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PHOSPHORUS UTILIZATION BY SIX BRASSICA CULTIVARS (*BRASSICA JUNCEA* L.) FROM TRI-CALCIUM PHOSPHATE; A RELATIVELY INSOLUBLE P COMPOUND

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Abstract

Phosphorus deficiency is a serious concern for agriculture productivity around the globe. It readily forms insoluble compounds by reacting with calcium after addition to calcareous soils. We evaluated six Brassica cultivars for P utilization from mono-ammonium phosphate (a soluble source) and a relatively insoluble P compound (Tri-calcium phosphate). Plants were germinated in sand and transplanted in a P-free Johnson's nutrient solution contained in two iron tubs. Phosphorus (@ 0.2 mM P) was applied in each tub using both sources. Cultivars differed significantly for their biomass grown with either source of P. Biomass was significantly ($p<0.05$) lower in plants grown with TCP than those grown with MAP. Relative shoot dry matter of plants grown with TCP compared to those grown with MAP varied from 45% in BARD-1 to 96% in 19-H. However 19-H exhibited lowest dry matter with both P sources indicating its inefficiency. Root:shoot ratio differed significantly among cultivars, however it was not much affected by P source. Cultivars RL-18, Raya Anmol, and KS-74 produced maximum shoot dry matter grown with TCP, however in plants grown with MAP, Poorbi Raya produced maximum dry matter indicating its high responsiveness but lower P solublization. Phosphorus uptake in both shoots and roots of plants grown with MAP was significantly more than those in TCP. Total P uptake in plants grown with TCP varied between 8.56 mg/plant in 19-H to 22.86 mg/plant in Poorbi Raya. However, relative P uptake was highest in 19-H indicating that its poor performance in MAP treatment was not because of lower P availability in growth medium but it was due to its lower P utilization efficiency. Phosphorus utilization efficiency was maximum in Poorbi Raya, RL-18 and BARD-1 when grown with MAP, while in plants grown with TCP, Raya Anmol and RL-18 exhibited maximum P use efficiency. However, P efficiency mechanisms of these cultivars must be studied in further physiological and morphological studies.

Keywords: Phosphorus utilization, Brassica, Tri-calcium phosphate, Calcareous soils.

Introduction

Phosphorus deficiency is a common nutritional problem affecting crop production globally (Fairhust *et al.*, 1999). Billions of hectares worldwide (> 30% of world's arable land) are considered to contain too little P to sustain adequate plant growth (Vance *et al.*, 2003). Despite having rich total P contents, the available P contents in even most fertile soils are too low to meet most plants' demands due to precipitation with Ca in alkaline soils (Rahmatullah *et. al.*, 1994) and with Al and Fe oxides in acid soils (Plaxton & Carswell, 1999; Raghothama, 1999). Low use efficiency of applied P (15-

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20%) in soils makes P fertilization not only uneconomical but also environmentally unsafe practice (Vance *et al.*, 2003). The ever rising prices of P fertilizers in the world market besides increasing concern of environmental degradation (Vance *et al.*, 2003) call for multidimensional solutions to tackle the problem, instead of relying upon conventionally available high input approaches.

Nature has bestowed higher plants with a number of morphological and physiological strategies to explore P under limiting conditions (Hinsinger, 1998; Vance *et al.*, 2003). The adaptive mechanisms include decreased growth rate, increased growth per unit of P uptake, remobilization of internal P (Plaxton & Carswell, 1999), increased production and secretion of phosphatases, exudation of organic acids (Raghothama, 1999; Hinsinger *et al.*, 2003) and increased root surface area due to more root growth, and changes in root morphology (Lynch & Brown, 2001; Vance *et al.*, 2003).

Plant species and even cultivars within species differ greatly in one or more of these adaptations (Gill *et al.*, 2002; Aziz *et al.*, 2005) and hence, differ in P acquisition and/or utilization. Plants that are efficient in absorption and utilization of nutrients greatly enhance fertilizer use efficiency, reducing cost of inputs, and preventing losses of nutrients. These genetic differences can be exploited to develop crop cultivars efficient in P acquisition and/or utilization. (Fageria & Baligar, 1997; Kosar *et al.*, 2002). In case of P, this strategy will not only help in categorizing the existing genetic material for increased P efficiency but will also provide database for future breeding ventures (Gill *et al.*, 2004).

Brassica is known to utilize P more efficiently than most of other crops mainly by increased exudation of organic acids in rhizosphere (Hoffland, 1992). However, very little work has been done on the uptake and internal utilization of P by *Brassica* cultivars. The present paper reports genetic differences for P acquisition and its onward utilization for producing biomass among six *Brassica* cultivars. In alkaline calcareous soils, P is generally present in form of tri-calcium phosphate (TCP) which is a relatively insoluble P compound. We used TCP as P source in this nutrient solution experiment for representing the unavailable P in growth medium and for its comparison with available P used as mono-ammonium phosphate (MAP).

Material and Methods

Growth conditions: The experiment was conducted under natural conditions in a rain protected wire house of Institute of Soil & Environmental Sciences, University of Agriculture, Faisalabad. The temperature of wire house varied from a minimum of 9°C to a maximum of 22°C with a mean value of 14°C. Relative humidity in greenhouse ranged from 45 to 85% at day and night, respectively. Light intensity varied between 300 and 1400 µmol photon m^2S^{-1} depending upon day and cloud conditions during the growth period.

Plant growth, harvesting and basic analysis: Seeds of 6 Brassica cultivars (*Brassica juncea* L.) were sown in river bed sand contained in polyethylene lined iron trays. After one week of germination, uniform sized seedlings were transplanted in foam-plugged holes on thermophore sheets floating on continuously aerated half strength modified Johnson’s nutrient solution (Johnson *et al.*, 1957). The solution contained 6 mM N, 3 mM K, 2 mM Ca, 1 mM Mg, 3 mM S, 50 µM Cl, 25 µM B, 2 µM Mn, 2 µM Zn, 1 µM Cu, 0.05 µM Mo and 50 µM Fe. Two P sources i.e., tricalcium phosphate (TCP, a least soluble P source) and monoammonium phosphate (MAP, a soluble P source) were used to maintain 0.2 m*M* of total P in nutrient solution. There were five replications of each cultivar grown on both P sources and completely randomized design was followed. Hydrogen ion activity (pH) of nutrient solution was monitored daily in both treatments and adjusted daily at 6.0 ± 0.5 in solution containing MAP as P source only. In other treatment, pH of solution was not adjusted to 6.0 daily to prevent dissolution of TCP by acid.

Plants were harvested 40 d after transplanting. They were washed in distilled water and blotted dry using filter paper sheets and separated into shoots and roots before air drying for 2 d. The samples then were oven dried at 75 °C in a forced air driven oven for 48 h to record dry matter yield (g plant^{-1}). Relative biomass production by *Brassica* cultivars grown with TCP compared to those grown with MAP was calculated by using following formula.

$$\text{Relative dry matter production (\%)} = \frac{DW_{\text{TCP}}}{DW_{\text{MAP}}} \times 100$$

DW_{TCP} is dry matter of shoot or root of plants grown with TCP and DW_{MAP} is dry matters of shoot or root of plants grown with MAP.

Dried samples of shoots and roots were ground in a mechanical grinder (MF 10 IKA, Werke, Germany) to pass through a 1 mm sieve. A 0.5 g portion of uniformly mixed plant sample was digested in diacid mixture of nitric acid and perchloric acid (3:1) at 150°C (Miller, 1998). Phosphorus concentration in shoot and root digest was estimated using vanadate-molybdate colorimetric method (Chapmann & Pratt, 1961). Phosphorus contents (mg P plant^{-1}) were calculated in root and shoot by multiplying P concentration in the respective tissue with its dry matter and on whole plant basis by adding up shoot and root P contents. Phosphorus utilization efficiency (g SDM mg^{-1} P) was calculated by the following formula (Siddiqui & Glass, 1981)

$$\text{Phosphorus Utilization Efficiency} = \frac{1}{\text{Shoot P concentration}} \times \text{Dry matter}$$

The data were subjected to statistical treatments using computer software “MS-Excel” and “MSTAT-C” (Russell & Eisensmith, 1983). Completely randomized factorial design was employed for analysis of variance (ANOVA). Least square method of regression / linear correlation was used to calculate regression and correlation coefficients among different parameters.

Results

Biomass production: There were significant main and interactive effects of cultivars and P sources on shoot dry matter (SDM) and root dry matter (RDM) of Brassica cultivars (Table 1). Shoot dry matter of cultivars was significantly lower (>2 folds) in plants grown with TCP than those grown with MAP. Shoot dry matter of plants varied significantly ($p<0.05$) among cultivars grown with either TCP or MAP in nutrient solution. In plants

Table 1. Shoot dry matter, root dry matter, and root:shoot ratio of six brassica cultivars (*B. juncea*) grown with monoammonium phosphate (MAP) and tri-calcium phosphate (TCP).

Cultivars	**Shoot dry matter (g/plant)**		**Root dry matter (g/plant)**		**Root:shoot ratio**	
	MAP	**TCP**	**MAP**	**TCP**	**MAP**	**TCP**
KS-74	2.23 ± 0.16	1.31 ± 0.16	0.25 ± 0.03	0.15 ± 0.02	0.11 ± 0.01	0.12 ± 0.02
Poorbi Raya	2.86 ± 0.36	1.06 ± 0.11	0.24 ± 0.02	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.01
Raya Anmol	2.09 ± 0.25	1.41 ± 0.08	0.17 ± 0.02	0.12 ±0.01	0.08 ± 0.01	0.09 ± 0.01
RL-18	2.56 ± 0.42	1.42 ± 0.13	0.24 ± 0.01	0.15 ± 0.04	0.09 ± 0.01	0.04 ± 0.02
BARD-1	2.23 ± 0.17	1.01 ± 0.09	0.15 ± 0.02	0.10 ± 0.01	0.07 ± 0.01	0.10 ± 0.01
19-H	1.00 ± 0.13	0.96 ± 0.09	0.14 ± 0.02	0.11 ± 0.02	0.14 ± 0.01	0.11 ± 0.01
Probability						
Treatments	**		**		NS	
Cultivars	*	*	*	**	*	**

Whereas * = significant at p<0.05, ** = significant at p<0.01 and ns= non significant

Table 2: Phosphorus concentration and utilization efficiency of six brassica cultivars grown with monoammonium phosphate (MAP) and tri-calcium phosphate (TCP).

Cultivars	**Shoot P concentration (mg Pg^{-1})**		**Root P concentration (mg Pg^{-1})**	
	MAP	**TCP**	**MAP**	**TCP**
KS-74	0.87 ± 0.02	0.75 ± 0.08	0.80 ± 0.12	1.12 ± 0.25
Poorbi Raya	0.73 ± 0.03	0.71 ± 0.02	0.86 ± 0.04	2.42 ± 0.11
Raya Anmol	0.74 ± 0.04	0.63 ± 0.03	0.96 ± 0.09	1.78 ± 0.11
RL-18	0.76 ± 0.04	0.62 ± 0.06	0.75 ± 0.05	1.11 ± 0.07
BARD-1	0.73 ± 0.03	0.84 ± 0.17	0.87 ± 0.06	2.65 ± 0.22
19-H	0.74 ± 0.06	0.63 ± 0.09	0.85 ± 0.01	2.16 ± 0.17
F value				
Treatments	NS		*	
Cultivars	*	**	*	**

Whereas * = significant at p<0.05, ** = significant at p<0.01 and ns= non significant

Table 3: Phosphorus uptake in shoot and roots of six brassica cultivars (*B. juncea*) grown with monoammonium phosphate (MAP) and tri-calcium phosphate (TCP).

Cultivars	**Plant P contents (mg/plant)**		**Shoot P contents (mg/plant)**	
	MAP	**TCP**	**MAP**	**TCP**
KS-74	21.53 ± 1.36	11.57 ± 2.19	19.51 ± 1.33	10.07 ± 1.88
Poorbi Raya	22.86 ± 2.40	9.45 ± 1.12	20.78 ± 2.36	7.52 ± 0.88
Raya Anmol	17.13 ± 1.86	10.97 ± 0.95	15.48 ± 1.76	8.88 ± 0.75
RL-18	21.20 ± 4.12	10.53 ± 0.85	19.42 ± 4.00	8.79 ± 0.97
BARD-1	17.67 ± 1.12	11.28 ± 0.86	16.38 ± 0.98	7.98 ± 0.65
19-H	8.56 ± 1.50	8.45 ± 1.16	7.39 ± 1.41	6.17 ± 1.00
F Value				
Treatments	*		**	
Cultivars	**	*	*	*

Whereas * = significant at p<0.05, ** = significant at p<0.01 and ns= non significant

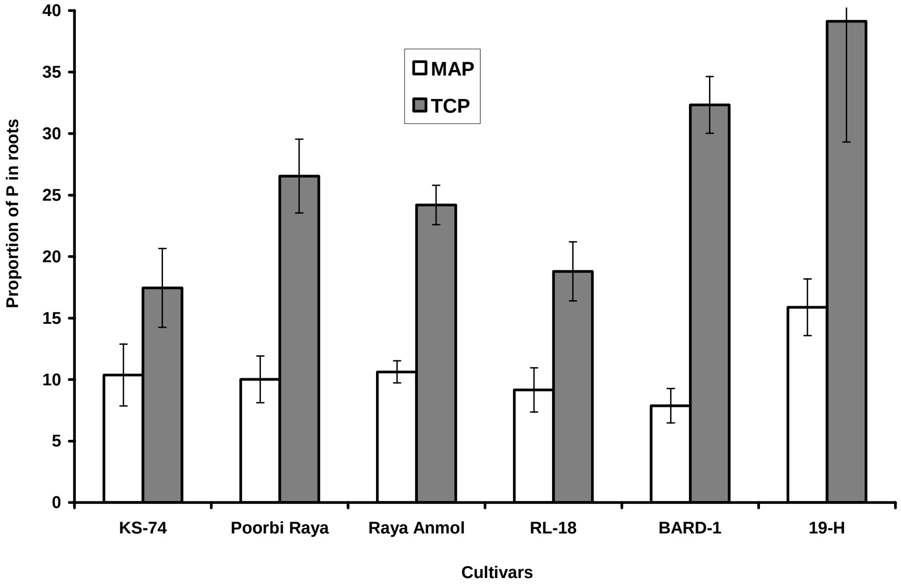

Fig. 1. Proportion of total P in roots of Brassica cultivars grown with MAP and TCP

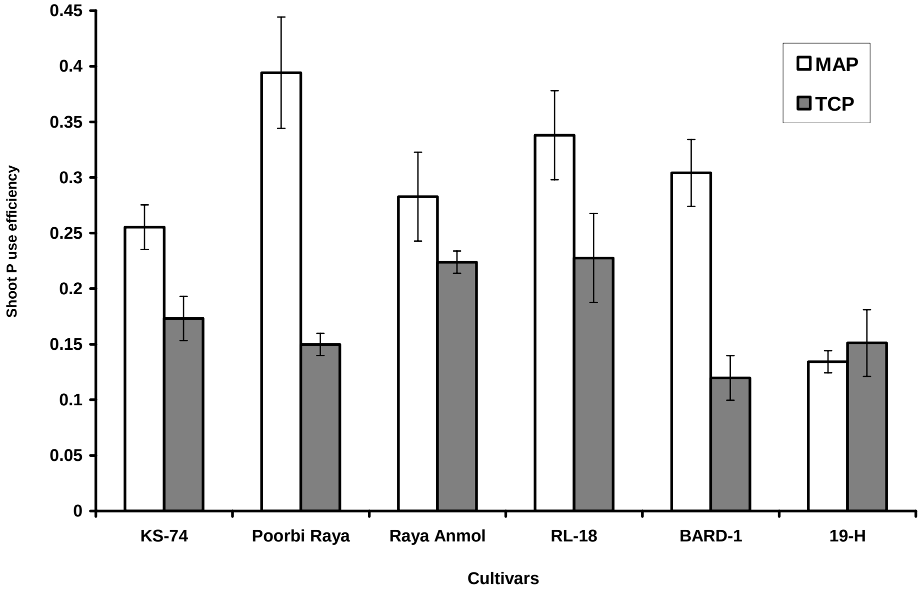

Fig. 2 Shoot P use efficiency of Brassica cultivars grown with MAP and TCP

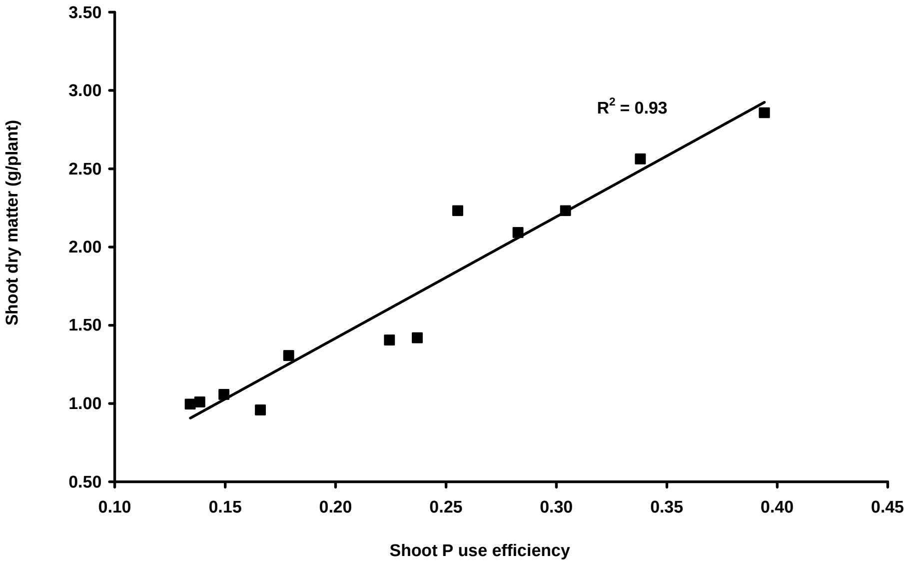

Fig. 3. Correlation among shoot dry matter and P use efficiency of Brassica cultivars

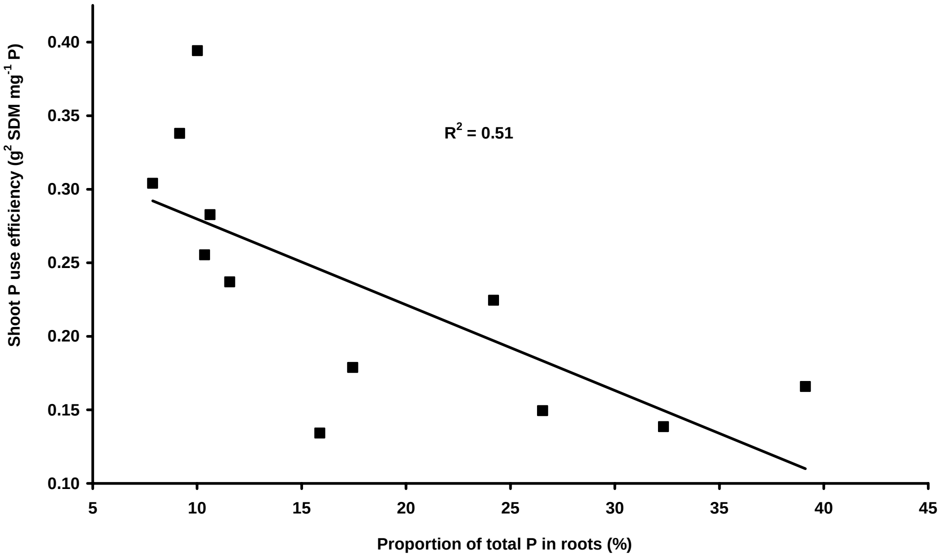

Fig. 4 Correlation between Propotion of P contents in roots with shoot P use efficiency

receiving MAP, SDM varied from 1.0 g/plant in 19-H to 2.86 g/plant in Poorbi Raya. Shoot dry matter of plants grown with TCP varied from 0.96 g/plant in 19-H to 1.42 g/plant in RL-18.

Root dry matter (RDM) of plants grown with MAP was significantly higher (>2 folds) than those grown with TCP. Maximum RDM of plants grown with MAP was produced by KS-74, Poorbi Raya and RL-18 (>0.20 g/plant). Variations among cultivars for RDM were more pronounced in plants grown with TCP. Minimum RDM was produced by RL-18 and maximum RDM was produced by KS-74. Root shoot ratio (RSR) of cultivars did not differ significantly among P sources. Cultivars varied significantly ($p<0.05$) for RSR grown either with MAP and TCP. Maximum RSR was observed in 19-H and KS-74 in plants grown with MAP. In plants grown with TCP, lowest RSR was observed in RL-18.

Phosphorus concentration, uptake and use efficiency: Phosphorus concentration in Brassica shoots was significantly ($p<0.05$) more in plants grown with MAP than those grown with TCP (Table 2). Cultivars also differed significantly for shoot P concentration when grown either with MAP or TCP; however variations in shoot P concentration were more pronounced in TCP treatment. Maximum P concentration was observed in KS-74 (0.87 mg g^{-1}) and BARD-1 (0.84 mg g^{-1}) in the plants grown with MAP and TCP, respectively.

Root P concentration was significantly affected by P sources (Table 2). It was >2 folds higher in plants grown with TCP than those grown with MAP. Cultivars did not differ significantly for root P concentration when grown with MAP, however, significant ($p<0.01$) variations were exhibited among cultivars when grown with TCP. Minimum root P Concentration was observed in KS-74 when grown with TCP in root medium.

There were significant ($p<0.01$) main and interactive effects of P sources and cultivars on shoot and total P contents (Table 2). Total P contents were significantly more in plants grown with MAP than those grown with TCP. Cultivars differed significantly ($p<0.01$) for total P contents in only MAP treatment. Shoot P contents were also significantly more in plants grown with MAP than those grown with TCP. Variations for shoot P contents were more pronounced in MAP treatment, where it ranged from 7.39 mg/plant in 19-H to 20.78 mg/plant in Poorbi Raya. Root P contents were significantly more in plants grown with TCP than those grown with MAP (Fig. 1). Maximum P contents in roots were accumulated by Poorbi Raya and KS-74 when grown with MAP. In plants grown with TCP, root P contents were significantly more in BARD-1 and 19-H. Minimum root P contents in plants grown with TCP, were accumulated by RL-18.

There were significant main and interactive effects of cultivars and P sources on P use efficiency in shoots of Brassica cultivars (Fig. 2). Phosphorus use efficiency was significantly more in Poorbi Raya, RL-18 and BARD-1 than other cultivars in MAP treatment. In plants grown with TCP, Raya Anmol and RL-18 produced more biomass per unit P absorbed.

Discussion

Application of P fertilizers is recommended to cope wide spread P deficiency in agricultural soils around the globe. However, ever-rising prices of P fertilizers and its low use efficiency makes this practice both uneconomical and environmentally unsafe. A wide variation exists among crop species and even cultivars for P acquisition form soil and its utilization within plant body. These variations can be exploited through selection and breeding for more P efficient crop species to sustain crop productivity and soil health (Gill *et al.*, 2004; Aziz *et al.*, 2005). The present solution culture study was planned to evaluate genetic variations among six Brassica cultivars which is an important oilseed crop of Pakistan.

The cultivars exhibited wide variations for biomass as well as P contents when grown either with MAP or TCP. Significant interaction ($p<0.05$) among cultivars and P sources (environment) is very important for recombinant breeding and producing more efficient cultivars (Kang *et al.*, 1998). Poorbi Raya, Raya Anmol and RL-18 were efficient in biomass production grown in both P sources. Cultivar 19-H produced lowest biomass grown in both of P treatments indicating its lower efficiency. Significant reduction in biomass and total P contents in plants grown with TCP clearly indicated low P availability (generally prevalent in calcareous soils) in nutrient solution.

Relative biomass production by plants grown with low P availability (TCP) (if plants grown with MAP are considered as control, 100 %) is a good indicator of P deficiency tolerance and/or solubilization of P (Kosar *et al.*, 2002; Aziz *et al.*, 2005). Cultivars varied significantly for relative biomass production in TCP, but this parameter was not of much significance in this study for relative P efficiency. Cultivar 19-H grown with TCP produced 96% of its maximum potential when grown with MAP. But its lower absolute value of SDM (0.96 g/plant) than other cultivars makes it unsuitable for cultivation on low P soils. Raya Anmol produced only 67% of its maximum potential with TCP, but it produced significantly more biomass than 19-H when grown with MAP or TCP. Hence absolute values of biomass must be considered in addition to their relative performance under P deficiency before selecting crop cultivars for P efficiency.

Roots had to perform better under P deficiency to explore more soil volume for P acquisition hence, increased RDM and changes in root morphology are well reported in many of crop species under P deficiency (Lynch, 1995; Aziz, 2006). However, in present study, root:shoot ratio was not affected significantly by low P availability in TCP treatment. These contrasting results were attributed to the differences in growth medium as most of earlier studies, showing increased RSR, were conducted in low P soils (Lynch & Brown, 2001). Changes in root morphology and architecture is greatly affected by a number of edaphic factors which are usually absent in solution culture studies.

Low shoot P concentration clearly indicated low P availability in TCP treatment than in MAP treatment. However, plants have solublized TCP too, as P concentration in plants grown with TCP was quite sufficient for plant growth at this ontogenic stage (Reutor *et al.*, 1997). Root P concentration was significantly more in plants grown with TCP treatment, which was attributed to negative growth dilution effect because of low RDM production. Phosphorus contents in shoots and roots is an important parameter indicating relative acquisition efficiencies of these cultivars from TCP. Low P contents in shoots grown with TCP indicated low P availability from TCP in nutrient solution, however efficient cultivars such as KS-74, Poorbi Raya and RL-18 accumulated more P contents than other cultivars.

Phosphorus use efficiency is the amount of dry matter produced per unit of P absorbed (Siddiqui & Glass, 1981; Aziz *et al.*, 2005). Differences in P use efficiency have been reported in various crops such as in wheat (Kosar *et al.*, 2002), rice (Aziz *et al.*, 2005), and cotton (Ahmad *et al.*, 2001). Present results also indicated significant differences in *Brassica juncea* cultivars for P utilization efficiency. Phosphorus use efficiency was positively correlated with dry matter production (Fig. 3). Cultivars KS-74, Raya Anmol and RL-18 exhibited maximum P use efficiency when grown with TCP. These cultivars were also efficient in biomass production. These cultivars must be selected for future breeding programs and for recommendations to the farmers in soils low in available P. Cultivar 19-H accumulated minimum P contents grown either with MAP or TCP. It indicated its low acquisition and low utilization efficiency. Translocation of P within plant body from inactive to metabolically active sites (young and growing leaves) is also an adaptive response to P deficiency (Adu-Gyamfi *et al.*, 1990). Plants efficient in translocation of P from roots to shoots such as RL-18, KS-74, Poorbi Raya were efficient in biomass production. Phosphorus contents in roots was negatively correlated with P use efficiency (Fig. 4) indicating that cultivars accumulating more P in their roots such as 19-H were inefficient in biomass production.

Conclusions

Useful genetic variations existed among Brassica cultivars for P acquisition (differences in P uptake) and its onward utilization to produce biomass (use efficiency). Cultivars efficient in P acquisition and its utilization (RL-18, KS-74, Poorbi Raya and Raya Anmol) produced more growth than inefficient cultivars (19-H). Efficient cultivars translocated more P from roots towards shoot while inefficient cultivars retained more proportion of total P in their roots. Efficient P acquisition, utilization and translocation from roots towards shoot were the major adaptive mechanisms identified in P efficient cultivars which must be studied further under pot and field conditions.

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