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Phosphorus Deficiency Induced Physiological and Antioxidant Response in Mungbean

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ABSTRACT

Background: Phosphorus (P) is the essential nutrient required for the growth and development of plants. P deficiency mainly leads to dark green foliage, alteration of root architectural traits and higher root to shoot ratio in plants. Further P deficiency results in enhancement of reactive oxygen species (ROS), thereby it leads to oxidative damage to plant cells. Plants have developed the mechanisms like production of antioxidants to overcome this effect.

Methods: In the present study, 18 genotypes were evaluated under a hydroponic system with normal and low P levels. After 21 days, the seedlings were used for investigating the physiological and antioxidant activity response of genotypes under normal and low P condition.

Result: The mean values of traits chlorophyll concentration, root dry weight, root to shoot ratio, H_2O_2, FRAP and DPPH were significantly higher under low P condition compared to normal P condition. The correlation and principal component analysis revealed that the traits RDW and TDW are major contributors to variation and could be used for P deficiency screening in mungbean. Based on major contributing traits of variation, the genotypes PUSA 1333 was identified as an efficient genotype and could be used in P use efficiency improvement in mungbean.

Key words: Antioxidants, Phosphorus, P deficiency, Physiological traits, Mugbean.

INTRODUCTION

Mungbean (*Vigna radiata* L.) is a fast growing, annual, herbaceous and thriftily most significant legume crop in the *Vigna* group. Globally, it is mainly cultivating in East Asia, Southeast Asia and Indian Subcontinent. The reported cultivated area and production of mungbean are 7 m ha and 5 m tones respectively in the world (Nair *et al*., 2019). In India, during 2018-19, it is cultivated in the area of 4.25 m ha area with 2.41 m tones of production (AICRP on MULLaRP 2018-19). Mungbean is a rich source of digestible protein and its seeds are palatable, non-flatulent and more nutritive than other legume crop seeds (Sadeghipour *et al*., 2010). Mungbean seeds mainly contain around 25% protein, 60% carbohydrates, 1.5% oil, 2% fibre and 4% ash on a dry weight basis. It is mainly consumed as dhal, salad and sprouts. The antioxidant activity of sprouts is six times higher than the normal seeds (Guo *et al*., 2012).

Phosphorus (P) is the second most macronutrient essential for plant growth and development. P deficiency mainly leads to dark green foliage with higher chlorophyll concentration in leaves (Pandey, 2015). Low P mainly results in higher root biomass than shoot biomass, whereas high P results in higher leaf number and area (Kim and Li, 2016). Besides, P deficiency mainly affects the rate of electron transfer in the electron transport chain thereby it leads to the accumulation of reactive oxygen species (ROS) that induces the oxidative damage in plants (Bargaz *et al*., 2013). This leads to cellular damage, change in permeability, electrolyte leakage and alteration in cellular metabolism (Blokhina *et al*., 2003). However, to cope with P deficiency, plants have evolved several strategies including alteration of root morphology, increasing root to shoot ratio, enhanced expression of P transporters and exudation of organic acids (Shen *et al*., 2011). To protect the cells from oxidative damage, plants have evolved the defense system like the production of antioxidant and protective molecules (Cruz de Carvalho, 2008). The antioxidant activity of the sample can be measured by invitro methods like diphenyl-picrylyhydrazyl (DPPH) scavenging assay, ferric reducing antioxidant power (FRAP) assay and hydrogen peroxide (H_2O_2) scavenging assay (Alam *et al*., 2012).

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The antioxidant activity response to abiotic stress has been well documented. However, the physiological and antioxidant activity response to P deficiency is scarce. Therefore, the present study was designed to study the genetic variability among the genotypes for physiological and antioxidant activity response to P deficiency and genetic analysis of the physiological and antioxidant activity responsive traits to identify ideal traits for screening P deficiency in mungbean.

MATERIALS AND METHODS

Plant material and experimental conditions

The present investigation was carried out by using eighteen diverse genotypes including PUSA 1033, PUSA 1333, PUSA 1031, PUSA 0672, OLRM 24, IPM 02-17, M1319B, Prakash Nephal, Bhutan Lm2, DMS 4, KM 12-29, SM 11-75, TM 9725, ML 1464, SML 668, MH 565, PLM 271 and ML 512 under the hydroponic system at Indian Agricultural Research Institute (IARI), New Delhi. The controlled environmental conditions maintained in the greenhouse during the experimental period include 28/18°C temperature, 12 hr photoperiod and 90% relative humidity. Initially, the seeds of all genotypes were treated with 0.1% (w/v) mercuric chloride and kept for germination. On the fifth day, the uniformly emerged seedlings were transferred to hydroponic trays containing modified Hoagland's solution with normal (250 µM) and low (3 µM) P levels (Reddy *et al*., 2020). The composition of modified Hoagland's solution used was $MgSO_4$ (1 mM), K_2SO_4 (0.92 mM), $CaCl_2$. $2H_2O$ (0.75 mM), Fe-EDTA (0.04 mM), Urea (5 mM) and micronutrients [H_3BO_3 (2.4 µM), $MnSO_4$ (0.9 µM), $ZnSO_4$ (0.6 µM), $CuSO_4$ (0.62 µM) and Na_2MoO_4 (0.6 µM)] (Sivasakthi *et al*., 2017). The nutrient solution was replaced on every alternate day interval and pH of the solution was maintained around 6.0 using 1 M KOH and 1M HCL.

Physiological traits measurement

After 21 days, the hydroponically grown seedlings were used for the evaluation of physiological and antioxidant activity response under both normal and low phosphorus conditions. The chlorophyll concentration (CHL) (µmol/m^2) and total leaf area (TLA) (m^2/plant) were measured using chlorophyll concentration meter (MC-100, Apogee Instruments, Inc., USA) and leaf area meter (LI-COR 3000, Lincoln, NE), respectively. The detached root and shoot portions were dried at 60°C until obtaining of constant dry weight and used for estimation of root dry weight (RDW) (g/plant) and shoot dry weight (SDW) (g/plant) using precision weighing balance. The root and shoot dry weights of each plant were summed and divided to get the total dry weight (TDW) (g/plant) and root to shoot ratio (RSR), respectively. The dried root and shoot portions were ground to obtain a fine powder and used for estimation of antioxidant activity response and P concentration.

Antioxidants measurement

The amount of H_2O_2 was measured as per the method described by Loreto and Velikova (2001). The H_2O_2 content of the solution was measured by a comparison of the absorbance values at 390 nm to a standard calibration curve in the range of 100 to 1000 µmol/mL and the concentration was expressed as µmol/g dry weight. The FRAP assay was performed by the following method given by Wong *et al*. (2006). The intensity of formation of blue colour was measured spectrophotometrically at 593 nm and the results are expressed in µmolTrolox Equivalent (TE)/dry weight. The DPPH radical method was used to determine the radical scavenging activity according to the Devi *et al*. (2019). The DPPH inhibition percentage was estimated by using the formula:

$$\text{Inhibition (\%)} = \frac{A_o - A_1}{A_1} \times 100$$

Where A_o and A_1 are initial and final absorbance of sample extract at 515 nm and methanol (95%) was used as blank. The P content of the sample was estimated as per the method of Murphy and Riley (1962). The P concentration was measured by comparing the absorbance at 660 nm with a standard P solutions curve and expressed as mg P/g dry weight. In the present study, descriptive statistics, analysis of variance, heritability, correlation analysis and principal component analysis were performed by using STAR (Statistical Tool for Agricultural Research) 2.1.0 software (Gulles *et al*., 2014).

RESULTS AND DISCUSSION

Trait response to P deficiency

The descriptive statistical values of physiological and antioxidant responsive traits under normal phosphorus (NP) and low phosphorus (LP) conditions were presented in Table 1. The mean values of physiological traits like CHL, RDW and RSR were significantly higher under LP condition than NP condition. Whereas traits TLA, SDW and TDW were higher under NP condition than LP condition. Under P deficiency condition, significant higher root dry weight and root to shoot ratio and lower total leaf area, shoot dry weight and total dry weight were observed in rice (Li *et al*., 2009), wheat (Pearse *et al*., 2006) and mungbean (Pandey *et al*., 2014). The dark green foliage with higher chlorophyll concentration under the LP condition is due to the accumulation of sugars and starch (Pandey, 2015). The antioxidant measurement traits like H_2O_2, FRAP and DPPH were significantly higher under LP condition than NP condition. Under P deficient conditions, a significant increase in antioxidant activity was observed in rice (Guo *et al*., 2012) and maize (Tewari *et al*., 2004). The P concentration was found to be higher under NP condition. These results showed the effect of P treatment on physiological and antioxidant responsive traits in tested

Table 1: Descriptive statistics of physiological and antioxidant response traits under normal and low phosphorus conditions.

Traits	P level	Mean+SD	Range	Heritability	CV
CHL	NP	244.94±24.98	210.37-292.37	0.91	3.22
	LP	321.36±41.24	240.1-380.97	0.94	3.23
TLA	NP	67.14±26.98	41.75-136.9	0.93	10.57
	LP	32.72±7.98	18.62-47.19	0.78	12.46
RDW	NP	0.047±0.021	0.023-0.11	0.88	13.52
	LP	0.054±0.024	0.016-0.107	0.85	13.99
SDW	NP	0.227±0.072	0.12-0.44	0.88	11.53
	LP	0.143±0.03	0.07-0.19	0.73	11.37
RSR	NP	0.204±0.054	0.122-0.311	0.87	10.29
	LP	0.373±0.129	0.194-0.588	0.90	11.35
TDW	NP	0.273±0.089	0.143-0.547	0.90	11.26
	LP	0.197±0.05	0.09-0.297	0.82	11.14
H_2O_2	NP	12.41±3.19	7.23-22.64	0.82	11.51
	LP	16.12±2.32	12.62-21.35	0.62	10.26
FRAP	NP	27.54±7.48	16.36-38.41	0.93	7.60
	LP	40.54±3.85	33.04-48.72	0.80	4.56
DPPH	NP	13.65±7.78	4.82-35.11	0.93	15.56
	LP	26.52±8.36	17.24-47.76	0.95	6.94
PC	NP	7.96±1.54	5.69-11.01	0.70	11.83
	LP	2.18±0.56	1.34-3.6	0.53	21.26

CHL, Chlorophyll concentration; TLA: Total leaf area; RDW: Root dry weight; SDW: Shoot dry weight; RSR: Root to shoot ratio; TDW: Total dry weight; H_2O_2: Hydrogen peroxide concentration; FRAP: Ferric reducing antioxidant power; DPPH: Diphenylpicrylhydrazyl; PC: Phosphorus concentration; SD: Standard deviation; CV: Coefficient of variation.

genotypes. The obtained results are in good agreement with the previous reports in rice (Wissuwa *et al*., 2015) and wheat (Yuan *et al*., 2017).

Among the studied traits, TLA (0.93), DPPH (0.93) and FRAP (0.93) under NP condition and DPPH (0.95) under LP condition recorded the highest broad sense heritability. Whereas, the P concentration was recoded lowest heritability under both P conditions. The coefficient of variation values was ranged from 3.22 (CHL) to 15.56 (DPPH) and 3.23 (CHL) to 21.26 (PC) under NP and LP conditions, respectively.This shows the presence of a significant amount of variability among the genotypes for investigated traits under P limiting condition. Silva *et al*. (2016) in common bean and Wang *et al.* (2019) in maize reported that traits with higher genetic variation with high heritability are reliable selection criteria for P use efficiency improvement.

Correlation analysis between traits

Pearson's correlation coefficients between the investigated traits under NP and LP conditions were presented in Table 2. Under NP condition, the highest positive and significant correlations were observed between SDW and TDW (0.989) followed by TLA and SDW (0.907) and RDW and TDW (0.873). Whereas none of the traits showed a positive correlation with P concentration. Under the LP condition, the highest positive and significant correlations were observed between SDW and TDW (0.936) followed by RDW and TDW (0.9) and RSR and RDW (0.878). The PC showed significant and positive correlations with RDW, RSR, TDW, H_2O_2 and DPPH. Interestingly, among physiological traits, the correlations between SDW and TDW, TLA and SDW, RDW and TDW, RDW and RSR, TLA and TDW were found to be significant and positive under both P conditions. Whereas, among the antioxidant measurement traits, the correlation between H_2O_2 and DPPH was positive and significant under LP condition only. In soybean, Kakiuchi and Kamuji, (2015) reported a significant and positive correlation between shoot dry matter and P content accumulation. The activity of antioxidants was significantly increased under P deficiency condition in tested genotypes of Brassica (Chen *et al*., 2015) and rice (Veronica *et al*., 2016).

Traits explaining higher percentage of variation

Principal component analysis (PCA) was carried out to identify the most contributing traits of variation under both normal and low P conditions. The first three principal components (PC) having more than one eigen value explained the variation of 76% and 77% under NP and LP conditions, respectively (Table 3 and Fig 1). Under NP condition, the most contributing traits of variation are TDW, SDW and RDW in PC1 and RSR, H_2O_2 and FRAP in PC2. Whereas the traits TDW and RDW in PC1 and H_2O_2 and FRAP in PC2 are the most contributing traits of variation under LP condition. Overall from PCA analysis, physiological traits RDW and TDW and antioxidant measurement traits H_2O_2 and FRAP are the most contributing traits of variation under both P conditions. Under P deficiency, the root and

shoot dry weights and root to shoot ratio of genotypes contribute a higher percentage of variation among P efficient traits in soybean (Xiang wen *et al*., 2008). P deficiency leads to increase of H_2O_2 concentration (Yao *et al*., 2007) and thereby results in significant enhancement of antioxidant activity measured through FRAP assay in tomato (Koleska *et al*., 2017)

Identification of efficient genotypes

The 18 genotypes evaluated in the present investigation showed significant variation for both physiological and antioxidant measurement traits (Fig 2 and 3). Among the physiological traits, for the most contributing traits of variation, the genotype PUSA 1333 showed the highest RDW, SDW and TDW under both NP and LP conditions. Whereas the genotype SML 668 showed lowest RDW, SDW and TDW under both NP and LP conditions. For antioxidant measurement traits, the genotypes PUSA 1031 under NP condition and PUSA 0672 under the LP condition recorded the highest H_2O_2 concentration. Whereas the genotype, ML 512 under NP condition and Prakash Nephal under LP condition recorded lowest H_2O_2 concentration among the 18 genotypes. For FRAP assay, the genotype PUSA 0672 under NP condition and PUSA 1333 under LP condition recorded the highest FRAP values. Whereas the genotype Bhutan Lm2 under NP condition and MH 565 under LP condition recorded the lowest values for FRAP assay. For the DPPH assay, the genotype PUSA 1333 recorded the

Table 2: Pearson's correlation coefficients between investigated traits under normal P (NP) and low P (LP) conditions.

Traits	CHL	TLA	RDW	SDW	RSR	TDW	H_2O_2	FRAP	DPPH	PC
CHL	1	-0.185	-0.039	-0.050	-0.037	-0.049	-0.037	-0.032	0.303	-0.235
TLA	0.231	1	0.565*	0.907***	-0.179	0.861***	0.009	-0.315	0.275	0.096
RDW	0.644**	0.322	1	0.795***	0.657**	0.873***	0.421	-0.162	0.415	0.346
SDW	0.544*	0.557*	0.689***	1	0.088	0.989***	0.146	-0.343	0.339	0.166
RSR	0.555*	0.198	0.878***	0.301	1	0.225	0.585**	0.169	0.201	0.393
TDW	0.642**	0.493*	0.900***	0.936***	0.608**	1	0.216	-0.312	0.369	0.212
H_2O_2	0.140	-0.075	0.317	0.417	0.119	0.402	1	0.387	0.057	0.287
FRAP	0.184	0.202	0.249	0.294	0.126	0.298	0.417	1	0.066	-0.049
DPPH	0.455	0.111	0.701***	0.557*	0.513*	0.675***	0.502*	0.437	1	0.283
PC	0.197	0.321	0.630**	0.436	0.523*	0.567**	0.462*	0.459	0.654***	1

Correlation coefficients under NP and LP conditions were listed in the upper right and lower left respectively. The diagonal indicatesthe correlation coefficients between the same traits under both phosphorus conditions. *, ** and *** significant at $p<0.05$, $p<0.01$ and $p<0.001$ respectively. CHL: Chlorophyll concentration; TLA: Total leaf area; RDW: Root dry weight; SDW: Shoot dry weight; RSR: Root to shoot ratio; TDW: Total dry weight; H_2O_2: Hydrogen peroxide concentration; FRAP: Ferric reducing antioxidant power; DPPH: Diphenylpicrylhydrazyl; PC: Phosphorus concentration.

Table 3: Principal component analysis of investigated traits under two phosphorus conditions.

Traits	Normal phosphorus			Low phosphorus		
	PC1	PC2	PC3	PC1	PC2	PC3
CHL	-0.05	-0.02	-0.79	-0.29	0.31	-0.17
TLA	0.35	-0.34	0.08	-0.20	0.31	0.68
RDW	0.42	0.15	-0.06	-0.41	0.17	-0.23
SDW	0.42	-0.22	-0.02	-0.36	0.09	0.37
RSR	0.18	0.54	-0.02	-0.31	0.25	-0.46
TDW	0.43	-0.14	-0.03	-0.41	0.14	0.11
H_2O_2	0.15	0.50	0.05	-0.22	-0.57	-0.03
FRAP	-0.12	0.41	-0.08	-0.20	-0.48	0.25
DPPH	0.21	0.08	-0.54	-0.35	-0.24	-0.20
PC	0.20	0.29	0.24	-0.32	-0.27	-0.01
EigenValues	4.94	2.14	1.30	5.22	1.43	1.13
%Variance	0.45	0.19	0.12	0.52	0.14	0.11
Cumulative Variance%	0.45	0.64	0.76	0.52	0.66	0.77
Most contributingtraits	TDW, SDW, RDW	RSR, H_2O_2, FRAP	PC	TDW, RDW	H_2O_2, FRAP	TLA, RSR

NP: Normal phosphorus; LP: Low phosphorus; PC1: Principal component 1; PC2: Principal component 2; PC3: Principal component 3; CHL: Chlorophyll concentration; TLA: Total leaf area; RDW: Root dry weight; SDW: Shoot dry weight; RSR: Root to shoot ratio; TDW: Total dry weight; H_2O_2: Hydrogen peroxide concentration; FRAP: Ferric reducing antioxidant power; DPPH: Diphenylpicrylhydrazyl; PC: Phosphorus concentration.

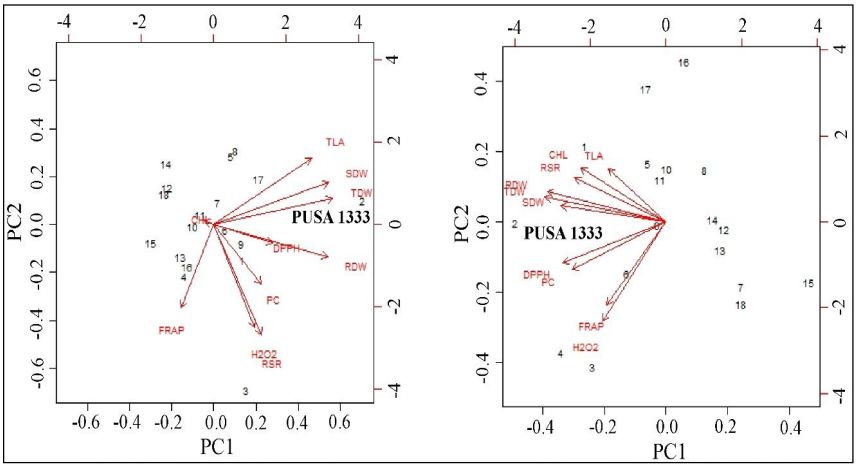

Fig 1: Biplots of first two principal components showing variation among the P uptake and utilization efficiency traits under normal P (NP) and low P (LP) condition.

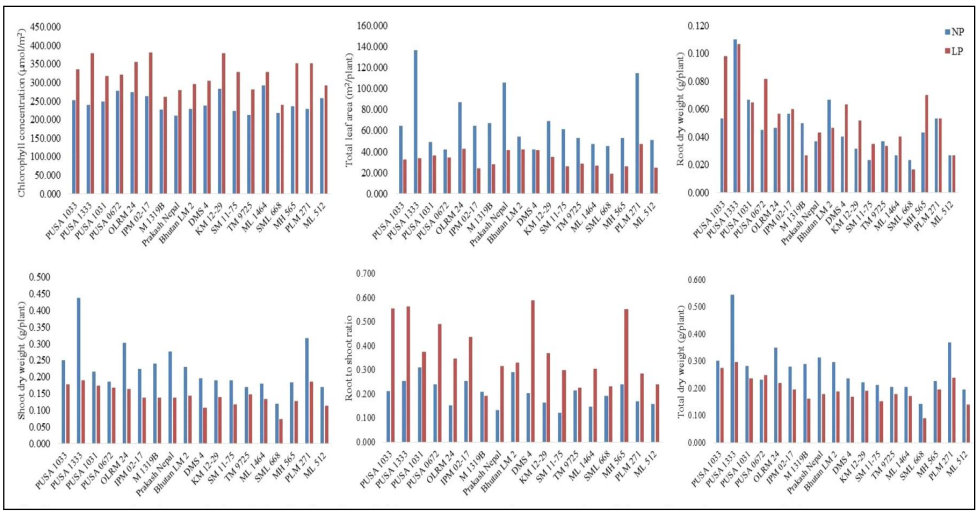

Fig 2: Measured values of physiological traits of 18 genotypes under normal and low phosphorus condition.

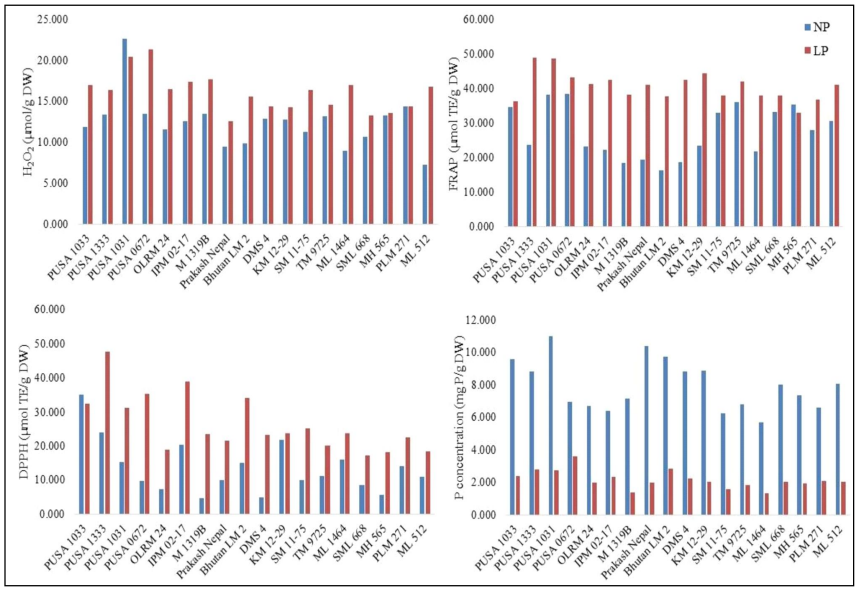

Fig 3: Measured values of antioxidant measurement traits and phosphorus concentration of 18 genotypes under normal and low phosphorus condition.

highest value under the LP condition. The genotypes PUSA 1031 and PUSA 0672 recorded the highest concentration for P content under NP and LP conditions, respectively. The efficient genotype must give the best performance under both normal and stress conditions, but not in either of the situation (Bilal *et al*., 2018). Overall the genotype PUSA 1333 showed the best performance for most contributing traits of variation and it is found to be efficient under P limiting condition among the studied genotypes.

CONCLUSION

From the experiment, it can be inferred that the higher mean values of CHL, RDW, RSR, H_2O_2, FRAP and DPPH under LP condition indicating that these are highly responsive traits to P deficiency. Further, the traits RDW and TDW explained the most percentage of variation among the genotypes. This suggests that these are ideal selection criteria for screening the large number of germplasm under P limiting environment. The genotype PUSA 1333 was identified as efficient genotype and it could be used in future breeding programmes to improve P use efficiency in mungbean.

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