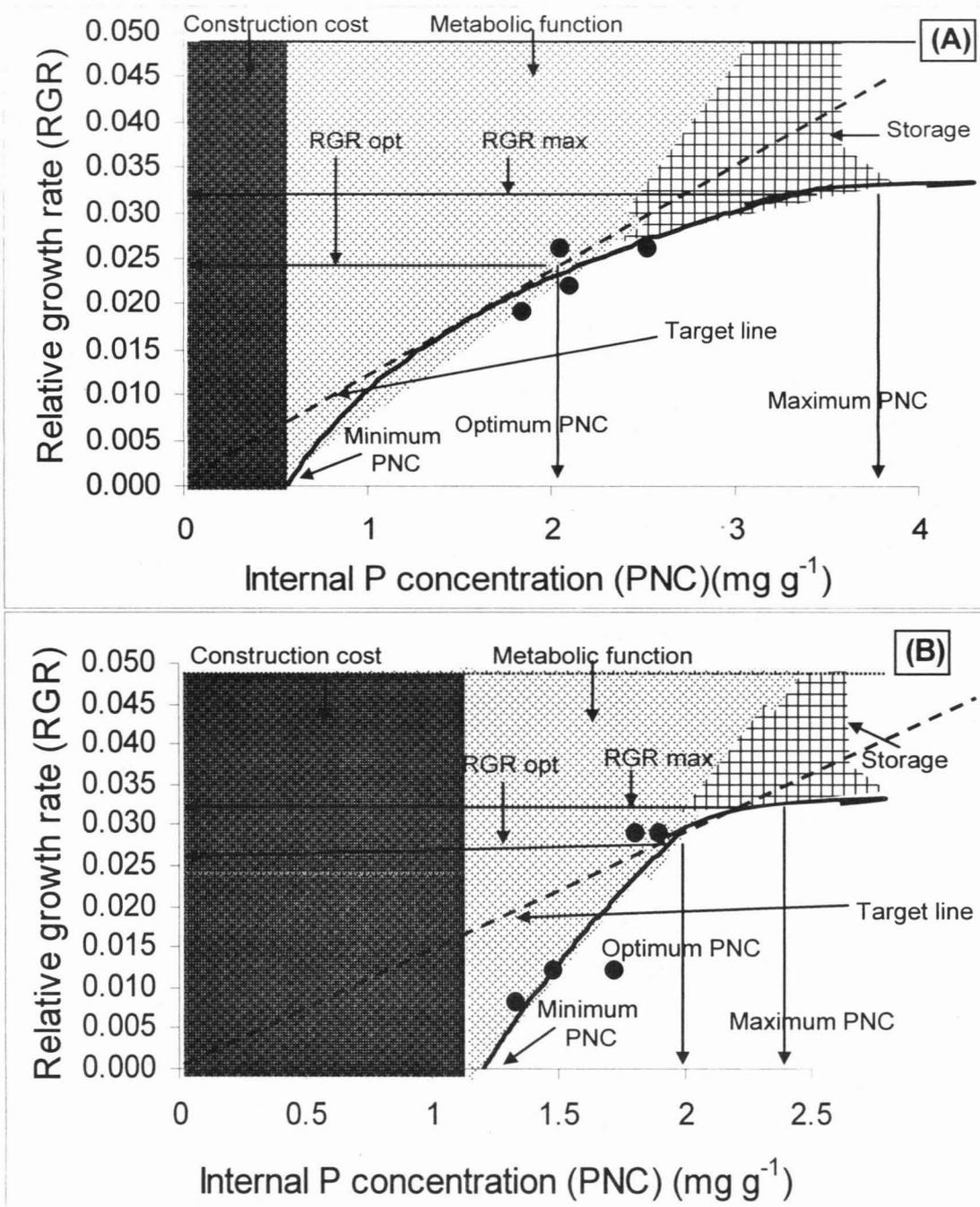
Root Absorbing Power (RAP) =  $V/C_r$  ( $\mu\text{mol g}^{-1} \text{h}^{-1}$ ) / ( $\mu\text{mol L}^{-1}$ )  
(= slope of line through the origin)

RAP decreases with increasing  $C_r$  due to saturation of ion carriers

$I_{\max}$  = maximal influx (maximal rate of uptake)

When  $I = 1/2 I_{\max}$ . The value is characteristic for a particular ion crossing the specific membrane. The concentration ( $C_r$ ) required for  $1/2 I_{\max}$  equals the Michaelis Menten constant.

**Fig. 2** Nutrient uptake kinetics. (A) Michaelis Menten equation of ion uptake ('hyperbolic saturation curve') (B) and the relationship between the ion concentration and the rate of uptake by two contrasting *Brassica* (P efficient-'Con-1' and P inefficient-'Gold Rush') cultivars.



**Fig. 3** Schematic illustration of the 3 functional fractions of internal P concentration (PNC): ‘structural, metabolic, storage’ of (A) P-tolerant group I (Brown Raya, Con-1, Rainbow, Dunkled and Peela Raya) cultivars and (B) P-sensitive group II (Torla, Sultan Raya, B.S.A, Torla Selection and Gold Rush) cultivars.  $RGR_{opt}$  = optimum relative growth rate;  $RGR_{max}$  = maximum relative growth rate. Efficient cultivars showed large metabolic fraction and small structural fraction than inefficient cultivars.

unit biomass. Internal use efficiency can be simply defined as the output/input or result/investment. Result = growth rate, biomass, reproduction, whereas, investment = cost of nutrient uptake, nutrient status, internal concentration. Internal use efficiency has 3 components:

‘construction efficiency’ (biomass present per unit nutrient in plant) ‘nutrient productivity’ (biomass production rate per unit nutrient in plant) ‘conservation efficiency’ (nutrient loss rate per unit nutrient in plant). Plot between RGR vs. PNC can give us the clear understanding of these three

components as outlined in Fig. 3. Construction efficiency is highest when  $PNC = PNC_{min}$  and nutrient productivity (tangent): zero at  $PNC_{min}$ , max at  $PNC_{opt}$ ; less at  $PNC_{max}$ . High construction efficiency  $\rightarrow$  low PNC  $\rightarrow$  low nutrient productivity while high conservation efficiency  $\rightarrow$  protection  $\rightarrow$  low construction efficiency.

#### 4 CONCLUSIONS

The P-tolerant cultivar 'Con-1' exhibited high  $I_{max}$  or  $V_{max}$  and low  $K_m$  or  $\frac{1}{2} I_{max}$  than P-sensitive cultivar 'Gold Rush', indicating that this cultivar had favorable characteristic for P-uptake and adapt well in otherwise P-stress environment. Efficient cultivars (group I) showed large metabolic fraction and small structural and storage fractions, whereas P-sensitive cultivars (group II) cultivars showed large structural and storage fractions and small metabolic fraction. This provides basis that P-efficient cultivars had more capacity to survive under P-starvation.

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