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ORIGINAL PAPER

Root carboxylate exudation capacity under phosphorus stress does not improve grain yield in green gram

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Abstract

Key message **Genetic variability in carboxylate exudation capacity along with improved root traits was a key mechanism for P-efficient green gram genotype to cope with P-stress but it did not increase grain yield.**

Abstract This study evaluates genotypic variability in green gram for total root carbon exudation under low phosphorus (P) using ^{14}C and its relationship with root exuded carboxylates, growth and yield potential in contrasting genotypes. Forty-four genotypes grown hydroponically with low (2 μM) and sufficient (100 μM) P concentrations were exposed to $^{14}CO_2$ to screen for total root carbon exudation. Contrasting genotypes were employed to study carboxylate exudation and their performance in soil at two P levels. Based on relative ^{14}C exudation and biomass, genotypes were categorized. Carboxylic acids were measured in exudates and root apices of contrasting genotypes belonging to efficient and inefficient categories. Oxalic and citric acids were released into the medium under low-P. PDM-139 (efficient) was highly efficient in carboxylate exudation as compared to ML-818 (inefficient). In low soil P, the reduction in biomass was higher in ML-818 as compared to PDM-139. Total leaf area and photosynthetic rate averaged for genotypes increased by 71 and 41 %, respectively, with P fertilization. Significantly, higher root surface area and volume were observed in PDM-139 under low soil P. Though the grain yield was higher in ML-818, the total plant biomass was significantly higher in PDM-139 indicating improved P uptake and its efficient translation into biomass. The higher carboxylate exudation capacity and improved root traits in the later genotype might be the possible adaptive mechanisms to cope with P-stress. However, it is not necessary that higher root exudation would result in higher grain yield.

Keywords ^{14}C shoot labelling · Carboxylic acid · Grain yield · Phosphorus · Root morphology · *Vigna radiata*

Abbreviations

ER	Efficient and responsive
ENR	Efficient and non-responsive
IER	Inefficient and responsive
IENR	Inefficient and non-responsive
P	Phosphorus

Introduction

Phosphorus (P) deficiency is one of the major limiting factors to crop production in most of the soils throughout the world (Hinsinger 2001). About 49.3 % of Indian soils are in low-P category, 48.8 % in medium and only 1.9 % are in high-P category (Hasan 1994). The use of phosphatic fertilizer in India has increased from 0.009 million tons (Mt) in 1950–1951 to 8.05 Mt in 2010–2011 and with the

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current rate of P fertilization, the projected demand would be 9–12 Mt by 2020 (FAI 2011). According to the demand for diammonium phosphate and single super phosphate during the year 2011–2012, and considering P-use efficiency as 10 %, the monetary loss of P_2O_5 was calculated to the tune of INR 7.81 billions. This is a huge loss for the resource poor agricultural sector in India. The major problem with P nutrition is not the soil P content but its bioavailability to plants. To improve crop yields, large amounts of phosphatic fertilizers are applied which not only cause serious environmental pollution, but also a huge drain in terms of foreign exchange.

P is an integral component of high-energy molecules and biomembranes and involved in several metabolic reactions and signal transduction pathways. Therefore, the availability of P has profound consequences on plant growth and physiology, and ultimately yield. Plants possess potential adaptive mechanisms to cope with long-term P-stress which include modification in root system architecture, enhanced exudation of organic acids and enzymes into the rhizosphere, increased expression of high-affinity P transporter, RNase and phosphoenolpyruvate carboxylase in plant tissues (Raghothama 1999). Out of the above mechanisms, rhizosphere exudation provides maximum exploration of soil volume around the rooting zone leading to conversion of non-available nutrients into available forms, thus resulting in enhanced P-uptake efficiency.

The organic compounds released as root exudates are mainly composed of organic acids, sugars, amino acids, phenolics and phytosiderophores (Marschner 1995). The most common organic acids released under P-stress include carboxylic acids such as acetic, aconitic, citric, fumaric, gluconic, lactic, malic, oxalic and succinic acids (Neumann and Römheld 1999; Osaki and Shinano 2000). These carboxylic acids dissociate into organic anions and release P into soil solution by chelating iron or aluminium bound in metal-phosphate complexes. On the other hand, carboxylates and P compete for the same adsorption site on metal-hydrous oxides in soil resulting in displacing P (Jones 1998; Gerke et al. 2000). Therefore, a particular crop species/cultivar releasing higher amount of carboxylates in the rhizosphere may suffice its P requirement using the residual fertilizer or bound minerals which otherwise remain unutilized. It is considered that the increased concentrations of carboxylates in root apices might lead to its enhanced efflux from roots. However, the dependence of carboxylic acid efflux on internal concentrations has not been rigorously tested. In *Brassica* genotypes, higher internal concentration of malate and citrate in roots resulted in increased exudation under P deficiency (Hoffland et al. 1989). Genetic engineering for improved carboxylate exudation was shown to increase P-acquisition efficiency in transgenic tobacco (Lü et al. 2012). Overexpression of mitochondrial malate dehydrogenase (MDH) from *Panicum oxalicum* in tobacco plants leads to increased root exudation of malate induced by P-stress. In soybean, however, no relationship between carboxylate exudation and internal concentration was observed (Dong et al. 2004).

Among grain legumes, green gram (*Vigna radiata* (L.) Wilczek) is the third important conventional crop. Its total P uptake is highest amongst the grain legumes which removes 48.1 kg P_2O_5 per ton of grain produced (FAI 2011). Sub-optimal P supply in legumes restricts root growth, photosynthesis, translocation of sugars and other processes like nodule development and nitrogen fixation. Selection of genotypes based on total root carbon exudation could be one of the easily assessed parameters for improved P-uptake efficiency as shown previously (Pandey et al. 2013). Breeding crop plants for higher root exudation may be an effective strategy to increase utilization efficiency of P fertilizers. Thus, experiments were carried out with three major objectives to (1) screen green gram genotypes for total root carbon exudation under low-P using ^{14}C, (2) characterization and quantification of carboxylic acids released in contrasting genotypes, and (3) establish the relationship between carboxylic acid exudation and physiology and yield of green gram under different P levels in soil.

Materials and methods

Experiment 1: screening genotypes for total carbon exudation using ^{14}C

Seeds of 44 green gram [*Vigna radiata* (L.) Wilczek] genotypes (Online Resource 1) were procured from the Division of Genetics, Indian Agricultural Research Institute, New Delhi and employed in the screening experiment. Treated seeds with 0.1 % $HgCl_2$ (w/v) were wrapped in germination towel for 2 days, and after germination seedlings were transferred to modified Hoagland solution. The composition of nutrient solution was as follows: $Ca(NO_3)_2$ 4.0 mM, KNO_3 4.0 mM, $(NH_4)_2SO_4$ 0.4 mM, $MgSO_4$ 2.0 mM, H_3BO_3 10.0 μM, $MnSO_4$ 2.0 μM, $ZnSO_4{\cdot}7H_2O$ 0.8 μM, $CuSO_4{\cdot}5H_2O$ 0.3 μM, Na_2MoO_4 0.1 μM, NaCl 3.0 μM and $FeCl_3$ + EDTA 3.0 μM. Concentration of P was maintained as low (2 μM) and sufficient (100 μM) by adding 1.0 M orthophosphoric acid. The pH of solution was maintained at 6.0 throughout the experiment. Plants were supported on Styrofoam sheet (50 mm thickness) with holes made at 5 × 5 cm plant-to-plant and row-to-row distance. The Styrofoam sheet was fitted into plastic containers (30 × 45 × 15 cm) holding 10 L solution. Forty-five seedlings were raised in one such container.

Fifteen genotypes were screened at a time with three replicates for each genotype. The solution was aerated continuously and changed every alternate day. The whole experimental setup was maintained in controlled environment chambers (Model PGW 36, Conviron, Winnipeg, Canada) at National Phytotron Facility, IARI, New Delhi. In these chambers, the growth conditions were maintained as: 30/18 °C day/night, photoperiod of 12 h with photon flux density of 450 μmol m^{-2} s^{-1} (PAR) and relative humidity at 90 %.

After the plants unfolded two trifoliate leaves (20 days after germination), individual plants were transferred to 100-mL Erlenmeyer flasks containing 50 mL of basal nutrient solution with sufficient or low-P. They were transferred to an airtight chamber for exposing the shoots to $^{14}CO_2$ as per the method described by Pandey et al. (2013). The ^{14}C counts were measured using liquid scintillation counter (Packard 1900 Tricarb). The ^{14}C count (disintegration per minute) was converted to Becquerel (Bq) and the rate of total ^{14}C exudation was expressed as Bq g^{-1} root FW h^{-1}. On the basis of relative total root ^{14}C exudation and biomass at sufficient and low-P, the genotypes were grouped into four categories: (a) efficient and non-responsive (ENR), genotypes with low biomass but higher exudation at low-P, (b) efficient and responsive (ER), genotypes with high biomass and higher exudation at low-P, (c) inefficient and non-responsive (IENR), genotypes with low biomass and exudation at low-P and (d) inefficient and responsive (IER), genotypes with higher biomass but low exudation at low-P. The relative changes were calculated as follows:

$$\text{Relative total root exudation } (\%) = \frac{\text{Total root exudation at low} - \text{P}}{\text{Total root exudation at sufficient P}} \times 100$$

$$\text{Relative total biomass}(\%) = \frac{\text{Total biomass at low} - \text{P}}{\text{Total biomass at sufficient P}} \times 100$$

Experiment 2: carboxylic acids in contrasting genotypes

Out of the above four categories, we selected two contrasting genotypes belonging to ER (PDM-139) and IENR (ML-818) category for further study. Carboxylic acids exuded into the medium were considered as the external or root exudate while those in the root apices represented the internal concentration. 20-Day-old plants raised as mentioned above were used for collection of root exudates by following the method of Dong et al. (2004). Plants were removed from nutrient solution and roots briefly rinsed in double distilled water. After blotting excess moisture, five plants were grouped and the roots were immersed in 50 mL of 0.5 mM $CaCl_2$ solution (pH 4.5) in an Erlenmeyer flask covered with black paper. The whole setup was kept in growth chamber, and after 4 h (from 0800 to 1,200 h) plants were removed to record the root fresh weight. The exudate was collected and eluted through Amberlite resin IR 120(H) (Sigma) filled in a glass column (10 × 1.8 cm). The eluant was filtered through 0.4 μm filter before injecting into high-pressure liquid chromatography (Agilent Technologies, 1200 Infinity Series).

Internal concentration of carboxylates was quantified by homogenizing 0.1 g root apices excised approximately 1 cm from the tip, in 0.2 mL of 0.1 M HCl (Pandey et al. 2013). Volume was made to 2.0 mL with double distilled water and was incubated at 80 °C in water bath for 40 min, cooled at room temperature and centrifuged at 12,000×*g* for 10 min. Supernatant was diluted 5 times and passed through a syringe filter before analysis using HPLC. The column used was Hamilton PRP-1 and the mobile phase was 0.1 % orthophosphoric acid with 1 mL per minute flow rate, at wavelength 210 nm using VWD (visual wavelength detector). The standards of oxalic, citric, maleic, succinic and lactic acids were used and the concentrations of carboxylates were expressed as μmol $plant^{-1}$.

Experiment 3: response of contrasting genotypes to soil P levels

Seeds of PDM-139 and ML-818 were sown in earthen pots (12 inches diameter) containing 8 kg air-dried sieved soil during summer season (March–June). Pots were exposed to natural environmental condition. The soil texture was sandy loam with pH 8.3 (in water). Available soil N and P were 193 and 14 $mgkg^{-1}$ of dry soil, respectively. Two levels of P were created, 'sufficient P' by adding 17.7 mg P_2O_5 kg^{-1} soil (0.575 mg pot^{-1} single super phosphate) at the recommended rate of 40 kg P_2O_5 ha^{-1}, and 'low-P' without adding P fertilizer. Recommended dose of N and K_2O corresponding to 100 mg pot^{-1} urea and 200 mg pot^{-1} muriate of potash was added. Before sowing, seeds were soaked in water for 6 h followed by treatment with *Rhizobium* culture. Thinning was done at the appearance of first trifoliate leaf to maintain one healthy plant in each pot. All observations were made at anthesis stage corresponding to 35 days after germination, and data on yield attributes were recorded at maturity.

Measurement of growth and photosynthesis

Plants were carefully uprooted and separated into root, stem and leaf for estimating growth attributes. Total leaf area was measured using leaf area meter (Li-COR 3000, Lincon Nebraska, USA) and expressed as cm^2 $plant^{-1}$. The

plant material was kept in hot air oven at 65 °C for drying till a constant weight was obtained, and dry weight was recorded. Photosynthetic rate was measured on the terminal leaflet of second fully expanded trifoliate leaf in the forenoon (from 0800 to 1,100) using Portable Photosynthesis System (Li-COR 6400*xt*) and expressed as μmol CO_2 m^{-2} s^{-1}. Plants were irrigated a day prior to taking measurements.

Root morphology

Plants were uprooted and washed carefully to remove adhering soil particles. Roots were scanned using root scanner (Regent Instruments Inc., Canada). Total root length (cm $plant^{-1}$), surface area (cm^2 $plant^{-1}$), volume (cm^3 $plant^{-1}$) and root diameter (cm) were computed from the scanned images using image analysis software WinRhizo.

Tissue P concentration and uptake

Concentration of P was estimated in root, stem and leaf tissues (Murphy and Riley 1962) after wet digestion with diacid mixture (HNO_3:$HClO_4$ in the ratio of 9:4) and measuring the absorbance of blue colour phospho-molybdate complex at 660 nm. P uptake per plant was calculated by multiplying tissue P concentration (%) with dry weight of the respective organ and expressed as mg P $plant^{-1}$.

Statistical analysis

All three experiments were conducted under 2-factor completely randomized design (F-CRD) with three replications each and mean values were used for comparisons. Variance analysis (ANOVA) was performed with statistical software (SAS version 9.1.3) (SAS Institute 2004). The means were compared by the critical difference (CD) at 5 and 1 % level of significance.

Results

Screening genotypes for total carbon exudation into the rhizosphere

Significant ($P < 0.05$ and 0.01) genotypic variation was observed in terms of total carbon exudation and biomass in seedlings grown under sufficient and low-P. The relative change in total carbon exudation and biomass at 24, 48 and 96 h after exposure to $^{14}CO_2$ were used to categorize the forty-four genotypes into four groups (Fig. 1). The genotype PDM-139 (ER) and ML-818 (IENR) showed consistently higher and lower relative total root

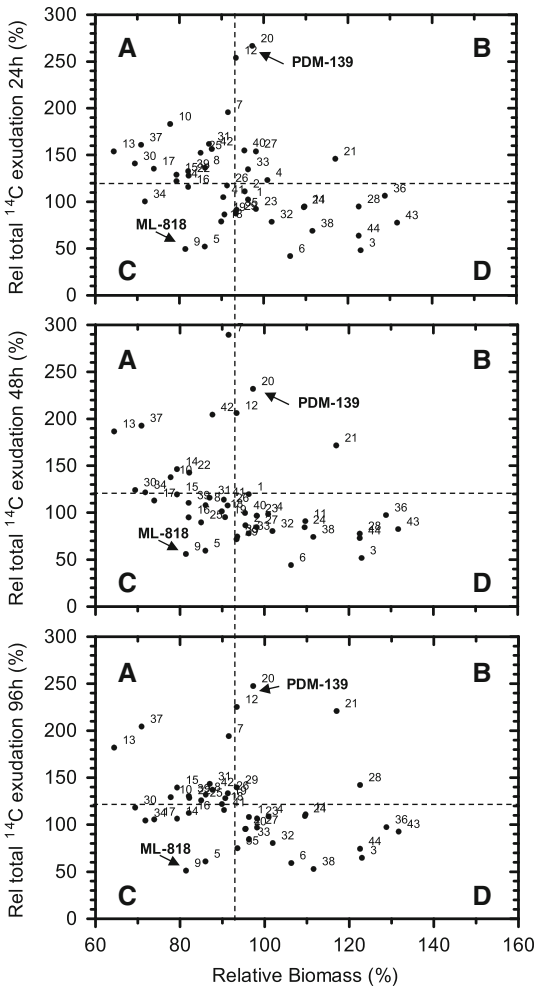

Fig. 1 Forty-four green gram genotypes categorized on the basis of relative values of total root ^{14}C exudation and biomass under sufficient (100 μM) and low (2 μM) P concentration recorded at 24, 48 and 96 h after $^{14}CO_2$ exposure. Genotypic mean ± SEM on *X* and *Y* axes demarcates four quadrants: *a* efficient and non-responsive, genotypes with lower biomass but higher exudation under low-P, *b* efficient and responsive, genotypes with maximum biomass and higher exudation at low-P, *c* inefficient and non-responsive, genotypes with low biomass and exudation at low-P and *d* inefficient and responsive, genotypes with low exudation but higher biomass under low-P. Data are means of three replicates and were compared with the critical difference at $P < 0.05$ and 0.01. Numbers correspond to the genotype name presented in Online Resource 1

^{14}C exudation, respectively, under P-stress at all time intervals. These two contrasting genotypes were selected for further study.

Characterization and quantification of carboxylic acids in contrasting genotypes

P-stress induced carboxylate exudation into the media and their concentration in root tissues differed significantly among genotypes (Online Resource 2). The major carboxylates in root exudate were oxalic and citric acid while

Table 1 Characterization and quantification of carboxylic acids (μmol plant^{-1}) in root tissues and exudate of contrasting green gram genotypes grown hydroponically at low (2 μM)- and sufficient (100 μM) P levels

Variety	Oxalic acid		Citric acid		Maleic acid		Succinic acid		Lactic acid		Total carboxylic acid	
	Low-P	Sufficient P	Low-P	Sufficient P	Low-P	Sufficient P	Low-P	Sufficient P	Low-P	Sufficient P	Low-P	Sufficient P
Carboxylates in root exudates												
ML-818	3.3 ± 0.11	5.3 ± 0.18	3.3 ± 0.14	2.3 ± 0.08	nd	nd	nd	nd	nd	nd	6.6 ± 0.23	7.6 ± 0.26
PDM-139	12.5 ± 0.43	2.2 ± 0.08	0.3 ± 0.01	1.4 ± 0.05	nd	nd	nd	nd	nd	nd	12.8 ± 0.44	3.6 ± 0.13
Carboxylates in root tissues												
ML-818	26.8 ± 0.93	24.9 ± 0.86	nd	nd	0.06 ± 0.003	0.10 ± 0.005	76.7 ± 2.66	25.1 ± 0.87	35.6 ± 1.23	38.1 ± 1.32	139.2 ± 4.82	88.2 ± 3.06
PDM-139	5.2 ± 0.18	18.9 ± 0.92	nd	nd	0.03 ± 0.002	0.03 ± 0.002	5.8 ± 0.20	4.7 ± 0.16	8.1 ± 0.28	7.4 ± 0.26	19.1 ± 0.66	31.0 ± 1.34

Data are mean ± SEM of three replicates

nd not detected

the root apices possessed higher concentration of oxalic, succinic and lactic acids (Table 1). Though a very less quantity of maleic acid was also synthesized in root tissues, it was not released into the rhizosphere. In root exudates of PDM-139, the concentration of oxalic acid per plant was 5.7-fold higher at low-P as compared to sufficient P. On the other hand, exudates of ML-818 had 38 % less oxalic acid while citric acid was 43 % higher at low-P in comparison to sufficient P. Maleic, succinic and lactic acid were not detected in root exudate of both genotypes.

The internal concentration of total carboxylic acid in root apices was higher in ML-818 as compared to PDM-139. When averaged over P level and genotypes, the maximum synthesized carboxylic acid in root tissue was in the order of succinic > lactic > oxalic > maleic. However, citric acid was not detected in root tissue of both genotypes indicating the possibility that the maximum amount being exuded into the media.

Physiological response of contrasting genotypes to soil P

The application of P significantly increased leaf, stem and total plant dry matter in both genotypes compared to low-P. Leaf dry matter (Fig. 2a) increased by 1.9- (ML-818) and 1.6 (PDM-139)- fold in plants grown with sufficient P in comparison with low-P soil. Dry matter accumulation in stem (Fig. 2b) was also higher in sufficient P grown plants resulting in 1.6- and 1.4-fold in ML-818 and PDM-139, respectively, as compared to low-P. Under low-P, root dry matter decreased by 40 % in ML-818 whereas PDM-139 did not show any significant reduction (Fig. 2c). However, the interaction between variety and P level in terms of dry matter accumulation in leaf, stem and root recorded an increase by 69, 59 and 12 %, respectively, with sufficient P.

The whole plant dry matter significantly increased by 59 % due to P application when averaged over genotypes (Fig. 2d). The reduction in whole plant dry matter was 82 % in ML-818 and 44 % in PDM-139, when grown under low-P over sufficient P. Further, PDM-139 produced maximum leaf (2.15 g plant^{-1}), stem (2.65 g plant^{-1}) and root (0.36 g plant^{-1}) dry matter as compared to ML-818 (leaf 1.55, stem 1.99 and root 0.33 g plant^{-1}) when averaged over P treatments. When averaged over P treatment, PDM-139 recorded 33 % increase in whole plant biomass over ML-818.

Whole plant leaf area was significantly influenced by P treatment and variety (Fig. 3a). Leaf area averaged over varieties increased by 71 % due to P application as compared to plants grown in low-P soil. Both varieties showed similar percentage of reduction in whole plant leaf area

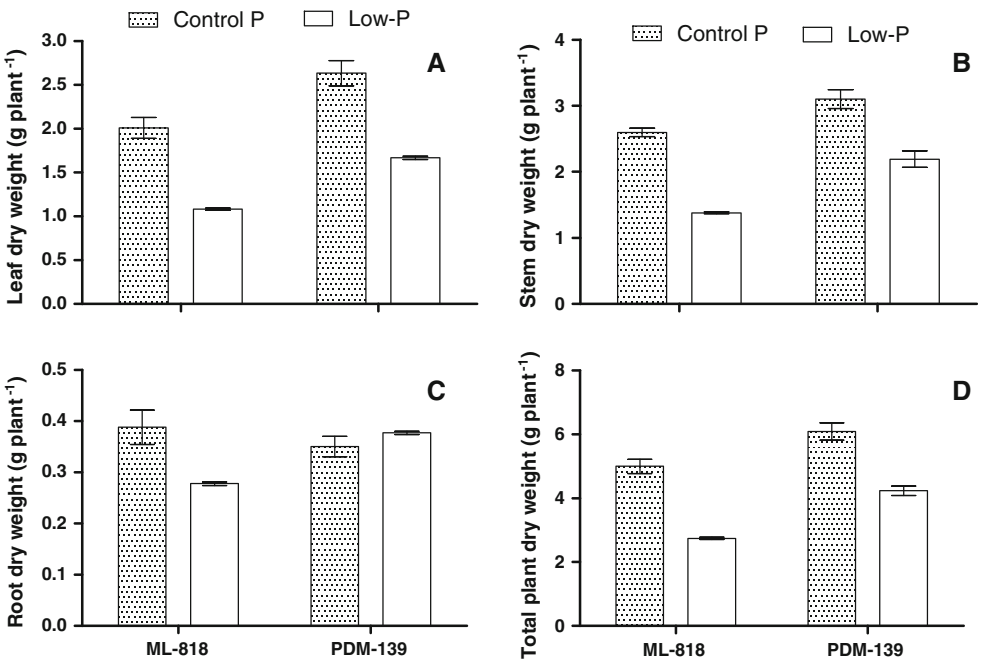

Fig. 2 Biomass accumulation of contrasting green gram genotypes grown in soil with two levels of P—14.0 (low) and 41.8 (control) mg P kg^{-1} soil. **a** Leaf dry weight, **b** stem dry weight, **c** root dry weight, and **d** total plant dry weight. Data are mean ± SEM of three replicates

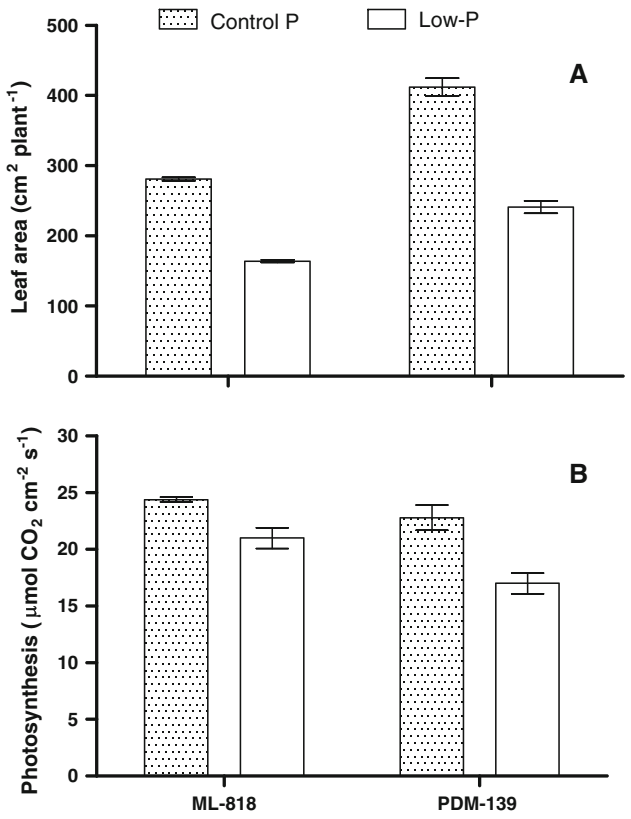

Fig. 3 **a** Total leaf area, and **b** photosynthetic rate of contrasting green gram genotypes grown in soil with two levels of P—14.0 (low) and 41.8 (control) mg P kg^{-1} soil. Data are mean ± SEM of three replicates

under low-P than sufficient P. However, among genotypes, maximum leaf area was produced in PDM-139 (326.6 cm^2 $plant^{-1}$) as against ML-818 (222.4 cm^2 $plant^{-1}$).

The rate of photosynthesis reduced significantly by 24 % in low-P treatment (Fig. 3b). External P application resulted in 16 and 41 % increase in photosynthetic rate in ML-818 and PDM-139, respectively, as compared to low-P treatment. Photosynthetic rate averaged over P levels was higher in ML-818 (22.7 µmol CO_2 m^{-2} s^{-1}) as compared to PDM-139 (19.9 µmol CO_2 m^{-2} s^{-1}).

Root morphology

The root parameters viz. length, volume, surface area and average root diameter were significantly influenced by P treatment and genotypes but the response did not follow a specific trend (Fig. 4a–d). There was no effect of soil P level on total root length but among the genotypes, ML-818 produced significantly longer roots as compared to PDM-139. Root surface area was significant only for the P x genotype interactive effect, with 12 % increase (averaged over P) in PDM-139 and 7 % increase (averaged over genotypes) due to P application. Total root volume increased by 20 % (averaged over genotypes) due to P application as compared to low-P, though the genotypic difference was not significant. The average root diameter was significant only between varieties. The root diameter averaged over P level was 0.39 and 0.15 cm in PDM-139 and ML-818,

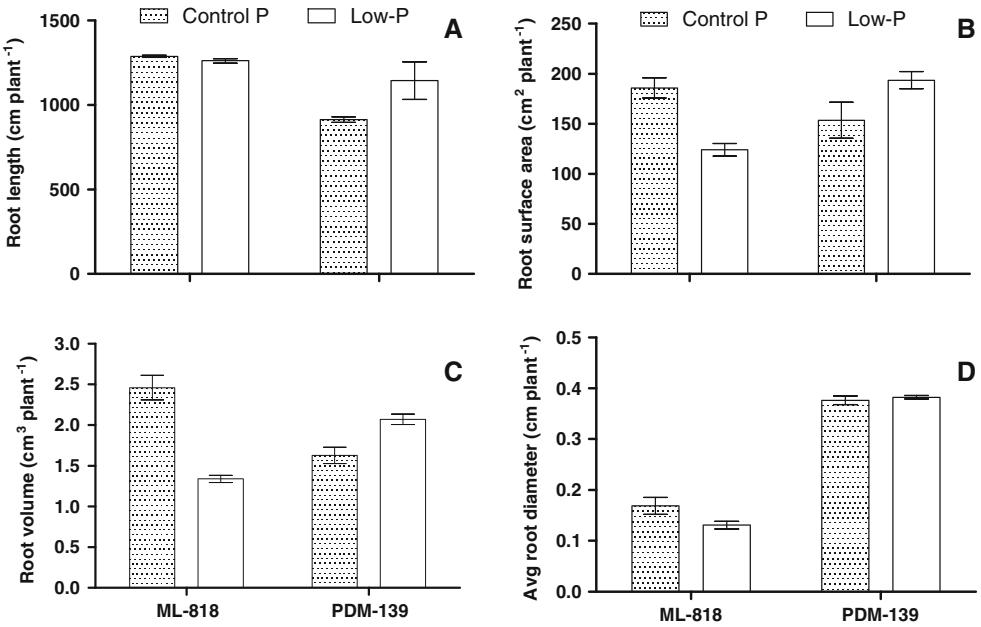

Fig. 4 Root morphology of contrasting green gram genotypes in response to levels of soil P—14.0 (low) and 41.8 (control) mg P kg^{-1} soil. **a** Total root length, **b** root surface area, **c** root volume, and **d** average root diameter. Data are mean ± SEM of three replicates

respectively, which indicates PDM-139 has 2.5 times thicker roots than the latter.

Phosphorus concentration and uptake

The P concentration of leaf, stem and root was significantly influenced by soil P levels (Fig. 5a–c). Leaf P concentration averaged over P levels was higher in PDM-139 (0.53 %) as compared to ML-818 (0.42 %) (Fig. 5a). Under sufficient P, there was 30 % increase in leaf P concentration in PDM-139 as compared to low-P while it was not significant in ML-818. However, the stem and root tissue P concentration averaged over P levels were significantly higher in ML-818 as compared to PDM-139 (Fig. 5b, c).

Total plant P uptake was significantly higher in soil with sufficient P in both genotypes which was 62 % in ML-818 and 88 % in PDM-139 (Fig. 5d). Nevertheless, total plant P uptake averaged over P level was higher in PDM-139 (23.3 mg plant^{-1}) compared to ML-818 (16.6 mg plant^{-1}).

Yield response of contrasting genotypes

Table 2 presents yield data indicating no significant effect of soil P level on number of pods per plant, grains per pod and 100-seed weight while grain yield was significantly affected by low soil P (Online Resource 2). Genotypic variation was noted in terms of 100-seed weight which was higher in ML-818 as compared to PDM-139. Grain yield decreased by 14 and 19 % in ML-818 and PDM-139, respectively, under low-P as compared to sufficient P.

Discussion

Differential response of genotypes to low-P for total root carbon exudation

It is well established that P deficiency induces exudation of organic compounds into the rhizosphere which includes high (ecto-enzymes, mucilages) and low (organic acids, sugars, amino acids) molecular weight compounds in a range of plant species (Marschner 1995). This characteristic phenomenon of exudation of carbon compounds under low-P was taken into account to screen green gram genotypes by exposing them to labelled carbon. Based on the relative amount of total ^{14}C exudation and biomass (Fig. 1), the genotypes were grouped into four categories. Genotypes falling under ER category were considered efficient in terms of total carbon exudation and biomass accumulation under low-P. However, those belonging to IER category would also perform better provided their root exudation is enhanced. The contrasting genotypes from ER (PDM-139) and IENR (ML-818) categories were used to quantify the carboxylic acid in root exudate.

External and internal concentration of carboxylic acids in contrasting genotypes

Major acids exuded into the rhizosphere were oxalic and citric while the internal concentration of oxalic, succinic and lactic acids were higher. A very less quantity of maleic acid was also synthesized in root apices but not released into the rhizosphere. Maleic and succinic acids were not detectable in the exudates while citric acid was not

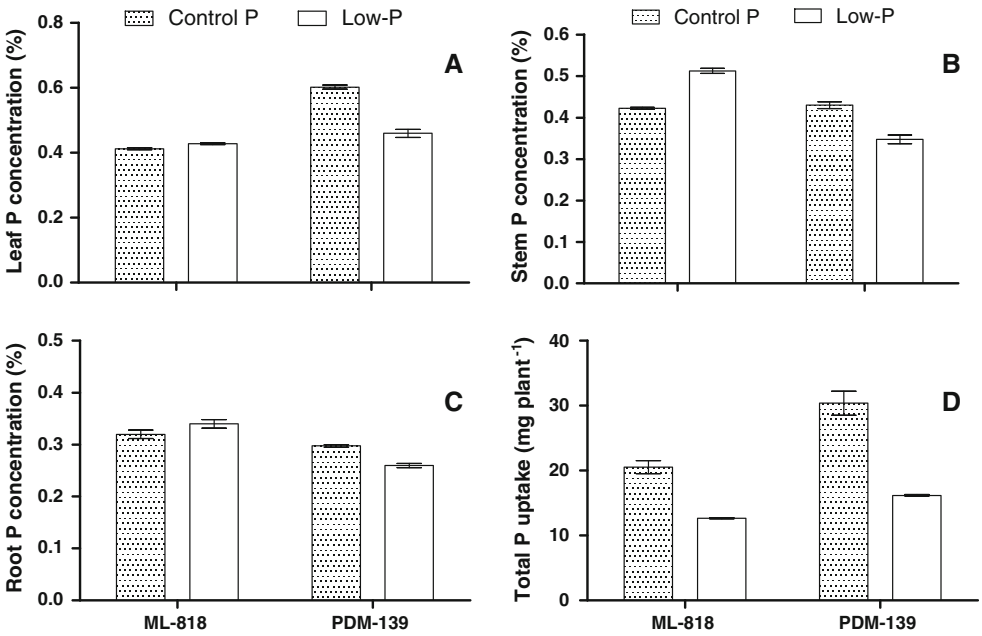

Fig. 5 Tissue P concentration and uptake in contrasting green gram genotypes grown in soil with two levels of P—14.0 (low) and 41.8 (control) mg P kg^{-1} soil. **a** Leaf P concentration, **b** stem P concentration, **c** root P concentration, and **d** total plant P uptake. Data are mean ± SEM of three replicates

Table 2 Effect of phosphorus level on yield attributes of green gram grown in soil

Variety	Treatment	Number of pods $plant^{-1}$	Number of grains pod^{-1}	100-seed wt (g)	Grain yield (g $plant^{-1}$)
ML-818	+P	14.41 ± 2.60	11.23 ± 0.46	3.67 ± 0.09	7.27 ± 1.19
	−P	14.72 ± 1.23	10.50 ± 0.23	3.70 ± 0.01	6.28 ± 0.37
	Mean	14.57 ± 0.15	10.87 ± 0.37	3.69 ± 0.02	6.78 ± 0.50
PDM-139	+P	15.61 ± 0.14	10.27 ± 0.07	2.97 ± 0.07	5.33 ± 0.23
	−P	13.22 ± 0.28	9.43 ± 0.18	2.77 ± 0.03	4.30 ± 0.25
	Mean	14.42 ± 1.20	9.85 ± 0.42	2.87 ± 0.10	4.82 ± 0.52

Data are mean ± SEM of three replicates

Sufficient P, 41.8 mg P kg^{-1} soil; Low-P, 14.0 mg P kg^{-1} soil

detectable in the root apices. This indicates the probabilities of maximum exudation of citric acid synthesized in roots, while succinic and lactic acids were synthesized but not exuded. Effective exudation of carboxylates into the rhizosphere depends primarily on the induction of membrane transporter/channel protein which allows efflux of carboxylates or organic anions into the root apoplast and rhizosphere. Among the genotypes, PDM-139 released almost double the amount of total carboxylic acid as compared to ML-818 while the root internal concentration was higher in the latter. Similar genotypic variation in chickpea (*Cicer arietinum*) for carboxylate exudation in rhizosphere under low-P was reported (Wouterlood et al. 2004). This genotypic variation could be due to the difference in efflux activity of organic anion transporters in response to P-stress.

The exudation of maleic acid into rhizosphere was not detected which may be attributed to the fact that maleic acid being a less stable dicarboxylic acid may be immediately converted to fumaric acid. Another possibility may be that in green gram, maleic acid may not be the dominant carboxylic acid involved in P solubilization. Plant species differ in efflux of carboxylic acids in terms of quality and quantity. A twofold increase in exudation of citrate was observed under P starvation in alfalfa (Lipton et al. 1987). *Brassica napus* (Zhang et al. 1997) and *Cicer arietinum* (Veneklaas et al. 2003; Gahoonia et al. 2007) were found to release large amounts of carboxylates from their roots under P-stress. Carboxylate exudation under low-P availability has also been reported in maize (Pellet et al. 1995), wheat (Sasaki et al. 2004) and soybean (Liao et al. 2006). The carbon exuded into rhizosphere is mainly contributed by non-photosynthetic carbon fixation in roots. Under P-stress, enhanced activity of phosphoenol pyruvate carboxylase accompanied by higher malate dehydrogenase and citrate synthase activities in roots of white lupin contributed about 25 and 35 % of the carbon exuded as citrate and malate, respectively (Johnson et al. 1994, 1996).

In this study, mostly oxalic acid was synthesized and exuded into the rhizosphere. Total ^{14}C exudation was found to be positively correlated with oxalic acid concentration while citric acid was negatively correlated at low-P

(Pandey et al. 2013). Oxalate being the simplest dicarboxylic acid with high acidity exhibits strong chelating power for some cations such as Ca^{2+}, Al^{3+}, Cu^{2+} and Fe^{3+} (Ryan et al. 2001). Oxalate concentration ranges from 6 to 10 % of tissue dry weight in several plant species (Zindler-Frank 1976). Several authors have shown that oxalate could detoxify metals or solubilize P as effectively as the other carboxylic acids such as citrate, malate, etc. (Kpomblekou and Tabatabai 1994; Ginting et al. 1998). In rapeseed, roots released citric and malic acids into the rhizosphere which solubilized P from rock phosphate (Hoffland et al. 1989). Therefore, oxalate exudation may have a greater advantage over others as far as physiological efficiency is concerned: (1) less carbon and energy consumption when being exuded, and (2) being amenable to metabolic engineering since it is not an intermediate metabolite like citrate and malate.

Growth and yield of contrasting genotypes under low-P

Reduction of biomass at low soil P compared to sufficient P was noted in both genotypes. However, PDM-139 produced highest biomass at both sufficient and low-P in comparison to ML-818. Similar biomass reduction under low-P supply in green gram has been reported by other workers (Chaudhary et al. 2008; Ali et al. 2010). Further, a reduction in whole plant leaf area and the rate of photosynthesis was observed in plants grown at low soil P. P deficiency restricts leaf expansion caused due to reduction in the rate of cell division and cell expansion (Assuero et al. 2004). To maintain the optimum photosynthetic rate, sufficient supply of P is necessary in green gram (Chaudhary and Fujita 1998; Chaudhary et al. 2008). Therefore, reduction in leaf expansion and photosynthate accumulation in green gram grown at low-P resulted in less biomass production.

Root morphological characters such as architecture (spatial arrangement), branching, density and length of root hairs have profound effect on acquisition of nutrients from soil. PDM-139 produced higher root surface area and root volume under low-P as compared to sufficient P. Variation in average root diameter seemed to be a varietal trait rather than the effect of P level. There are several reports on increase in root surface area, volume and root hair length under P-stress (Gahoonia and Nielsen 2004; Pandey and Gahoonia 2004; Magalhães et al. 2011). Higher lateral root density and root hairs contribute to increased root surface area resulting in higher exudation into the rhizosphere (Gahoonia et al. 2000). Plant size (biomass) also determines the rate of production and exudation of organic compounds into the rhizosphere (Thorsos 2011). Increased root growth in response to nutrient deficiency thereby improves acquisition of nutrients by plants.

The tissue P concentration did not show any specific trend in contrasting genotypes, however, total plant P uptake was significantly higher in PDM-139 as compared to ML-818 under both soil P levels. Higher P uptake in PDM-139 might be due to the increased root surface area and root volume, and higher amount of carboxylate exudation into the rhizosphere. Our previous study showed that carboxylate exudation in green gram plants grown hydroponically was linearly correlated with total plant P uptake and biomass at low-P (Pandey et al. 2013). Similarly, Shujie and Yunfa (2011) reported that the soybean genotype with improved root parameters together with high H^+ extrusion by roots led to increased relative P absorption efficiency to cope with low-P conditions.

Addition of P increases grain yield in green gram as reported by Kywe et al. (2007). Although PDM-139 translated its carboxylate exudation capacity into higher P uptake and its efficient utilization to produce biomass, yield traits were not improved. The grain yield of PDM-139 was less as compared to the inefficient genotype ML-818. The main reason was grain size, a varietal trait. PDM-139 has smaller size grains as compared to ML-818 resulting in lower grain weight as evident from 100-seed weight.

In conclusion, this study suggests that the genotype exuding higher total carbon compounds into the rhizosphere under low-P might contain higher amounts of carboxylic acid. Root exudate of green gram was primarily composed of oxalic and citric acids while root apices possessed higher concentration of oxalic, succinic and lactic acids. This suggests the possible role of oxalic and citric acids in solubilizing fixed P. Thus, higher total root exudation capacity of a genotype may not necessarily result in increased grain yield at low-P.

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