Genetic Diversity in *Brassica* Cultivars under Deficiently Buffered P-Stress Environment: I. Biomass Accumulation, P-Concentration, P-Uptake, and Related Growth Parameters

M. Shahbaz Akhtar[*1,2], Yoko Oki[1], Tadashi Adachi[2]

[1]Department of Environmental Management Engineering, Faculty of Environmental Science and Technology, Graduate School of Environmental Science, Okayama University, Japan
[2]CADGK, and Institute of Soil & Environmental Sciences, University of Agriculture, Faisalabad, Pakistan
Telephone: +81-90-7546-1975; E-mail: shahbazak46@gmail.com
and Adachi

Abstract: *Brassica* is known as an effective, non-mycorrhizal user of sparingly soluble phosphorus (P) sources and its importance in edible oil production cannot be overemphasized. Global P reserves are being depleted, with half depletion predicted to occur between 2040 and 2060. More efficient utilization of inorganic phosphate (Pi) by plants is needed to extend the useful life of the world P-reserves, to reduce the cost of producing crops, and to improve the value of the end product. To investigate the genetic variations in P-acquisition from Jordon rock-P (RP), biomass accumulation, root-shoot ratio of dry matter yield, P-concentration and uptake and other related growth parameters, fourteen genetically diverse *Brassica* cultivars were grown in solution culture experiment conducted under climatically controlled growth chamber. 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 Jordon RP @ 2 g L^{-1} in a bid to maintain deficiently buffered P-stress environment. Tested cultivars showed substantial genetic variability in relation to biomass accumulation, P-acquisition, P-concentration, and P-uptake. P-stress markedly reduced shoot dry matter (SDM), root dry matter (RDM) and total dry matter (TDM) productions. Highly significant positive correlation was found between SDM, RDM, TDM and shoot P-uptake. Control of whole plant dry matter by plant P-contents under low P-starvation suggest an internal regulation in addition to influence excreted by exogenous P-supply. Efficient cultivars have potential for tolerating P-stress culture environments due to better rooting system, which provided basis for tolerance under P-starved environment. [The Journal of American Science. 2007;3(2):55-63]. (ISSN: 1545-1003).

Key words: *Brassica*, Biomass accumulation, $NH_4H_2PO_4$, P-uptake, P-stress, rock-P, RSR,

Introduction

Phosphorus is required as a structural component in nucleic acids and phospholipids, as an element in intermediates in carbon metabolism, and to allow (in) activation of wide range of enzymes. Major biochemical processes such as photosynthesis and respiration are activated by inorganic phosphate (Pi) or its organic derivatives. Phosphate esters in general act as energy carriers in various metabolic pathways. Phospholipids play an important role in membrane integrity and function. After nitrogen (N), P is quantitatively the most important inorganic nutrient for plant growth, and often limits primary productivity in natural systems as well as cropping systems, unless supplied as fertilizer (Vance *et al.*, 2003; Raghothama and Karthikeyan, 2005).

Despite its wide distribution in nature, it is deficient nutrient in most soils. Many soils are defined as having high P-fixation capacity, since a substantial amount of any applied fertilizer is rendered unavailable and frequent applications of soluble forms of Pi are needed to maintain adequate levels for plant productivity. There is great disparity in distribution of Pi between plant cells (mM) and soil solution (µM) because of its strong reactions with soil components. Pi is principally supplied to plant roots by diffusion rather than mass flow and diffusion co-efficient of Pi in soils is slow (10^{-12} to 10^{-15} m^2 S^{-1}; Rausch and Bucher, 2002), hence, P is one of the most dilute and inaccessible macronutrients in the soil. Heavy fertilization is a traditional approach to increase the crop production by maintaining high solution P (Tisdale *et al.*, 2002). However, transformation of added P to the plant unavailable forms (Hinsinger, 2001) has restricted the effectiveness of the heavy fertilization. Furthermore good quality phosphate ore are being depleted owing to heavy fertilization and would last for about 200 years (Bohn *et al.*, 2004). Anticipate phosphate crisis in the 21st century for agriculture (Abelson, 1999) coupled with farmers' inability to purchase expensive phosphatic fertilizers and notorious transformation of the added P to plant unavailable forms has threatened agricultural productivity and demands the effective strategy to deal with this problem. With the current tendency for a reduced use of agrochemicals and efficient application of natural materials in agroecosystems, a renewed interest in direct application of rock phosphate (RP) has arisen. P is non-renewable resource and global P reserves are rapidly being depleted; depending on the assumed scenario, current P reserves will be halved (relative to the reserves at the turn of the twentieth century) by 2040 or, more likely, by 2060 (Steen, 1998; Lambers *et al.*, 2006). With decreasing global P reserves, P-fertilizer prices are bound to increase. There is an urgent need to develop crops that are highly effective at acquiring inorganic P (Pi) from P-sorbing soils and sparingly soluble P-sources, and/or at using P more efficiently. Nevertheless, plant species and cultivars differ genetically in their ability to absorb translocation, accumulate and redistribute minerals to adapt to mineral stress environments. Selection and breeding of crops for P-uptake efficiency that can reduce P-fertilization and sustain high yield in P-deficient soils is good strategy. Plant root geometry and morphology are important for maximizing P uptake, because root systems that have higher ratios of surface area to volume will more effectively explore a larger volume of soil. Among the physiological mechanisms which may explain the existing varietal and species differences related to low P-tolerance are differential rates of P-absorption and translocation. Therefore, understanding of P-acquisition would be very helpful in screening/breeding crop cultivars with high ability of P-uptake from sparingly soluble P-sources.

The use of RP for direct application is an attractive cost-effective alternative to expensive water soluble P-fertilizers, especially under resource poor environments, where indigenous RP deposits exists and water soluble P-fertilizers are imported. Among the crop species, rape is reported to be very efficient in utilizing not only reactive RP but also RP sources of low reactivity, generally not suitable for direct application (Hoffland *et al.*, 1989; Bekele and Hofner, 1993; Hinsinger, 2001; Montenegro and Zapata, 2002; Akhtar *et al.*, 2006).

The objective of the present work was to investigate the effect of P-stress induced with Jordon RP on the biomass accumulation, differential growth response, RSR, and P-concentration and uptake by cultivars in a solution culture rooting media. For comparison, we applied $NH_4H_2PO_4$ as an adequate (control) treatment.

Materials and Methods

Plant material and growth 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.

Biomass and orthophosphate assay, and measurements of related growth parameters

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 and root-shoot ratio of P. 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). P-uptake (mg plant^{-1}) was calculated on dry matter basis by multiplying P-concentrations in the respective tissue with its dry matter, and on whole plant basis by summing up the shoot-[P] and root-[P].

P-uptake = [P] (mg g^{-1}) X dry matter (g plant^{-1})

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

Effect of P-stress on biomass accumulation and root-shoot ratio of dry matter produced by *Brassica* cultivars

Biomass is an important plant trait in growth analysis. It is the key parameter in many allometric relationships (West *et al.*, 1999; Niklas and Enquist, 2002). Repeated measurements of biomass are the basis for the calculations of net primary production and growth rates (Poschlod *et al.*, 2003; Cornelissen *et al.*, 2003), and thus a basis for quantifying physiological responses of plants to environmental conditions and their developmental processes.

Biomass accumulation by tested *Brassica* cultivars is presented in Figure 1. Cultivars showed significant genetic variability in shoot growth, root development and total biomass accumulation. P-starvation affects significantly ($P < 0.01$) reduced the shoot dry matter (SDM) and on an average more than 2 fold (53%) reduction in SDM was observed due to P-stress. Cultivar 'Con-1' produced maximum SDM (1.98 g plant^{-1}) at stress P-level while minimum SDM (0.20 g plant^{-1}) was produced by 'Gold Rush' (Figure 1A). Production of SDM is generally considered a good indicator of ultimate economic yield (Römer and Schenk, 1998; Ahmad *et al*., 2001; Gill *et al*., 2002). Cultivars differences for SDM production indicated that SDM can be used as reliable parameter for screening efficient cultivars (Liao *et al.*, 2004; Gill *et al.*, 2005). *Brassica* cultivars differed significantly ($P < 0.01$) for root dry matter (RDM). Cultivar 'Con-1' and 'Brown Raya' produced the maximum RDM (0.70 g plant^{-1} in case of both cultivars) followed by 'Poorbi Raya' and 'Rain Bow' (0.69 and 0.59 g plant^{-1}, respectively) while minimum RDM (0.12 g plant^{-1}) was produced by 'Gold Rsuh' under stress environment induced with RP (Figure 1B). Total dry matter (TDM) was 1.8 fold (45%) lower in plants grown with RP compared with control treatment.

Root shoot ratio of dry matter (RSR) was significantly higher (2.5 fold) in plants grown at RP compared to AP (Figure 2). Higher RSR in P-starved plants compared to P-sufficient plants can be attributed to higher export rates of photosynthates to the roots and use of photoassimilates in the roots and increasing root surface area for P-absorption. Preferential root growth helps the stressed plants to acquire more P from the surrounding environment in response to P-starvation.

P-concentration, P-uptake and root-shoot ratio of P-contents in *Brassica* cultivars

Phosphorus levels significantly ($P < 0.05$) affected the P-concentration [P] (Figure 3) and P-uptake among the tested cultivars (Figure 4) and [P] in roots of cultivars was higher than that in shoots at RP (Figure 3b). P-stress reduced the [P] in shoot by 75% and in roots by 48 %. Mollier and Pellerin (1999) in maize and Gill *et al.* (2005) in cotton observed the similar results. Cultivar 'Gold Rush' accumulated maximum shoot-[P] (1.81 mgg^{-1}) while 'Con-1' had the minimum shoot-[P] (0.75 mg g^{-1}) (Figure 3a). P-stress reduced the root-[P] by 48 % compared with AP. Cultivars showed differential root-[P] and maximum root-[P] (2.42 mg g^{-1}) was found in 'Con-1' and minimum root-[P] (1.39 mg g^{-1}) was found in

'Shiralle' at stress P-level (RP) (Figure 3b). Strong positive correlation (r = 0.89**) between root-[P] and shoot-[P], indicates that roots transfer P to the shoot. 'Brown Raya' transferred maximum P to the shoot evidencing its higher transfer rate and adaptability under P-stress environment.

P-sources significantly affected the P-uptake among all the cultivars and reduction due to P-stress was 88% in shoot and 47% in root (Figure 4a,b). Cultivar 'Con-1 uptake maximum total P (3.18 mg $plant^{-1}$), while 'KS-75' and 'B.S.A' uptake minimum total P (0.64 mg $plant^{-1}$) (Figure 5). Minimum reduction in total P-content was observed in 'Con-1' (64%) followed by 'KS-74' (76%). Shoot P-uptake was significantly correlated with SDM (r =0.93**). At RP, root P-uptake was less affected due to P-stress as compare to the shoot P-uptake. This cab be ascribed to the more root growth under P-stress (Mollier and Pellerin, 1999) and more distribution of P to the roots under P-stress as root are good sink for P under P-stress (Mengel and Kirkby, 2001). Tested cultivars showed variability in for root-shoot P ratio (Figure 6) and maximum root-shoot P-content was observed at stress P level compared to AP level. Under P-starvation plants retain more P in their roots than shoots (Adu-Gyamfi *et al.*, 1990; Akhtar *et al.*, 2006). To develop more efficient root system, P-starved plants retain relatively larger amount of stressed element.

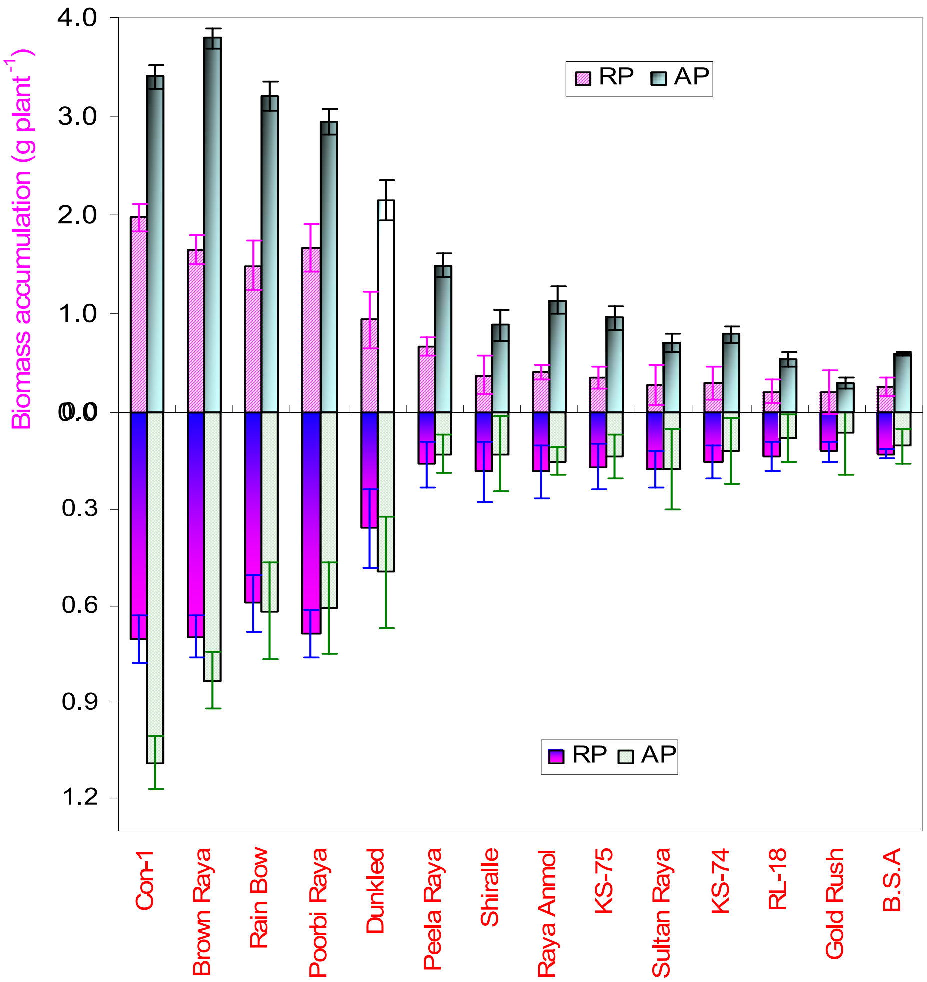

Figure 1. Biomass accumulation by 14 *Brassica* cultivars grown in nutrient solution containing $NH_4H_2PO_4$ (AP) and sparingly soluble rock-P (RP); error bars show ± SE.

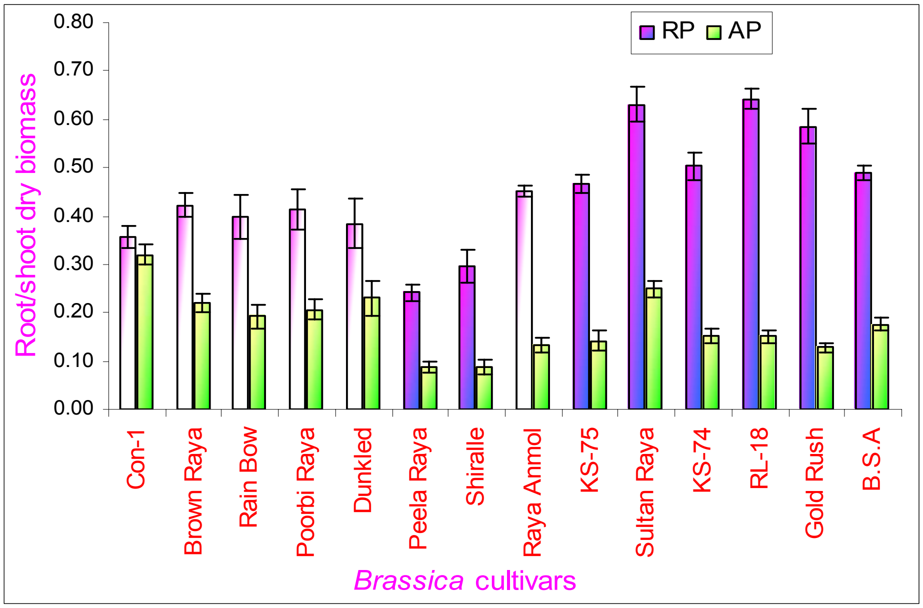

Figure 2. Root-shoot ratio of dry matter of 14 *Brassica* cultivars grown in nutrient solution containing $NH_4H_2PO_4$ (AP) and sparingly soluble rock-P (RP); error bars show ± SE.

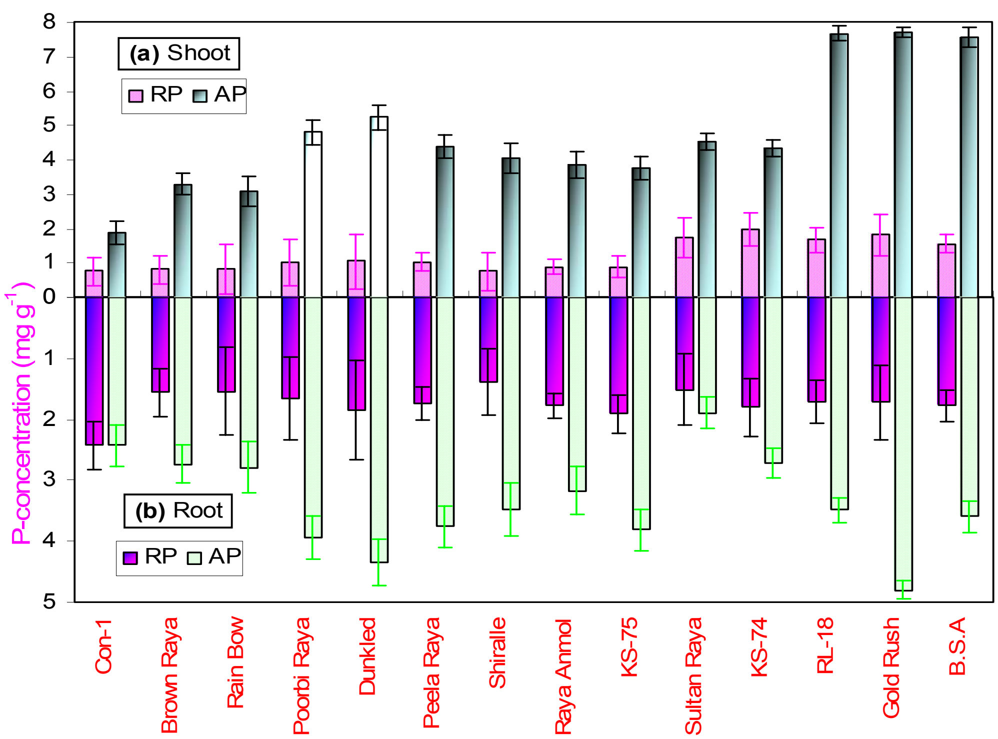

Figure 3. Tissue P-concentration in 14 *Brassica* cultivars grown in nutrient solution containing $NH_4H_2PO_4$ (AP) and sparingly soluble rock-P (RP); error bars show ± SE.

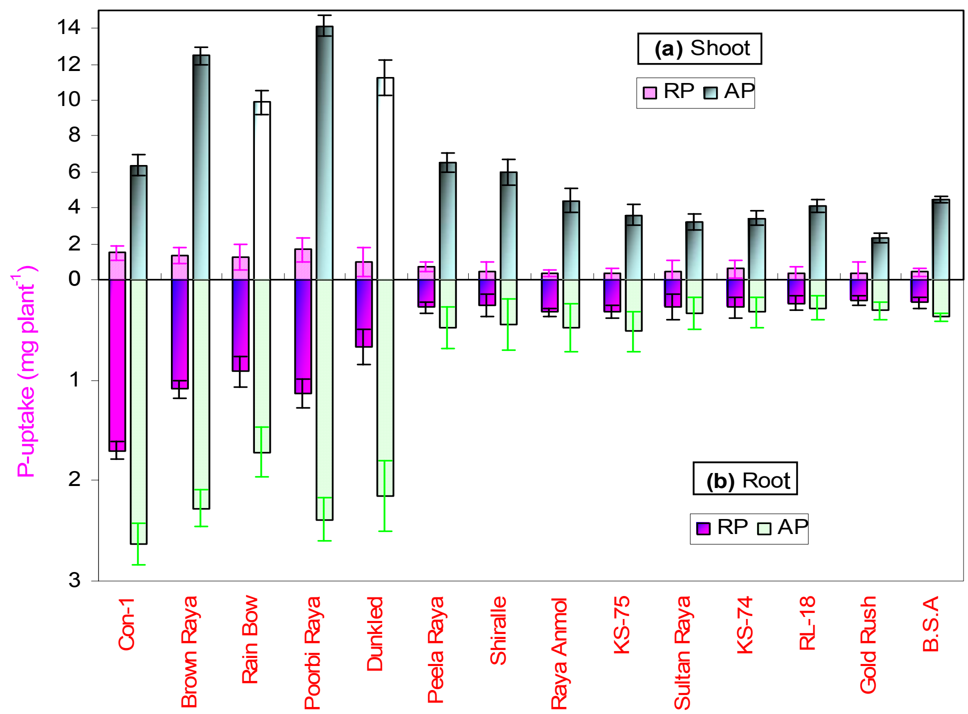

Figure 4. Tissue P-uptake by 14 *Brassica* cultivars grown in nutrient solution containing $NH_4H_2PO_4$ (AP) and sparingly soluble rock-P (RP); error bars show ± SE.

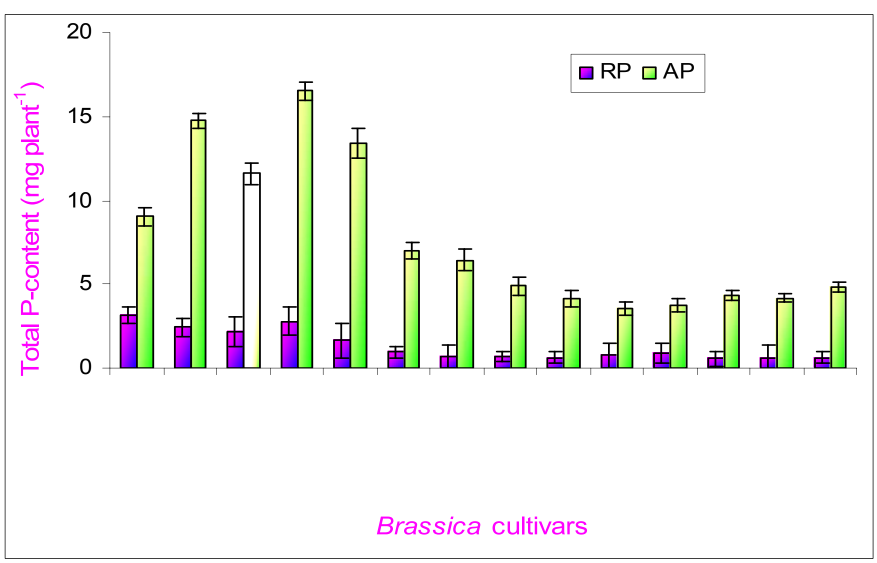

Figure 5. Total P-content of 14 *Brassica* cultivars grown in nutrient solution containing $NH_4H_2PO_4$ (AP) and sparingly soluble rock-P (RP); error bars show ± SE.

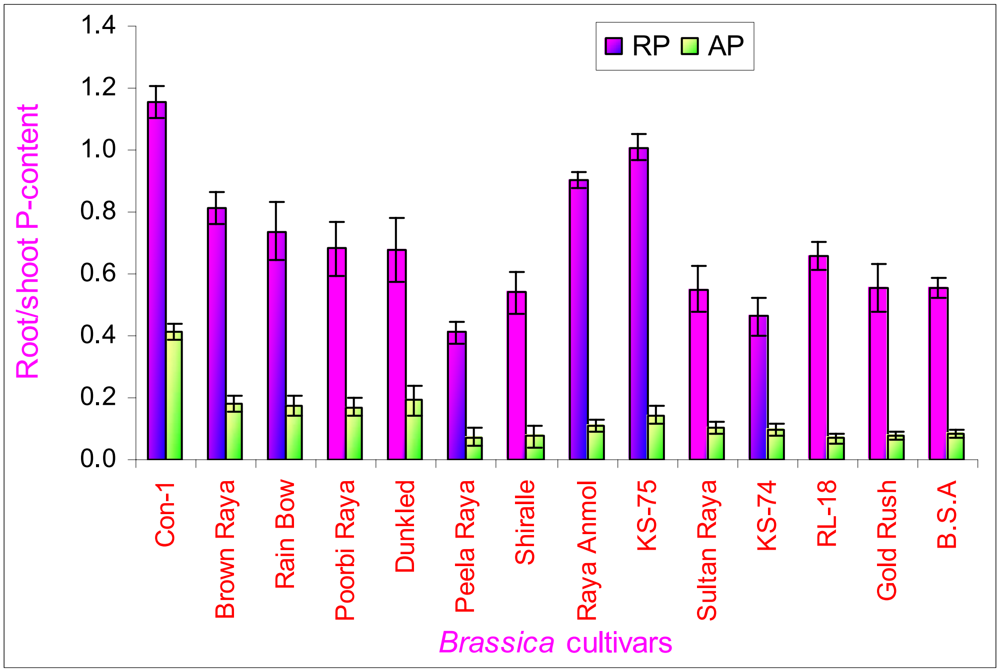

Figure 6. Root/shoot P content ratio of 14 *Brassica* cultivars grown in nutrient solution containing $NH_4H_2PO_4$ (AP) and sparingly soluble rock-P (RP); error bars show ± SE.

Conclusions

Considerable intraspecific genetic diversity was observed in *Brassica* cultivars in terms of shoot, root and total dry matter accumulation, root-shoot ratio of dry matter, P-concentration and P-contents under stress and adequate P-supply. Biomass of tested cultivars was correlated significantly with P-content. Cultivars such as 'Con-1', 'Brown Raya', 'Poorbi Raya', 'Rain Bow', and 'Dunkled' were efficient cultivars in terms biomass accumulation compared to other cultivars. These cultivars proved to be the most efficient P-user and therefore seem to have the highest potential for growth on soils with poor P-availability. Higher biomass accumulation of the cultivars was related to their better P-uptake efficiency which in turn was related to better P-acquisition ability from sparingly soluble rock phosphate.

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.

Correspondence author:
Dr. M. Shahbaz Akhtar
Department of Environmental Management Engineering
Faculty of Environmental Science and Technology
Graduate School of Environmental Science
Okayama University,
3-1-1 Tsushima-naka, Okayama, 700-8530, Japan.
Telephone: +81-90-7546-1975

E-mail: shahbazak46@gmail.com

References

1. Abelson. 1999. A potential phosphate crisis. Science 283: 2015.
2. Adu-Gyamfi, J. J., Fujita, K., and Ogata, S. 1990. Phosphorus fractions in relation to growth in pigeon pea at various levels of P supply. Soil Science and Plant Nutrition 36: 531-543.
3. Ahmad, Z, Gill, M. A, Qureshi, R. H., Rehman, H., Mahmood, T. 2001. Phosphorus nutrition of cotton cultivars under deficient and adequate levels in solution culture. Communications in Soil Science and Plant Analysis 32: 171-187.
4. Akhtar, M. S., Oki, Y., Adachi, T., Murata, Y., and Khan, Md. H. R. 2006): Phosphorus starvation induced root-mediated pH changes in solublization and acquisition of sparingly soluble P sources and organic acids exudation by *Brassica* cultivars. Soil Science and Plant Nutrition 52: 623-633.
5. Bekele, T., and Hofner, W. 1993. Effects of different phosphate fertilizers on yield of barley and rape seed on reddish brown soils of ethiopion highlands. Fertilizer Research 34: 243-250.
6. Chapmann, H. D., and Pratt, P. F. 1961. Phosphorus. In: Methods of Analysis for Soils, Plants and Waters. pp 160-170. Berkeley: University of California, USA.
7. Cornelissen, J. H. C., Lavorel, S., Garnier, E., Dı´az, S., Buchmann, N., Gurvich, D. E., et al. 2003. A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. Australian Journal of Botany 51: 335–380.
8. Gill, M. A., Mansoor, S., Aziz, T., Rahmatullah, Akhtar, M. S. 2002. Differential growth response and phosphorus utilization efficiency of rice genotypes. Pakistan Journal of Agricultural Sciences 39: 83-87.
9. Gill, M. A., Sabir, M., Ashraf, S., Rahmatullah, and Aziz, T. 2005. Effect of P-stress on growth, phosphorus uptake and utilization efficiency of different cotton cultivars. Pakistan Journal of Agricultural Sciences 42: 42-47.
10. Gomez, K. A., and Gomez, A. A. 1984. Statistical Procedures for Agricultural Research. New York: Jhon Willey & Sons, U.S.A.
11. Hinsinger, P. 2001. Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. Plant and Soil 237:173–195.
12. Hoffland, E., Findenegg, G. R., and Nelemans, J. A. 1989. Solubilization of rock phosphate by rape I. Evaluation of the role of the nutrient uptake pattern. Plant and Soil 113: 155–160.
13. Lambers, H., Shane, M. W., Cramer, M. D., Pearse, S. J., and Veneklaas, E. J. 2006. Root structure and functioning for efficient acquisition of phosphorus: Matching morphological and physiological traits. Annals of Botany 98: 693–713.
14. Liao, M. T., Hocking, P., Dong, B., Delhaize, E., and Ryan, P. R. 2004. Screening for genotypic variation in phosphorus uptake efficiency in cereals on Australian soils. 4th 1ntl. Congress, Soil Science.
15. Mengel, K., and Kirkby, E. A. 2001. Principle of Plant Nutrition. Kluver academic pub., London.
16. Mollier, A., and Pellerin, S. 1999. Maize root system growth and development as influenced by phosphorus deficiency. Journal of Experimental Botany 50: 487-497.
17. Montenegro, A., and Zapata, F. 2002. Rape genotypic differences in P-uptake and utilization from phosphate rocks in an andisol of chile. Nutrient Cycling in Agroecosystems 63: 27-22.
18. Niklas, K. J., Enquist, B. J. 2002. On the vegetative biomass partitioning of seed plant leaves, stems, and roots. American Naturalist 159: 482–497.
19. Poschlod, P., Kleyer, M., Jackel, A. K., Dannemann, A., and Tackenberg, O. 2003. BIOPOP – a database of plant traits and Internet application for nature conservation. Folia Geobotanica 38: 263–271.
20. Raghothama, K. G., and Karthikeyan, A. S. 2005. Phosphate acquisition. Plant and Soil 274: 37–49.
21. Rausch, C., and Bucher, M. 2002. Molecular mechanisms of phosphate transport in plants. Planta 216: 23–37.
22. Römer, W., and Schenk, H. 1998. Influence of genotype on phosphate uptake and utilization efficiencies in spring barley. European Journal of Agronomy 8: 215-224.
23. Steel, R. G. D., and Torrie, J. H. 1980. Principles and Procedures of Statistics. New York: McGraw Hill Book Co. Inc.

24. Steen, I. 1998. Phosphorus management in the 21st century. Management of a non-renewable resource. Phosphorus and Potassium 217: 25–31.
25. Tisdale, S. M., Nelson, W. L., Beaton, J. D., and Havlin, J. L. 2002. Soil Fertility and Fertilizer. pp. 189-190. Prentice-Hall of India, Private Limited, New Delhi.
26. Vance, C. P., Uhde-Stone, C., and Allan, D. L. 2003. Phosphorus acquisition and use: critical adaptations by plants securing a nonrenewable resource. New Phytologist 157: 423-457.
27. West, G. B., Brown, J. H., and Enquist, B. J. 1999. A general model for the structure and allometry of plant vascular systems. Nature 400: 664–667.