

Genetic Diversity in *Brassica* Cultivars under Deficiently Buffered P-Stress Environment: III. Leaf Area (LA), P-Stress Induced Percent Reductions in LA, P-Absorption, Transport and Utilization Rates

M. Shahbaz Akhtar^{*1,2}, Yoko Oki¹, Tadashi Adachi²

¹Department of Environmental Management Engineering, Faculty of Environmental Science and Technology, Graduate School of Environmental Science, Okayama University, Japan

²CADGK, and Institute of Soil & Environmental Sciences, University of Agriculture, Faisalabad, Pakistan
Telephone: +81-90-7546-1975; E-mail: shahbazak46@gmail.com

Abstract: Plant growth in natural and agricultural ecosystems is frequently limited by P availability. Plants constantly sense the changes in their environment when minerals elements are scarce and may evolve a number of plastic responses resulting in increased P-uptake. These responses include a decrease in overall growth, an increase in the proportion of resources devoted to root growth, and enhanced uptake rate of the limiting nutrient, and/or a high nutrient efficiency, the inverse of tissue concentration. To investigate the possible adaptive traits in terms of various growth related parameters and P-starvation induced % reduction in leaf area per plant, fourteen genetically diverse *Brassica* cultivars were grown hydroponically in a solution culture experiment conducted under climatically controlled growth conditions using modified Hoffland's nutrient solution. One week old uniform sized pre-germinated seedlings were transplanted in solution containing $\text{NH}_4\text{H}_2\text{PO}_4$ (AP) @ $200 \mu\text{M P L}^{-1}$ as an adequate (control) level and Jordan rock-phosphate (RP) @ 2 g L^{-1} in a bid to maintain deficiently buffered P-stress environment. Orthophosphate concentration [P] and uptake in plant parts, leaf area per plant (LA) and P-absorption, transport and utilization rates were substantially different in *Brassica* cultivars evidencing considerable genetic diversity is present in tested cultivars. Highly significant correlation was observed between LA and accumulated biomass by cultivars. Relative growth rate of root (RGRR) was correlated significantly with root and total dry matter production. Mean P-transport rate increased about 2-fold with AP compared to RP. On an average, P-stress caused 56% reduction in LA among cultivars grown with RP compared with AP. Percent reduction in leaf area per plant ranged from 30% (Con-1) to 72% (B.S.A), and cultivars exhibiting lower values are considered more suitable for growing under P-starved environment. [The Journal of American Science. 2007;3(2):73-82]. (ISSN: 1545-1003).

Key words: *Brassica*, Leaf area, P-transport rate, Relative growth rate, Specific utilization rate

Introduction

Theoretical considerations on plant nutrient requirements suggest that sparingly soluble nutrients such as P in the rhizosphere soil solution must be renovated 20-50 times per day (Marschner, 1995; Neumann and Romheld, 2002). This is not simply explainable on base of diffusion processes and requires additional adaptive traits of higher plants for acquisition of nutrients with limited solubility such as P. Paradoxically, although total P is abundant in lithosphere, limited orthophosphate (Pi) availability is a major culprit in natural and agricultural contexts. Due to notorious slow diffusion coefficient of Pi in soils (10^{-12} to $10^{-15} \text{ m}^2 \text{ S}^{-1}$; Rausch and Bucher, 2002), there is great disparity in distribution of Pi between plant cells (mM) and soil solution (μM) because of its strong reactions with soil components (Vance *et al.*, 2003) in addition to fixation of Pi in soils with metals and/or bound to soil particles and with organic compounds.

Agricultural intensification has greatly increased the productive capacity of agroecosystems, but has had unintended environmental consequences including degradation of soil resources and nutrient pools. The unintended consequences of intensive modern agriculture extend well beyond agricultural landscapes themselves (Matson *et al.*, 1997; Pimentel *et al.*, 1991; Drinkwater and Snapp, 2007). To develop crops that can thrive nutrient stress environments, selection of cultivars that are able to access a range of nutrient pools will be critical. Current nutrient management strategies aim to deliver soluble inorganic nutrients directly to crops and have uncoupled carbon, nitrogen, and phosphorus cycles in space and time. As a result, agricultural ecosystems are maintained in a state of nutrient saturation and are inherently leaky because chronic surplus additions of nitrogen and phosphorus are required to meet yield goals leading to a store or 'bank' in agricultural soils. Selection and breeding of crops for P-uptake and use efficiency that can reduce P-fertilization and sustain high yield in P-deficient soils is good alternative ecologically sound strategy and

will reduce the eutrophication threat of pooled P. Differential response of genotypes in nutrient stress environments may be related to morphological root features, efficiency of ion uptake mechanisms, nutrient movement across roots and delivery to the xylem, and nutrient use in metabolism and growth processes (Gill and Ahmad, 2003).

Phosphorus stress reduces the leaf area and no more required assimilates for leaf area expansion, are directed towards roots (Wissuwa *et al.*, 2005), ultimately increasing root surface area to absorb more P from P-deficient medium (Valizadeh *et al.*, 2003). Radin and Edinbock (1984, 1986) suggested that the decline in leaf elongation rate with P-stress is a consequence of decreased hydraulic conductance leading to reduced water transport and therefore turgor necessary for leaf enlargement. Another possibility is that the effect of P-stress on leaf expansion may be mediated by growth hormones. Changes in leaf phosphate status have been shown to be correlated with changes in leaf epidermal cell area. Since leaf epidermal cell expansion is apparently a critical process controlling the expansion of plant's leaf blade, and low P leads to dramatic decreases in Pi concentrations in the upper epidermis (Treeby *et al.*, 1987), the effect of low P on leaf expansion may also have been mediated through the supply of Pi to the epidermal cells.

As a part of a effort to understand the physiological basis of P-uptake and effect of P-stress on plant growth, we evaluated the effect of P-starvation induced with Jordon rock-P (RP) and surfeit on hydroponically grown fourteen *Brassica* cultivars to elucidate genetic diversity among cultivars in terms of Leaf area plant⁻¹ (LA), P-stress induced % reduction in LA, and P-absorption, transport and utilization rates using NH₄H₂PO₄ as control treatment. This will open the possibility to understand the effect of P-stress on the above motioned parameters and will provide information regarding the direct use of RP and to conserve the rapidly depleted world reserves of inexpensive RP around the globe. This will also provide the data base for selection/development of smart plants with the promise of sustainable cropping especially under resource limited environments.

Materials and Methods

Plant material and culture conditions

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]: KNO₃ [2], NH₄NO₃ [1], Ca(NO₃)₂.4H₂O [2], MgSO₄.7H₂O [0.5], K₂SO₄ [0.5] and [in μM]: Fe(III)-EDTA [50], H₃BO₃ [25], MnSO₄.H₂O [2], ZnSO₄.7H₂O [2], CuSO₄.5H₂O [0.5], KCl [50], H₂MoO₄ [0.5]. The solutions in these pots were modified by adding 200 μM P using NH₄H₂PO₄ (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 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, orthophosphate assay, and measurements of growth related parameters

Plants were harvested at two stages [24 and 34 day after transplanting (DAT)] to facilitate calculations of rate-related parameters such as absolute and relative growth rates, specific P-absorption and utilization rates, and P-transport rate. 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 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 [P] 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 and root-P contents. Leaf area (cm² plant⁻¹) (LA) of each plant was measured using a leaf area meter. The following growth related parameters were determined by using various expressions.

Relative reduction in leaf area plant⁻¹ (%LA; %PSF) was calculated by the formula given below:

$$\text{Relative reduction in LA (\%)} = \{LA_{(\text{high/Adequate P})} - LA_{(\text{Stress P})} / LA_{(\text{Adequate P})}\} \times 100 \quad [1]$$

Specific P uptake (SPU) was calculated as below:

$$\text{Specific P-uptake (SPU) (mg P g}^{-1} \text{ RDM)} = \text{Total P-uptake} / \text{RDM} \quad [2]$$

where RDM is root dry matter (g plant⁻¹) in the respective treatment.

The following growth parameters were calculated using data recorded at 24 and 34 DAT.

(a) Absolute growth rate (AGR) was calculated in terms of biomass produced per day using the following expression.

$$\text{AGR (g day}^{-1}\text{)} = \frac{M_2 - M_1}{T_2 - T_1} \quad [3]$$

Where 'M₂' refers to biomass recorded at time 'T₂' (second harvest) and 'M₁' refers to biomass recorded at time 'T₁' (first harvest). (b) Relative growth rate (RGR) was calculated by assuming growth in exponential terms using the following formula.

$$\text{RGR ([g g}^{-1}\text{] day}^{-1}\text{)} = \frac{\text{Log } M_2 - \text{Log } M_1}{T_2 - T_1} \quad [4]$$

(c) Specific absorption rate (SAR) was determined as the amount of P absorbed by whole plant per unit root dry matter per day during the time interval between two harvests, using the following formula assuming exponential growth rate.

$$\text{SAR (mg P g RDM}^{-1} \text{ day}^{-1}\text{)} = \frac{P_2 - P_1}{T_2 - T_1} \times \frac{\text{Log RDM}_2 - \text{Log RDM}_1}{\text{RDM}_2 - \text{RDM}_1} \quad [5]$$

where P refers to the total P-content recorded at time=T and RDM is root dry matter. (d) Specific utilization rate (SUR) was calculated on the basis of biomass produced per unit of P absorbed assuming an exponential growth rate.

$$\text{SUR (g biomass mg}^{-1} \text{ P day}^{-1}\text{)} = \frac{M_2 - M_1}{T_2 - T_1} \times \frac{\text{Log } P_2 - \text{Log } P_1}{P_2 - P_1} \quad [6]$$

P-transport rate (PTR) was calculated on the basis of SDM by the following expression:

$$\text{PTR (mg P g}^{-1} \text{ SDM day}^{-1}\text{)} = \{(P_2 - P_1) / (S_2 - S_1)\} \times \text{RGR (shoot)} \quad [7]$$

Where P₁ and P₂ represent total P-uptake in shoot, S₁ and S₂ at first and second harvest, respectively and RGR (shoot) is relative growth rate of shoot.

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 Discussions

Leaf area plant⁻¹ (LA) produced by *Brassica* cultivars

P-stress affects the leaf area plant⁻¹ (LA) significantly ($P < 0.01$) and reduced the leaf area by 56 % compared with P-adequate level (AP) and cultivars such as 'Con-1', 'Brown Raya', 'Poorbi Raya', 'Rain Bow', and 'Dunkled' exhibited more leaf area compared to other cultivars at stress P-level (RP) (Figure 1). *Brassica* cultivar 'Con-1' produced maximum LA (328 cm² plant⁻¹), followed by 'Brown Raya' (293 cm² plant⁻¹) at RP (Figure 1). Phosphorus stress reduces the leaf area by limiting the cell division at meristematic apex of the shoot (Chiera *et al.*, 2002; Radin and Eidenbock, 1986). P-starvation is reported to inhibit leaf expansion via their inhibitory effects on hydraulic conductance of roots. Radin and Eidenbock

(1984) assumed that reduced leaf area per plant due to P-stress was related to the accumulation of total dry matter (TDM) per plant. Therefore, it can be assumed that cultivars which can resist P stress on leaf area development per plant can better adapt to low P conditions. In the present experiment, significant positive correlations ($r > 0.95^{**}$) observed between LA and shoot dry matter (SDM), RDM, and TDM of cultivars under P-stress support this assumption and cultivars having high LA are P-tolerant under P-stress environment.

Relative reduction in LA due to P-stress (%PSF)

Relative reduction in LA due to P-stress (PSF) can also be used as a useful parameter in assessing relative tolerance of *Brassica* cultivars to P-stress conditions. P-stress factor (%PSF) (calculated according to equation 1) was significantly ($p < 0.01$) different among cultivars indicating their relative tolerance to low P conditions (Figure 2). The cultivars showing relatively lower PSF values or % relative reduction in LA are considered suitable for growing under P limiting conditions. Relative reduction in LA of the cultivars ranged between 30% (Con-1) and 74% (Gold Rush) indicating considerable genetic diversity for adaptability to P-starved conditions. Since PSF only indicates relative magnitude of reduction in LA due to P-stress compared to the control, it cannot be used as a sole criterion for selecting cultivars to be grown under P-stress. In the present experiment, for example, although 'Shiralle' and 'Peela Raya' was also less affected due to P-stress (PSF value is 49 for both cultivars; Figure 2), their low biomass production at each level of P-supply (biomass data is presented in part I) makes them as an unacceptable choice for farmers for agricultural production. The P-tolerant selected cultivars on the basis of PSF should exhibit acceptable biomass production under sufficient as well as P-deficient conditions.

Specific absorption, uptake and transport rates, and absolute and relative growth rates

Rate of biomass production between 24 and 34 DAT was lower in case of RP treatment compared to control, in absolute (biomass production per day) as well as relative (biomass accumulated per unit of existing biomass per day) terms. The effect of cultivars on these parameters was, however, statistically non-significant in case of SAR (Figure 3) and SPU (Figure 4) at RP, while phosphorus x cultivar interaction was statistically significant for RGR, AGR and PTR. The cultivars, nevertheless, showed significant differences among one another, for SUR (Figure 5) AGR (Figure 6), RGR (Figure 7) and PTR (Figure 8), when compared in solution under P-stress as well at AP. Simple correlation matrix among various *Brassica* growth and P-uptake parameters after 34 DAT in solution containing RP in the rooting media is presented in Table 1.

Rate-related growth parameters are important for identifying varietal or species differences in tolerance to P-stress (Salinas and Sanchez, 1976; Ahmad *et al.*, 2001). SAR can be defined as the amount of P absorbed per unit RDM per unit time while SUR may be defined as the amount of P utilized per unit TDM per unit time. In the present experiment, SAR during 24 to 34 DAT (Figure 3) increased significantly with decreasing P supply. This observation is in agreement with results reported by Ahmad *et al.* (2001) who observed increased P-influx rate of cotton cultivars under P-stress compared to adequate P-supply. A negative correlation was observed between SAR and P-contents of roots ($r = -0.32^*$) and shoots ($r = -0.26$) of P-stressed cultivars suggesting an internal regulation of SAR in addition to the influence exerted by external P supply (Salinas and Sanchez, 1976). It is interesting to note that 'Con-1', which accumulated maximum SDM and TDM under P-starvation, had a mean SAR value of 0.0085 between 24 and 34 DAT in the nutrient solutions. This cultivar ranked first with respect to whole plant P content in this treatment, which might explain its zero SAR, whereas its SUR was higher than other cultivars, perhaps contributing to the high biomass accumulation by this cultivar. Apparently, 'Dunkled' produced less SDM and TDM under P-stress at 24 DAT (data of 25 DAT not shown as data of 34 DAT is presented here) while it improved its position between the two harvest interval time and recorded SDM higher than 9 out of 14 cultivars after growth of ten more days in solution, and higher AGR and RGR than some other cultivars. This cultivar also had a SAR higher than some other cultivars, which might have been triggered by its relatively lower whole plant P-contents. The SUR value of 'Con-1' was highest among all the cultivars, which implied its ability to adapt to P-stress with time and growth owing to higher absorption and utilization rates.

Mean PTR increased about 2-fold with AP compared to RP. Maximum PTR was in 'Con-1' while minimum was of 'B.S.A' at both P-sources. A significant correlation between PTR and shoot P-concentration and uptake (Table 1) indicated that cultivars with higher PTR were able to retain higher P in their shoots under P-starvation and reliable for growing under P-stress environments.

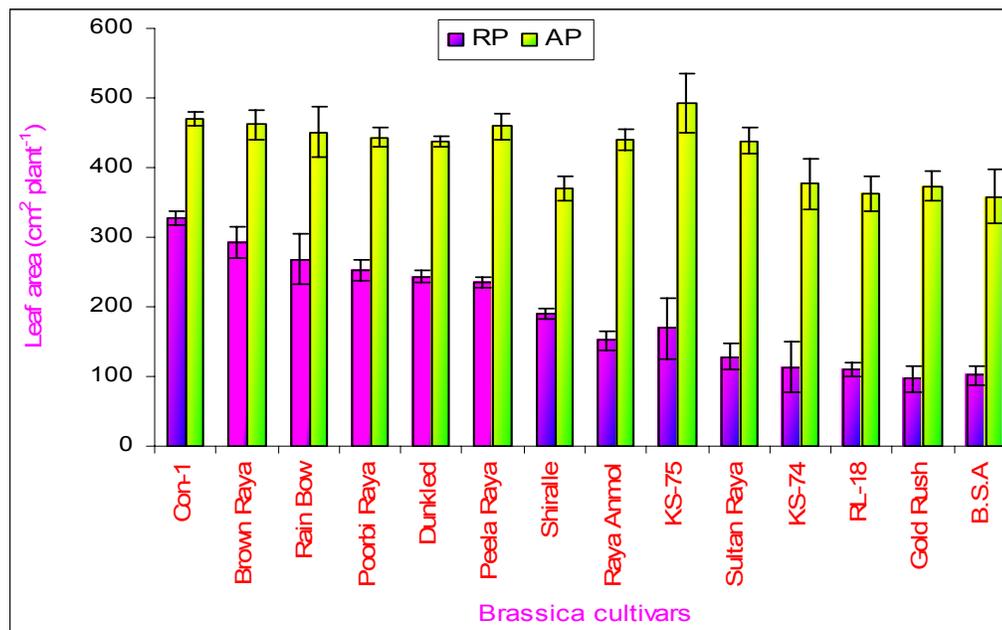


Figure 1. Leaf area per plant ($\text{cm}^2 \text{ plant}^{-1}$) of 14 *Brassica* cultivars grown in nutrient solution containing $\text{NH}_4\text{H}_2\text{PO}_4$ (AP) and sparingly soluble rock-P (RP); error bars show \pm SE.

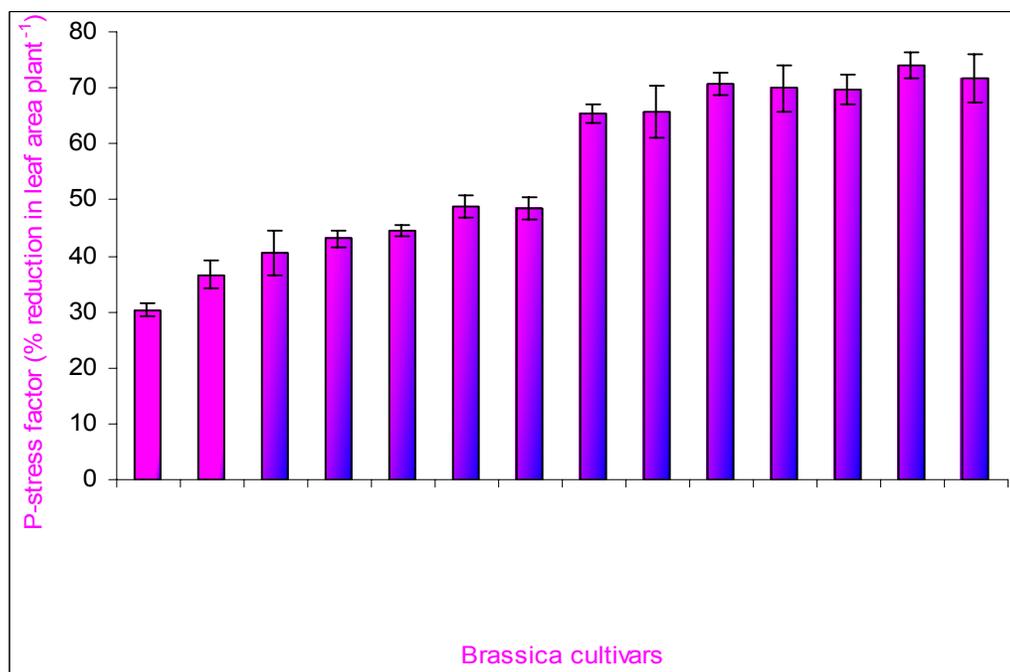


Figure 2. Phosphorus stress factor [% reduction in leaf area plant^{-1} ($\text{cm}^2 \text{ plant}^{-1}$; %PSF)] (calculated according to equation 1) of 14 *Brassica* cultivars grown in nutrient solution containing $\text{NH}_4\text{H}_2\text{PO}_4$ (AP) and sparingly soluble rock-P (RP); error bars show \pm SE.

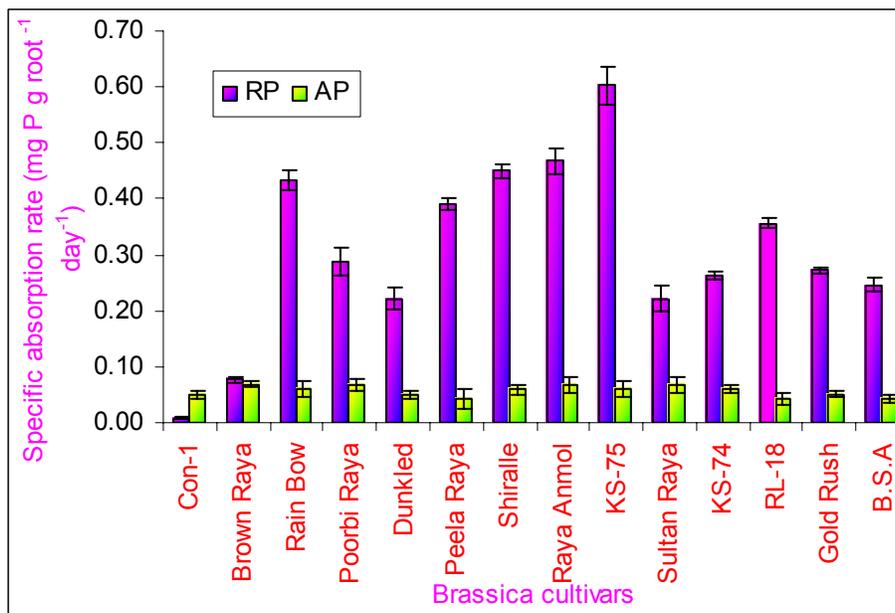


Figure 3. Specific absorption rate of 14 *Brassica* cultivars grown in nutrient solution containing $\text{NH}_4\text{H}_2\text{PO}_4$ (AP) and sparingly soluble rock-P (RP); error bars show \pm SE.

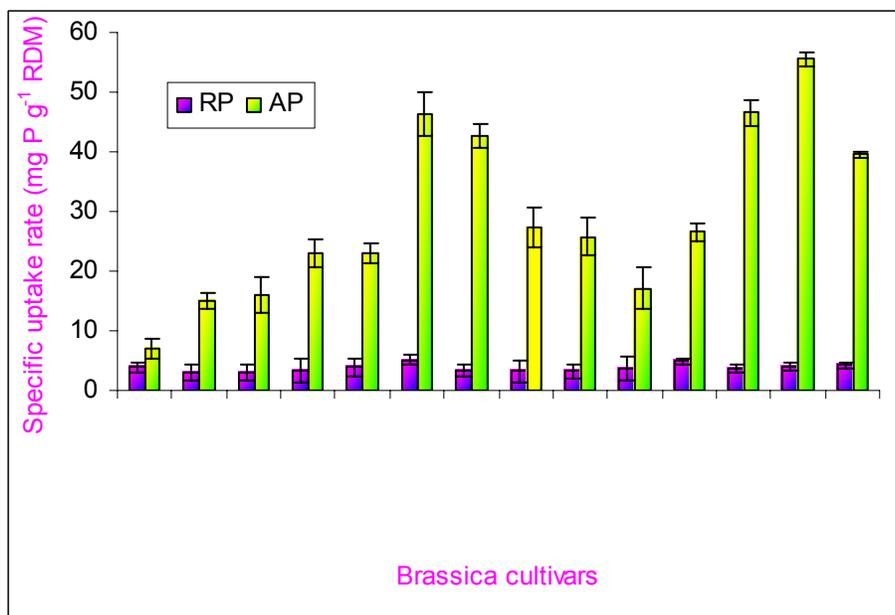


Figure 4. Specific uptake rate of 14 *Brassica* cultivars grown in nutrient solution containing $\text{NH}_4\text{H}_2\text{PO}_4$ (AP) and sparingly soluble rock-P (RP); error bars show \pm SE.

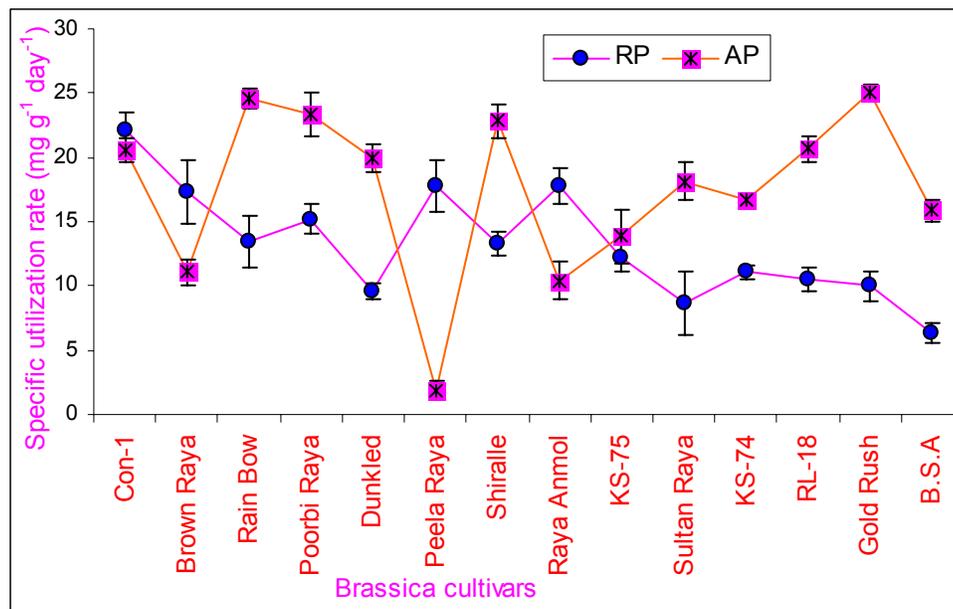


Figure 5. Specific utilization rate of 14 *Brassica* cultivars grown in nutrient solution containing NH₄H₂PO₄ (AP) and sparingly soluble rock-P (RP); error bars show ± SE.

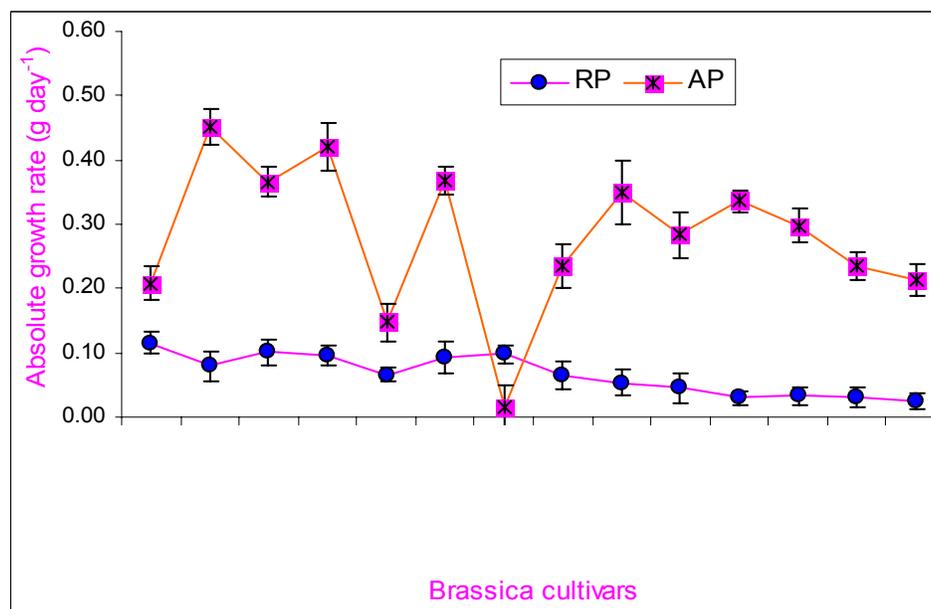


Figure 6. Absolute growth rate of 14 *Brassica* cultivars grown in nutrient solution containing NH₄H₂PO₄ (AP) and sparingly soluble rock-P (RP); error bars show ± SE.

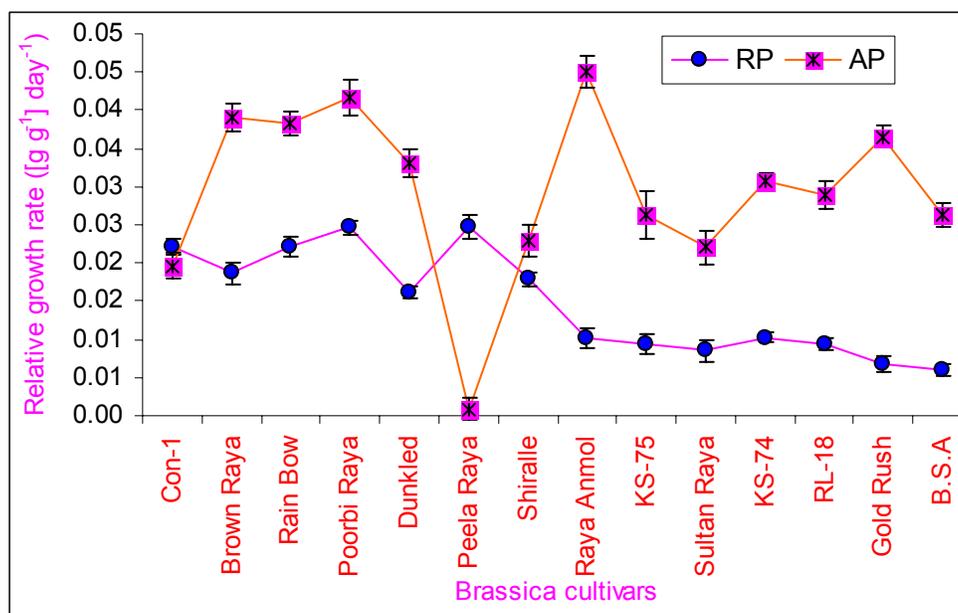


Figure 7. Relative growth rate of 14 *Brassica* cultivars grown in nutrient solution containing $\text{NH}_4\text{H}_2\text{PO}_4$ (AP) and sparingly soluble rock-P (RP); error bars show \pm SE.

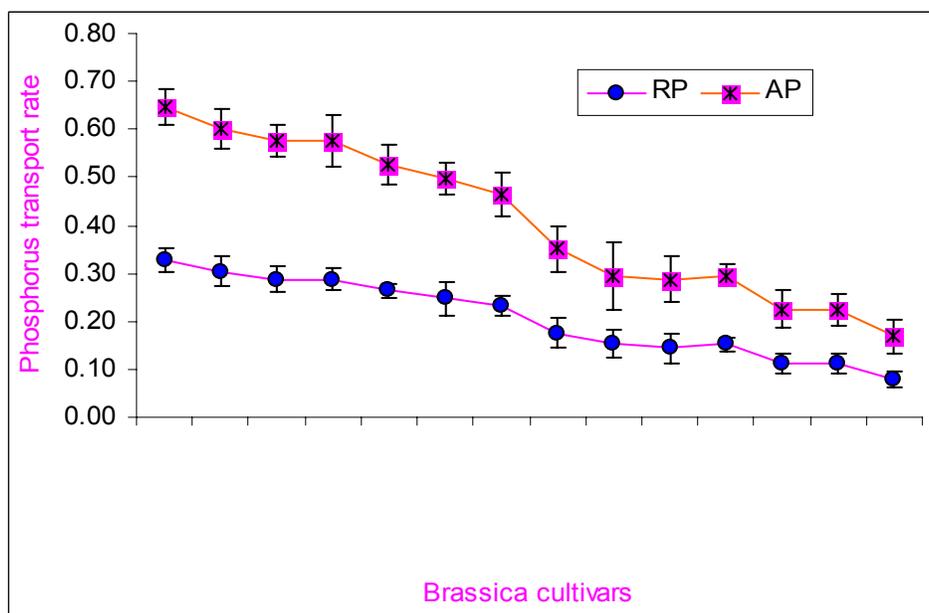


Figure 8. Phosphorus transport rate of 14 *Brassica* cultivars grown in nutrient solution containing $\text{NH}_4\text{H}_2\text{PO}_4$ (AP) and sparingly soluble rock-P (RP); error bars show \pm SE.

TABLE 1. Simple correlation matrix among various *Brassica* growth and P-uptake parameters after 34 DAT in solution containing RP in the rooting media.

	SDM	RGS	RDM	RGRR	TDM	RSR	TPS	TPR	PUE	PTR
RGS	-0.24*									
RDM	0.75**	0.12 ^{NS}								
RGRR	-0.23*	0.53**	0.78**							
TDM	0.93**	-0.31*	0.81**							
RSR	-0.71**	0.16 ^{NS}	0.85**	0.78**	-0.53**					
TPS	0.88**	-0.52**	0.87**	0.45**	0.91**	-0.49**				
TPR	0.21 ^{NS}	0.73**	0.69**	0.42**	0.86**	-0.15 ^{NS}	0.86**			
PUE	0.85**	0.46**	0.78**	0.39*	0.81**	-0.09 ^{NS}	0.13 ^{NS}	0.29*		
PTR	0.07 ^{NS}	0.09 ^{NS}	-0.04 ^{NS}	0.52**	0.11 ^{NS}	-0.31*	0.61**	0.32*	-0.41*	
SAR	0.26 ^{NS}	0.26*	-0.13 ^{NS}	0.46**	0.31*	-0.56**	-0.32*	0.23 ^{NS}	-0.2 ^{NS}	0.93**

SDM: Shoot dry matter; RGS: Relative growth of shoot; RDM: Root dry matter; RGRR: Relative growth rate of root; TDM: Total dry matter; TPS: Total P-content in shoot; TPR: Total P-content in root; PUE: P-utilization efficiency; PTR: P-transport rate; RSR: Root shoot ratio; SAR: Specific absorption rate. ** = significant at $P = 0.01$, * = Significant at $P = 0.05$, ^{NS} = Non-significant at $P = 0.05$.

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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

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