# Phosphorus and Biomass Distribution, and P-efficiency by Diverse *Brassica* Cultivars Exposed to Adequate and P-stress Environment

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To acclimate under orthophosphate (Pi) starved environment, plant species and cultivars display an elegant myriad of Pi-adaptive and rescue responses via reprioritizing internal Pi use and maximizing external Pi acquisition by reprogramming metabolism and restructuring root system architecture. Exploitation of considerable genetic diversity both between and within crop species and harnessing of these genetic variations can lead us to develop smart plants with improved P-acquisition, growth and yield under P-deprivation. To elucidate the effect of P-stress on plant growth, and P-efficiency under P-starvation, 14 diverse *Brassica* cultivars were grown hydroponically in a climatically controlled chamber using sufficient (200 and 400 μM) and stress (10 and 20 μM) P-levels using ammonium phosphate ($NH_4H_2PO_4$) as a P source. Cultivars showed differential growth behaviour in terms of biomass accumulation (shoot and root dry matter partitioning), percent distribution of Pi-concentration ([P]) and P-contents in plant parts (roots and shoots), and P-efficiency ratio (%PER) (relative shoot growth) indicating considerable genetic diversity among the tested *Brassica* cultivars. PER and the proportional increases in shoot dry matter (SDM) accumulation ($SDM_{max}/SDM_{min}$) in response to the P levels assisted in categorizing the cultivars into efficient and inefficient utilizers of the absorbed P from an ambient environment. Cultivars were classified into efficient responsive (ER), efficient non-responsive (ENR), non-efficient responsive (NER) and non-efficient non-responsive (NENR) by plotting ordination plots between PER and $SDM_{max}/SDM_{min}$ under P-stress environment. Differential PER values at stress P levels corresponds to high P levels suggest that P efficiency mechanisms can be different from one cultivar to another within a give plant species and cultivars exhibiting high PER values are better choice to thrive under P-starvation.

***Key words***: *Brassica cultivars, biomass and P-distribution, P-contents, P-efficiency ratio*

## 1 NTRODUCTION

The assimilated and fully oxidized form of phosphorus (P), inorganic phosphate (Pi), is indispensable for coupling the light and dark reactions in photosynthesis, and thus plays a pivotal structural and regulatory role at the nexus of energy conservation and carbon assimilation. Metabolism depends on inorganic phosphate (Pi) as reactant, allosteric effector and regulatory moiety in covalent protein modification (Ticconi and Abel, 2004). The chemical stability and cellular retention of nucleic acids, phospholipids and many metabolites are dependent on Pi as a building block because Pi is the only inorganic anion that forms charged diesters at physiological pH (Epstein et al., 2004). It is therefore not surprising that Pi availability profoundly affects plant metabolism and crop performance and is one of the major reason of malnutrition in tropics and subtropics especially under resource poor environments. Phosphorus is a non-renewable resource and global P reserves are rapidly being depleted; depending on assumed scenario, current P reserves will be halved assumed scenario, current P

reserves will be halved (relative to the reserves at the turn of the 20th 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 (Vance et al., 2003).

Nevertheless, phosphorus is abundant in the lithosphere; the physicochemical properties and notorious soil chemistry of Pi render it one of the least available and least mobile nutrients present in soils. Soil concentrations of soluble Pi are often up to a 1000 times lower than those of other required ions (Vance et al., 2003; Raghothama, 2000). High sorption capacity for P in the soil (*e.g.* on metal oxides), P complexation (*e.g.* Ca-P salts such as apatite), and/or fixation of P in organic forms result in low availability of P for uptake into plants. Phosphorus malnutrition poses a severe threat to agriculture that is typically averted in affluent countries by extensive application of concentrated Pi fertilizers. However, crop parts of the world with poor soils and limited economic with P-starvation, plants activate a set of adaptive responses that reprioritize internal Pi use and maximize

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external Pi acquisition (Ticconi and Abel, 2004). Such countermeasures include adjustment of metabolism to protect intracellular Pi homeostasis and redesign of root system architecture to accelerate soil exploration. P-starvation is remedied by the application of P fertilizers produced commercially by the solubilization of P in phosphate rocks by treatment with $H_2SO_4$ or $HNO_3$. The application of P fertilizers has undoubtedly contributed to greater crop production, even though little of the applied P is taken up by plants in the year of application (Laegrid et al., 1999). However, the balance of the P in the crop has been taken up from soil reserves and must be replaced if soil fertility is not to be jeopardised. Thus, it is not surprising that the global use of P-fertilizers increased dramatically during the past century (FAO, 2005). However, there are financial and environmental consequences to the use of P fertilizers. It has been estimated that their production, packaging, transport and application represents more than 2% of global energy use (White et al., 2005). Phosphate rocks are a diminishing natural resource, although there are great differences in estimates of how soon existing resources will be exhausted (Isherwood, 2003; Denison and Kiers, 2005).

There is an urgent need to develop crop plants that are more efficient at acquiring inorganic P (Pi) from soil and/or at using P more efficiently (Lambers et al., 2006). 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 in soil. 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: i.e., slow diffusion coefficient of P is notorious ($10^{-12}$-$10^{-15}$ $m^2$ $s^{-1}$; Rausch and Bucher, 2002). 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 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 toward 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. However, theoretical considerations on plant nutrient requirements suggest that sparingly soluble nutrients such as P in the rhizosphere soil solution must be replenished 20-50 times per day (Neumann and Römheld, 2002). This is not simply explainable on simple diffusion processes but requires additional adaptive traits by plants to thrive under P stress. All these adaptive traits enhance P-acquisition and P-use efficiency under P-starvation. In industrialized countries, low P-availability in agricultural soils is compensated by a high input of P fertilizer to guarantee high crop productivity. Water run-off, soil erosion and leakage in highly fertilized soils may cause environmental problems such as eutrophication of lakes, rivers and marine estuaries. Tilman et al. (2001) forecasted that during the next 50 years, conversion of natural ecosystems to agriculture for global food demand will be accompanied by an approximate 2.5-fold increase in N and P-driven eutrophication of terrestrial, freshwater, and near-shore marine ecosystems. The adverse effects of eutrophication of surface water bodies are of concern and in many cases P is the limiting nutrient. Thus, the transfer of P from agricultural soils to water is a serious issue. Johnston and Dawson (2005) concluded that the greatest risk of P transfer is in eroded soil and in management practices such as the inappropriate application of slurry to land. Little P is lost by leaching analogous to nitrate leaching, but P can be transported in drainage when attached to mineral and organic particles. The problematical loss of P in eroded soil, whether in surface runoff or drainage, implies that if acceptable yields of crops can be grown on soils with less plant available P than is currently recommended, then the risk of eutrophication will be lessened (White et al., 2005). However, P off-take in crops will still have to be replaced to maintain soil P status. The higher the level of extractable soil-P that must be maintained for good crop growth, the greater is the amount of P fertilizer that must be applied to the land, because of a more rapid chemical conversion of P into forms that are unavailable to plants (Greenwood et al., 2001; Karpinets et al., 2004). As the acquisition of P by plants and the efficient use of P in the plant are genetically determined, this can be exploited to explore cultivars that utilize soil P, whether derived from fertilizers or manures, more effectively so that they could be grown on low P soils. To alleviate the forecasted adverse negative effects of agricultural expansion and fear of depletion of world reserves of rock-P coupled with potential phosphate crisis in 21st centaury, classical strategies such as selection/screening of P-efficient cultivars or developing more precise methods to monitor crop P-status aiming at an improved crop yield with a lower input of exogenous hazardous soluble P-fertilizers are cheaper and environmental friendly alternatives.

The aim of the present work was to elucidate the differential growth response, percent distribution of P in plant parts, and P-efficiency by *Brassica* cultivars exposed to adequate and stress P levels. Screening/selection programs directed towards identification of efficient and or inefficient genome should target the quantitative processes and take into control of the environmental factors that modify them (Smith et al., 1993). Selection, categorization and exploitation of P-efficient/superior genome can mitigate the off-site risks of P-eutrophication of streams and rivers ensuring sustainable cropping.

## 2 MATERIALS AND METHODS

### 2.1 Plant Material and Culture Environment

Different cultivars of *Brassica* tested were: 'Con-1',

'Brown Raya', 'Poorbi Raya', '2B5-98016', 'Dunkled', 'RB5-96026', 'Sultan Raya', 'KS-75', 'Shiralle', 'Raya Anmol', 'KS-74', 'Gold Rush', 'B.S.A', and 'RL-18'. Seeds were germinated in polyethylene-lined iron trays containing pre-washed riverbed sand and irrigated with distilled water for seed germination and seedling establishment in a dark chamber at $25°C$. The experiment was conducted in a controlled-climate chamber 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. Treatments were fourteen *Brassica* cultivars, grown under four rates of P in nutrient solution, with three replications. The experimental design was a randomized complete block arranged in split-plots. The main plots were the P rates and the sub-plots, the *Brassica* cultivars. The main plots consisted of the following P rates: 10; 20; 200 and 400 µmol $L^{-1}$. Each plot consisted of four 12.5-L plastic recipients connected to a 50-L recipient (a total of 100 L nutrient solution). The experiment consisted of 12 plots and 48 subplots. Half-strength modified Hoffland's solution (Hoffland et al., 1989) was used as a base nutrient solution. The composition of the solution was; [in mM]: $KNO_3$ [2], $NH_4NO_3$ [1], $Ca(NO_3)_2.4H_2O$ [2], $MgSO_4.7H_2O$ [0.5], $K_2SO_4$ [0.5] and [in µM]: Fe(III)-EDTA [50], $H_3BO_3$ [25], $MnSO_4.H_2O$ [2], $ZnSO_4.7H_2O$ [2], $CuSO_4.5H_2O$ [0.5], KCl [50], $H_2MoO_4$ [0.5]. The solutions were modified by adding P at four rates [10 (P1); 20 (P2); 200 (P3), and 400 (P4) µmol $L^{-1}$] using ammonium phosphate ($NH_4H_2PO_4$) as P source. The pH of the continuously aerated solutions was monitored daily and maintained at $5.5 \pm 0.5$ by addition of HCl or NaOH. The nutrient solution was sampled for chemical analysis at the beginning of the experiment and at regular intervals. The nutrient solution was replenished regularly when nutrients were depleted to about 65% of the initial concentrations, using proportional volumes of the balanced stock solutions. The nutrient depletion due to plant uptake occurred slowly because of the large volume of solution available to the plants.

## 2.2 Plant Handling, Biomass Assay and Measurements of Various Growth Parameters

One week old uniform-sized seedlings were transplanted to the 12.5-L recipients. In each recipient, two seedlings / hole were suspended in the solution from lids holding plants of five cultivars. Each plot had 100 L of solution for 40 plants, corresponding to 2.5 L of solution per plant or 5 L per cultivar. At the end of the experiment, plants were harvested 31 days after imposing treatments, separated into shoots and roots, rinsed with deionized water, blotted dry with tissue papers, put in craft paper bags, and dried at $70°C$ in a forced-air oven.

The shoot and root samples were ground to pass through a 0.42 mm screen (40-mesh) and the samples were digested in 2N HCl after dry ashing in a muffle furnace for 7 hr at 550°C. P concentrations in shoot and root were measured by the vanadate-molybdate yellow color method (Chapman and Pratt, 1961) using a

spectrophotometer (Hitachi, U-1100). P uptake (mg $plant^{-1}$) was calculated by multiplying P concentrations with biomass on the whole plant basis. Biomass, [P] and P-contents distribution in roots and shoots were estimated among various cultivars. P-efficiency ratio (PER) was determined as described by Ozturk et al. (2005).

P-efficiency (PER) (relative shoot growth) = (SDM $_{(low\ or\ stress/\ 0P)}$/ SDM $_{(high/+\ P)}$) X 100

For the classification, two variables were plotted for each cultivar: in the Y-axis, the PER in the lower P levels (10 and 20 µmol $L^{-1}$, respectively) and in the X-axis, the ratio between the maximum and the minimum shoot dry matter yield obtained for each cultivar. The average value in the Y and X-axis defined the four groups: efficient responsive (ER), non-efficient responsive (NER), efficient non responsive (ENR) and non-efficient non-responsive (NENR). The term ER means that cultivars can be classified as P efficient (higher yielding than other cultivars under low P supply) and/or responsive (higher yielding than other cultivars under high P supply).

## 2.3 Statistical Analysis

Data were analyzed according to standard procedures (Steel and Torrie, 1980) using 'MSTAT-C' computer program and the methods described by Gomez and Gomez (1984). Data were subjected to analysis of variance and correlation coefficient (r) values were determined among various parameters using the treatment means.

## 3 RESULTS AND DISCUSSION

### 3.1 Biomass and P Distribution in Plant Parts

*Brassica* cultivars showed differential behavior in terms of biomass and P-distribution in shoots and roots indicating considerable diversity among the tested genome.

*Brassica* cultivars differed in distribution of biomass between root and shoot exposed to adequate (200 and 400 µM) and stress (10 and 20 µM) P-levels (Fig. 1A-D). On an average, cultivars 'Shiralle', 'RB5-96026', 'Raya Anmol', 'KS-75', 'Sultan Raya', 'KS-74', 'RL-18', 'B.S.A' and 'Glold Rush' were most affected due to P-stress as these cultivars exhibited more reduction in root dry matter (RDM) compared to other cultivars ('Brown Raya', 'Poorbi Raya', '2B5-98016', 'Dunkled', 'RB5-96026', 'Sultan Raya', 'KS-75') and this reduction in RDM indicates less tolerance of these cultivars to P-stress.

Phosphorus stress significantly affected the dry matter (DM) distribution between shoot and root at four P-levels. Vance et al. (2003) reported that most of the cultivars distributed more assimilates towards the roots under P-stress, thereby increasing absorptive surface area of the roots. Mollier and Pellerin (1999) stated that P-stress caused an accumulation of 16% more assimilates towards the roots compared to the sufficient P-level, and the roots
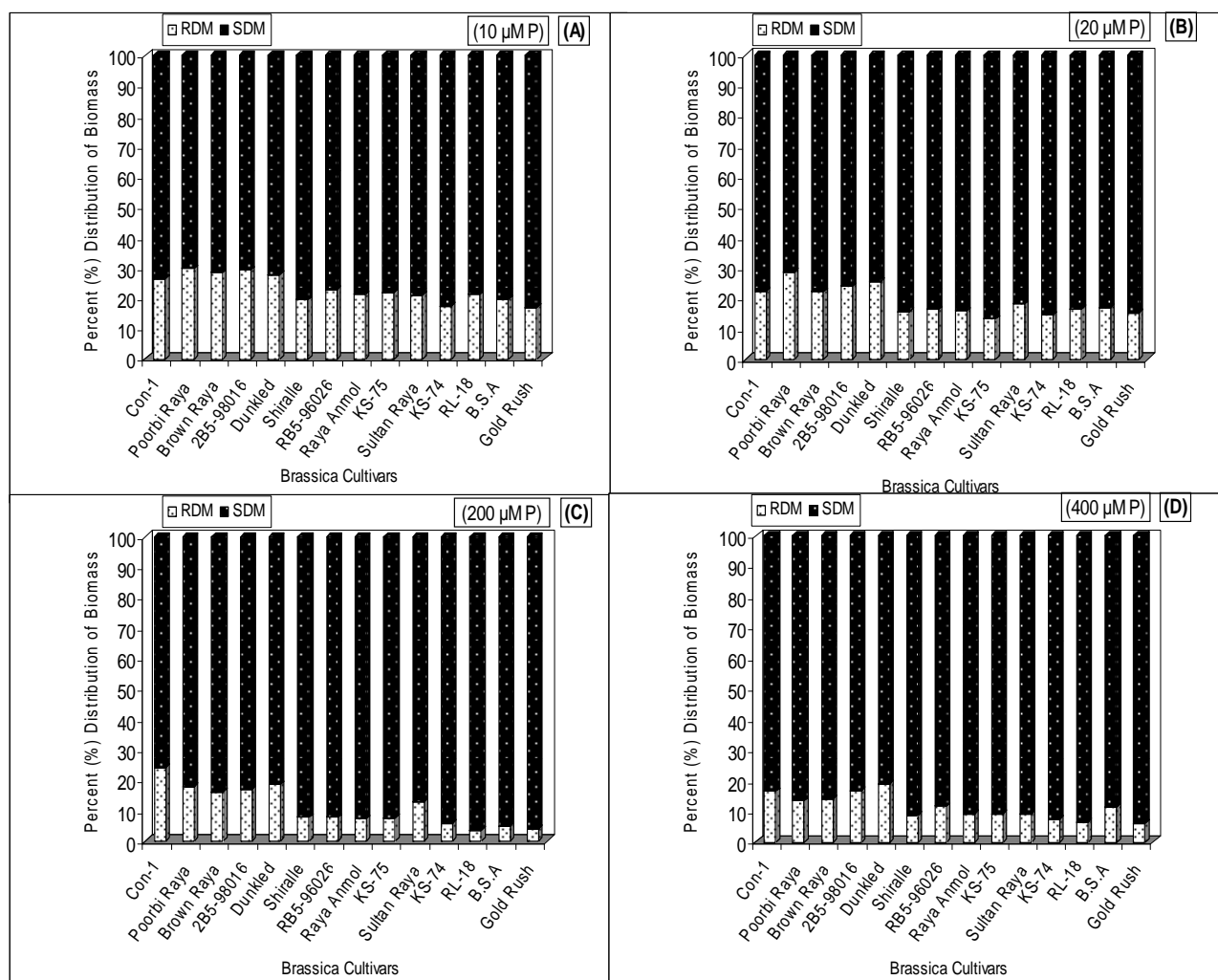
**Fig. 1** Percent (%) biomass distribution in shoots and roots under low/stress (10 and 20 µM P, respectively) (A-B) and adequate (200 and 400 µM P, respectively) (C-D) P-levels by14 *Brassica* cultivars grown in solution containing $NH_4H_2PO_4$ as a P-source

were strong sinks of assimilates under P stress, and thus increases the RSR. Same observation was confirmed in our present experiment. Differences for distribution of DM between shoot and root were more pronounced under low P-levels or P-starved environment (10 and 20 µM) as compared to sufficient ((200 and 400 µM) P-levels, respectively, indicating that cultivars elicit physiological and morphological adjustments for enhanced P acquisition under P-starvation (Raghothama and Karthikeyan, 2005; Hinsinger, 2001; Akhtar et al., 2006a,b; 2007; Lambers et al., 1998; Machado and Furlani; 2004). Cultivar 'Con-1' had only 2% and 5% more assimilates distributed to the roots at low P (10 and 20 µM, respectively) as compared to high P (200 and 400 µM, respectively) and it had maximum SDM under P-stress indicating its ability to grow efficiently under P-starvation without decreasing shoot growth.

*Brassica* cultivars differed significantly in distribution of [P] between root and shoot (Fig. 2A-D). P-starvation cause the preferential distribution of P to the roots (on average, 65% at 10 µM P-level in solution media) compared with adequate P-level (on average, 54% at 200 µM P-level) (Fig. 2A,C) and roots act as good sink for P under P-stress environment, which is in agreement

with the results reported by Mengel and Kirkby (2001). Tested cultivars showed considerable diversity in terms of percent P-contents in various plant parts (Fig. 3A-D). On an average, more P is retained in shots at adequate (P3-level) compared to stress P-levels. Under P-starvation, roots exposed to P-stress (P1 and P2 levels) retained approximately 2-fold more P than the roots exposed to sufficient P-levels (P3 and P4). This is in agreement with the results reported by Adu-Gyamfi et al., (1990) who stated that plants exposed to P-stress environment retain more P in their roots than shoots. Cultivars ('Con-1', 'Poorbi Raya', 'Brown Raya', '2B5-98016', and 'Dunkled' retained more P their roots compared to other cultivars under P-deprivation (low P-levels) indicating their more ability to combat P-stress by establishing better root system. Snapp and Lynch (1996) reported that efficient genotypes retain relatively larger amounts of stressed element in their roots, probably in a bid to develop more efficient root system.

**3.2 P-efficiency ratio (relative shoot growth) by tested *Brassica* cultivars**

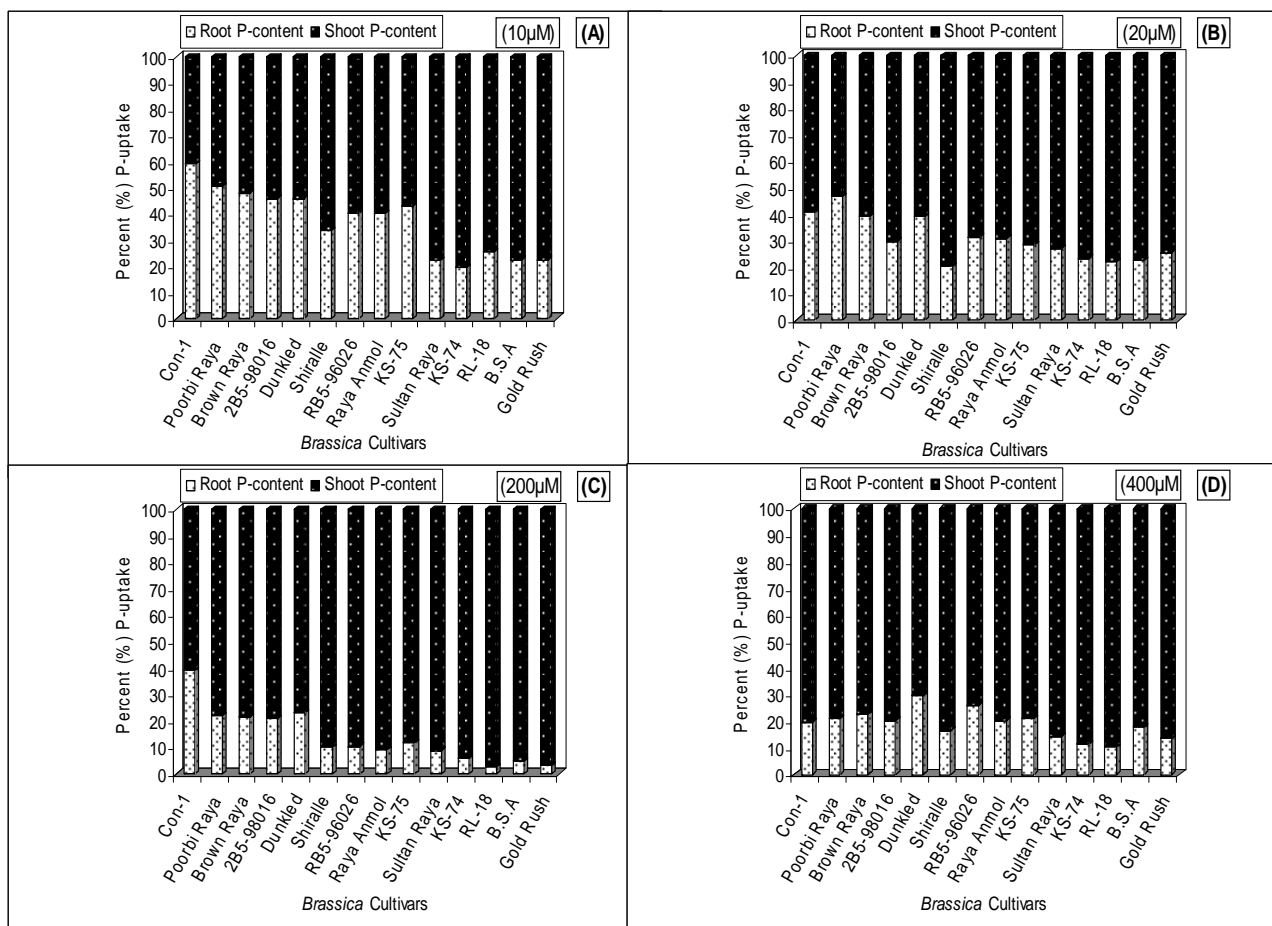Phosphorus efficiency ratio (%PER) used in the
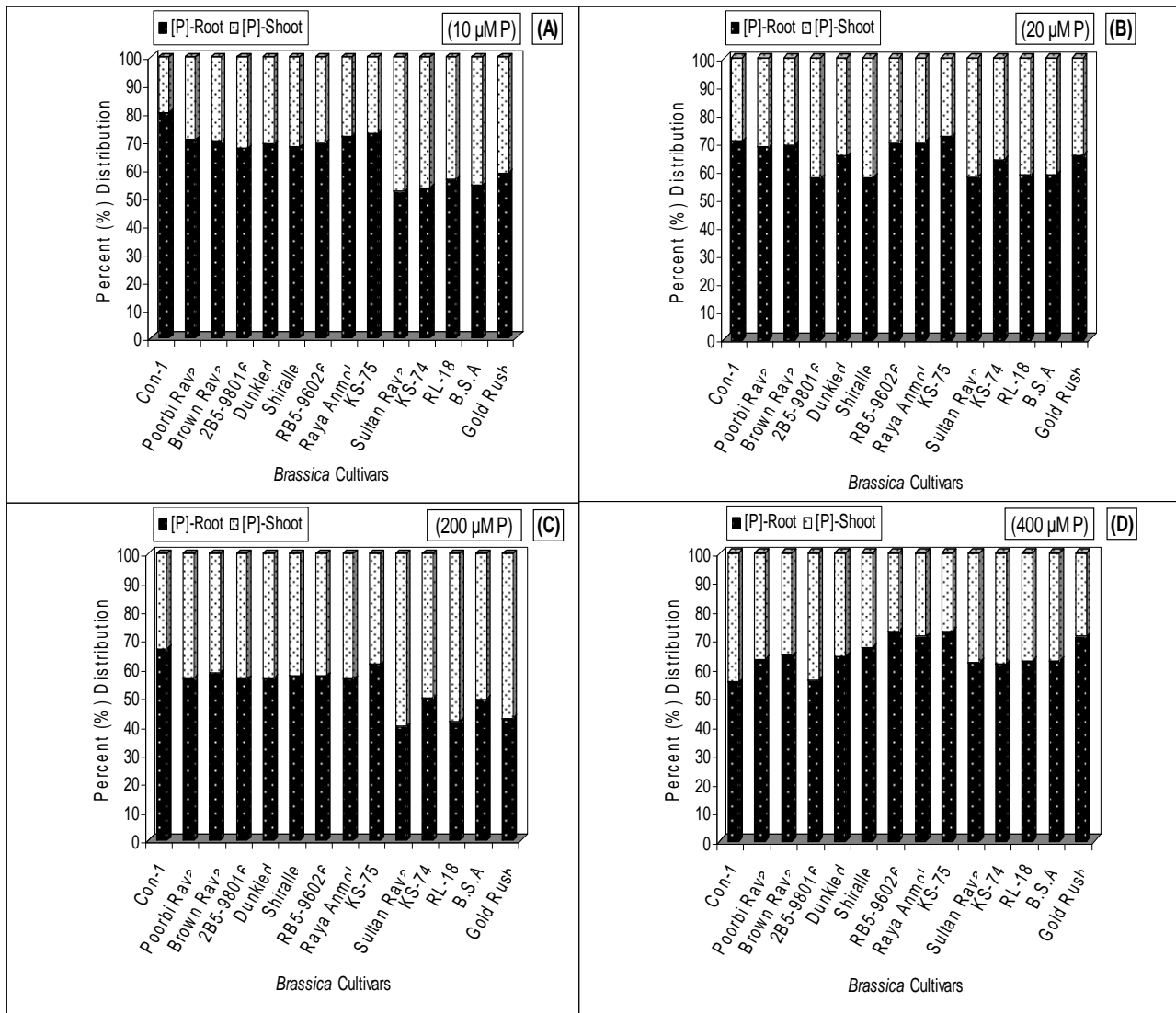
**Fig. 2** Percent (%) distribution of P-concentration in roots and shoots under low (10 and 20 µM P, respectively) (A-B) and adequate (200 and 400 µM P, respectively) (C-D) P-levels by 14 *Brassica* cultivars grown in solution containing $NH_4H_2PO_4$ as a P-source

present study is defined as the ability of cultivars to yield better under P-stress environment, and was calculated as the ration of SDM at the stress P supply to that obtained under adequate P-supply (Graham 1984; Ozturk et al., 2005; Gunes et al., 2006). Phosphorus efficiency is a very complex phenomenon and affected by large number of plant mechanisms associated with P acquisition from soil and P utilization at cellular level (Gourley et al., 1994; Marschner, 1995; Ciarelli et al., 1998; Schachtman et al., 1998; Lynch and Ho, 2004; Ozturk et al., 2005). Wissuwa (2003) suggested that large differences in tolerance to P deficiency within rice genotypes are caused by small changes in mechanisms affecting P deficiency tolerance, and such small changes are generally difficult to detect. Nevertheless, there is no general mechanism that determines the extent of P efficiency in crop plants and many definitions are proliferating in the literature. To date, a large number of mechanisms for P efficiency have been reported, operating at a cellular and at the soil-root interface (Gourley et al., 1994; Vance et al., 2003; Raghothama

and Karthikeyan, 2005). According to the results obtained in wheat cultivars Ozturk et al. (2005) reported that P efficiency mechanisms can be totally different from one genotype to other. Therefore, to elucidate and categorize genome on the basis of PER, tested *Brassica* cultivars were exposed to different P-levels. PER was significantly ($P < 0.01$) was significantly different among tested cultivars (Fig. 4). On an average, cultivars showed 16% more PER values at P2-level compared to P1-level corresponding to high P-levels (P3 and P4-levels, respectively) in the rooting environment. Cultivar 'Con-1' exhibited more PUE at 10 µM P-level corresponds to high P-levels than other cultivars, and proved to be more efficient in terms of biomass accumulation, whereas 'RL-18' showed low PER at P1-level and proved to be least inefficient cultivar. This differential behavior of cultivars can be used to identify the P-efficient/superior genome and then can be exploited to grow superior cultivars under P-stress environments.

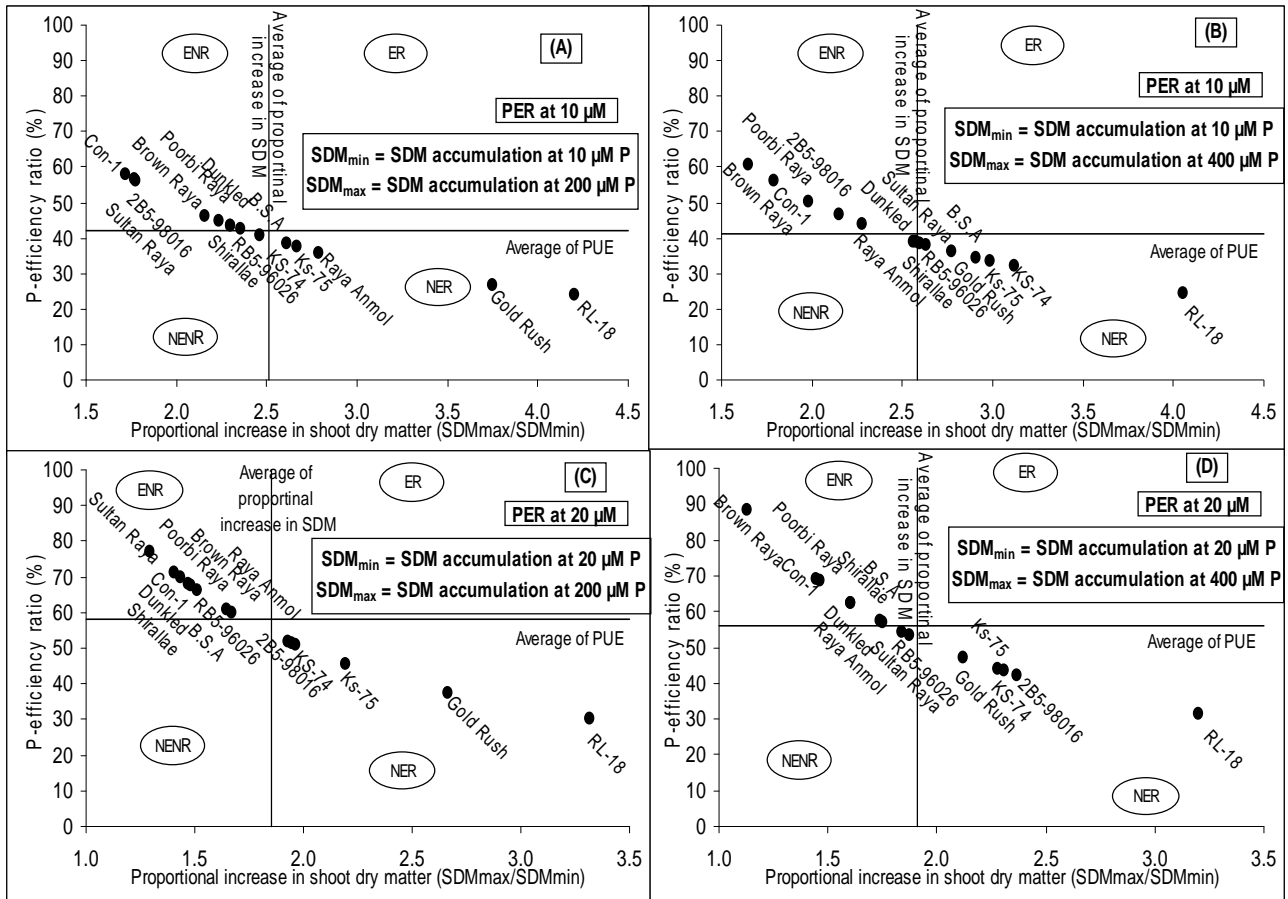To categorize the tested *Brassica* cultivars, each of the

**Fig. 3** Percent (%) uptake of P by *Brassica* cultivars grown under low/stress (10 and 20 µM P, respectively) (A-B) and adequate (200 and 400 µM P, respectively) (C-D) P-levels using $NH_4H_2PO_4$ as a P-source
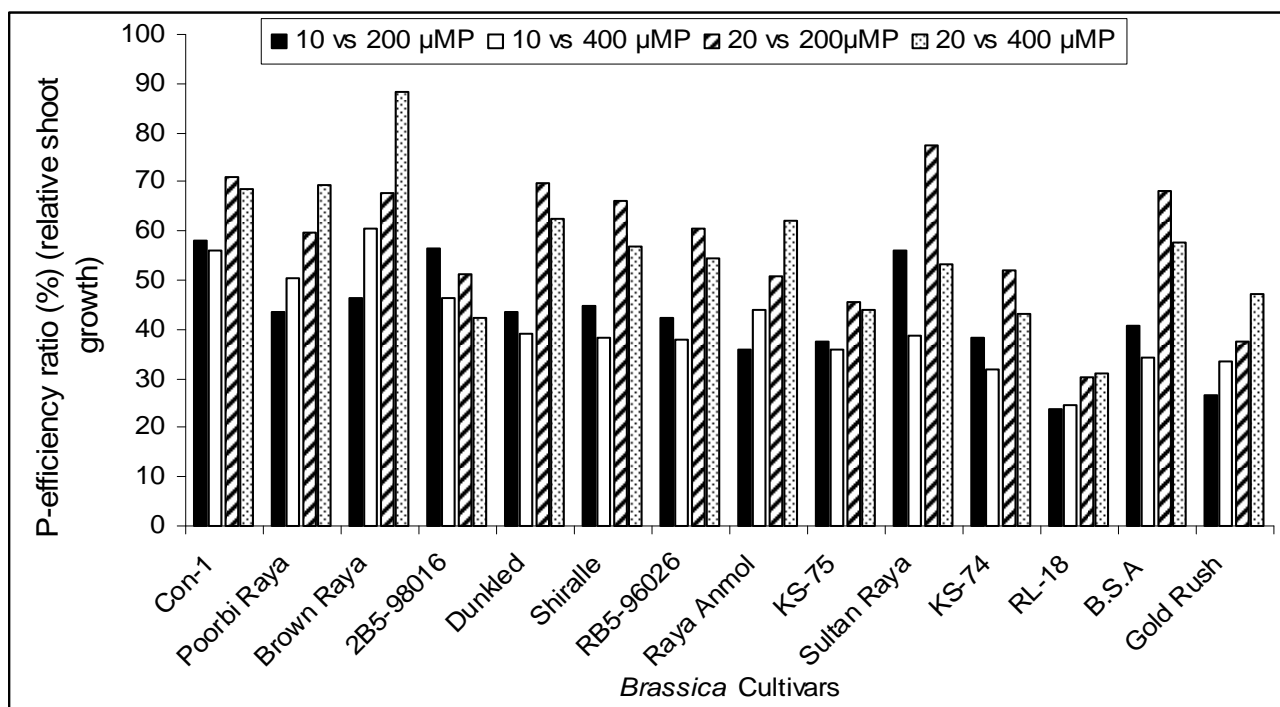


**Fig. 4** Phosphorus efficiency ratio (%PER) of 14 *Brassica* cultivars grown in nutrient solution with four P concentrations

**Fig. 5** Ordination plots to classify cultivars for P-efficiency ratio (PER) at low P levels (10 and 20 µM P) as a function of proportional increase in shoot dry matter (SDM) production ($SDM_{max}/SDM_{min}$) in response to the P levels (A-D) by14 *Brassica* cultivars grown in nutrient solution with four P concentrations. ER: Efficient and responsive; NER: Non-efficient but responsive; ENR: Efficient but non-responsive; NENR: Non-efficient and non-responsive

fourteen cultivars was plotted comparing the PER in the lower P-levels (10 and 20 µmol P L$^{-1}$, respectively) in the Y-axis, against the proportional increase in SDM yield obtained for each cultivar ($SDM_{max}/SDM_{min}$) in the X-axis in an ordination plot (Fig. 5A-D). The average values for the means in the Y and X axes defined the four cultivars groups: 'ER' = efficient responsive, 'NER' = non-efficient responsive, 'ENR' = efficient non-responsive and 'NENR' = non-efficient non-responsive. Cultivars were considered to be efficient, when the PER values were above the average for low P-levels and responsive those that had value of $SDM_{max}/SDM_{min}$ above the average. A very strong positive correlation between PER and total P uptake (r = 0.98$^{**}$) is specifically important as it indicates lack of interaction between these two traits and thus efficient use of P taken up by the plant for production of SDM. Thus, under P-stress, better P-acquisition and P-efficiency by the efficient cultivars for biomass synthesis collectively formed the basis of higher SDM production, evidencing that P-uptake and P use efficiency are important plant traits for selecting low P tolerant cultivars.

## 4 CONCLUSIONS

Conclusively, tested *Brassica* cultivars showed considerable genetic diversity in terms of biomass and P distribution in shoot and root dry mass. Cultivars exposed to P-stress environment retained more P in their roots compared to cultivars grown under high P supply. *Brassica* cultivars showed variations in P-efficiency ratio. Cultivars showing high PER values are categorized as efficient cultivars, and are desirable to grow under P-starvation. However, validation of these results is warranted under field conditions.

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