Indian J. Genet., 80(4) 412-418 (2020)
DOI: 10.31742/IJGPB.80.4.6

Unravelling the phosphorus use efficiency associated traits in mungbean (*Vigna radiata* L.) under low phosphorus condition

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(Received: August 2020; Revised: November 2020; Accepted: November 2020)

Abstract

Phosphorus (P) deficiency is one of the serious problems affecting plant growth in mungbean in different parts of the world. The root, shoot and biomass related traits were investigated for identifying P-efficient genotypes in 54 mungbean genotypes under low-P (LP) and normal-P (NP) conditions. In this study, the membership function value of P use efficiency of studied traits was used as a compendious index for studying P use efficiency (PUE) in mungbean. Among the studied traits, mean values of total root volume, chlorophyll concentration, root dry weight (RDW) and root to shoot ratio increased >25% under LP condition indicating that these traits are highly responsive to P deficiency. Correlation and stepwise regression analysis revealed that RDW explained most of the variation and could be used as a clear indicator of PUE. The five highly P-efficient genotypes namely, MH 805, M 42, PUSA 9531, EC 398885 and M 209 with high MFVP values may be used for PUE improvement in mungbean.

Key words: Green gram, membership function value of P use efficiency, phosphorus use efficiency, root traits, regression analysis

Introduction

Mungbean (*Vigna radiata* L.) or green-gram is an important grain legume, after chickpea and pigeonpea. Mungbean is thought to be native of the Indian subcontinent and from here it spread to Southeast Asia (Mogotsi et al. 2006). Cultivated mungbean was introduced into Eastern and Southern Asia, West Indies, Australia, South and North America (Oplinger et al. 1990). Globally, mungbean is cultivated in nearly 7 m ha, which fetches production to the tune of approximately 7 m tones (Nair et al. 2019). In India, during 2018-19, the mungbean was cultivated in 4.25 m ha area with 2.41 m tones of production (AICRP on MULLaRP 2018-19), which is more than 50% of the world acreage. The mungbean seeds contain approximately 25% protein, 62% carbohydrates, 1.5% oil, 3.5% fibre and 4.5% ash on a dry weight basis (Mubarak 2005). Mungbean seeds are more nutritive, non-flatulent, digestible and palatable in comparison with other legume seeds (Sadeghipour et al. 2010; Paul et al. 2018). Mungbeans are slightly sweet and are consumed as *dhal* or as sprouts or as dried beans. Especially sprouted mungbeans have a more antioxidants profile than normal mungbean seeds. Thus, mungbean is a nature's bestowal to mankind and can be used as a potential economic food supplement for malnutrition (Ganesan and Xu 2017).

Phosphorus (P) is one of the essential macronutrients for plant development and is required mainly for seed germination, flowering and fruit formation (Maharajan et al. 2018; Pandit et al. 2018). Further, it is also constituent of nucleic acids, ATP and plant hormones and it determines the quality and yield of crop plants to a large extent (Yun et al. 2001; Qiu et al. 2013). In plants, P-deficiency mainly activates a range of mechanisms including modifications of root architectural traits, changes in root physiology like exudation of organic acids, enhanced expression P transporters, increased root to shoot ratio and root microbial association (Shen et al. 2011; Meena et al. 2020). Among these, change in root morphology plays an important role in increasing P acquisition as greater root surface area explore given soil volume very effectively (Lynch 1995). The significant contribution of root length, surface area, volume and lateral roots

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Published by the Indian Society of Genetics & Plant Breeding, A-Block, F2, First Floor, NASC Complex, IARI P.O., Pusa Campus, New Delhi 110 012; Online management by www.isgpb.org; indianjournals.com

in P acquisition was observed in blackgram (Jakkeral et al. 2009). P deficiency causes accumulation of sugars and starch results in dark green foliage with higher chlorophyll concentration in leaves (Pandey 2015). In lantana (*Lantana camara*), a higher P supply is known to result in increasing the leaf number and leaf surface area whereas, low P supply results in higher root biomass than shoot biomass (Kim and Li 2016).

Mungbean is one of the biological N fixing legumes that can acquire 80-90% of N requirement through fixation (Heuer et al. 2017). Hence, small dose of N (15-25 kg N/ha) is enough to meet up the N requirement (Thiyagarajan 2003). Apart from N, mungbean requires 48 kg P_2O_5 per ton of grain production (FAI 2011). Keeping the importance of this pulse crop concerning P use efficacy (PUE), the present work was designed to study the genetic variation for root, shoot and biomass traits among mungbean genotypes in response to P-deficiency, to identify the ideal traits for screening of PUE in mungbean and to select the superior genotypes with high P-efficiency concerning screened PUE traits.

Materials and methods

The present investigation was carried out using 54 diverse mungbean genotypes under the hydroponic system to find the effect of P-deficiency on various traits at seedling stage. The experiment was conducted under partially controlled greenhouse conditions, at IARI, New Delhi, wherein various weather parameters were kept as 12 h photoperiod, $30^oC/18^oC$ day/night temperature and 90% relative humidity. For all genotypes, seeds were treated with $HgCl_2$ (0.1% w/v) and kept for germination in a paper towel. After the appearance of cotyledonary leaves, uniformly germinated seedlings were transferred to hydroponic trays with two P-levels i.e. low-P (LP) (3 µM) and normal-P (NP) (250 µM). The composition of modified Hoagland solution used was $MgSO_4$ (1mM), K_2SO_4 (0.92 mM), urea (5mM), Fe-EDTA (0.04 mM), $CaCl_2.2H_2O$ (0.75mM) and micronutrients [Na_2MoO_4 (0.6µM), H_3BO_3 (2.4µM), $ZnSO_4$ (0.6µM), $MnSO_4$ (0.9µM), $CuSO_4$ (0.62µM)] (Reddy et al. 2020). The pH of the nutrient solution was maintained around 6.0 using 1M KOH or 1M HCL and the solution was replaced every alternate day.

The experiment was performed in a completely randomized design (CRD) using three replications per genotype. The 21 day old seedlings grown under two P conditions were used for studying the root, shoot and biomass related traits. Root traits include total root length (TRL), total surface area (TSA), total root volume (TRV), root average diameter (RAD) and total root tips (TRT). These were recorded using a root scanner with WhiRhizo software (Pro version 2016a; Regent Instrument Inc., Quebec, Canada). Moreover, the primary root length (PRL), Chlorophyll concentration (CHL) and total leaf area (TLA) were measured using meter scale, MC-100 chlorophyll concentration meter (Apogee Instruments, Inc., USA) and leaf area meter (LI-COR 3000, Lincoln, NE), respectively. Separated root and shoot portions were dried at 60^oC until obtaining constant mass and then root dry weight (RDW), shoot dry weight (SDW), total dry weight (TDW) and root to shoot ratio (RSR) were recorded.

The PUE coefficient (PC) was computed as the ratio of data derived from LP to NP conditions of the same genotype for each trait using the formula $PC_{ij} = X_{ijLP}/X_{ijNP}$; where PC_{ij} is the PUE coefficient of j^{th} trait for i^{th} genotype. X_{ijLP} and X_{ijNP} are the value of the j^{th} trait for i^{th} genotype grown in LP and NP regimes, respectively. Mungbean P-deficiency tolerance was estimated by the Membership Function Value of PUE (MFVP). The MFVP value was estimated by using the formula $U_{ij} = (PC_{ij}-PC_{jmin}/PC_{jmax}-PC_{jmin})$ and $Ui = \frac{1}{n}\sum_{j=1}^{n} Uij$, where U_{ij} is the membership function value of the j^{th} trait for i^{th} genotype for P efficiency. PC_{jmax} is the maximum value of PC_{ij}. PC_{jmin} is the minimum value of PC_{ij}. U_i is the average membership function value of all the traits for the genotype (i) for P efficiency. Based on the mean value ($\bar{x}$) and standard deviation (SD) of MFVP, genotypes were divided into three categories of P efficiency. When $U_i \geq \bar{x} + SD$, high P efficient; $U_i \geq (\bar{x} - SD)$ to ($\leq \bar{x} + SD$), medium P efficient and $U_i \leq \bar{x} - SD$, low P efficient groups. Descriptive statistics, analysis of variance (ANOVA), heritability and correlation analysis were performed by using STAR (Statistical tool for Agricultural Research) 2.1.0 software (Gulles et al. 2014). Frequency distribution and multiple stepwise linear regression analyses were performed by using SPSS 16.0 software.

Results and discussion

The response of traits to P deficiency

The descriptive statistics of all the traits were explored as explanatory variables of P-deficiency tolerance (Table 1). Among 12 traits, TRL, TSA, TLA, and SDW

Table 1. Mean value, standard deviation (SD) of traits investigated under two phosphorus regimes and the phosphorus use efficiency coefficient (PC) of each trait

Traits	Mean ± SD (LP)	Mean ± SD (NP)	Mean ± SD (PC)
PRL	37.05 ± 6.87	32.73 ± 6.17	1.16 ± 0.24 A
TRL	699.84 ± 184.97	941.19 ± 191.85	0.75 ± 0.17 B
TSA	71.98 ± 18.87	99.99 ± 21.85	0.74 ± 0.20 B
TRV	0.87 ± 0.24	0.59 ± 0.16	1.57 ± 0.57 C
RAD	0.35 ± 0.03	0.32 ± 0.02	1.07 ± 0.05 A
TRT	722.53 ± 224.62	948.73 ± 208.05	0.76 ± 0.16 B
CHL	341.63 ± 37.10	236.46 ± 25.66	1.34 ± 0.15 C
TLA	31.07 ± 6.73	46.69 ± 12.79	0.70 ± 0.18 D
RDW	0.05 ± 0.02	0.03 ± 0.01	1.50 ± 0.54 C
SDW	0.12 ± 0.02	0.16 ± 0.04	0.80 ± 0.14 B
TDW	0.17 ± 0.04	0.19 ± 0.05	0.90 ± 0.15 B
RSR	0.36 ± 0.15	0.19 ± 0.06	1.93 ± 0.74 E

LP = Low phosphorus; NP = Normal phosphorus; PRL = Primary root length; TRL = Total root length; TSA = Total root surface area; TRV = Total root volume; RAD = Root average diameter; TRT = Total root tips; CHL = Chlorophyll concentration; TLA = Total leaf area; RDW = Root dry weight; SDW = Shoot dry weight; TDW = Total dry weight; RSR = Root to shoot ratio. In fourth column same letter indicates no significant difference at $p<0.01$ as determined by least significant difference test (LSD)

decreased >25% under LP compared to NP condition. Whereas, traits TRV, CHL, RDW, and RSR increased >25% under LP compared to NP condition. This indicates that these traits are highly responsive to P-deficiency and might be used to study the PUE in mungbean. Li et al. (2009) observed the increase of primary root length and root to shoot ratio and inhibition of shoot biomass and total biomass of seedlings in response to P-deficiency in rice. Similar observations were also recorded by Calderon-vazquez et al. (2009) in maize, Pearse et al. (2006) in wheat and Pandey et al. (2014) in mungbean.

The PC values of tested traits ranged from 0.70 (TLA) to 1.93 (RSR) with six trait values being higher than 1.00. Further, the least significant difference (LSD) test showed that significant differences ($p<0.01$) were present between the PC of TRV, CHL, RDW and RSR with other traits. The analysis of variance showed existence of highly significant variation among the genotypes for all 12 studied traits at $p<0.001$ (Table 2). Except for RAD, significant ($p<0.001$) interaction between genotype and P treatment was observed indicating the effect of P treatment. The coefficient of variation and broad-sense heritability ranged from 2.93 (CHL) to 14.33 (RDW) and 0.37 (TRV) to 0.88 (RAD), respectively among tested traits. This shows that

Table 2. Analysis of variance, coefficient of variation (CV) and broad sense heritability (h^2) of each trait under low phosphorus (LP) and normal phosphorus (NP) regimes

Variables	Means of squares			CV (%)	h^2
	Genotype (G) (df = 53)	Phosphorus (P) (df = 1)	G × P (df = 53)		
PRL	180.03***	1514.51***	75.71***	8.91	0.58
TRL	158545.42***	4730161.01***	54516.42***	11.94	0.66
TSA	1605.00***	63561.13***	894.95***	11.91	0.44
TRV	0.152***	6.409***	0.096***	14.08	0.37
RAD	0.003***	0.044***	0.000	5.14	0.88
TRT	240263.52***	4144617.36***	40965.90***	12.49	0.83
CHL	4592.77***	495052.96***	1489.4***	2.93	0.68
TLA	426.83***	19759.30***	200.58***	9.90	0.53
RDW	0.002***	0.019***	0.001***	14.33	0.67
SDW	0.006***	0.097***	0.001***	12.24	0.81
TDW	0.011***	0.030***	0.002***	11.08	0.85
RSR	0.048***	2.235***	0.028***	13.01	0.41

df = degree of freedom; PRL = Primary root length; TRL = Total root length; TSA = Total root surface area; TRV = Total root volume; RAD = Root average diameter; TRT = Total root tips; CHL = Chlorophyll concentration; TLA = Total leaf area; RDW = Root dry weight; SDW = Shoot dry weight; TDW = Total dry weight; RSR = Root to shoot ratio. *** Significant at p<0.001 respectively sufficient variability exists among the tested traits under the P limiting environment. The presence of traits with sufficient genetic variation and high heritability is a prerequisite for making genetic gain under selection. In this study, traits with higher genetic variation and high heritability are realistic selection criteria for mungbean breeding concerning PUE as also reported in common bean (Silva et al. 2016) and maize (Wang et al. 2019).

Identification of P efficient genotypes among tested genotypes

The large-scale screening for PUE is difficult as it is a complex trait under the control of many plant architectural traits. Thus, a compendious index called MFVP was derived using the PC of all studied traits to evaluate PUE in 54 mungbean genotypes (Table 3). The distribution of MFVP showed nearly normal distribution with a mean 0.41 and a standard deviation of 0.088. The highest and lowest values of MFVP were 0.69 (MH 805) and 0.17 (M1319B). Out of 54, five and eight genotypes were categorized into high (> $\bar{x}$ + SD) and low (< $\bar{x}$ – SD) P efficient groups, respectively. These five highly P-efficient genotypes (MH 805, M 42, PUSA 9531, EC 398885 and M 209) could be used in the mungbean breeding program for improving the PUE. Thus, MFVP can be used for the comprehensive assessment of PUE traits in mungbean as it mainly explains the degree of membership between traits values to PUE (Liu et al. 2017). A similar type of comprehensive approach was also used to estimate various traits such as drought tolerance in wheat (Chen et al. 2012) and maize (Li et al. 2015), and for salt tolerance in *Brassica* (Wu et al. 2019).

Correlation between MFVP and PC of each trait

Pearson's correlation coefficients between MFVP and PC of all tested traits and within the PC of each trait were estimated (Table 4). Of all the tested traits, PC of seven traits including PRL (r=0.44), TRV (r=0.54), CHL (r=0.71), RDW (r=0.78), TDW (r=0.73) and RSR (r=0.56) showed positive and significant correlations with MFVP. The correlation between TRL and TSA (r=0.94) was observed to be the highest, positive and significant which was followed by RDW and RSR (r=0.91). In addition, positive and significant

Table 3. Estimated membership function value of phosphorus use efficiency (MFVP) values of 54 mungbean genotypes based on traits tested under two phosphorus regimes.

S. No.	Genotype	MFVP	S. No	Genotype	MFVP	S. No	Genotype	MFVP
1	11/395	0.396	19	M 1393	0.426	37	M 989	0.392
2	BMGP 1	0.449	20	M 1435	0.434	38	MH 421	0.447
3	DMS 10	0.417	21	M 1443	0.351	39	MH 521	0.424
4	DMS 4	0.425	22	M 186	0.425	40	MH 805	0.695
5	EC 398885	0.543	23	M 201	0.421	41	ML 1451	0.409
6	EC 520034	0.489	24	M 209	0.534	42	ML 1666	0.489
7	HUM 12	0.320	25	M 260	0.324	43	ML 512	0.311
8	IC 436636	0.367	26	M 322	0.413	44	PM-5	0.450
9	KM 11-10	0.314	27	M 409	0.306	45	PUSA-0672	0.421
10	KM 12-29	0.394	28	M 418	0.428	46	PUSA-0831	0.362
11	KPS 3	0.356	29	M 42	0.614	47	PUSA-1031	0.363
12	LGG 450	0.457	30	M 450	0.427	48	PUSA-105	0.348
13	M 1129	0.347	31	M 460	0.473	49	PUSA-831	0.375
14	M 1209	0.381	32	M 512	0.306	50	PUSA-9072	0.472
15	M 1316	0.375	33	M 660	0.400	51	PUSA-9531	0.583
16	M 1319B	0.177	34	M 729	0.389	52	SM 11-75	0.318
17	M 1334	0.484	35	M 739	0.342	53	SML 1115	0.220
18	M 1354	0.366	36	M 961	0.413	54	SML 1815	0.496

Table 4. Pearson's correlation coefficients between the membership function value of phosphorus use efficiency (MFVP) and the phosphorus use efficiency coefficient (PC) of each trait

	PC_{PRL}	PC_{TRL}	PC_{TSA}	PC_{TRV}	PC_{RAD}	PC_{TRT}	PC_{CHL}	PC_{TLA}	PC_{RDW}	PC_{SDW}	PC_{TDW}	PC_{RSR}
PC_{TRL}	-0.12											
PC_{TSA}	-0.10	0.94***										
PC_{TRV}	0.25	-0.71***	-0.72***									
PC_{RAD}	0.00	-0.04	-0.02	0.25								
PC_{TRT}	-0.00	0.44***	0.46***	-0.49***	-0.05							
PC_{CHL}	0.24	-0.32*	-0.28*	0.61***	0.30*	-0.27						
PC_{TLA}	0.09	-0.29*	-0.29*	0.28*	0.18	-0.23	0.29*					
PC_{RDW}	0.28*	-0.29*	-0.29*	0.64***	0.36**	-0.20	0.64***	0.26				
PC_{SDW}	0.11	-0.22	-0.22	0.34*	-0.02	-0.14	0.21	0.20	0.05			
PC_{TDW}	0.25	-0.43**	-0.43**	0.67***	0.17	-0.28*	0.53***	0.35**	0.56***	0.83***		
PC_{RSR}	0.21	-0.19	-0.19	0.47***	0.32*	-0.13	0.52***	0.16	0.91***	-0.35**	0.19	
MFVP	0.44***	-0.07	-0.05	0.54***	0.47***	-0.02	0.71***	0.43**	0.78***	0.42**	0.73***	0.56***

PRL = Primary root length; TRL = Total root length; TSA = Total root surface area; TRV = Total root volume; RAD = Root average diameter; TRT = Total root tips; CHL = Chlorophyll concentration; TLA = Total leaf area; RDW = Root dry weight; SDW = Shoot dry weight; TDW = Total dry weight; RSR = Root to shoot ratio. *, ** and *** significant at p<0.05, p<0.01 and p<0.001 respectively.

correlations (p<0.001) were found between TRL and TRT (r=0.44), TSA and TRT (r=0.46), TRV and CHL (r=0.61), TRV and RSR (r=0.47), CHL and RDW (r=0.64), CHL and TDW (r=0.53), CHL and RSR (r=0.52), RDW and TDW (r=0.56) RDW and RSR (r=0.91) and SDW and TDW (r=0.83). In total, RDW found to be a more important trait among all investigated traits to mungbean PUE. The highly significant and positive correlations between MFVP and PC of seven traits indicate that these traits including PRL, TRV, CHL, RDW, TDW, and RSR could be utilized for evaluation of mungbean P efficiency. Also, Sandhu et al. (2019) while studying the correlations between physiological traits and nitrogen, phosphorus, iron, and zinc efficiency, reported that roots are important and play a complementary functional role in nutrient uptake in rice. On a similar note, in wheat, Nahar (2017) reported positive and significant correlations between shoot P uptake, total root length, and root hair length.

Multiple linear regression analysis with MFVP as a dependent variable

Since MFVP showed positive and significant correlations with most of the traits, stepwise multiple linear regression analysis was carried out for identifying the traits contributing most to the MFVP variability. The partial and cumulative R^2 values of five accepted PC of traits were estimated. It was found that 60.8%, 14.3%, 6.9%, 5.3% and 3.8% of MFVP variation was explained by PC of RDW, SDW, TSA, TLA, and PRL, respectively. Together, these five traits explained 90.1% of the total MFVP variation. For these identified traits, regression coefficients were also estimated. Following standardized regression coefficients, RDW determines the majority of variation in MFVP in comparison with other traits. From the PC of five identified traits, the best-derived equation for MFVP was as follows:

$$Y_{MFVP} = -0.247 + 0.121X1 + 0.248X2 + 0.143X3 + 0.122X4 + 0.074X5$$

where Y_{MFVP} was the membership function value of PUE, X1, X2, X3, X4, and X5 are the PC value of traits RDW, SDW, TSA, TLA, and PRL, respectively.

From the stepwise regression analysis, it was observed that the PC of RDW followed by SDW explained most of the variation of MFVP. Root dry weight probably combining TRL, TSA, TRV, and TRT are being easily measured under hydroponics system and can be explored for improvement of PUE. Further, by combining RDW and SDW i.e., source and sink capacity, can be exploited to develop more P efficient genotypes under P deficient condition. This stepwise regression was carried out by previous workers in cotton (Chen et al. 2019) and rice (Mori et al. 2016) to study the variation explained by PUE traits. Mori et al. (2016) found that root dry mass in rice was significantly associated with plant performance under

P deficient soils.

Using twelve-traits for the selection of P-efficient genotypes in mungbean and MFVP value as a compendious measurement to evaluate PUE, five genotypes (MH 805, M 42, PUSA 9531, EC 398885 and M 209) could be identified as high P-efficient genotypes. These genotypes could be used in the future breeding programme for PUE improvement in mungbean. From both correlation and stepwise regression analysis, it is clear that root dry weight is a clear indicator of P efficiency and explains the most percentage of variation. Moreover, the tested highly efficient mungbean genotypes need to be evaluated at field conditions under varied P levels. Besides, changes in root architectural traits in response to P deficiency needs to be studied in the large number of genotypes for confirming their contribution towards PUE. In future, detailed investigations should be aimed to understand the association between seedling stage traits with that of adult plant traits for PUE in mungbean.

Authors contribution

Conceptualization of research (HKD, RP, GPM); Designing of the experiments (VRPR, HKD); Contribution of experimental materials (HKD, MA); Execution of field/lab experiments and data collection (VRPR, MA, RP, MPS); Analysis of data and interpretation (VRPR, HKD, GPM); Preparation of the manuscript (VRPR, HKD).

Declaration

The authors declare no conflict of interest.

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