Title
Phosphorus Acquisition Efficiency from Sparingly Soluble P-sources by Brassica Cultivars under P-stress Environment

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Introduction

Total soil P-content typically varies from 500 to 2000 mg Kg^{-1}, total bioavailable P may be only a few mg Kg^{-1} (Vance et al. 2003). The negligible Pi- transport to the plant root surface by mass flow of water (1-5% of plant's P-demand; Lambers et al. 2006), notoriously slow diffusion coefficient of orthophosphate (Pi) (10^{-12}–10^{-15} m^2 s^{-1}; Rausch and Bucher 2002), high sorbing capacity for P in the soil (sorbtion to Fe/Al oxides), P-complexation (Ca-P salts such as apatite), and/or fixation into organic forms results in P-starvation in highly weathered and alkaline calcareous soils (Ragothama and Karthikeyan 2005; Vance et al. 2003). To combat Pi-starvation, > 30% of world's arable land requires exogenous application of Pi fertilizers produced commercially from rock phosphate (RP). Whilst the application of Pi fertilizers has undoubtedly increased crop productivity, overuse of Pi fertilizers in industrialized countries has also posed many serious problems such as eutrophication and hypoxia of lakes, rivers and surrounding water bodies. Moreover, with decreasing global P reserves, prices of P-fertilizers are bound to increase and farmers of tropical and subtropical countries are reluctant to apply costly imported Pi fertilizers. Therefore, to extend the useful life of the world's P-reserves, to reduce the cost of producing crops, and to sustain agriculture production systems, more efficient utilization of inorganic Pi by plants is direly needed. The use of RP for direct application and/or in combination with P-tolerant genome is a cost-effective alternative to expensive P-fertilizers, especially in resource-poor environments, where indigenous RP deposits exist and water soluble P-fertilizers are imported. It is a research priority to develop/select plants that are highly effective at acquiring Pi from soil-bound P reserves and/or at using P more efficiently; or develop more precise P monitoring methods aiming at an improved yield with low input of soluble Pi-fertilizers. 'Tailoring the plant to fit the soil instead of tailoring the soil to fit the plant' is an alternative ecologically sound strategy ensuring sustainable cropping with less exogenous Pi-fertilizers applications.

Materials and Methods

Seeds of fourteen *Brassica* cultivars were germinated in polyethylene-lined iron trays containing riverbed sand for seedling establishment in a dark chamber at 25°C. Solution culture experiments were conducted under controlled-climate chamber using modified Hoffland's solution 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. The solutions were renovated at 3-day intervals. The solutions in the tubs were modified by adding 200 µM P using $NH_4H_2PO_4$ as a control treatment (AP), and $Ca_3(PO_4)_2$ (TCP)(0.2 g L^{-1}), powdered Jordan rock-P (RP) (2 g L^{-1}), respectively as sparingly soluble P-sources. Plants were harvested 36 days after transplanting (DAT) for plant measurements. In soil culture, a pot experiment was conducted in a glasshouse using soil texturally sandy loam and categorized as P-deficient having Mehlich-III-extractable P 6.13 mg kg^{-1}, EC_e 1.4 dS m^{-1}, pH in water (1:5 ratio) 7.8. The soil was air-dried and coarse-ground before glazed china clay pots were filled (30 cm deep and 22 cm diameter) at the rate of 6 kg per pot. To half the pots, P was applied at the rate of 60 mg P kg^{-1} soil as $NH_4H_2PO_4$ (+P) while no P was applied to remaining half (0P). N and K were applied at 175 and 75 mg kg^{-1} soil, respectively. Plants were harvested 41 days after sowing. Harvested plants were separated into shoots and roots, dried at 70°C for 48 hours and oven dry mass (g $plant^{-1}$) were recorded. Ground samples

(0.42 mm) were digested in 2N HCl after dry ashing at 550°C for 7 hours. P-concentrations ([P]s) in samples were estimated by the vanadate-molybdate yellow color method. P-uptake (mg plant^{-1}) was calculated by multiplying [P]s in the respective tissue with its dry matter as below:

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

P-stress factor (%PSF) for shoot dry matter (SDM; g plant^{-1}) was calculated as below:

PSF = ($SDM_{(adequate/sufficient\ P)}$ – $SDM_{(deficient/stress\ P)}$ / $SDM_{(adequate\ P)}$) X 100 [2]

P-utilization efficiency (PUE) was determined according to Siddiqi and Glass (1981).

PUE (g^2 SDM mg^{-1} shoot-P) = SDM (g plant^{-1}) / [P] (mg g^{-1}) [3]

P-efficiency ratio (PER) at stress/low P was calculated as below.

P-efficiency ratio (g DM mg^{-1} P) = Dry matter (g plant^{-1}) / P-uptake (mg plant^{-1}) [4]

P-efficiency (relative shoot growth) was calculated as described by Ozturk et al. (2005).

P-efficiency (PE) (relative shoot growth) = ($SDM_{(low\ or\ stress\ P)}$/ $SDM_{(high/sufficient\ P)}$) X 100 [5]

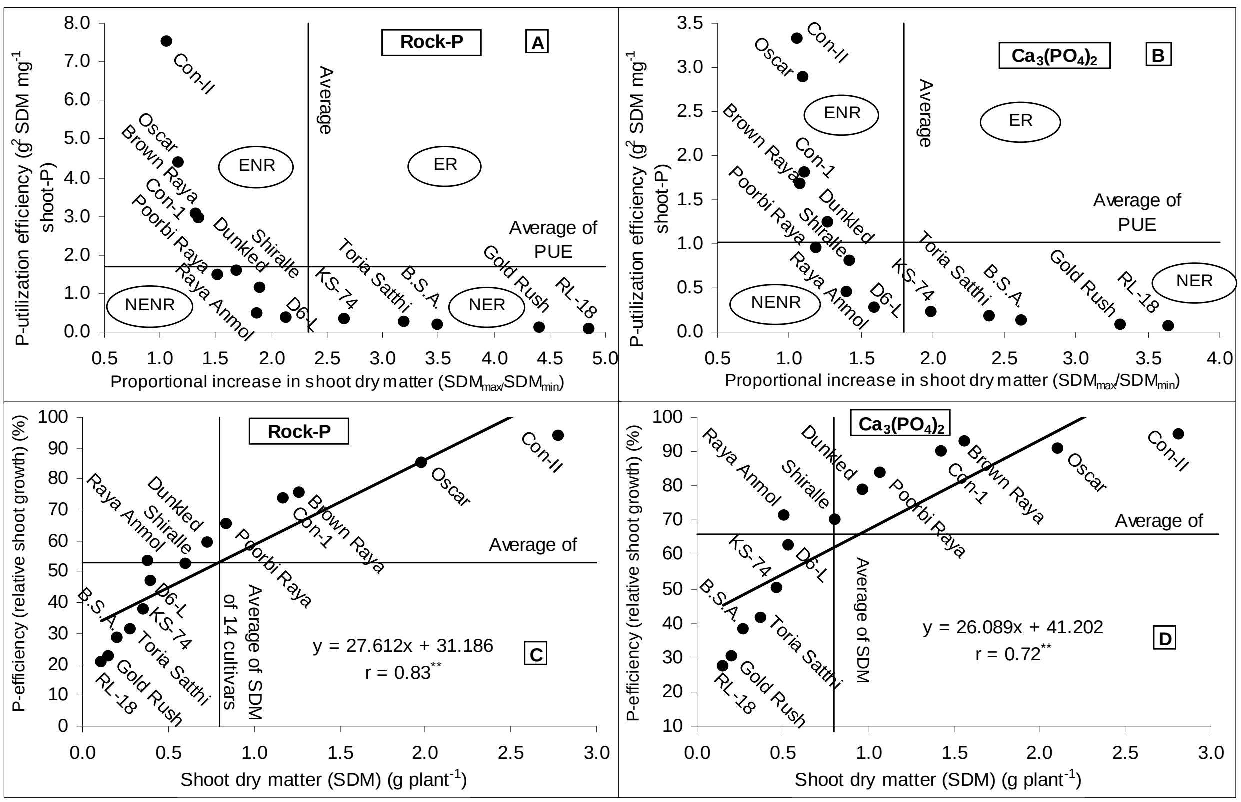

Figure 1. Ordination plots to classify cultivars for PUE as a function of proportional increase in SDM (SDM_{max}/SDM_{min}) in response to sparingly soluble P sources (A, B), and relations between PE and SDM (C, D). Efficient & responsive; NER: Non-efficient but responsive; ENR: Efficient but non-responsive; NENR: Non-efficient & non-responsive; ** = significant at $P < 0.01$.

Results and Discussion

Different P sources and *Brassica* cultivars had a significant main and interactive effect on shoot growth, root development, total biomass production, P and Ca concentrations and contents, and P efficiency characteristics, indicating the existence of useful genetic differences among cultivars for solublization of P from sparingly soluble P-sources. Cultivars depicting low PSF values were tolerant to thrive under P-starvation. To classify cultivars into efficient and inefficient utilizers of the absorbed P, two variables were plotted for each cultivar: in the Y-axis, the PUE under P-stress (RP and TCP) and in the X-axis, the ratio between the maximum and the minimum SDM obtained for each cultivar (Figure1A, B). The most undesirable cultivars are the 'NENR' type as per their poor performance. Significant correlation between PE and SDM at TCP and RP (Figure 1C,D), indicates that cultivars showing higher PE produced higher SDM under P-stress. In soil culture, significant correlations between PE, PER, and SDM at 0P (Figure 2A,B) indicates that differences in P-use efficiency were largely due to differences in yields. Ordination plots between PUE, PE and PSF revealed that class-I cultivars showed high PUE, PE and low PSF values compared to class-II cultivars (Figure 2C, D), and are considered to be more P-tolerant than class-II cultivars. SDM production and total amount of P per shoot at stress P were the most reliable parameters in assessing *Brassica* cultivars for their P efficiency at the vegetative stage.

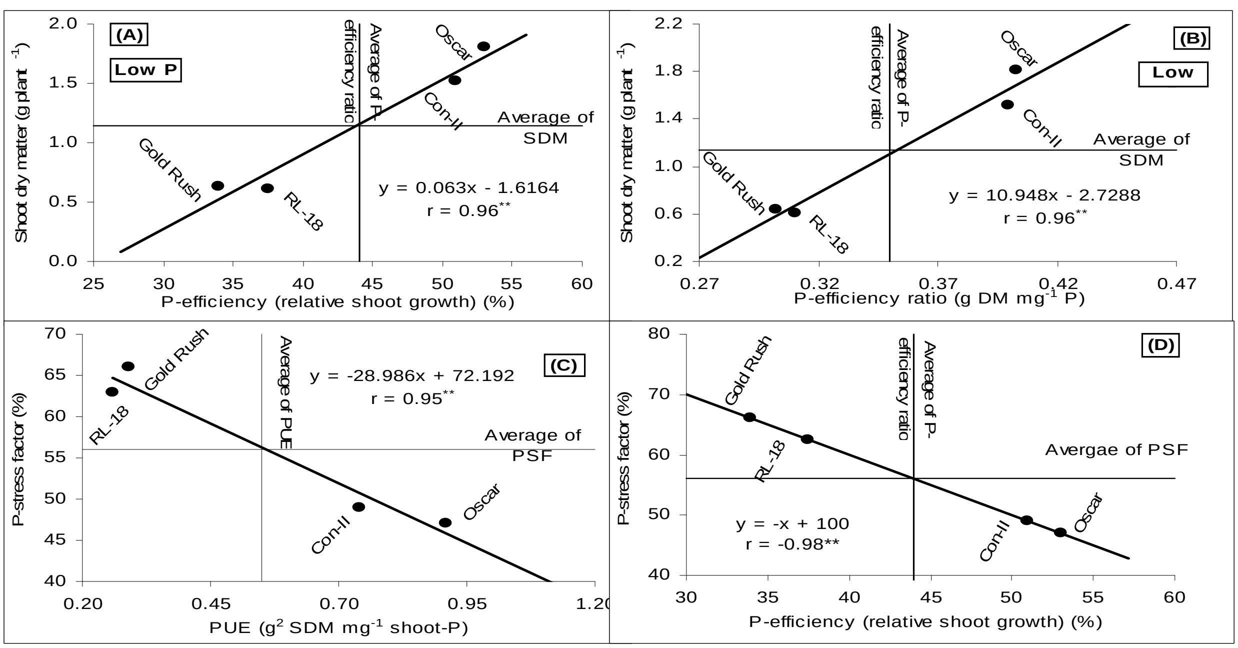

Figure 2. Relationship between biomass accumulation and various growth parameters of four *Brassica* cultivars grown in soil culture. ** = significant at $P = 0.01$.

Conclusions

Tested cultivars grown with sparingly soluble P sources showed considerable genetic variations in growth behavior, P-acquisition and P-use efficiency. Significant correlations between various growth parameters and biomass accumulation indicate that cultivars efficient in P-acquisition and internal utilization accumulated maximum biomass under P-starvation. It is plausible to conclude that cultivars depicting high PUE and PE, and low PSF values were better choice under P-starved environments. Systematic analysis and deployment of the plant rescue traits underlying the nutrient acquisition, assimilation and utilization will bring more sparingly soluble P into cropping systems and will help to scavenge more P from plant unavailable bound P-reserves.

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