Vol. 21(10), pp.472-482, October 2022
DOI: 10.5897/AJB2022.17521
Article Number: 06B5B8A69873
ISSN: 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Screening for phosphate deficiency tolerance and expression of phosphate uptake genes in Nigerian local rice landraces

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Received 14 September 2022; Accepted 17 October 2022

Twenty-one Nigerian rice landraces were screened for tolerance to phosphate deficiency in nutrient medium using selected morphological indices from which Phosphate Deficiency Tolerance Index (PDTI) was evaluated. All landraces were analyzed for the presence of four *phosphate uptake 1* (*Pup-1*) gene-linked markers while relative expression levels of two *Pup-1* genes were evaluated in selected landraces under zero, normal and excess phosphate. PDTI analysis grouped the landraces into negative, low positive or high positive PDTI categories depending on whether performance at zero P was better than normal P, comparable to normal P, or less than normal P, respectively. However, irrespective of PDTI grouping, each landrace had at least one *Pup-1* gene marker. Under zero P, *Phillipine* landrace showed no superior expression of *OsPupK05-1* and *OsPupK04-1* genes despite belonging to negative PDTI group. Thus, PDTI-based characterization of the landraces was not completely consonant with the presence or expression levels of the *Pup-1* genes, suggesting a possible influence of other P deficiency tolerant genes. However, with a combination of negative PDTI, superior performance in root and shoot traits under zero P, and possession of at least 3 *Pup 1* genes, *Dantala Mass*, *Ankulyan*, and *Variety 44* may be regarded as P tolerant landraces.

Key words: *Oryza sativa*, phosphate deficiency tolerance index, RT-PCR, *Pup* genes.

INTRODUCTION

Phosphorus (P) is one of the essential macronutrients required for plant growth. Its deficiency in soil affects about 50% of rice-cultivated areas worldwide (Pariasca-Tanaka et al*.,* 2014). Together with drought and salinity, they constitute the major abiotic stresses confronting rice production in the growing areas (GRiSP, 2013; Uyoh et al., 2019; Umego et al*.,* 2020). Phosphorus is usually added to the soil in the form of fertilizer for enhanced yield. However, many peasant farmers in sub-Saharan Africa including Nigeria cannot afford this extra cost. Concerns are also expressed that fertilizer usage is not sustainable (Cordell et al., 2009).

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Furthermore, if applied in excess it could be washed into the water bodies leading to environmental pollution and eutrophication. Thus, the use of cultivars with good yield in both P- deficient and P- rich soils will help ameliorate these problems and improve food security. One way of achieving this would be through marker-assisted introgression of Phosphorus Starvation Tolerance-1 gene (*PSTOL1*) in rice varieties with high yield potential but which perform poorly under phosphate deficient conditions (Pariasca-Tanaka et al., 2014). This protein kinase-encoding gene was identified within the *Phosphorus uptake 1* (*Pup-1*) QTL, located on chromosome 12 of rice (*Oryza sativa*) and has been reported to confer tolerance to phosphate deficiency by enhancing phosphate uptake (Gamuyao et al., 2012). However, genetic instability of the *Pup-1* loci was earlier reported by Heuer et al. (2009), who attributed it to the presence of transposons and truncated elements. This was later corroborated by Chin et al. (2011) when they found that many of the developed markers for these loci were not informative when tested in a wider range of rice germplasm. There is need to authenticate these reports using local rice varieties grown by peasant farmers in Nigeria. The present study was aimed at screening twenty-one local rice cultivars in Nigeria for phosphate deficiency tolerance using morphological indices and validating the result obtained with phosphate uptake 1 (*Pup1*)-linked markers. The levels of *Pup1* gene expression under normal, excess and phosphate-deficient (zero) conditions were also determined using reverse transcriptase-polymerase chain reaction (RT-PCR) to further ascertain the role of this gene in phosphate tolerance in rice.

MATERIALS AND METHODS

Morphological studies

Seed collection and germination

Twenty-one (21) rice land races were obtained from local rice farmers in Benue and Cross River States, Nigeria (Supplementary Table 1). The rice seeds were soaked in water for 24 h to break seed dormancy and thereafter incubated in Petri dishes containing wet filter paper for 7 days for germination.

Preparation of nutrient solution

The modified Hoagland's solution described by Hoagland and Arnon (1950) was used for the present study with slight modification. The main adjustment made was addition of dipotassium hydrogen phosphate (K_2HPO_4) in different concentrations in place of monopotassium dihydrogen phosphate (KH_2PO_4) (Supplementary Table 2).

Transplanting and treatment application

Three (3) seedlings per landrace were transplanted into 50 ml labeled Falcon tubes containing water and kept for two days for acclimatization. The Falcon tubes were wrapped with foil paper to protect them from direct sunlight. After two days, water was drained from the labeled Falcon tubes and replaced with the modified Hoagland solutions containing 0.00 mM, 0.4 mM and 0.8 mM K_2HPO_4 (zero phosphate, normal phosphate and excess phosphate, respectively). The experiment was a 21 X 3 factorial, laid out using the Completely Randomized Design. At 6 weeks of age, the seedlings were transplanted to more spacious Polyvinyl Chloride (PVC) pipes with the same treatment solutions continued (Supplementary Figure 1). In all cases, the treatment solution was changed every four days to prevent a build-up of ions released by the plants.

Data collection and analysis of morphological parameters

At ten weeks after planting, data were collected in triplicates on plant height, number of tillers, root length, shoot and root dry mass in all the 21 landraces at the 3 phosphate concentrations. The means from these were used in estimating Phosphorus Deficiency Tolerance Index (PDTI) or Phosphorus stress factor for each landrace in all the traits using the formula: Trait under sufficient Pi - Trait under deficient Pi / Trait under sufficient Pi, that is (Trait under Pi^N - trait under Pi^-/ trait under Pi^N) (Chankaew et al., 2019; Irfan et al., 2020).

Molecular studies

DNA extraction and amplification of Pup markers

DNA was extracted from the twenty-one rice landraces using modified cetyltrimethylammonium bromide (CTAB) method as described by Uyoh et al. (2019). Four (4) gene-based *Pup* markers selected from the improved set of *Pup-1* markers developed by Chin et al. (2011) based on stable and conserved protein-coded genes verified through gene expression and sequencing data, were used in this study. The reaction was constituted in a total of 50 µL made up of 40.75 µL distilled water, 2.5 µL 10X Ex Taq buffer (1X), 2.5 µL $MgCl_2$ (1.5 mM), 1 µL deoxynucleoside triphosphate (dNTPs) (200 µM), 1 µL forward primer (0.2 µM), 1 µL reverse primer (0.2 µM), 0.25 µL Ex Taq polymerase enzyme (1.25 units), and 1 µL DNA (100 ng/µL). The polymerase chain reaction (PCR) conditions were as follows: Initial denaturation at 95°C for 30 s, 35 cycles of denaturation at 95°C for 20 s, annealing at 55°C for 55 s, extension at 68°C for 1 min, followed by a final extension at 72°C for 5 min and holding at 4°C. The amplified products were separated by electrophoresis in 1.3% agarose gel at 100 V for 30 min, stained with ethidium bromide and visualized under UV trans-illuminator.

RNA extraction and cDNA synthesis

To assess gene expression level and responsiveness to P, reverse transcriptase-PCR (RT-PCR) analyses were done using roots and shoots from rice samples germinated from the same seed stock used previously for the morphological analyses. They were grown in Falcon tubes for 2 weeks with (Pi^N) or without P (Pi^-) as described previously. RNA was extracted from the roots and shoots of selected samples (Phillipine, Ayange and Obasanjo) using the E.Z.N.A.® Total RNA kit (Omega Bio-tek), according to the manufacturer's protocol manual. The landraces were selected randomly from those that showed PCR amplicons for all genes tested. The final RNA was eluted with 40 µL diethyl pyrocarbonate (DEPC) water and stored at -80°C. cDNA was synthesized using LunaScript™ RT SuperMix kit (New England BioLabs Inc.) according to the instructions in user manual. Five µL of the synthesized cDNA was used for PCR analysis to detect the base pairs of the phosphate uptake gene

using the primers *OsPupK04-1* and *OsPupK05*-1. Ubiquitin gene was used as internal control to check the quality of the cDNA using the primers OsUbq_F and OsUbq_R (Supplementary Table 3). The reaction was constituted in a total of 50 µL volume containing 5 µL of 10X Ex Taq buffer (1x), 1 µL dNTPs (200 µM), 1 µL of 10 µM forward primer (0.2 µM), 1 µL of 10 µM reverse primer (0.2 µM), 0.25 µL of Ex Taq polymerase enzyme (1.25 units), 5 µL of cDNA, and 36.75 µL of nuclease-free water. PCR amplification was performed as follows: Initial denaturation set at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 45 s, annealing at 52°C for 1 min, extension at 72°C for 1 min, final extension at 72°C for 7 min. After amplification, 10 µL of the PCR products were resolved on 1% agarose gel stained with ethidium bromide.

RESULTS

Morphological studies and phosphate deficiency tolerance indices (PDTI)

The mean responses of the 21 rice landraces in terms of shoot length, tiller number, root length, wet and dry biomass under Pi^+, Pi^N, Pi^- conditions, along with the calculated PDTI values are presented in Table 1. The mean shoot length ranged from 20.3 cm in Togo to 51.33 cm in OC for those treated with Pi^N; it ranged from 19.5 cm in Togo to 49.43 cm in Election 3 for those given deficient P (Pi^-) and 17.71 cm in Variety 1.4 to 56.33 cm in Election 3 for those given excess P (Pi^+). The mean tiller number ranged from 2.00 in Variety 1.4 to 5.33 in Sedi 1, Election 2 and Dantala Mass under Pi^N; it ranged from 1.00 in variety 1.4 to 3.67 in Sedi1 and Achancha for those treated with Pi^-; and from 1.33 in Variety 1.4 to 5.03 in Sidi 3 for those given Pi^+. Wet biomass ranged from 0.76 g in Togo to 3.99 g in Election 3 for those treated with Pi^N; those treated with Pi^- gave a range from 0.69 g in variety 45 to 2.37 g in Election 3 and for those treated with Pi^+, it ranged from 0.46 g in Variety 1.4 to 2.91 g in Obasanjo. Dry biomass ranged from 0.25 g in Varieties 1.4 and 45 to 0.59 g in Ayange for those treated with Pi^N; it ranged from 0.22g in Variety 1.4 and Election 2 to 0.38 g in Variety 44 for those treated with Pi^-; for those treated with Pi^+ it ranged from 0.22 g in Togo to 0.49 g in Election 3. About 90% of the landraces showed increased root length in Pi^- condition compared with the values for Pi^N and Pi^+. Based on the mean PDTI values obtained, the 21 landraces can be seen to fall into 3 groups (Table 2): (a) those with negative PDTI values ranging from -0.29 to -2.18, indicating that their mean performance under Pi^- was better than that under Pi^N; (b) those with low positive values (≤ 1.50) signifying that their mean performance under Pi^N and Pi^- were quite similar and (c) those with high positive values (>1.5) indicating their mean performance under Pi^N was much higher than at Pi^-.

Validation of Pup-1 linked markers in the rice landraces

Four *Pup-1* linked markers were validated on the 21 rice landraces (Figure 1). The presence of any of the PCR products in a land race was taken as an indication that the land race possessed the *Pup-1* gene (Table 3). At least, one of the four *Pup-1* genes was confirmed to be present in each of the 21 landraces, but none of the genes was present in all samples.

Expression of Pup genes in the rice landraces

Expression of 2 phosphate uptake genes (*OsPupKO4-1 and OsPupKO5-1*) under zero (Pi-), normal (Pi^N) and excess (Pi^+) phosphate levels was tested in 3 out of the 21 rice land races using RT-PCR. The 3 land races were selected randomly from those that showed PCR products for all the 4 genes tested. Expression profiles of the 2 genes and the control (Ubiquitin gene) are given in Figure 2a, b and c. The highest expression of *OsPupKO5-1* gene was seen in the shoot of *Ayange* under normal and excess phosphate conditions (Figure 2aii). The gene was also strongly expressed in the root of this landrace under excess phosphate but poorly expressed under normal and zero phosphate levels (Figure 2ai). It was poorly expressed in the shoots of *Philippine* landrace but showed slightly better expression in the roots. For *Obasanjo* landrace, there was no expression of this gene in the shoots, but it was strongly expressed in the roots under low and normal phosphate levels.

DISCUSSION

The 21 rice landraces evaluated in this study revealed different responses under Pi^+, Pi^N and Pi^- conditions. Tiller number, wet and dry biomass of shoot and root were generally higher when the plants were raised under sufficient P than when raised under deficient P, except in a few (5) landraces. On the other hand, all the landraces except Togo showed increased root length to different levels under deficient P condition, an adaptation for increased nutrient uptake under stressful situations. Wang et al. (2015) reported that different crops show divergent morphological and physiological responses to low P availability, including specific traits of root morphology and root exudation which enhance their P-uptake capacity under low-P conditions. Nirubana et al. (2020) also observed a wide range of varied responses to P deficiency for all the traits they studied in the 30 rice genotypes from India. They reported increased mean performance of root length and enzyme activity under P-condition compared to P+ condition. Vejchasarn et al. (2016) observed an increase in root hair length and density and a reduction in tiller number and shoot length under low P availability in all the 15 rice accessions they studied.

The banding pattern of the four *Pup* markers used in the present study suggests that these genes, especially

Table 1. Mean morphological features and phosphate deficiency tolerance indices (PDTI) at 8 weeks after planting for 21 rice landraces treated with 3 phosphate concentrations.

Landrace	Phosphate level and PDTI	Shoot length (cm)	Root length (cm)	Number of tillers	Wet biomass (g)	Dry biomass (g)	Mean PDTI
Achancha	P^N	30.90	8.00	4.67	1.07	0.29	0.11
	P^-	29.83	8.83	3.67	0.75	0.26	
	P^+	30.67	6.33	4.00	0.93	0.28	
	PDTI	0.03	-0.1	0.21	0.30	0.10	
Sedi 1	P^N	41.50	7.17	5.33	2.77	0.31	0.15
	P^-	31.83	9.17	3.67	2.18	0.23	
	P^+	33.00	7.67	4.67	2.27	0.24	
	PDTI	0.23	-0.28	0.31	0.21	0.26	
Election 3	P^N	55.00	10.10	2.33	3.99	0.45	0.17
	P^-	49.43	12.60	1.67	2.37	0.30	
	P^+	56.33	9.00	1.33	2.60	0.48	
	PDTI	0.10	-0.25	0.28	0.41	0.33	
Ayange	P^N	44.87	7.30	2.67	3.52	0.59	0.17
	P^-	35.50	11.90	2.33	1.19	0.31	
	P^+	42.03	8.50	2.33	1.87	0.42	
	PDTI	0.21	-0.63	0.13	0.66	0.47	
Ankulyan`	P^N	28.70	8.43	3.33	1.51	0.33	-0.71
	P^-	32.77	11.63	3.50	1.54	0.37	
	P^+	26.67	7.0	2.33	1.34	0.28	
	PDTI	-0.14	-0.38	-0.05	-0.02	-0.12	
Faro15	P^N	47.30	6.90	2.67	3.59	0.52	0.15
	P^-	36.67	11.07	2.33	1.89	0.24	
	P^+	47.33	9.83	2.50	2.37	0.28	
	PDTI	0.22	-0.60	0.13	0.47	0.54	
OC	P^N	51.33	6.33	5.00	2.58	0.43	0.14
	P^-	43.17	10.70	3.33	1.12	0.29	
	P^+	47.33	9.83	5.33	2.37	0.29	
	PDTI	0.16	-0.69	0.33	0.57	0.33	
Zomuje	P^N	46.83	8.33	4.67	2.03	0.30	2.47
	P^-	44.17	11.17	3.00	1.24	0.24	
	P^+	47.83	7.33	5.00	2.35	0.25	
	PDTI	0.06	-0.34	0.36	0.39	0.20	
Dantala mass	P^N	39.00	4.70	3.33	2.03	0.27	-2.18
	P^-	46.83	10.53	5.33	2.23	0.28	
	P^+	44.83	5.00	4.33	2.05	0.33	
	PDTI	-0.20	-1.24	-0.6	-0.1	-0.04	
Bnarda Sipi	P^N	43.43	6.70	4.00	2.80	0.31	0.04
	P^-	38.67	10.67	3.33	1.83	0.26	
	P^+	45.33	6.97	4.67	2.84	0.30	
	PDTI	0.11	-0.59	0.17	0.35	0.16	

Table 1. Cont'd

Gadanwaye	P^N	55.83	5.0	4.33	2.47	0.37	
	P^-	37.83	11.07	3.0	2.34	0.35	
	P^+	56.07	7.33	4.67	2.92	0.31	
	PDTI	0.32	-1.2	0.31	0.05	0.05	
Sipi3	P^N	41.83	6.83	4.33	1.81	0.28	0.14
	P^-	36.00	10.67	2.67	0.83	0.23	
	P^+	43.00	10.67	5.03	1.85	0.33	
	PDTI	0.14	-0.56	0.38	0.54	0.18	
Variety1.4	P^N	25.07	8.43	2.67	0.93	0.25	0.13
	P^-	26.83	10.77	1.00	0.72	0.22	
	P^+	17.73	9.33	1.33	0.46	0.26	
	PDTI	-0.07	-0.28	0.63	0.23	0.12	
Variety44	P^N	39.90	9.83	2.00	1.03	0.31	-0.29
	P^-	43.67	10.52	2.67	1.77	0.38	
	P^+	31.3	8.93	2.00	0.71	0.28	
	PDTI	-0.09	-0.07	-0.34	-0.72	-0.23	
Sipi2	P^N	52.2	5.60	2.33	3.63	0.56	0.19
	P^-	35.53	11.53	1.33	0.88	0.28	
	P^+	38.9	3.93	1.67	1.10	0.25	
	PDTI	0.32	-1.06	0.43	0.76	0.50	
Togo	P^N	20.3	12.10	2.33	0.76	0.29	0.12
	P^-	19.5	10.83	1.33	0.74	0.29	
	P^+	33.9	6.67	2.67	1.22	0.22	
	PDTI	0.04	0.10	0.43	0.03	0.0	
Election2	P^N	31.07	5.67	5.33	1.81	0.34	0.20
	P^-	29.50	6.83	3.67	0.95	0.22	
	P^+	30.67	5.67	5.00	1.42	0.23	
	PDTI	0.05	-0.2	0.31	0.48	0.35	
Phillipine	P^N	33.17	5.33	4.17	1.76	0.29	-1.03
	P^-	27.67	14.83	3.33	1.36	0.24	
	P^+	33.53	6.67	4.33	1.75	0.30	
	PDTI	0.17	-1.8	0.20	0.23	0.17	
Obasanjo	P^N	47.50	5.33	5.00	2.19	0.39	0.33
	P^-	34.00	10.00	2.33	1.20	0.23	
	P^+	45.33	7.17	5.00	2.91	0.28	
	PDTI	0.28	-0.88	0.53	0.45	0.41	
Sedi2	P^N	48.50	7.50	4.67	1.76	0.31	0.22
	P^-	31.67	10.17	3.00	0.85	0.24	
	P^+	33.80	7.67	4.33	0.88	0.23	
	PDTI	0.35	-0.36	0.36	0.52	0.23	
Variety45	P^N	35.77	5.9	3.67	3.31	0.29	1.79
	P^-	22.83	10.53	2.33	0.69	0.25	
	P^+	32.33	5.83	2.33	1.96	0.23	
	PDTI	0.36	-0.79	0.37	0.79	0.50	

Source: Author

Table 2. Mean phosphate deficiency tolerance index (PDTI) grouping of 21 rice land races at 8 weeks after planting.

Negative PDTI (*)	***Low positive PDTI ≤1.50 (**)***	***High positive PDTI >1.50 (***)***
	Achancha	
	Sedi 1	
	Election 3	
	Ayange	
Ankulyan	*Faro15*	
Dantala mass	*OC*	
Gadanwaye	*Bnarda Supi*	*Zomoje*
Variety 44	*Supi 3*	*Variety 45*
Phillipine	*Variety 1.4*	
	Supi 2	
	Togo	
	Election 2	
	Obasanjo	
	Sedi 2	

*Landraces performed better under deficient P conditions at least in some traits (Good P deficiency tolerance); **Landraces performed similarly under both deficient and sufficient P conditions (reduced P deficiency tolerance); ***Landraces performed better under sufficient P conditions (weak P deficiency tolerance). In each landrace, PDTI was evaluated individually for each morphological trait using the formular: (Trait under sufficient Pi – Trait under deficient Pi)/ (Trait under sufficient Pi) following Chankaew et al. (2019). Mean PDTI for all the traits was used as a basis for categorization in Table 1.
Source: Author

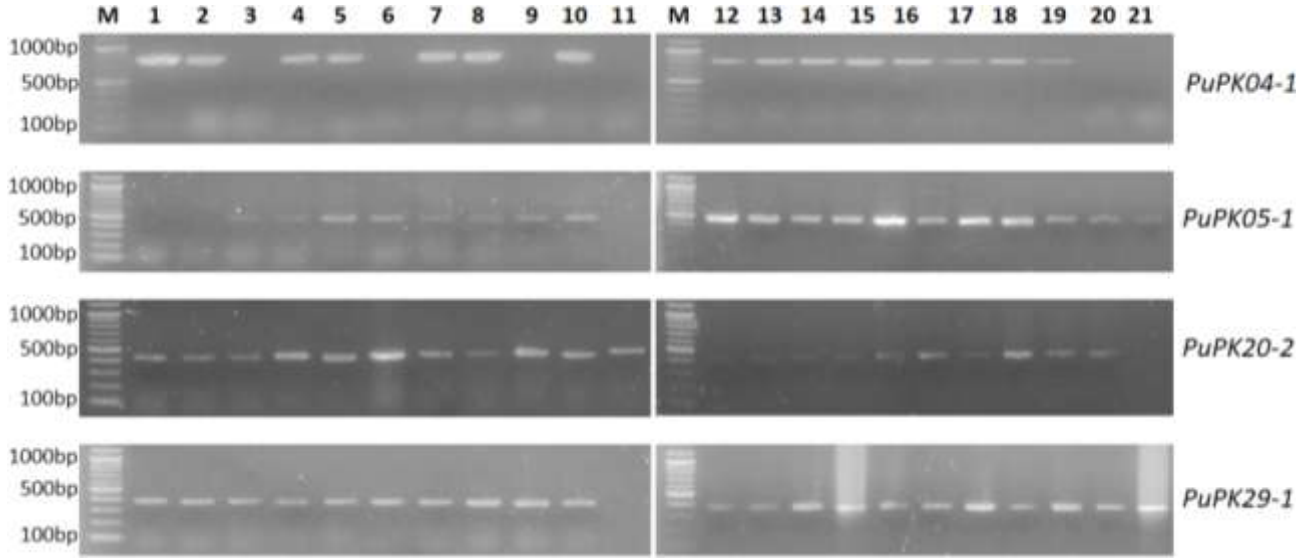

Figure 1. PCR amplification of *PupK04-1, PupK05-1, PupK20-2 and PupK29-1* in 21 rice landraces. 1=Achancha; 2=Sedi1, 3=Election 3, 4=Ayange, 5= Ankulyan,` 6=Faro15, 7=OC, 8= Zomoje, 9= Dantala max, 10 = Bnarda Supi, 11=Gadanwaye, 12= Supi3, 13=Variety1.4, 14= Variety44, 15=Supi2, 16=Togo, 17=Election2, 18=Phillipine, 19=Obasanjo, 20 = Sedi2, 21= Variety45, M= 100 bp DNA marker
Source: Author

those of *OsPup*K29-1 and *OsPup*K20-2 which showed up in over 95% of the landraces are reasonably conserved in the 21 upland rice landraces. Previous reports also indicated that *Pup-1* gene family was highly conserved in stress-adapted rice accessions (Chin et al., 2010, 2011). Heuer et al. (2009) added that *Pup-1* locus increases P uptake under adverse conditions rather than increasing internal P-use efficiency. Chankaew et al. (2019) however reported that the presence or absence of the tolerant allele at the *Pup*-1 locus only showed a slight relationship

Table 3. Summary of the genotyping results[1] of 21 rice landraces with 4 *Pup-1* linked markers.

Landraces	OsPupK04-1	OsPupK05-1	OsPupK20-2	OsPupK29-1
Achancha	1	0	1	1
Sedi 1	1	0	1	1
Election 3	0	1	1	1
Ayange	1	1	1	1
Ankulyan	1	1	1	1
Faro15	0	1	1	1
OC	1	1	1	1
Zomoje	1	1	1	1
Dantala mass	0	1	1	1
Bnarda Supi	1	1	1	1
Gadanwaye	0	0	1	0
Supi 3	1	1	1	1
Variety 1.4	1	1	1	1
Variety 44	1	1	1	1
Supi 2	1	1	1	1
Togo	1	1	1	1
Election 2	1	1	1	1
Phillipine	1	1	1	1
Obasanjo	1	1	1	1
Sedi 2	0	1	1	1
Variety 45	0	1	0	1

[1]Expected PCR product of *Pup-1* linked markers present (1), absent (0)
Source: Author

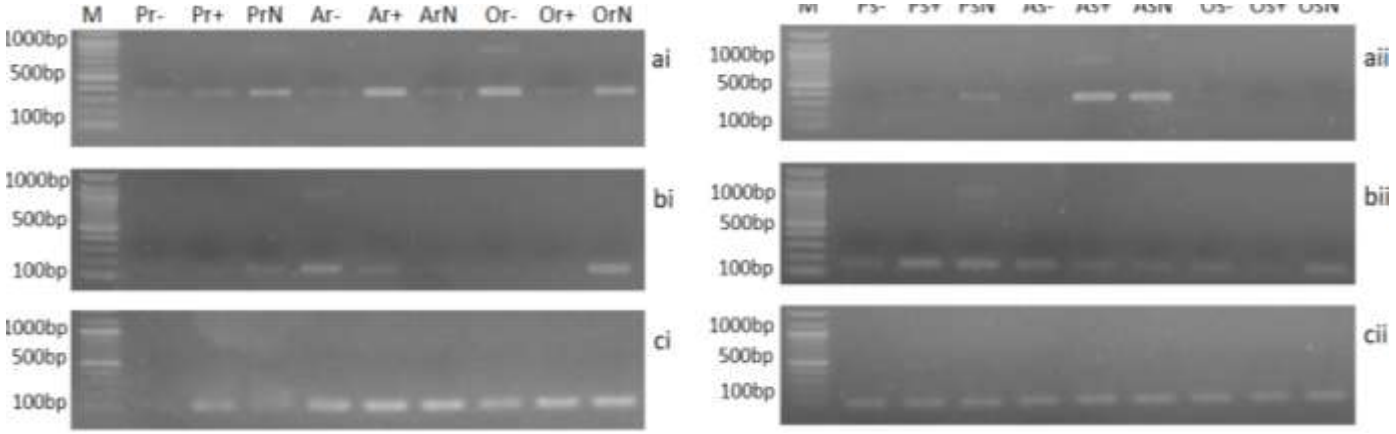

Figure 2. RT-PCR analysis of *OsPupK05-1*(ai and aii), *OsPup04-1* (bi and bii) and *OsUbiqPCR* (ci and cii) genes in the root (r) and shoot (s) of 3 rice landraces (P = *Phillipine*, A = *Ayange*, O = *Obasanjo*) under low (-), normal (N) and excess (+) phosphate conditions.
Source: Author

with the tolerance indices in Thai indigenous upland rice germplasm. Moreover, some lines expressed high tolerance without the *Pup*-1- linked gene product. These lines, they suggested, may carry unknown P-deficient tolerance genes. Sarkar et al. (2011) reported that haplotyping of *Pup*-1-K42 markers showed the expected 918 bp amplification in 9 out of the 31 genotypes studied, but among them, only 3 genotypes showed higher P-uptake and dry-matter weight in P-limiting condition. Thus, the gene may be present but not expressed. This differential expression of the *Pup-1* genes was also observed in the present study. Even though at least one of the genes was present in all the 21 landraces, their expression levels varied with the landrace and the

phosphate concentration in the medium. The highest expression of *OsPupK05-1* gene was seen in the roots and shoots of *Ayange* under excess (Pi^+) and normal phosphate (Pi^N) rather than under Pi^- when it is most needed. This perhaps resulted in the better performance of this land race under Pi^N compared to that at deficient P. Moreover, *OsPupK04-1* gene was weakly expressed in the roots and shoots of this landrace under deficient P. *OsPupK05-1* gene was well expressed in the root of *Obasanjo* under normal (Pi^N) and deficient (Pi^-) levels which also explains the enhanced performance of Obasanjo seen under Pi^N compared to Pi^- (the medium had zero P). It was also observed that Phillipine had the longest root under low P relative to the length under normal P, as reflected in the PDTI value of -1.8 for this trait. However, this did not reflect in the overall plant biomass for this landrace as all the other traits performed better under normal P level. Furthermore, *OsPupK04-1* gene was well expressed in the shoot of Philippine under excess and normal phosphate which explains the better performance of this land race under Pi^N compared with Pi^-. The average tolerance index for this landrace was, however, negative because of the very long root produced under Pi^-. Since this *OsPupKO4-1* gene was not expressed under Pi^- condition in this landrace, the possibility of another phosphate uptake gene operating under this stress condition cannot be ruled out.

The present report showed that only 3 out of the 21 landraces (Variety 44, Ankulyan and Dantala Mass) had higher dry-matter weight in P-limiting conditions which supports the report by Chankaew (2019) that the presence of the tolerant allele at the *Pup* locus does not have significant correlation with the tolerance indices. The choice of *OsPupK04-1* and *OsPupK05-1* for gene expression analysis in the present study was based on previous report of their ubiquitous expression in both roots and shoots of P- and P+ rice accessions by Chin et al. (2011) unlike *OsPup20* which is restricted to roots with higher transcript abundance under P deficient conditions. Chin et al. (2011) observed that most of the *Pup* markers were not informative when tested in a wider range of rice germplasm and that *OsPup-K29* was unstable. This marker, however, gave very consistent bands in 95% of the landraces used in the present study even though some were not expressed.

Conclusion

The study indicated that although low phosphate concentration causes adverse effects on the growth and productivity of these rice plants, the presence and adequate expression of phosphate deficiency tolerance genes in such plants improves adaptation to the stress by producing much longer roots in search of phosphate in the growth medium. Three genotypes namely Dantala Mass, Ankulyan, and Variety 44 may be regarded as tolerant to phosphate deficiency as they each had mean negative PDTI value with better performance in desirable roots and shoots traits under zero phosphate, in addition to possessing at least 3 of the 4 *Pup 1* genes used. Also, the landraces that had good biomass with PDTI values close to zero (which means they performed well in both P-deficient and P-sufficient media) such as Achancha and which invariably also contained *the Pup-1* gene(s), may be further explored along with the other three for possible inclusion in the breeding of this crop for P deficiency tolerance.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors are indirectly grateful to Bill and Melinda Gates Foundation as well as Nigerian Tertiary Education Trust Fund (TETFund) for equipping the laboratory used for the molecular aspect of the study.

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Supplementary Table 1. Rice *(Oryza sativa)* landraces used for the experiment and their sources.

Code	Local name	Source	Coordinates
BN-001	Achancha	Agasha, Benue State	7°58'0''N8°54'0''E
BN-002	Sedi one	Makurdi, Benue State	7.732°N8.5391°E
BN-003	Election 3	Agasha, Benue State	7°58'0''N8°54'0''E
BN-004	Ayange	Gbajimba, Benue State	7°49'0''N8°51'0''E
BN-005	OC	Makurdi, Benue State	7.732°N8.5391°E
BN-006	Zomuje	Gbajimba, Benue State	7°49'0''N8°51'0''E
BN-007	Dantala	Makurdi, Benue State	7.732°N8.5391°E
BN-008	Election 2	Agasha, Benue State	7°58'0''N8°54'0''E
BN-009	Mass	Agasha, Benue State	7°58'0''N8°54'0''E
BN-010	Bnarda Sipi	Naka, Benue State	7°34'59.9''N8°12.264'E
BN-011	Sipi 2 (Faro 44)	Naka, Benue State	7°34'59.9''N8°12.264'E
BN-012	Sipi 3 (Faro 44)	Naka, Benue State	7°34'59.9''N8°12.264'E
BN-013	Variety 45	Agasha, Benue State	7°58'0''N8°54'0''E
BN-014	Togo	Agasha, Benue State	7°58'0''N8°54'0''E
BN-015	Variety 1.4	Naka, Benue State	7°34'59.9''N8°12.264'E
BN-016	Variety 44	Naka, Benue State	7°34'59.9''N8°12.264'E
BN-017	Obasanjo	Makurdi, Benue State	7.732°N8.5391°E
BN-018	Election 3	Makurdi, Benue State	7.732°N8.5391°E
CR-001	Philippine	Adim, Cross River State	5º44' 0" N8º2' 0" E
FARO 15	-	Adim, Cross River State	5º44' 0" N8º2' 0" E

Supplementary Table 2. Modified Hoagland solution used in the study.

S/N	Components	Concentration (mM)	Molecular wt. (g/mol)	Stock (g/L)	Stock (500 mL)	Working vol. (mL)
	Macronutrients					
1	NH_4NO_3	5.6	80.04	448.224	224.1	1
	K_2HPO_4	0.4 (P^N)	174.18	69.672	34.84	1
2	K_2HPO_4	0.8 (Pi^+)	174.18	139.34	69.67	
	K_2HPO_4	0.0 (Pi^-)	-	-	-	
3	$MgSO_4.7H_2O$	0.8	246.48	197.184	98.592	1
	K_2SO_4	0.8	174.25	139.4	69.7	
4	$FeSO_4.7H_2O$	0.18	278.0	50.04	25.02	1
	$Na_2EDTA.2H_2O$	0.18	374.2	67.36	33.68	
5	$CaCl_2.2H_2O$	1.6	147.0	235.2	117.6	1
6	KNO_3	0.8	101.10	80.88	40.44	1
	Micronutrients					
	H_3BO_3	0.023	61.811	1.422	0.711	
	$MnCl_2.4H_20$	0.0045	197.84	0.890	0.445	
7	$CuSO_4.5H_2O$	0.0003	249.61	0.075	0.037	1
	$ZnCl_2$	0.0015	136.29	0.204	0.102	
	$Na_2MoO_4.2H_2O$	0.0001	241.93	0.024	0.012	

Supplementary Table 3. Oligonucleotides used in the study.

Primer name	Sequence 5'-3'	Application
OsPUpK04-1 F	GGGATATCAAGCTTGTGGTG	PCR, RT-PCR
OsPUpK04-1 R	GAATGCTGTTTCGCTTATGG	
OsPUpK05-1 F	AGTACAGTCCGGCGTCATAC	PCR, RT-PCR
OsPUpK05-1 R	CCGAGATCTGGTCCTCAATA	
OsPUpK20-2 F	CTGGACTTGACCCCAATGTA	PCR
OsPUpK20-2 R	TCTGATGGAGTGTTCGGAGT	
OsPUpK29-1 F	CCAATGCATCCAATTCTTGT	PCR
OsPUpK29-1 R	ATGAGCCCAGATTACGAATG	
*OsUbq*_F	GCCCAAGAAGAAGATCAAGAAC	RT-PCR
*OsUbq*_R	AGATAACAACGGAAGCATAAAAGTC	

Supplementary Figure 1. Rice seedlings at P-, P+ and P^N respectively, in each of the PVC pipes at 7 weeks after planting.