Genetic Diversity in *Brassica* Cultivars under Deficiently Buffered P-Stress Environment: II. Percent Distribution of Biomass and P-Concentration, P-Stress Factor and P-Utilization Efficiency

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Abstract: Phosphorus (P) availability is notoriously low and after nitrogen often limits primary productivity in many natural systems as well as cropping systems, unless applied as fertilizer. P-deficiency is more critical in highly withered soils as well as alkaline calcareous environments. Plants growing in apparently infertile environments are often classified as 'efficient' rather than being viewed in the ecological context as the plant best capable of the utilizing the available resources. In addition to improved P-acquisition efficiency, there are possibilities to enhance P-utilization efficiency (PUE). According to an estimate, PUE can be enhanced 25%, by employing the current knowledge of PUE traits. Nevertheless, Plants species and cultivars display classical array of adaptive traits under P-starvation and rational analysis of these traits highlight the ability of plants to adapt and thrive under P-stress environment by sensing, solublizing and scavenging of Pi from sparingly soluble P sources or from P-sorbing soils. To elucidate the genetic diversity among *Brassica* cultivars in relation to percent distribution of biomass and P-concentrations [P] in plant parts, P-stress factor (PSF) and P-utilization efficiency (PUE), 14 hydropnoically grown cultivars were evaluated in solution culture experiment conducted under climatically controlled growth chamber. One week old uniform sized pre-germinated seedlings were transplanted in solution containing $NH_4H_2PO_4$ (AP) @ 200 µM P L^{-1} as an adequate (control) level and Jordan rock-phosphate (RP) @ 2 g L^{-1} in a bid to maintain deficiently buffered P-stress environment throughout entire growth period. Tested cultivars showed substantial genetic variations in percent distribution of biomass and [P] in plant parts, PSF and PUE. P-stress caused preferential distribution of dry matter and [P] to roots as compared to the shoot indicated by strong positive correlation between RDM and root-[P]. P-stress induced with RP caused 16% more assimilate partitioning to the roots as compared to control. Cultivars were characterized and classified in terms of efficiency and responsiveness by using shoot dry matter, PUE and PSF. From ordination plot, it is plausible to conclude that efficient-responsive cultivars such as 'Con-1', 'Brown Raya', Rain Bow' 'Poorbi Raya', and 'Dunkled' are suitable for growing under P-stress environments. [The Journal of American Science. 2007;3(2):64-72]. (ISSN: 1545-1003).

Key words: *Brassica*, Efficient-responsive, $NH_4H_2PO_4$, P-use efficiency, P-stress factor, Rock-P

Introduction

Phosphorus (P) is needed in virtually all metabolic processes such as energy transfer, signal transduction, macro-molecular biosynthesis, photosynthesis, respiration. In addition, phosphorylation and dephosphorylation of proteins, P is crucial for signal-transduction pathways in plants. Furthermore phosphate homeostasis in the chloroplast regulates the transport of phosphorylated sugars across the membrane and synthesis of starch (Raghothama and Karthikeyan, 2005). Phosphorous (P) is one of the least available, least mobile, mineral nutrient to the plants in many cropping environments, based on its contribution to the biomass as a macronutrient (Goldstein *et al.*, 1988). Orthophosphate (Pi), the fully oxidized form of P 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. In this context P-deficiency is considered to be the major limitations for crop production, particularly in the tropics. Interestingly the ability of plants to acquire Pi increases significantly under Pi-deficiency.

Amelioration attempts to cope with Pi-deficiency by the application of costly and environmentally hazardous soluble P-fertilizers such as single and triple super-phosphate and $(NH_4)_2HPO_4$ is not an ideal solution and problematic for both the intensive and extensive agriculture of the developed and developing worlds, respectively. 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 and rivers. 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. Modern agricultural soils are almost universally maintained at high fertilization. Selection of new cultivars is usually made under such conditions and will not normally distinguish between plants varying in nutrient efficiency. In contrast, in many developing tropical countries, subsistence farmers can not buy enough fertilizer due to limited financial capacities, disappearance of subsidies for fertilizers or poor infrastructure. As a consequence, P-deprivation dramatically limits crop yield and is one of the reasons for poverty and malnutrition. To alleviate the forecasted adverse negative effects of agricultural expansion and fear of depletion of world reserves of rock-P coupled with increasing price of soluble P-fertilizers, classical strategies such as selection/breeding 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 P-fertilizers are cheaper and environmental friendly alternatives. Differences in growth and elemental uptake among cultivars have been related to absorption, translocation, shoot demand and dry matter production potentials per unit of nutrient absorbed. Phosphorus utilization efficiency (PUE) is the ability of cultivars to function well under low available P-concentrations.

Under Pi-stress, plant species and cultivars evolved elegant array of morphological physiological, biochemical and molecular adaptations that enable them to scavenge P from sparingly soluble P-soil fractions. These adaptations include modifications in root architecture, altered root-shoot ratio, occurrence of proteiod roots(Lambers *et al.*, 2006; Shane and Lambers, 2005), regulation of root hairs, increased root hair density and elongation of lateral roots (Vance *et al.*, 2003; Wu *et al.*, 2005), increased organic acids exudation (Jones, 1998; Ryan *et al.*, 2001; Akhtar *et al.*, 2006), rhizosphere acidification (Rengel, 2002; Hinsinger *et al.*, 2003; Pearse *et al.*, 2006), increased production of phosphatases and RNAses (Radersma and Grierson, 2004), enhanced phosphate uptake rate (Gilory and Jones, 2000) and an increase in the synthesis of Pi transporters (Hammond *et al.*, 2004; Raghothama and Karthikeyan, 2005). The genetic characterization of these traits in crop plants is difficult because of the high cost and large amount of time required for the analyses. Furthermore, the analyses of these mechanisms are complicated by the existence of several potential sites of control and also by the presence of adaptive responses (Narang *et al.*, 2000; Shenoy and Kalagudi, 2005). Screening programs directed towards identification of the adaptations in efficient genotypes should target the quantitative processes and take into account the environmental factors that modify them (Smith *et al.*, 1993).

Efficient cultivars can increase their capacity to access nutrients (to convert unavailable forms into available forms) by altering root morphology and enhance nutrient availability and uptake. While we have known for a long time that crops species differ in their capability to exploit soil-P pool and utilize the acquired P, we still have a rudimentary understanding of the basis of such differences at intra-specific level. Therefore, exploitation of intra-specific variation to identify and characterize efficient genoma is an area of research priority for managing P efficiently and providing excellent resource for genetic model system to open the cork of gene bottle to hunt and isolate genes that determine the PUE traits.

The objective of the present work was to evaluate, characterize and classify the available genetic pool of *Brassica* cultivars for genetic diversity in percent distribution of biomass and [P], PSF and PUE from applied Jordon RP in a solution culture system. By doing so, superior germplasm can be seprated and can be used for sustainable cropping with less fertilizer inputs and will provide a database for breeders for their future ventures.

Materials and Methods

Plant material and culture environment

Different cultivars of *Brassica* tested were: 'Con-1', 'Brown Raya', 'Rain Bow', 'Poorbi Raya', 'Peela Raya', 'Dunkled', '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. Experiment was conducted under climatically controlled conditions in a growth 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. Seven-day-old pre-germinated uniform sized seedlings were transplanted in foam-plugged holes in thermopal sheets floating on continuously aerated 200-L half strength modified Hoffland's solution (Hoffland *et al.,* 1989) in two polyethylene lined iron tubs (1 X 1 X 0.3 m). 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 in these pots were modified by adding 200 µM P using $NH_4H_2PO_4$ (AP) as a control treatment and powdered rock phosphate (RP) (@ 2 g L^{-1}), respectively. The RP imported from Jordon was finely ground (0.15 mm) contained 13.6 % total P, 4.5 % citrate-soluble (2 % citric acid) P and no water soluble P. This is one of the medium reactive RP's known. The nutrient solutions were renewed regularly at 3-day intervals to maintain concentrations being exhausted because of plant uptake. Fourteen cultivars were grown in each P-level by using factorial completely randomized design (CRD) with six repeats of each cultivar. The pH of the continuously aerated solutions of control treatment was monitored daily and maintained at 5.5 ± 0.5 by addition of HCl or NaOH. In RP treatment, solution was stirred mechanically twice a day.

Plant measurements and orthophosphate assay

Plants were harvested at two stages [24 and 34 day after transplanting (DAT)] to facilitate calculations of rate-related parameters. As the data obtained were similar at both the harvests, only the results obtained after 34 DAT are being presented here. Plants separated into shoots and roots, rinsed in demineralized water (taking care that no grain of RP attached with roots), blotted dry with tissue papers, put in craft paper bags, and dried at 70°C for 48 hrs in a forced-air driven oven. The following measurements were made: dry matter (DM) yield of plant parts; root-shoot ratio (RSR) of DM, P-concentrations [P] and P-contents in plant parts; root-shoot ratio of P-contents; percent (%) distribution of [P] and DM as affected by stress, P-stress factor (PSF), and P-utilization efficiency (PUE). 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 estimated by the vanadate-molybdate yellow color method (Chapman and Pratt, 1961) using a spectrophotometer (Hitachi, U-1100).

PSF for SDM was calculated by the formula given below:

$$PSF = (SDM_{(adequate/sufficient\ P)} - SDM_{(deficient/stress\ P)} / SDM_{(adequate/\ sufficient\ P)}) \times 100 \qquad [1]$$

Where SDM is shoot dry matter (g $plant^{-1}$) in the respective treatment.

PUE was determined according to Siddiqi and Glass (1981) as below:

$$PUE\ (g^2\ SDM\ mg^{-1}\ shoot\text{-}P) = SDM\ (g\ plant^{-1}) / [P]\ (mg\ g^{-1}) \qquad [2]$$

For the classification, two variables were plotted for each cultivar: in the Y-axis, the PUE in the lower P-level (stress P-level induced with Jordan RP) and in the X-axis, the SDM yield obtained for each cultivar to corresponding P-level. 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), respectively.

Statistical Analysis

Data were analysed according to standard procedures (Steel and Torrie, 1980) using 'MSTAT-C' computer program and the methods described by Gomez and Gomez (1984). Factorial CRD was employed for analysis of variance (ANOVA). Correlation coefficient (r) values were determined among various parameters using treatment means.

Results and Discussion

Crop yield on 40% of the world's arable land is limited by P availability. Paradoxically, although total P is quite abundant in many soils, it is largely unavailable for root uptake. Rock-P is a limited resource in the world and estimated to be depleted in 60–80 years. Developing P-efficient cultivars may be one possible way of reducing the demand for rock-P. It has been suggested that P efficiency is a multi-gene controlled quantitative trait. Therefore, identification of P-efficient cultivars and understanding of underlying morphological and physiological mechanisms of efficiency are important for parent selection in a traditional breeding program, or for gene isolation in a transgenic approach. Exploring the genetic resource of crops is one alternative way to utilize the less available P in soils, and copy with the incoming shortage of rock phosphate (RP).

Percent distribution of biomass and P-concentrations in plant parts

Brassica cultivars differed in distribution of dry matter (DM) between root and shoot (Figure 1a,b). Most of the cultivars distributed more assimilates towards the roots (Vance *et al.*, 2003) thereby increasing absorptive surface area of roots. Cultivars 'Peela Raya' and 'Shiralle' were most affected due to P-stress as these cultivars exhibited maximum reduction in root dry matter (RDM) compared to other cultivars and reduction in RDM indicates less tolerance of the cultivars to P-stress. (Wissuva *et al.*, 2005). Phosphorus stress significantly affected the DM distribution between shoot and root at both P-sources (Figure 1a,b). P-stress caused 16% more assimilated towards the roots as compare to the sufficient P-level and roots are strong sinks of assimilates under P stress and thus increases the RSR (Mollier and Pellerin, 1999) and same observation was confirmed in our present experiment. Differences for distribution of DM between shoot and root were more pronounced under P-stress conditions as compared to AP indicating that cultivars elicit physiological and morphological adjustments for enhanced P acquisition under P-starvation (Raghothama and Karthikeyan, 2005). 'RL-18' distributed 39% assimilates to the roots at RP, 26 % more as compared to the AP level, while 'Con-1' had only 2% more assimilate at RP distributed to the roots as compared to AP and it had maximum SDM at RP 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. P-starvation cause the preferential distribution of P to the roots (71%) compared with adequate P-level (43%) (Figure 2a,b) and roots act as good sink for P under deficiently buffered P-stress environment which is in agreement with the results reported by Mengel and Kirkby (2001).

P-utilization efficiency, P-stress factor and classification of *Brassica* cultivars

Phosphorus use efficiency (PUE) was significantly ($P < 0.01$) affected by P-sources and increased the PUE 1.8-fold as compared to the adequate P-level (Figure 3). Reduction of PUE at AP indicating that less dry matter was produced for each additional unit of P absorbed. PUE ranged from 0.11 to 2.64 (g^2 SDM mg^{-1} shoot-P) at RP and 0.07 to 1.82 (g^2 SDM mg^{-1} shoot-P) at AP, respectively confirming the ability of *Brassica* cultivars to take up significant amount of P from RP. Cultivars 'Con-1', 'Brown Rya', 'Rain Bow' and 'Poorbi Raya' exhibited more PUE at RP and proved to be more efficient than other cultivars (Figure 3). PUE of tested cultivars was correlated significantly with P-content and accumulated biomass. P-stress factor (PSF) is a tolerance index of cultivars under P-starvation. PSF indicates relative reduction in SDM due to P-stress and cultivars exhibiting low PSF values are considered more P-tolerant under P-deprivation. Figure 4 shows considerable genetic variability among cultivars for relative tolerance under P-starvation. Cultivars 'Con-1' and 'Poorbi Raya' showing low PSF values (Figure 4) compared to other cultivars may be considered reliable to grow under P-starved environment.

For the classification, two variables were plotted for each cultivar: in the Y-axis, the PUE at RP and in the X-axis, the SDM yield obtained for each cultivar (Figure 5). 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 'efficient-responsive' 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), respectively. The cultivars classified as 'ER' were: 'Con-1', 'Brown Raya', 'Poorbi Raya', 'Rain Bow', and 'Dunkled' while all other cultivars classified as 'NENR' at RP (Figure 5). 'ER' cultivars are the most desirable due to their good performance and the most undesirable cultivars are the 'NENR' type as per their poor performance. PSF as a function of PUE of cultivars grown with RP revealed that 'ER' cultivars showing lower PSF values are considered more efficient than other cultivars (Figure 6). Cultivars showing lower PSF and higher PUE values are considered suitable to grow under P-stress environments with less fertilizer inputs. Thus, under P-stress, better P-acquisition and PUE by the efficient cultivars for biomass synthesis collectively formed the basis of higher SDM production, evidencing that P-uptake and PUE are important plant traits for selecting low P tolerant cultivars.

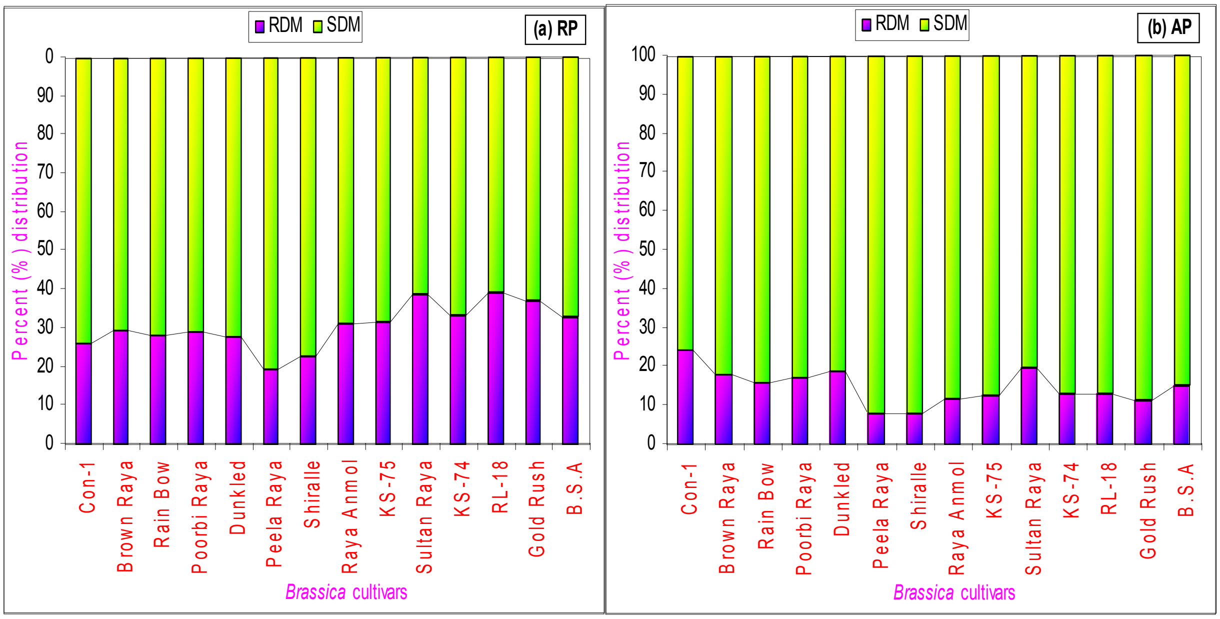

Figure 1. Percent (%) distribution of and dry matter as affected by stress (RP) (a) and adequate (AP) (b) P-levels of 14 *Brassica* cultivars grown in nutrient solution containing $NH_4H_2PO_4$ (AP) and sparingly soluble rock-P (RP).

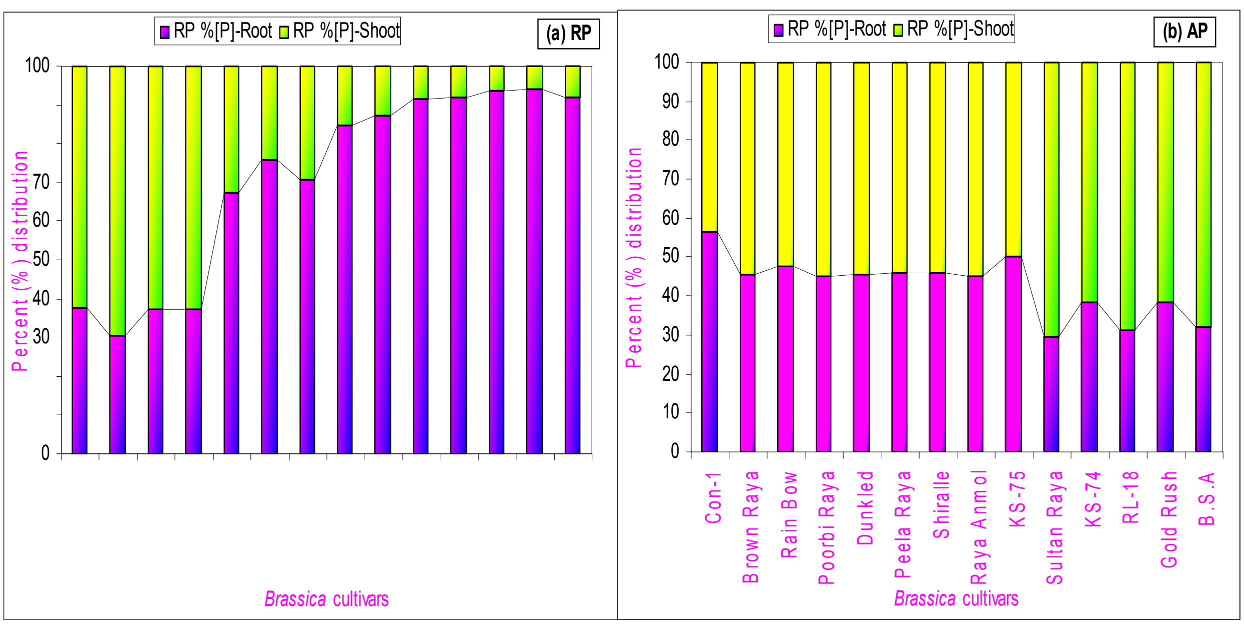

Figure 2. Percent (%) distribution of P-concentration [P] as affected by stress (RP) (a) and adequate (AP) (b) P-levels of 14 *Brassica* cultivars grown in nutrient solution containing $NH_4H_2PO_4$ (AP) and sparingly soluble rock-P (RP).

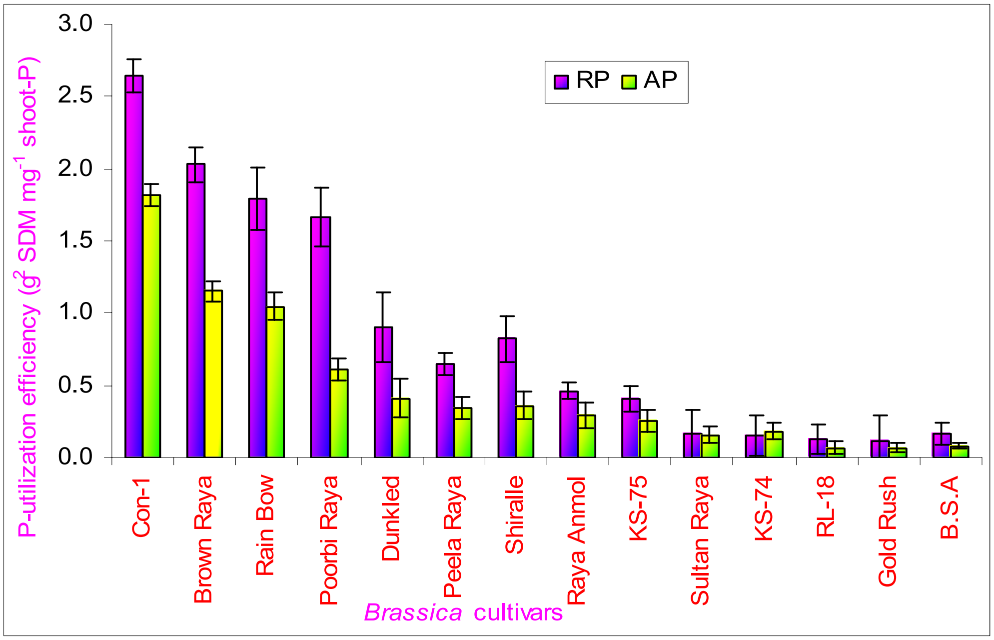

Figure 3. Phosphorus utilization efficiency (PUE) of 14 *Brassica* cultivars grown in nutrient solution containing $NH_4H_2PO_4$ (AP) and sparingly soluble rock-P (RP); error bars show ± SE.

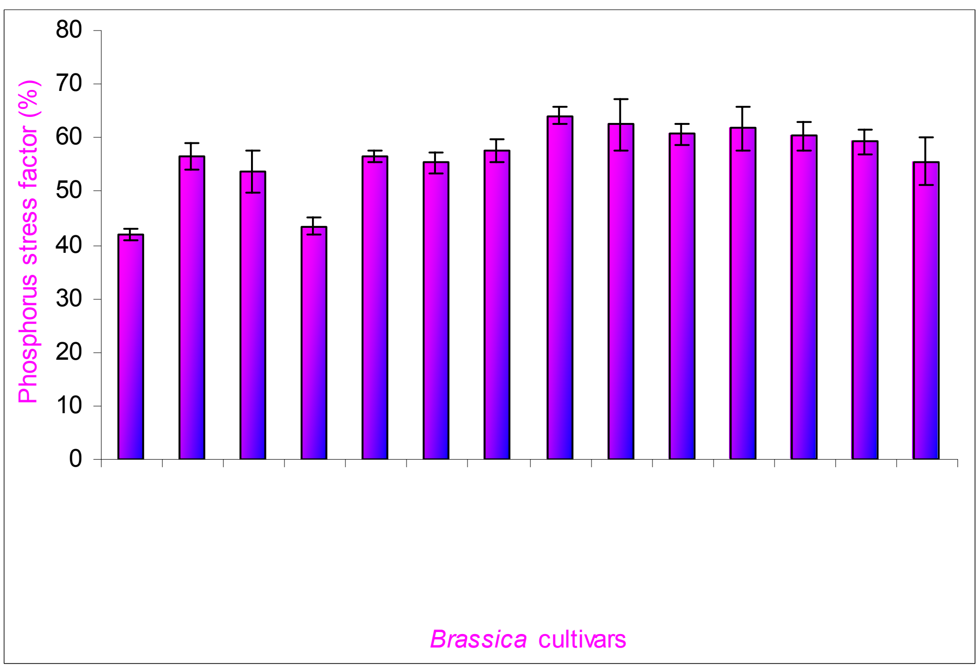

Figure 4. Phosphorus stress factor (%PSF) calculated (according to equation 1) of 14 *Brassica* cultivars grown in nutrient solution containing $NH_4H_2PO_4$ (AP) and sparingly soluble rock-P (RP); error bars show ± SE.

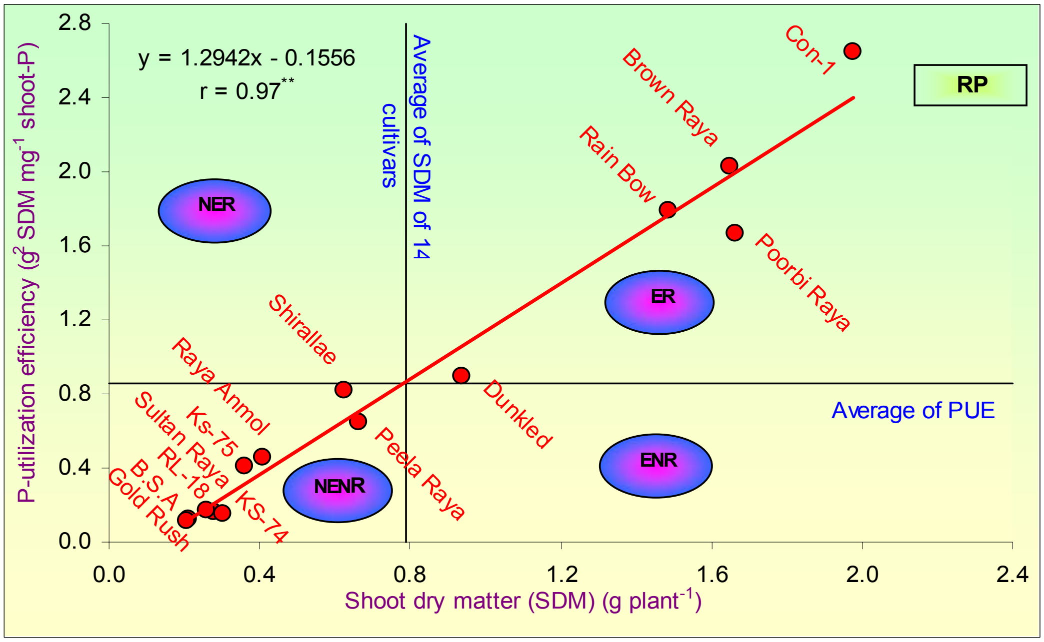

Figure 5. Ordination plot to classify cultivars for P-utilization efficiency (PUE) as a function of shoot dry matter (SDM) of 14 *Brassica* cultivars grown in nutrient solution containing sparingly soluble rock-P (RP). ER: Efficient and Responsive; NER: Non-efficient but Responsive; ENR: Efficient but non-responsive; NENR: Non-efficient and non-responsive; ** = significant at $P < 0.01$.

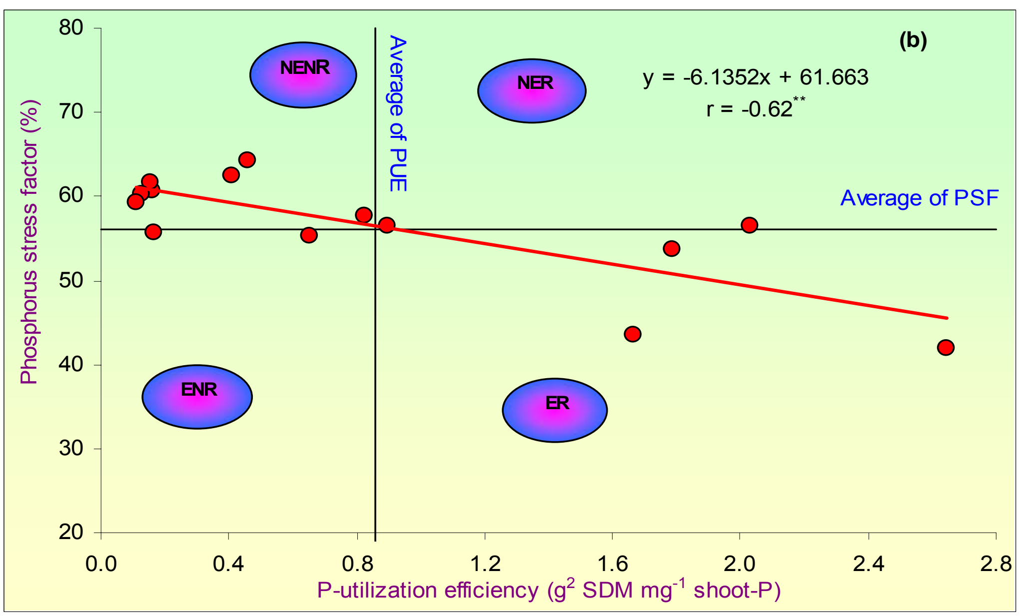

Figure 6. Ordination plot to classify cultivars for P-utilization efficiency (PUE) as a function of P-stress factor (%relative reduction in shoot dry matter of cultivars due to P-stress) PUE of 14 *Brassica* cultivars grown in nutrient solution containing $NH_4H_2PO_4$ (AP) and sparingly soluble rock-P (RP). ER: Efficient and Responsive; NER: Non-efficient but Responsive; ENR: Efficient but non-responsive; NENR: Non-efficient and non-responsive; ** = significant at $P < 0.01$.

Conclusions

Conclusively, tested cultivars grown under deficient buffered P-stress conditions induced with Jordon rock phosphate (RP) showed considerable genetic variations in percent distribution of biomass and [P] in plants parts, P-stress factor (PSF), and P-utilization efficiency (PUE). PUE of tested cultivars was correlated significantly with P-content and accumulated biomass. Cultivars showed variable tolerance in terms of PSF and cultivars showing lower PSF and higher PUE values are desirable to grow under P-starved environment. Cultivars such as 'Con-1', 'Brown Raya', 'Poorbi Raya', 'Rain Bow', and 'Dunkled' showing higher PUE values are also efficient accumulator of biomass at both P-sources (Biomass data is presented in part I). Efficient gemona elicit intricate adaptive traits in accessing, solublization, acquisition and utilization of P from sparingly soluble RP. Use of P-efficient cultivars in combination of with direct application of the Jordon rock phosphate or other RPs with low water solubility seems to be an attractive strategy for sustainable agricultural productions in resource poor environments.

Acknowledgments

The principal author M. Shahbaz Akhtar gratefully acknowledges the Ministry of Education, Science, Sports and Culture (MEXT), Japan, for partial financial support which enabled him to pursue this research work.

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