Intra Specific Variations of Phosphorus Absorption and Remobilization, P Forms, and their Internal Buffering in Brassica Cultivars Exposed to P Stress Environment

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Abstract

Translocation of absorbed phosphorus (P) from metabolically inactive sites to active sites in plants growing under P deprivation may increase its P utilization efficiency (PUE). Acclimation to phosphate (Pi) starvation may be caused by a differential storage pool of vacuolar P, its release, and the intensity of re-translocation of absorbed P as P starvation inducible environmental cues (PSIEC) from ambient environment. Biomass assay and three P forms i.e. inorganic (Pi), organic (Po), and acid-soluble total (Ptas) were estimated in Brassica cultivars exposed to ten days of P deprivation in the culture media. By considering that \( \frac{\delta \text{Pi}}{\delta t} \) denotes the rate of Pi release, Pi release velocity (RSPi) was determined as the tangent to the equations obtained for Pi f(t) at the mean point in the period of greatest Pi decrease, whereas the inverse of the RSPi was an estimate of the internal Pi buffering capacity (IBCPi). Inter cultivar variations in size of the non-metabolic Pi pool, RSPi, re-translocation of Pi from less to more active metabolic sites, and preferential Pi source and sink compartments were evaluated under P starvation. The cultivar ‘Brown Raya’ showed the highest Pi storage ability under adequate external P supply, and a more intensive release than ‘Rain Bow’ and ‘Dunkled’ under P stress. Cultivar ‘B.S.A’ was inferior to ‘Con-1’ in its ability to store and use Pi. Roots and upper leaves were the main sink of Pi stored in the lower and middle leaves of all cultivars and showed lower IBCPi and larger RSPi values than lower and middle leaves. In another trial, six cultivars were exposed to P free nutrition for 29 days after initial feeding on optimum nutrition for 15 days. With variable magnitude, all the cultivars re-translocated P from above ground parts to their roots under P starvation, and [P] at 44 days after transplanting was higher in developing leaves compared to developed leaves. Under P deprivation, translocation of absorbed P from metabolically inactive to active sites may have helped the tolerant cultivars to establish a better rooting system, which provided basis for tolerance against P starvation and increased PUE. A better understanding of the extent to which changes in flux of P absorption and re-tanslocation under PSIEC will help to scavenge Pi from bound P reserves and will bring more sparingly soluble P into cropping systems and obtain capitalization of P reserves.

Key words: Brassica, IBCPi, P absorption and remobilization, Pi homeostasis, RSPi, Vacuolar P

Phosphorus (P) (the ‘bearer of light’ in Greek mythology, and the ‘conveyor of light’ in plant biology; Ticconi and Abel 2004) is one of the most essential elements for biological organisms, and is indispensable for diverse metabolic processes such as energy transfer, signal transduction, macro-molecular biosynthesis, respiration, enzyme activation, and coupling the light
and dark reactions in photosynthesis. Therefore, direct availability of P determines the plant growth and profoundly affects plant metabolism and crop performance. Nevertheless, total P is abundant in the lithosphere (500 to 2000 ppm in soil), the physicochemical properties and elusive soil chemistry of inorganic phosphate (Pi) render it one of the least available and least mobile nutrients (Abel et al. 2002; Raghothama and Karthikeyan 2005). Soil concentrations of soluble Pi are often up to a 1000 times lower than those of other required ions (Schachtman et al. 1998; Raghothama 2000; Raghothama and Karthikeyan 2005; Vance et al. 2003). There is great disparity in distribution of Pi between plant cells (mM) and soil solution (µM) with an average [Pi] of 1 µM in soil solution which is well below \(K_m\) for plant uptake (Vance et al. 2003). 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. 2006). The rest of all required Pi must reach the root surface via diffusion; diffusion coefficients for Pi in soil are notoriously low i.e. \(10^{-12}\) to \(10^{-15}\) m² s⁻¹ (Rausch and Bucher 2002), and particularly slow in dry soil. Phosphorus malnutrition poses a severe threat to agriculture that is typically averted in affluent countries by extensive application of concentrated Pi fertilizers. However, crop yields are severely depressed by Pi starvation around the globe (5.7 billion ha) with P-impoverished soils (highly weathered soils of tropics and subtropics (2 billion ha), as well as calcareous/alkaline soils of Mediterranean basin; Hinsinger, 2001), and further aggravated under limited financial capacities and resource poor environments.

P-starvation has resulted in the evolution in plants of a myriad of adaptive responses that maximize external Pi acquisition and reprioritize internal Pi use by reprogramming metabolism and restructuring root system architecture (Narang et al. 2001; Smith et al. 2003; Ticconi and Abel 2004; Vance et al. 2003). Such coping strategies include remodeling in root architecture (Lambers et al. 2006; Williamson et al. 2001), enhanced root-shoot ratio and root elongation (Gilroy and Jones 2000; Bhadoria et al. 2004)), formation of proteoid and dauciform roots (Shane et al. 2005), exudation of carboxylates or H⁺ (Hinsinger et al. 2003; Rengel 2002) and phosphatases (Neumann et al. 2000). Making better use of the absorbed P to produce more biomass is another adaptive mechanism to P-starvation (Marschner et al. 1997). Among the intrinsic plant mechanisms, internal allocation and use of nutrients in the metabolism and growth, which are dependent on re-translocation and re-use under stress, play a fundamental role. Another very important factor is the release of ions from the vacuoles under nutritional stress (Bieleski and Ferguson 1983; Massonneau et al. 2000; Martinez et al. 2005). When the P supply is high, the plant is able to accumulate Pi in the vacuoles. On the other hand, when the supply is limited either by nutrient
insufficiency or by low soil moisture (limited transport), the previously accumulated Pi is released to the metabolic pools to meet P demands (Bieleski and Ferguson 1983; Ratcliffe 1994; Raghothama 1999). Under sufficient P supply, 85 to 95 % of the cellular P is found in the vacuole, and only 5 to 15 % make up the metabolic or cytoplasmatic pool (Glass and Siddqi 1984; Natr 1992). The cytosolic Pi levels are maintained at the expense of vacuolar Pi during P starvation (Sakano et al. 1992; Raghothama and Karthikeyan 2005) and according to the later author, a minimum concentration of P in the cytoplasm is critical to maintain the normal plant metabolism. Cells have mechanisms to maintain cytosolic levels of Pi, in spite of large fluctuations in the external concentrations (Massonneau et al. 2000). Based on these considerations, it is believed that the size of the non-metabolic pool and the plant’s ability to translocate this pool from one compartment to another certainly affect its adaptability to and survival under stress environments.

Phosphorus is a relatively mobile element in plants, moving easily between organs (Marschner 1995). Cycling of mineral nutrients, i.e. re-translocation in the phloem from older leaves to the growing shoots and from shoot to roots, and recycling, i.e. translocation of cycled nutrients back to the shoot in the xylem, are significant for plant growth, especially under stressed conditions (Marschner et al. 1997). In Pi starved plants the restricted supply of Pi to the shoots from the roots via the xylem is supplemented by increased mobilization of stored P in the older leaves and re-translocation to both the younger leaves and growing roots. This process involves both the depletion of Pi stores and the breakdown of organic P in the older leaves. A curious feature of P-starved plants is that approximately one-half of the Pi translocated from the shoots to the roots in the phloem is then transferred to the xylem and recycled back to the shoots (Jeschke et al. 1997). In the xylem P is transported almost solely as Pi, whereas significant amounts of organic P are found in the phloem (Schachtman et al. 1998). In studies with sorghum (Wieneke 1990), tomato (Fujita et al. 2003), wheat (Batten and Wardlaw 1987; Peng and Li 2005) and soybean (Martinez et al. 2005), it was shown that P remobilization is not only a matter of senescence but occurred even in young non-matured tissues supporting the idea that a specific P fraction is cycled in the plant. Nevertheless, attempts have been made to study the acquisition and use of P in plant species (Alloush 2003; Machado and Furlani 2004; Akhtar et al. 2006a, 2006b) but there is a dearth of information on intraspecific variations in terms of P-remobilization, source-sink relationships, and internal buffering of P forms in various plant organs, particularly in Brassica.

P-utilization efficiency (PUE) is the ability of crop cultivars to function well under low available P concentrations. According to an estimate, PUE can be enhanced by 25%, employing
the current knowledge about the PUE of efficient genome. To increase P efficiency, plant traits such as P-acquisition (absorption/uptake), translocation (transport/partitioning/remobilization), and internal utilization must be improved. Inter- and intra-specific variations for these traits are known to be under genetic control, but modified by the plant–environment interactions. Logically, an evaluation of P efficiency in plants should include not only efficient absorption, but also an efficient internal use under P stress environment. The intra-specific P efficiency may be a function of a genotypically different compartmentation (either physical or metabolic) and direct use after its initial translocation into the tissue. Elliott and Läuchli (1985) suggested that P partitioning between inorganic and organic forms is the major determinate. In addition, an assumed genotypically higher vacuolar affinity for inorganic P could also be detrimental to P remobilization in the plant. It can be assumed that cultivars capable of efficiently retranslocating P from inactive to metabolically active sites under P starvation can better tolerate to low P rooting environments and seems imperative for establishing the basis for their differential growth under P deprivation. There is, therefore, need to undertaking relative studies on the patterns of P remobilization and redistribution within various plant parts of Brassica cultivars under P starvation.

Nevertheless, Brassica’s importance in edible oil, bio-diesel, Brassiodol, livestock feed, raw material for bio-composites, plastics, high value lubricants, condiments and production of derived compounds (antioxidants, vitamins, anti-carcinogenic etc.) cannot be overemphasized, but P starvation has prominent effect on limiting crop production, oil contents and seed yield. Our aim was to evaluate the size of the non-metabolic (vacuolar Pi) P pool, pattern of P redistribution, Pi release capacity when the cytoplasmatic P falls to a limiting value by Brassica cultivars, to estimate which organs are preferentially sources and sinks of Pi under P starvation and to relate the P remobilization to different P pools. A further aim was to categorize the existing Brassica cultivars for Pi remobilization and use efficiency under P stress that will open the possibility to bioengineer more P efficient cultivars, and provide database to breeders for their future ventures.

Materials and Methods

Plant material and culture environment

Different Brassica cultivars tested were; ‘Brown Raya’, ‘Con-1’, ‘Rainbow’, ‘Dunkled’, and ‘B.S.A’ in study 1 and ‘Peela Raya’, ‘Sultan Raya’, ‘Toria Selection’, ‘Brown Raya’, ‘Con-1’ and ‘Rainbow’ in study 2. Seeds were germinated in polyethylene lined iron trays containing pre-washed riverbed quartz sand and irrigated with ultra pure water for seed germination and seedling establishment. Experiment 1 was conducted in a glasshouse in winter and the temperature during
the entire growth period varied from a minimum of 5°C to a maximum of 25°C, while experiment 2 was carried out under controlled conditions and the culture conditions were as follows: temperature 25°C; light intensity 40 μmol m⁻² s⁻¹; relative humidity 50%; light/dark 14/10 hr. The composition of the used modified Hoffland’s solution (Hoffland et al. 1989) was; [mM]: KNO₃ [2], NH₄NO₃ [1], Ca(NO₃)₂.4H₂O [2], MgSO₄.7H₂O [0.5], K₂SO₄ [0.5] and [μM]: Fe(III)-EDTA (Ferric Dihydrogen Ethylene Diamine Tetra-acetic Acid) [50], H₃BO₃ [25], MnSO₄.H₂O [2], ZnSO₄.7H₂O [2], CuSO₄.5H₂O [0.5], KCl [50], H₂MoO₄ [0.5].

In experiment 1, Seven day old uniform sized seedlings were transplanted in foam plugged holes in thermopal sheets floating on continuously aerated polyethylene lined 10-L plastic pots containing half strength modified Hoffland’s solution. The solutions were modified to maintain stress (25 μM P) level using NH₄H₂PO₄, where cultivars were allowed to grow for 25 days after transplanting (DAT). After this period, the plants were transferred to pots of equal volume containing Hoffland’s solution with high P (250 μM P) for 72 h. They were then transferred back to pots containing a nutrient solution with low P (25 μM P), and after 4 h in this solution they were transferred to Hoffland’s solution with no P. On this occasion, four plots were collected to estimate tissue P concentrations. These samples corresponded to zero days of P omission (DPO = 0). The other tissue samples were extracted 1, 2, 4, and 10 days after transferring the seedlings to no P nutrient solution (DPO = 1, 2, 4 and 10, respectively). Each experimental plot consisted of one 10-L pot with two plants. The treatments were a 5 x 5 factorial (five Brassica cultivars and five P omission periods) in a randomized complete block design, with four replicates.

In experiment 2, seven day old uniform pre-germinated seedlings of cultivars were transplanted to foam plugged holes of a polyethylene sheet floating on continuously aerated 24-L of Hoffland’s solution, containing 200 μM P as NH₄H₂PO₄ in rectangular shaped plastic tubs (45 x 25 x 25 cm at the top). One plant was transplanted per hole and each cultivar was repeated 6 times, with two cultivars per tub. Half the plants were harvested at 15 days after transplanting (DAT), while remaining plants were allowed to grow for an additional period of 29 days by replacing the solutions in the tubs modified to contain no P.

The pH of the continuously aerated solutions of both experiments was monitored daily and maintained at 5.5 ± 0.5 by addition of HCl or NaOH. The solution was renewed every 3 days to maintain nutrient concentrations being exhausted due to plant uptake. Apparently, solutions were always renewed when K and, or, P decreased to a value that was 30% lower than the initial one.
**Biomass assay and analysis of P absorption and remobilization in plants**

In study 1, at the end of each P omission period, the plants of four plots were collected and sampled. For tissue sampling, the leaves of one plant per plot were divided in three equal parts [upper leaves (UL), middle leaves (ML) and lower leaves (LL)]. Stems (S) and roots (R) were also collected. These plant parts were washed instantly in deionized water, blotted dry using filter paper sheets and dried in a forced air oven at 70°C for 48 hr and weighed. The second plant of the plot was similarly divided, and samples of about 1 g of fresh matter of each component was collected, weighed, placed in test tubes containing 2 mL of HClO₄ (0.2 mol L⁻¹) and immediately frozen. These samples were later macerated in HClO₄ (0.2 mol L⁻¹) at 4 °C, centrifuged at 5,000 xg, and used for the determination of the phosphate fraction (Pi), soluble in acid, in the supernatant as outlined in Figure 1. The total acid soluble phosphate (Ptas) was obtained after nitroperchloric digestion of a 10 mL aliquot of the extract obtained by maceration. The acid-soluble organic P (Po) was calculated by the difference between the Ptas and Pi contents, based on Hogue et al. (1970) and Martinez et al. (2005). All tissue P concentrations ([P]) were determined using spectrophotometry by reduction of the phosphomolibdate complex by ascorbic acid.

In study 2, plants harvested at 15 DAT were separated into roots and shoots, while those harvested at 44 DAT into roots, young and old leaves, and stems. Samples were digested and analyzed for P using vanadate-molybdate method. P-uptake for the first harvest was calculated by multiplying [P] in roots and shoots by the dry matter mass and by adding up the two for total P contents. Root P uptake for the 2nd harvest was calculated by multiplying the root-[P] by root dry matter (RDM). Shoot P uptake at 44 DAT could not be determined due to separation of shoot into young and older leaves and stems. [P] in these tissues were, however, also determined at 44 DAT.

**Pi release velocity (RSPi) and internal Pi buffering capacity (IBCPi)**

In study 1, considering that -δPi/δt expresses the velocity of Pi release and that the greatest reduction of the Pi content in all plant parts occurred between 1 and 4 DPO, Pi release velocity (RSPi) was estimated as being the tangent to the equations obtained for Pi f(t) at the point t = 2 days (the mean point in the period of greatest Pi decrease). The internal Pi buffering capacity (IBCPi) was estimated as the inverse of RSPi. Plant organ mean values of RSPi and IBCPi were obtained as arithmetic average among cultivars (Novais and Smyth 1999; Martinez et al. 2005).

**Statistical Background**

Data were subjected to analyses using ‘MSTAT-C’ (Russell and Eisensmith 1983) according to standard procedures (Steel and Torrie 1980), and methods described by Gomez and Gomez (1984).
In experiment 1, Pi and Po concentration values, as dependent variables of DPO in the solution, were submitted to variance and regression analyses. The highest degree model was chosen based on the F test and on the determination coefficient. When no significant treatment effect was detected or no model fitted the data, it was considered that the Pi and Po concentration did not vary as a function of time (t) of P omission. A constant estimated by the mean of the observed values was then obtained. Data of leaves, stems, shoot, root, and whole plant dry matter were submitted to analyses of variance and the means were compared by the Duncan’s Multiple Range Test (DMRT) to assess cultivar differences.

Results and Discussion

Experiment 1: Phosphorus absorption, re-translocation and P forms in *Brassica* cultivars

Phosphorus is readily mobilized in plants, and under P deprivation, the element contained in the older tissues is transferred to active meristematic regions. Increased development of the plant parts associated with P acquisition from rooting environment, and efficient re-translocation and distribution of absorbed P within in the plants to metabolically active sites may enhance its utilization efficiency under P stress environment. In study 1, *Brassica* plants not submitted to P omission stress presented estimated Pi-concentration ([Pi]) values varying from 597 to 1274 mg kg\(^{-1}\) of fresh matter in different analyzed plant parts (Figures 2 to 4). Roots (R) were the compartment with highest [Pi], followed by the upper leaves (UL) and middle leaves (ML); lower leaves (LL) and stems (S) were the compartments with the lowest [Pi]. Decrement of [Pi] in all plant parts follows quadratic pattern, with a more drastic reduction at the onset of the P omission period (0 to 4 days). After one day of P omission (DPO = 1), there was a high percentage reduction of [Pi] (29, 33, and 26 %, respectively) in UL (939 to 666 mg kg\(^{-1}\)), LL (655 to 442 mg kg\(^{-1}\)), and R (1071 to 798 mg kg\(^{-1}\)). After 4 DPO, the Pi reduction in R (1071 to 330 mg kg\(^{-1}\)) and UL (939 to 346 mg kg\(^{-1}\)) was higher than that in LL (655 to 264 mg kg\(^{-1}\)); 69, 63 and 59 % on average, respectively). After 10 DPO, the greatest percentage of Pi reduction was found for the R (average of 1071 to 110 mg kg\(^{-1}\), 90 %), followed by S (654 to 85 mg kg\(^{-1}\)), UL (939 to 210 mg kg\(^{-1}\)), LL (655 to 149 mg kg\(^{-1}\)) and ML (743 to 199 mg kg\(^{-1}\)), averaging 87, 78, 77, and 73 %, respectively, in relation to the plants not exposed to P stress environment (Figures 2 to 4).

Based on the differential P fractions in the plant parts during period of P omission (PPO), it is plausible to infer that the Pi release from the nonmetabolic to the metabolic pool was initially more intensive in UL, LL, and R. As the PPO persisted, there were higher percentages of Pi reduction in the stems. Nonetheless, the role of the stems as Pi source is equivocal in view of their
small initial reserve compared to other plant parts, especially R and UL (Figures 2 to 4). Burauel et al. (1989) reported that the maintenance or even increase of the total P content in the roots in a period of P starvation might be related to an enhanced change of the assimilate distribution in favor of the roots in plants under P stress. Geloff (1987) stated that beside other factors, plant adaptability to nutrient deficiency stress depends on re-transport of deficient nutrient from older to younger leaves. Higher Pi contents in R and UL of cultivars under adequate P supply show that young tissues have a greater storage capacity. Also, their greater metabolic activity could make them stronger sinks for the absorbed P. This causes higher cytoplasmatic P concentrations and triggers the mechanisms that operate at the tonoplast level and provide regulation for the cytoplasm-vacuole and vacuole-cytoplasm fluxes (Glass and Siddqi 1984; Clarkson 1985; Abel et al. 2002). Transfer of Pi from the cytoplasm to the vacuole involves a different set of thermodynamic parameters to those applying to the plasma membrane, mainly because of the millimolar concentrations in the cytoplasm and vacuole compared with the micromolar concentrations in the soil. Few estimates of cytosolic and vacuolar Pi concentrations are available. However, when maize was grown at Pi concentrations similar to those found in soils (i.e. 10 µM), the root cell cytoplasmic Pi concentration was estimated to be higher than the vacuolar concentration (Lee and Ratcliffe 1993). Soybean leaf cell cytoplasmic Pi concentrations were also found to be higher than concentrations in the vacuole when plants were grown in solutions containing 50 to 100 µM Pi (Lauer et al. 1989). Since the membrane potential of the vacuole is usually slightly positive with respect to the cytoplasm under these realistic conditions, Pi transfer to the vacuole need not be energized. According to Glass and Siddqi (1984) and Clarkson (1985), fluxes are mediated by adjustments in the turnover rate or in the quantity of active carriers. Raghothama (1999) reported that this transport is ATP-dependent and that the existence of a specific Pi channel or of a symport-type transport mechanism cannot be disregarded. Multiple transporters may be involved in maintaining both cellular and whole-plant Pi homeostasis (Raghothama and Karthikeyan 2005). Maintenance of cytosolic Pi homeostasis is bound to involve bi-directional movement of the nutrient across the tonoplast. There is evidence for ATP-dependent Pi transport across the tonoplast (Mimura et al. 1990; Sakano et al. 1995; Mimura 2000; Vance et al. 2003). A decrease in the ATP levels in the cytosol results in suppression of Pi transport across the tonoplast membranes (Sakano et al. 1992). It is likely that the tonoplast-associated H+-translocating pyrophosphatase or H+-ATPase may provide required energy for maintaining an electrochemical potential gradient of H+ across the tonoplast to facilitate Pi transport. Kinetic
analysis of Pi uptake in intact vacuoles further confirmed the stimulation of transport process by both ATP and pyrophosphate (Massonnearu et al. 2000). The apparent high $K_m$ (5 mM) of the vacuolar Pi-uptake system indicates that low affinity, high-flux transport mechanisms are operating in the tonoplast. At present details about this transport process are lacking as none of the tonoplastic Pi transporters are characterized at the molecular or biochemical level.

After the elimination of P influx from the external solution to the roots ($DPO = 0$), it is possible to conceive that all subsequent growth was due to the mobilization and use of stored Pi. Thus the sharp decrease of Pi contents in R, S and UL was caused by the release of Pi and by the growth of new young tissues with a very small P reserve. For ML and LL, the release of the reserves must have been the determining event in the drop of the stored $[\text{Pi}]$. R, S and UL were, in this order, the preferential sinks for the stored Pi under conditions of interrupted P absorption. This is in agreement of Martinez et al. (2005), who reported that leaves and roots were the main sink of stored Pi in middle and lower leaves of soybean cultivars. Alves (1994) observed that roots are the strongest Pi and sugars sinks in maize plants under omission of this element. Bieleski and Ferguson (1983) stated that most of the P moves towards the young growing leaves, flowers, fruits, or buds. Raghothama (1999) reported that this behavior may be altered during plant growth under P stress, and that compartments which are sources can become sinks. Under moderate Pi starvation, the transport of Pi increased in the shoots of potato plants, while in severely stressed plants, Pi was retained in the roots (Cogliatti and Clarkson 1983). Under continuous P supply environment, cultivars ‘Brown Raya’ and ‘Con-1’ generally showed the highest $[\text{Pi}]$ and ‘Dunkled’ the lowest ones. The ‘Rain Bow’ and ‘B.S.A’ cultivars had $[\text{Pi}]$ in-between and not significantly different from these two groups (Table 1).

The estimated values of $[\text{Po}]$ in fresh matter of plant components well supplied with P varied from 233 in R to 1239 mg kg$^{-1}$ in UL and showed an increasing order for R, S, LL, ML, and UL (Figures 2 to 4). Organic P was the fraction with the smallest variation during 10 DPO, stable in the UL of all cultivars ($\tilde{Y} = \tilde{Y} = 1097$ mg kg$^{-1}$ for ‘Brown Raya’; $\tilde{Y} = \tilde{Y} = 1239$ for ‘Con-1’; $\tilde{Y} = \tilde{Y} = 1104$ for ‘Rain Bow’; $\tilde{Y} = \tilde{Y} = 1114$ for ‘Dunkled’ and $\tilde{Y} = \tilde{Y} = 830$ for ‘B.S.A’), ML of ‘Con-1’ ($\tilde{Y} = \tilde{Y} = 759$) and ‘B.S.A’ ($\tilde{Y} = \tilde{Y} = 490$), LL of ‘Con-1’ ($\tilde{Y} = \tilde{Y} = 698$), ‘Dunkled’ ($\tilde{Y} = \tilde{Y} = 707$) and ‘B.S.A’ ($\tilde{Y} = \tilde{Y} = 488$), and S and R of ‘Brown Raya’ ($\tilde{Y} = \tilde{Y} = 527$; $\tilde{Y} = \tilde{Y} = 504$), ‘Con-1’ ($y_c = y_b = 523$; $y_c = y_b = 557$), ‘Dunkled’ ($\tilde{Y} = \tilde{Y} = 467$; $\tilde{Y} = \tilde{Y} = 539$) and
‘B.S.A’ ($\tilde{Y} = \tilde{Y} = 312; \tilde{Y} = \tilde{Y} = 232$). The greatest mean relative reductions were observed in descending order for ML and LL, followed by S and R, respectively (Figures 2 to 4). R and UL compartments, with the highest metabolic activity, showed a greater stability of Po content, probably at the expense of their own and also of Pi content in the ML, LL, and S compartments. The observed behavior of Po corroborates earlier discussions on the Pi concentration. The Po in R was proportionally less reduced than Po in S, nevertheless, that in R was observed to represent a weaker sink for the Pi released by ML and LL than the S. This can be explained by diverting greater initial Pi reserve in R tissues, which was used to maintain the root cytoplasmatic pool, instead of exporting it to the shoot. This agrees with Bieleski and Ferguson (1983), Clarkson (1985) and Martinez et al. (2005) who pointed out that when absorption is limited, a small Pi influx is retained by the roots, maintaining their growth at the expense of other plant parts and of the reduction of the shoot-root ratio. Under deprivation, proportionally more Pi retained in the S compartment than the amount that was translocated to the R, thus representing a stronger sink than the R. Bieleski and Ferguson (1983) reported that $^{32}$P applied to a P starved leaf was retained to a greater extent than exported. Raghothama (1999) argued that the stress intensity could mould the plant response, altering the source-sink relationship. Clarkson (1985) stated that the highest retention in R under P starvation was due to the fact that the roots lie closer to the supply source.

Before the onset of P omission, [Po] in the UL, the plant part that had the highest metabolic activity, was the highest (mean of 1077 mg kg$^{-1}$), and it was the lowest in S (mean of 525 mg kg$^{-1}$) and R (mean of 492 mg kg$^{-1}$), with ML (mean of 781 mg kg$^{-1}$) and LL (mean of 741 mg kg$^{-1}$) representing intermediate concentrations (Figures 2 to 4). After 10 DPO, the UL continued with higher Po contents (mean of 1077 mg kg$^{-1}$) than those of the remaining organs (means of 497, 504, 435, and 423 mg kg$^{-1}$ for ML, LL, S, and R respectively), while the Po of ML and LL were close to that of S and R. It is important to note that the whole root system was analyzed, and the observed Po contents were therefore average concentrations that include parts with a high metabolic activity, such as new and thin tips, and parts with low metabolic activity, such as mature and thick parts of roots. The results stated above show that the younger shoot and root tissues of the tested cultivars had a higher ability to store the excess of Pi absorbed under sufficient P supply. Conversely, under interrupted supply, R, S, and UL were the main sink for the released Pi. In general, without P starvation, ‘Con-1’ and ‘Rain Bow’ showed the highest [Po], followed by ‘Brown Raya’, ‘Dunkled’ and especially ‘B.S.A’ cultivars showed the lowest [Po] (Table 1).
Estimation of Pi release velocity and internal Pi buffering capacity by Brassica cultivars

Considering that \(-\delta\text{Pi}/\delta t\) expresses the velocity of Pi release (RSPi), then inverse of RSPi is the internal buffering capacity (IBCPi). The concept of plant IBCPi was enunciated by Novais and Smyth (1999) in analogous to what occurs in soils. Pi content as a function of PPO may be considered a measure of the IBCPi. There is an inverse relationship between IBCPi and the slope of equation i.e. the IBCPi will be higher as the slope of the equation that expresses this relationship is lowered. In study 1, the compartment R had highest RSPi and lowest IBCPi values, followed by UL (Table 2). This confirms the observation that these compartments are, concomitantly, important sources and sinks of Pi. The ML and LL showed higher IBCPi and lower RSPi values than UL and R, while the S had the lowest observed RSPi and highest IBCPi values.

Cultivar ‘Brown Raya’ had the highest Pi reserve (Table 1) at the onset of P omission and also the lowest internal P buffering, that is, the highest Pi release velocity from its compartments in order to keep the metabolic pools under P starvation and hence, proved to be more efficient than other tested cultivars. Under short intervals of P deprivation, this can represent an adaptive advantage of this cultivar. ‘Con-1’ had a similar Pi reserve as ‘Brown Raya’, but its capacity to release reserves to the metabolic pool immediately after starting the stress was lower. Cultivar ‘Rain Bow’, in spite of a lower Pi reserve than ‘Con-1’, showed similar RSPi and IBCPi values to ‘Con-1’ and to ‘Dunkled’. The Pi reserve of ‘Dunkled’ was the smallest among the tested cultivars. Cultivar ‘B.S.A’ presented an intermediate Pi reserve, but the greatest IBCPi and the smallest RSPi value compared to the others, especially in the S and UL compartments (Tables 1 and 2). This indicates that B.S.A. is less efficient than other Brassica cultivars. Martinez et al. (2005) stated that cultivar ‘Santa Rosa’ of soybean had the highest Pi reserve at the beginning of P hunger and also the lowest IBCPi, that is, highest RSPi from different plant parts under short intervals of nutritional stress. Novais and Smyth (1999) reported the IBCPi of two eucalyptus species i.e. E. cloesiana and E. camaldulensis. The IBCPi of E. cloesiana, a species originating from fertile soil and high annual rainfall regions of Australia, was 0.0060 kg day mg\(^{-1}\) (RSPi = 166.67 mg kg\(^{-1}\) day\(^{-1}\)), while that of E. camaldulensis, which is found in semi-arid regions with low annual rainfall, was 0.0472 kg day mg\(^{-1}\) (RSPi = 21.19 mg kg\(^{-1}\) day\(^{-1}\)). This suggests that internal buffering is, even when accompanied by growth restriction due to limited supply, a mechanism that guarantees survival under P starved environment. The analysis of biomass accumulation (Table 3) revealed that there were no significant differences between the cultivars for any of the plant compartments or even in the whole plant at the beginning of P omission. At the end of the 10th day of P deprivation,
cultivar ‘B.S.A’ presented less leaf, shoot and whole plant biomass accumulation than efficient cultivar ‘Brown Raya’, and less root dry matter production than cultivar ‘Dunkled’, which was accompanied by a slower Pi release and higher internal buffering for Pi, resulting in a greater growth loss due to P omission (Table 2). Cultivar ‘Brown Raya’ presented the highest RSPi value and largest whole plant biomass accumulation at the end of 10 DPO stress and ranked as P-efficient cultivar compared to other tested *Brassica* cultivars. The latter value was not significantly different from those of ‘Dunkled’, ‘Con-1’ and ‘Rain Bow’ (Tables 2 and 3). On the basis of the above observations, it is plausible to infer that internal remobilization of absorbed P and its possible retranslocation from metabolically active to inactive sites under P deprivation form the basis of differential growth behavior of *Brassica* cultivars which may have helped the efficient cultivars to establish a better rooting system to cope with P stress environment.

**Experiment 2: Phosphorus absorption and Remobilization in *Brassica* Plants**

**Tissue P-concentration and uptake by *Brassica* cultivars**

Nutrition and growth can differ among plant species and cultivars even under similar soil fertility conditions. Better nutritional and growth status may be a result of a more efficient nutrient absorption and, or, use by a cultivar. The ability of plants to remobilize P from inactive to active sites is a strategy adopted by some plants to tolerate P stress (Gill and Ahmad 2003) and is held responsible for variations in nutrient utilization of crop species and cultivars (Ahmad et al. 2001).

*Brassica* cultivars used in study 2 had previously been reported to be genetically diverse with respect to PUE for biomass synthesis under sufficient as well as deficient conditions (Akhtar et al. 2006a, 2006b, 2007). Variations in P concentrations ([Ps]) of various plant organs under P starvation and any evidence of P re-translocation under such environment was therefore, the main point of interest in this study. [Ps] in different tissues of the tested cultivars decreased drastically during P deprivation i.e. between 15 and 44 DAT (Table 4) compared to those noted after initial growth of 15 days in complete nutrition. [Ps] were invariably lower in shoots than roots at both harvests. At 44 DAT, [Ps] were higher in young/developing leaves than older/developed leaves of cultivars. The P efficient cultivar ‘Brown Raya’ had lower [P] in shoots as well as roots at 15 DAT and in developing and developed leaves at 44 DAT compared to the sensitive cultivar ‘Sultan Raya’. It implied better efficiency of the tolerant cultivar to synthesize biomass per unit of absorbed P compared to sensitive cultivar, and differences in [Ps] of the two cultivars can be ascribed to dilution effect. Nevertheless, [P] was higher in the roots of ‘Brown Raya’ than those of ‘Sultan Raya’ at 44 DAT. This may be an indication of the ability of the tolerant cultivar to better
re-translocate P from aerial parts to roots under P deprivation. An overall trend of P-re-translocation from shoot to root in all the cultivars exposed to P deprivation was noted (Figure 5). Expected [P] ascertained on the basis of [P] at the start of P hunger (as no P available to plants thereafter) and the increase in root dry matter during growth under P free conditions by considering the dilution of P. P re-translocation under P deprivation may imply an inducible character of *Brassica* cultivars to adapt P starvation stress. This is in agreement with the results of Gill and Ahmad (2003), who evaluated cotton varieties for P absorption and re-translocation and the work reported earlier (Akhtar et al. 2007). Snapp and Lynch (1996) reported that tolerant genotypes retain relatively larger amounts of stressed elements in their roots, in a bid to develop more efficient root system similar to Martinez et al. (2005). [Ps] in all plant tissues at 44 DAT were lower than sufficiency limit for *Brassica* at this ontogenic stage, however, deficiency symptoms could be observed on developed leaves only. A higher concentration of P in developing leaves compared to developed leaves during exposure to P free nutrition may entail remobilization and re-translocation of P within shoots as well, in addition to its re-translocation to roots under P deprivation. At 44 DAT, [P] of roots of the cultivars was positively correlated to [P] of both developing and developed leaves (r > 0.91**), while its correlation with stem [P] was statistically non-significant. Adu-Gyamfi et al. (1990) stated that the rate of P translocation to leaves under low P forms the basis for increased dry matter production under such conditions. Gerloff (1987) reported plant adaptability to nutrient stress depends upon re-transport of the deficient nutrient from older to younger leaves. Variations in compartmentalization of P in various organs are reported to be inducible under conditions of P deprivation and are under genetic influence.

At 15 DAT, P uptake by various plant tissues is presented in Table 4. It can be conceived that on average, 17 % of the total P acquired was portioned into the roots and 83 % into shoots during exposure to complete nutrition in the culture media (1 to 15 DAT). Due to P omission from the solution, further uptake was not possible and plants had to redistribute to fulfill their metabolic requirements for sustaining growth (Gabelman and Gerloff 1983; Gill and Ahmad 2003; Peng and Li 2005). At the termination of the experiment, the observed data confirmed the redistribution of P within various plant tissues. Besides deviations of tissue [P] on 44 DAT from those recorded at the onset of hunger period, the proportion of total P in roots increased to 26 % from 17 % and P in shoots decreased, confirming P re-translocation from shoots to roots (Figure 5).

These results suggest that some mechanism exists to regulate assimilates and P redistribution and reutilization within plants. It has been reported that the differences in IAA levels
were correlated with the differences in dry matter accumulation in various positions within the wheat ear (Bangerth et al. 1985). Applied IAA in lanolin to decapitated internodes of *Pisum sativum*, *Phaseolus vulgaris* or *Populus robusta* enhanced accumulation of endogenous N, P and K in the IAA-treated areas (Phillips 1968). Recent molecular studies have revealed that special transport systems are essential for the uptake of Pi and for its internal redistribution within plants. The internal utilization of Pi within the plant requires transport through membranes wherever symplastic connections are lost. Thus, membrane transport systems are also required for the distribution and remobilization of Pi throughout shoot tissues. A number of the Pht1 family of plant phosphate transporters fulfills this role (Mudge et al. 2002). The Pht1 family of Pi transporters is important for Pi acquisition (Raghothama and Karthikeyan 2005; Vance et al. 2003). Most of the genes of the Pht1 family expressed in roots are up-regulated in P-stressed plants (Smith et al. 2003). Also together with at least one member of another family of Pi transporters, the Pht2 family, a number of members of the Pht1 family of Pi transporters are responsible for the remobilization and internal cycling of Pi throughout shoot tissue (Daram et al. 1999; Smith et al. 2003). The Pht2 family has similarities to some mammalian Na+/H₂PO₄⁻ cotransporter in plants (Smith et al. 2003). The Pht2;1 transporter, which possesses a long N-terminal tail, is primarily expressed in shoot tissues where it is postulated that it plays a role in the internal cycling of Pi. A chloroplast transporter, PHT2;1, was cloned from *Arabidopsis* and *Solanum*, and shown to function in Pi remobilization from senescing to young leaves (Rausch et al. 2004).

Based on the above observations from both experiments, it is plausible to conclude that *Brassica* cultivars tend to ration its resources under exposure to P starvation by re-translocating the absorbed P to metabolically active sites such as roots (the apparatus of P acquisition) and young/developing leaves from metabolically inactive sites such as old leaves and stems, which may increase its PUE. Tested cultivars presented differential non-metabolic P pools, while homeostasis of Po was at the expense of Pi, and roots and upper leaves were the main sink for the released Pi under P deprivation. Cultivars showed differential behavior in terms of growth, Pi release velocity and internal buffering capacity, and growth reduction was inversely related to RSPi and directly related to IBCPi under P starvation. Cultivars such as ‘Brown Raya’ capable of efficiently re-translocating P from inactive to metabolically active sites under P deprivation can better tolerate P starved rooting environments.
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References


Figure 1. Framework of inorganic P (Pi) fractionation flow chart in Brassica cultivars.
Figure 2. Phosphorus [inorganic (Pi) and organic (Po)] concentration in upper leaves (a) and middle leaves (b) of 5 Brassica cultivars as a function of the P omission period. **: significant at 1 %; fw: fresh weight.
Figure 3. Phosphorus [inorganic (Pi) and organic (Po)] concentration in lower leaves (a) and stems (b) of 5 Brassica cultivars as a function of the P omission period. **: significant at 1 %; fw: fresh weight.
Figure 4. Phosphorus [inorganic (Pi) and organic (Po)] concentration in roots of 5 *Brassica* cultivars as a function of the P omission period. **: significant at 1%; fw: fresh weight.

Figure 5. Expected and actual P concentration of 6 *Brassica* cultivars at 44 days after transplanting.
Table 1. Phosphorus [organic P (Po), inorganic P (Pi)] concentrations (mg Kg\(^{-1}\) fresh matter) in roots, stems, upper, middle and lower leaves of 5 Brassica cultivars grown in complete nutrition for 35 days

<table>
<thead>
<tr>
<th>P- fraction</th>
<th>Plant compartment</th>
<th>B.S.A</th>
<th>Dunkled</th>
<th>Rain Bow</th>
<th>Con-1</th>
<th>Brown Raya</th>
</tr>
</thead>
<tbody>
<tr>
<td>Po Roots (R)</td>
<td>211 C</td>
<td>439 AB</td>
<td>593 AB</td>
<td>670 A</td>
<td>519 AB</td>
<td></td>
</tr>
<tr>
<td>Stems (S)</td>
<td>301 C</td>
<td>556 B</td>
<td>817 A</td>
<td>619 B</td>
<td>607 B</td>
<td></td>
</tr>
<tr>
<td>Upper leave (UL)</td>
<td>743 C</td>
<td>922 A</td>
<td>1394 A</td>
<td>1221 AB</td>
<td>1120 ABC</td>
<td></td>
</tr>
<tr>
<td>Middle Leave (ML)</td>
<td>603 B</td>
<td>938 A</td>
<td>883 A</td>
<td>943 A</td>
<td>922 A</td>
<td></td>
</tr>
<tr>
<td>Lower Leave (LL)</td>
<td>533 B</td>
<td>910 A</td>
<td>969 A</td>
<td>763 A</td>
<td>875 A</td>
<td></td>
</tr>
<tr>
<td>Pi Roots (R)</td>
<td>1048 AB</td>
<td>1020 B</td>
<td>918 B</td>
<td>1076 AB</td>
<td>1267 A</td>
<td></td>
</tr>
<tr>
<td>Stems (S)</td>
<td>584 BC</td>
<td>670 C</td>
<td>689 AB</td>
<td>635 AB</td>
<td>728 A</td>
<td></td>
</tr>
<tr>
<td>Upper leave (UL)</td>
<td>883 B</td>
<td>878.5 B</td>
<td>1022 AB</td>
<td>1013 AB</td>
<td>1088 A</td>
<td></td>
</tr>
<tr>
<td>Middle Leave (ML)</td>
<td>834 A</td>
<td>654 AB</td>
<td>694 AB</td>
<td>845 A</td>
<td>796 AB</td>
<td></td>
</tr>
<tr>
<td>Lower Leave (LL)</td>
<td>694 AB</td>
<td>645 B</td>
<td>623 B</td>
<td>679 AB</td>
<td>780 A</td>
<td></td>
</tr>
</tbody>
</table>

Means with different letter(s) differ significantly according to Duncan’s Multiple Range Test (P < 0.05).

Table 2. Inorganic P release velocity (RSPi) and internal Pi buffering capacity (IBCPi) in roots (R), stems (S), upper (UL), middle (ML) and lower leaves (LL) of 5 Brassica cultivars

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Parameter</th>
<th>R (mg Kg(^{-1}) day(^{-1}))</th>
<th>S</th>
<th>UL (mg Kg(^{-1}))</th>
<th>ML (mg Kg(^{-1}))</th>
<th>LL (mg Kg(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>B.S.A</td>
<td>RSPi</td>
<td>77.84</td>
<td>31.46</td>
<td>53.90</td>
<td>54.48</td>
<td>29.64</td>
</tr>
<tr>
<td></td>
<td>IBCPi (Kg day mg(^{-1}))</td>
<td>0.013</td>
<td>0.032</td>
<td>0.019</td>
<td>0.018</td>
<td>0.034</td>
</tr>
<tr>
<td>Dunkled</td>
<td>RSPi</td>
<td>89.89</td>
<td>55.25</td>
<td>67.04</td>
<td>45.76</td>
<td>45.87</td>
</tr>
<tr>
<td></td>
<td>IBCPi (Kg day mg(^{-1}))</td>
<td>0.011</td>
<td>0.018</td>
<td>0.015</td>
<td>0.022</td>
<td>0.022</td>
</tr>
<tr>
<td>Rain Bow</td>
<td>RSPi</td>
<td>73.88</td>
<td>38.38</td>
<td>67.07</td>
<td>46.98</td>
<td>38.73</td>
</tr>
<tr>
<td></td>
<td>IBCPi (Kg day mg(^{-1}))</td>
<td>0.014</td>
<td>0.026</td>
<td>0.015</td>
<td>0.021</td>
<td>0.026</td>
</tr>
<tr>
<td>Con-1</td>
<td>RSPi</td>
<td>68.70</td>
<td>35.75</td>
<td>65.17</td>
<td>54.03</td>
<td>46.32</td>
</tr>
<tr>
<td></td>
<td>IBCPi (Kg day mg(^{-1}))</td>
<td>0.015</td>
<td>0.028</td>
<td>0.015</td>
<td>0.019</td>
<td>0.022</td>
</tr>
<tr>
<td>Brown Raya</td>
<td>RSPi</td>
<td>92.07</td>
<td>47.43</td>
<td>86.35</td>
<td>51.89</td>
<td>58.3</td>
</tr>
<tr>
<td></td>
<td>IBCPi (Kg day mg(^{-1}))</td>
<td>0.011</td>
<td>0.021</td>
<td>0.012</td>
<td>0.019</td>
<td>0.017</td>
</tr>
<tr>
<td>Mean</td>
<td>RSPi</td>
<td>80.48</td>
<td>41.65</td>
<td>67.91</td>
<td>50.63</td>
<td>43.77</td>
</tr>
<tr>
<td></td>
<td>IBCPi (Kg day mg(^{-1}))</td>
<td>0.013</td>
<td>0.025</td>
<td>0.015</td>
<td>0.020</td>
<td>0.024</td>
</tr>
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</table>
Table 3. Biomass accumulation (mg plant\(^{-1}\)) of 5 *Brassica* cultivars cultivated in nutrient solution for 35 days in complete nutrition or exposed to P omission for 10 days

<table>
<thead>
<tr>
<th>Plant compartment</th>
<th>P-omission period</th>
<th>Cultivar</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>B.S.A Dunkled</td>
</tr>
<tr>
<td>Root</td>
<td>0</td>
<td>708 A</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>2226 B</td>
</tr>
<tr>
<td>Shoot</td>
<td>0</td>
<td>3704 A</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>11903 B</td>
</tr>
<tr>
<td>Stems</td>
<td>0</td>
<td>1193 A</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>4693 A</td>
</tr>
<tr>
<td>Leaves</td>
<td>0</td>
<td>2511 A</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>7210 B</td>
</tr>
<tr>
<td>TDM</td>
<td>0</td>
<td>8118 A</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>26033 B</td>
</tr>
</tbody>
</table>

Means with different letter(s) differ significantly according to Duncan’s Multiple Range Test (P < 0.05).

Table 4. Tissue P-concentration and uptake by genetically diverse 6 *Brassica* cultivars exposed to P hunger for 29 days after initial feeding on optimum nutrition for 15 days

| Tissue P-concentration (mg g\(^{-1}\) dry matter) of Brassica cultivars |
|--------------------------|------------------|-----------------|-----------------|
|                          | After 15 days    | At the end of experiment |
| Cultivars                | Root | Shoot | Developing leaf | Developed leaf | Root | Stem |
| Con-1                    | 5.35 ab | 3.5 bc | 0.90 ab | 0.45 b | 1.41 ab | 0.21 a |
| Brown Raya               | 4.99 b  | 3.01 c  | 0.72 cd | 0.44 b | 1.16 ab | 0.20 a |
| Sultan Raya              | 5.38 ab | 4.10 a  | 0.77 bc | 0.47 ab | 0.99 b  | 0.15 ab |
| Rainbow                  | 5.91 a  | 4.06 a  | 1.01 a  | 0.57 a  | 1.50 a  | 0.16 ab |
| Toria Selection          | 4.97 b  | 3.90 ab | 0.60 a  | 0.4 b   | 0.86 b  | 0.13 b  |
| Peela Raya               | 5.03 b  | 3.87 ab | 0.81 bc | 0.40 b  | 0.90 b  | 0.14 ab |

P-uptake (mg plant\(^{-1}\)) by various Brassica cultivars

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>After 15 days</th>
<th>At the termination of experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Root</td>
<td>Shoot</td>
</tr>
<tr>
<td>Con-1</td>
<td>1.24(^{\text{NS}})</td>
<td>5.36 b</td>
</tr>
<tr>
<td>Brown Raya</td>
<td>2.59</td>
<td>8.51 a</td>
</tr>
<tr>
<td>Sultan Raya</td>
<td>2.21</td>
<td>7.93 ab</td>
</tr>
<tr>
<td>Rainbow</td>
<td>2.48</td>
<td>9.54 ab</td>
</tr>
<tr>
<td>Toria Selection</td>
<td>1.66</td>
<td>8.08 ab</td>
</tr>
<tr>
<td>Peela Raya</td>
<td>1.44</td>
<td>7.06 bc</td>
</tr>
</tbody>
</table>

Means with different letter(s) differ significantly according to Duncan’s Multiple Range Test (P < 0.05).