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GENETIC VARIATION IN PHOSPHORUS DEFICIENCY TOLERANCE AMONG BANGLADESHI RICE (*ORYZA SATIVA* L.) CULTIVARS AND INTROGRESSION LINES WITH IR 64 GENETIC BACKGROUND

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ABSTRACT

Phosphorus is vital nutrient for the crop yield, and Breeding rice for tolerant to low phosphorus, efficient in uptake and assimilation is the best way for sustainable production. This study aimed to evaluate Bangladeshi rice cultivars and introgression lines (INLs) under phosphorus deficient soil to understand the genetic variation in deficiency tolerance. A total of 28 rice genotypes from various ecotypes such as Aus, Aman, Boro and Jhum and INLs were collected and grown in pot contained highly phosphorus deficient soil in the rooftop polythene shed house during October 2019 to March 2020. A phosphorus deficiency susceptible variety, IR 64, was used as control, and experiment was conducted following randomized complete block design with two replications. Biomass related traits such as dry weight (DW) and relative dry weight (RDW, %) were analyzed at early vegetative stage. Visual score based on the responses to artificial drought occurred due to absence of water for 5 consecutive days because of government imposed Covid-19 lockdown were also evaluated in a scale of 0 to 4. Plants showed wide variation in the measured traits in both in the phosphorus added normal or phosphorus deficient conditions. Two patterns of responses were observed. One pattern was similar to susceptible control IR 64 and another is highly sensitive to P- deficiency. Cluster analysis resulted four groups (I to IV). Group I consist of four rice varieties including Pathar kuchi, Lal dhan, INL-9, and INL-30, and showed low DW and low tolerances to phosphorus deficiency and artificial drought. Group II contain nine accessions including IR 64, Murali, Kuti Agrani, Kernaicha, and five INLs, and showed higher DW and susceptibility to phosphorus deficiency and artificial drought. Group III had medium DW and highly sensitive to phosphorus deficient condition and the accessions Kali jira and Aus (Awned) were included. Two jhum variety, Renkhoa Dhan and Galongpru, and seven INLs belong to the group IV which showed medium DW but tolerant to phosphorus deficiency and artificial drought compare to other groups. The genetic variations of DW and RDW under phosphorus deficient and artificial drought conditions were clarified among rice varieties in Bangladesh and INLs with IR 64 genetic background, and several varieties and INLs were found as the promising materials for further breeding program.

Keywords: genetic variation, Phosphorus deficiency tolerance, rice, Relative Dry weight (RDW, %)

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INTRODUCTION

Rice is a staple food for more than half of the global population with 90% of the global production is from Asia (Fukagawa and Ziska, 2019). Phosphorus (P-) is second to nitrogen, the most restrictive nutrient in plant growth and development including rice. P- deficiency has been established as a significant limiting factor for the yield of rice worldwide (Gamuyao, *et al.,* 2012). Mined rock phosphate is used for the manufacture of P- fertilizer to improve crop yields, and its demand is increasing throughout the world including Bangladesh (Rose, *et al.,* 2013; BRRI, 2015). Given its non-renewable existence, past year's price of P- fertilizer rose several folds and further price increases seem unavoidable (Cordell, *et al*., 2009). Bangladesh government spent over 71 billion Bangladeshi taka in the year 2012-2013 to import P-fertilizer (BER, 2014; FEMU, 2015) and become the top P- import dependent country (Roy *et al.,* 2019). Developing cultivars with tolerance to P-deficiency may represent a more sustainable solution than sole reliance on fertilizer application. Besides, continual removal of P- from fields through rice grains at harvest results in lower soil fertility in low-input farming systems and drives the need for fertilizer in high-input system (Pariasca-Tanaka, *et al*., 2015). Previous study on maize resulted that the shoot biomass, biomass of primary and secondary roots and P- content of all the genotypes were reduced at P-deficiency while the P-efficient lines were less affected (Mi, *et al*., 2004). The genotypic variation of rice was found for tolerance to P-deficiency that can be used successfully in rice improvement (Aluwihare, *et al.,* 2016). However, for modern farming systems, P-deficiency can be mitigated by applying fertilizer, but there are financial difficulties with growing fertilizer prices for farmers and inefficient practices can cause environmental issues.

The present and anticipated global food demands necessitate a significant increase in crop productivity on these less favorable problem soils. Understanding plant diversity is relevant to assess plant behavior in relation to adaptation to P-deficient conditions, and designing an effective phenotyping strategy requires thorough understanding of plant growth under low P. Therefore, assessment of genotypic variability under low P-conditions is an important precondition for a successful screening program focused on P-deficiency tolerance.

Currently, there is no such cultivars found and Breeding P-efficient rice for Bangladesh is essential to reduce our dependency of phosphate fertilizer, and cut cultivation cost. Therefore, the present study aimed to evaluate the Bangladeshi rice germplasms and improved breeding lines such as INLs under higher P- deficient condition to know the tolerance levels as well as finding their morphological variations.

MATERIALS AND METHODS

Plant Materials: At the outset of the experiment, a total of 72 rice germplasms including 52 Bangladeshi rice accessions from the genetic resources division of Bangladesh rice research institute (BRRI), Gazipur, Bangladesh, and 20 improved introgressions lines (INLs) developed and reported by from Fujita, *et al.* (2009), through a standard material transfer agreement (SMTA) between NSU and JIRCAS, were collected. Bangladeshi accessions were selected from various varietal groups and ecotypes such as Aus, Aman, Boro and Jhum and stress tolerance levels as explained by Khan, *et al.,* (2017) and the INLs have much potential for yield improvement under problem soils (Uddin, *et al.,* 2016). However, only 28 cultivars in total could finally be used (Supplementary Table 1) for this experiment due to lack of germination of Aus varieties including P-deficiency tolerant variety "Kasalath" due to low temperature in the growing period as well as damage occurred by insect attack. Rice variety IR 64 was used as P-deficiency susceptible control (Roy, *et. al.,* 2021).

Collection of P-deficient soil: A total of six soil samples consisted with top and sub soils from three representative P-deficient areas such as Bagura sadar (Bagura), Chandina (Cumilla) and Chandra (Gazipur Sadar) were collected based on the information of phosphorus status of Bangladeshi soil by Egashira, *et al.,* (2003). Top and sub soil sample were collected from the unplanted rice field or after rice harvest field following the sampling procedures explained by Piegne, *et al*., (2009) and chemical analysis was done by the Soil Resources and Development Institute (SRDI) laboratory, Dhaka, and the report is shown in the Table 1. All the standard method such as micro kjeldal for nitrogen (N), olsen for P- (while pH is >7) or Bray and Kurtz for P- (pH<7) were followed (SRDI, 2020). Among the samples, the sub-soil from Chandra (Gazipur) was found most P-deficient (yellow brown) and used in the experiment. This soil was characterized as highly acidic (pH= 5.3), low organic matter (1%), low P- (1.7 mcg/gm), low total nitrogen (0.05%) (Table 1).

Table 1: Soil characteristics of the experimental site (Chandra, Gazipur, Dhaka).

Collected Soil		Parameters								
Soil sources	Soil Type	Reaction	PH	Organic Compound	Total Nitrogen	K	P	S	B	Cu
				%		meq/100g	mcg/gm (ppm)			
Chandra (Gazipur)	Top soil in rice field	Mild Acidic	5.7	1.1	0.055	0.24	3.65	24.14	0.35	3.66
Chandra (Gazipur)	Sub soil in rice field	Highly Acidic	5.3	1	0.05	0.12	1.7	7.14	0.28	2.18

Growth conditions: Selected rice genotypes were grown in the earthen pots of 20cm×25cm size at the rooftop shed house made from 200 µ polyfilm at the top on the bamboo structure for UV and rain protection at Uttara, Dhaka, Bangladesh (23°52'25.5036"N and 90° 23'47.2344"E) in the late Aman season from a period of November 2019 to March 2020 (Figure 2). Temperature was lower during the growing period which is around 18 to 26 ° C (Figure 1).

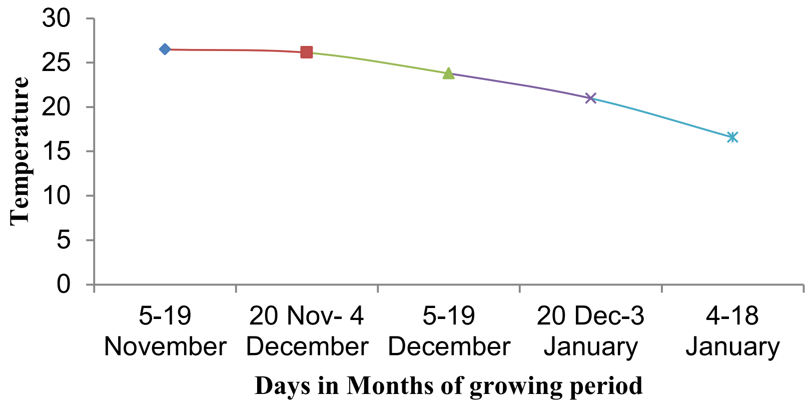

Figure 1: Temperature curve in 15 days' interval during growing season.

The temperature was recorded from Huafeng-AccuWeather mobile apps everyday around 9 to 10 am at the experimental site on the rooftop.

Seedlings development and treatment: A total 15 seeds of each genotypes were separated into small vinyl packs and disinfect them by fungicide Bavistin (Carbendazim) at a rate of 0.012g/pack for 24 hours according to the pack instructions. Before sowing seeds to the nursery tray seeds were soaked in distill normal water for 24 hours. After the seed sowing, the tray was fully covered with newspapers until the seeds started sprouting with regular watering. Twenty days old healthy seedlings (one for each accessions) were transplanted into the pot. Two replications of each treatment (P-added & without-P) were ensured and arranged in a randomized complete block design (RCBD) design. Bangladesh Agricultural Research Council's (BARC) recommended dose of Fertilizers for Aman rice (Chowdhury and Hassan, 2013) such as 195-52-82-60-0 for N-P-K-S were used and recalculated amount for one pot (0.975g-0.325g-0.41g-0.3g) were mixed with the soil and transplanted. In the P-control treatment P_{52} (52 kg P_2O_5 ha^{-1}) and other fertilizers were added and in the P- deficient treatment no P_0 (0 kg P_2O_5 ha^{-1}) added but other fertilizers were added. Watering was done in the morning and evening and non-stress conditions was maintained. Weeding and other related agricultural practices were done in a standard manner (Figure 2).

Figure 2: Photos showing the (A) differences of + P and - P responses of a representative plant, (B) variation in responses of cultivars in +P and –P pot soil condition

Measurement of dry weight (DW) and calculation of relative dry weight (RDW): Plant was harvested the plants at vegetative stage (68 days after sowing).

Harvesting was done by cutting the plants at the base at around 68 days, put them into the paper bag and keep for 10 days for sun dry. The dry weight (DW) were taken of the well dried plants and calculated the relative dry weight (RDW, %) (Namai, *et al.,* 2009).

$$\text{RDW (\%)} = \frac{DW\ of\ non\ P\text{-}\ treatment}{DW\ of\ P\ added\ control} \times 100$$

Where, DW is the dry matter weight of the leaves and stems (mg).

The visual score of the plants in a scale of 0 to 4 with a minor modification of standard evaluation system of IRRI (1980) and Roy, *et al.,* (2021) where 0 indicate no damage or dry symptom, 1 for up to 25% damage, 2 for up to 50% damage, 3 for up to 75%damage and 4 indicate up to 100% or death.

Statistical analysis: The descriptive statistics of the measured traits among the germplasms and data frequency distribution were calculated using Microsoft Excel (Redmond, WA, USA). The analysis of variance (ANOVA) with significance tests as well as cluster analysis were performed using statistical packages for social sciences (SPSS) version 19.0 respectively.

RESULTS AND DISCUSSION

A total of 28 rice genotypes including 10 Bangladeshi rice accessions and 18 improved INLs were used. Seedlings were grown under recommended dose P-added and without P- condition in the pot with P-deficient soil collected from Chandra (Gazipur). The growth condition was highly deficient of P. Plants showed wide variation in the measured traits in both P-added & P-deficient condition (Figure 2 and 3). The DW was ranged from 12 mg to 942 g (Supplemental Table 1 and Figure 3). Two patterns of P-deficiency susceptibility were observed. The first pattern was like very low DW in both conditions such as lal dhan and pathar kuchi and INL9 are seemed highly sensitive or susceptible to the P-deficiency stress and showed less growth (Figure 3). Second pattern was like some plants showed very high DW under P- added condition but less in non P- condition such as Kernaicha and these plants are P- susceptible too similar to IR 64 (Figure 3). Due to low temperature during the growing period as well as P-deficiency stress the growth of the plants were slower.

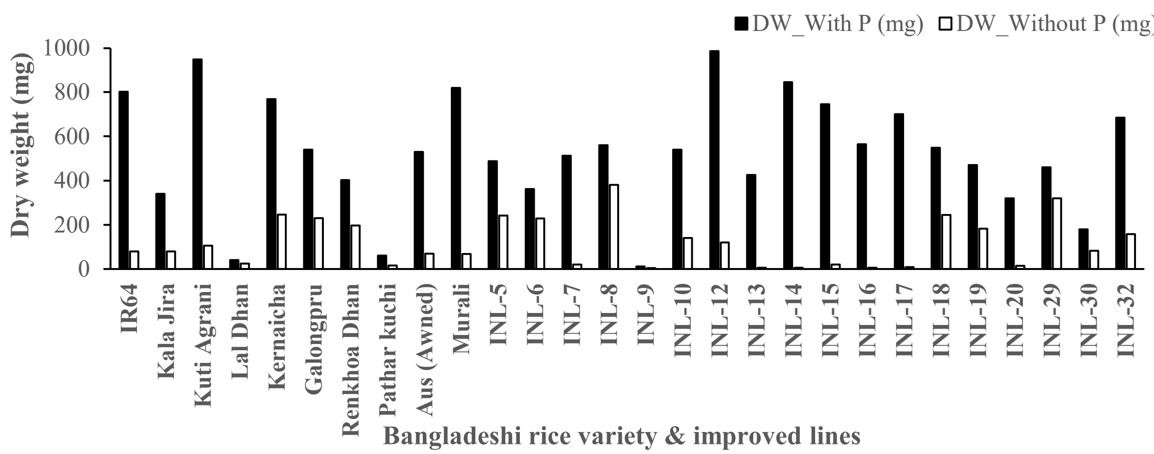

Figure 3: Variation of dry weight of the 28 Bangladeshi rice genotypes and improved lines in phosphorus and no phosphorus treated condition.

To understand more details, we further divided all the germplasms into four groups (I to IV) (Figure 4 and Table 2).

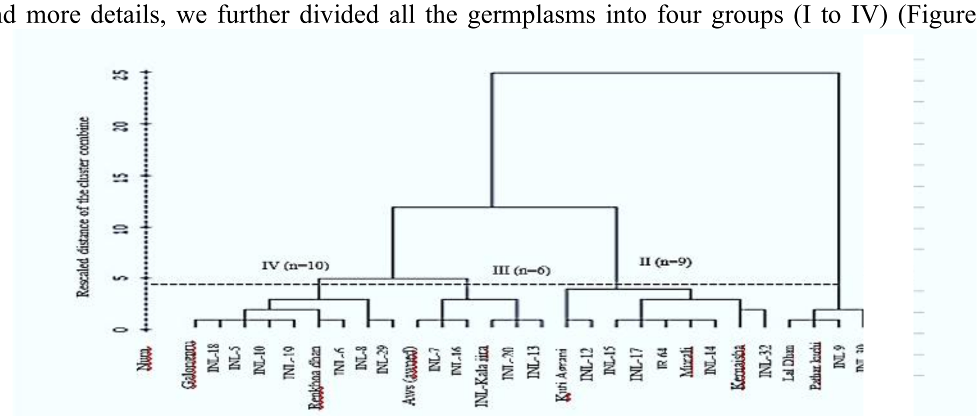

Figure 4: Classifications of 28 rice genotypes. Cluster analysis carried out by Ward`s Hierarchical analysis using SPSS. Rice varieties & improved lines were grown in the 20× 25 inch pot at the roof top in a building at Uttara, Dhaka, and the plants were treated with phosphorus and no phosphorus of recommended dose. Mean value of two replications for each accessions were calculated in each trait. DW, Dry weight; RDW, Relative dry weight

Table 2. Characteristics of four cluster groups

Cluster Group	DMP (mg)		RDW(%)	Score for drought tolerance	
	+P	-P		+P	-P
I (4)	73.0	32.0	42.2	1.8	1.1
II & IR 64 (9)	810.9	90.2	11.3	1.4	1.8
III (6)	448.5	32.5	7.9	1.8	0.3
IV (10)	485.8	240.3	50.1	2.3	2.8

Group I consist of 4 rice cultivar including Pathar kuchi, lal dhan, INL-9 and 30, and showed lower DW under both P-added and P-deficient conditions together with higher susceptibility to drought. IR 64 belongs to the Group- II together with Murali, Kuti Agrani, Kernaicha and other 5 INLs. They have higher DW in P-added condition but very low RDW in P-deficient condition and also susceptible to drought. We assume that overuse of P-fertilizer for long years and selection pressure may have contributed for this kind of response. Group III cultivars have medium DW under P-added and low RDW under P-deficient conditions and Kali jira and Aus (Awned) are belong to this group. Two jhum variety, Renkhoa Dhan and Galongpru and 7 INLs are belong to the group IV which has moderate to high RDW under both P-added and P-deficient conditions. Rice cultivars of this group showed higher levels of tolerance to drought and are promising for further breeding program to develop P-deficiency tolerant variety. Jhum is a shifting cultivation method practiced in the hilly terrace and these varieties are well known for various stress tolerance which corresponded with our results. This outcome will be useful in rainfed upland rice breeding (Roy, *et al.,* 2021). We cannot anticipate whether Pup-1 (Wissuwa and Ae*.,* 2001) or other gene responsible for this deficiency tolerance, however, Chin, *et al*., (2010) mentioned that Pup-1 is largely absent in modern variety but is highly conserved in stress tolerant breeding lines and upland varieties. This study allowed us to know about the response of Bangladeshi rice germplasms and selected lines under P-deficient soil and indicated the P-deficiency tolerance (use efficiency) levels. We paved the way to study P-deficiency tolerance in Bangladesh and this will help to breed P-efficient rice which can reduce our dependency of Phosphate fertilizer as well as cut cultivation cost. Evaluation of P-deficiency tolerance at mature stage as well as inclusion of molecular analysis would be good to understand p-status of Bangladeshi germplasms more clearly and we suggest for further studies by the inclusion of root traits.

Conflict of Interest: The authors declare that this experiment was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary Table 1. Detail of the 28 rice genotypes and improved INLs. Their DW, RDW for P-deficiency tolerance and visual score for artificial drought stress.

Sl. No.	Variety/Line name	YTH/ Line No	Origin (Acc. No.)	Ecotype	DW_ With P (mg)	DW_Without P (mg)	RDW(%)	Visual score of drought tolerance With P	Visual score of drought tolerance Without
1	IR64	YTH-1	JIRCAS. JP	Boro*	802.00	80.00	9.98	2.00	3.00
2	Kala Jira	L-14	BRRI-305	Aman	340.00	80.00	23.53	0.00	0.00
3	Kuti Agrani	L-27	BRRI-1588	Aman	948.00	106.00	11.18	0.00	1.00
4	Lal Dhan	L-29	BRRI-7441	Jhum	40.00	25.00	62.50	0.00	1.00
5	Kernaicha	L-30	BRRI-7442	Jhum	769.00	246.00	31.99	1.00	3.00
6	Galongpru	L-32	BRRI-7444	Jhum	540.00	230.00	42.59	1.00	3.00
7	Renkhoa Dhan	L-39	BRRI-7464	Jhum	403.00	197.00	48.88	0.00	2.00
8	Pathar kuchi	L-40	BRRI-7632	Aman	60.00	16.00	26.67	3.00	2.00
9	Aus (Awned)	L-41	BRRI-7473	Aus	530.00	69.00	13.02	4.00	2.00
10	Murali	L-42	BRRI-7475	Aus	819.00	68.00	8.30	2.00	1.00
11	IR84643-11-23-8-8-2-2-3-2-2-B	INL-5	JIRCAS. JP	Boro*	488.00	241.00	49.39	0.00	4.00
12	IR84643-11-47-6-11-2-2-4-2-2-4-	INL-6	JIRCAS. JP	Boro	362.00	228.00	62.98	3.25	4.00
13	IR84643-11-68-9-3-2-2-4-3-2-14-	INL-7	JIRCAS. JP	Boro	512.00	20.00	3.91	2.00	0.00
14	IR84643-11-68-9-6-2-3-2-2-B	INL-8	JIRCAS, JP	Boro	560.00	380.00	67.86	2.00	3.00
15	IR84643-11-68-11-6-2-3-2-3-B	INL-9	JIRCAS, JP	Boro	12.00	4.00	33.33	1.85	0.00
16	IR84643-11-68-11-8-3-2-4-2-2-3-	INL-10	JIRCAS. JP	Boro	540.00	140.00	25.93	5.00	0.00
17	IR84643-11-74-3-2-3-3-2-2-B	INL-12	JIRCAS. JP	Boro	986.00	120.00	12.17	0.00	1.00
18	IR84643-11-81-4-4-3-3-2-4-B	INL-13	JIRCAS, JP	Boro	425.00	6.00	1.41	3.00	0.00
19	IR84643-11-81-4-6-3-2-4-2-2-2-E	INL-14	JIRCAS. JP	Boro	845.00	6.00	0.71	3.00	4.00
20	IR84643-11-82-8-2-3-3--2-3-B	INL-15	JIRCAS. JP	Boro	745.00	20.00	2.68	0.00	2.00
21	IR84643-11-82-8-9-3-4-2-2-2-B	INL-16	JIRCAS. JP	Boro	564.00	5.00	0.89	0.00	0.00
22	IR84643-11-105-5-5-2-3-2-6-B	INL-17	JIRCAS. JP	Boro	700.00	8.00	1.14	2.00	0.00
23	IR84643-11-105-7-9-2-3-2-2-B	INL-18	JIRCAS, JP	Boro	549.00	245.00	44.63	3.00	4.00
24	IR84643-11-105-8-3-2-2-4-2-3-3-	INL-19	JIRCAS. JP	Boro	470.00	182.00	38.72	3.00	3.00
25	IR84643-11-105-11-11-3-2-4-2-2-	INL-20	JIRCAS. JP	Boro	320.00	15.00	4.69	2.00	0.00
26	IR84643-11-115-4-2-3-2-5-2-2-2-	INL-29	JIRCAS. JP	Boro	460	320	69.57	3.00	2.00
27	IR84643-11-115-4-5-3-2-4-2-2-2-	INL-30	JIRCAS. JP	Boro	180	83	46.11	2.50	1.50
28	IR84636-13-2-3-2-2-2-4-2-2-2-B	INL-32	JIRCAS. JP	Boro	684	158	23.10	3.00	1.00

DW, dry weight; RDW, Relative dry weight; P-deficiency tolerance was decided based on the degree of RDW. Higher degree of RDW indicate tolerance and lower RDW indicate susceptibility.