Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 25, No. (3) : 2023 : 535-545
© EM International
ISSN-0972-3005

DOI No.: http://doi.org/10.53550/AJMBES.2023.v25i03.024

PATH ANALYSIS REVEALS LINKAGE BETWEEN PHOSPHORUS-UTILIZATION EFFICIENCY WITH STEM BIOMASS IN RICE

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(Received 4 March, 2023; Accepted 15 May, 2023)

Key words: *Oryza sativa, Tissue-specific biomass, Phosphorus utilization efficiency, Path co-efficient analysis*

Abstract – Eighteen rice genotypes were utilised to decipher the relationship among traits especially phosphorus utilisation efficiency and tissue-specific biomass by using path-coefficient analysis; the first report suggested so far. Characters such as the relative value of root number, root surface area, root volume, ratio between root and shoot length, root and shoot biomass portrayed extensive proliferation leading to enhanced biomass accumulation in shoot and root including greater phosphorus uptake. Meanwhile, path co-efficient analysis unveils that the relative value of root phosphorus concentration followed by shoot phosphorus concentration, shoot dry weight and shoot length possess a higher positive direct effect; whereas, the relative value of total leaf dry weight followed by root phosphorus utilization efficiency, shoot phosphorus utilization efficiency and root phosphorus uptake had a higher negative direct effect on relative stem biomass subsequently providing a silver lining to our outcomes. In total, this study identifies promising low phosphorus associated traits with the true nature of each trait towards relative stem dry weight.

INTRODUCTION

Phosphorus (P), sustains crop economy by influencing metabolism from germination to yield. However, its non-renewability, reduced solubility, and soil rhizospheric concentration of around 0.05-0.3 μg/ml (Bolan, 1991) makes it the second most limiting macronutrient affecting global crop production including rice. Concurrently, rice (*Oryza sativa* L.) feeds a large share of the global population (Rose *et al*., 2013) and simultaneously provides a total of 30-75% of calories to more than 3 billion Asians (Krishnan *et al*., 2011). Yet, its P utilization efficiency (PUE) is only 25% (Dobermann and Frairhurst 2000) and it alone consumes 1.07M tonnes of P_2O_5 at the rate of 24.3 kg/ha instigating soil P deficiency further, one of the major yield-limiting factors in rice production, providing tremendous scope of development. Additionally, statistics reveal that 20Mha of world rice arable area is P deficient (Neue *et al*., 1990) and 61.02% of Indian soil is reeling under low P (Muralidharudu *et al*., 2011). So, it will be imperative to increase P fertiliser use efficiency for crop growth.

The enhancement in P fertilizer use efficiency can be accomplished possibly through the improved acquisition of phosphate from soil (P acquisition efficiency) (Mori *et al*., 2016) and increased biomass and/or yield per unit P acquire (internal P utilization efficiency) (Veneklaas *et al*., 2012). Comparing these two approaches, P acquisition efficiency albeit uplifts the yield, but it brings ecosystem discrepancy

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through greater soil P excavation causing off-site environmental problems (Childers *et al*., 2011). In contrast, adopting P utilization efficiency makes the agro-ecosystem both sustainable and productive by reducing injudicious rock phosphate mining and eutrophication. But, before utilising the concept of P utilization efficiency, a thorough understanding is inevitable for this complex trait (Veneklaas *et al*., 2012). Phosphorus utilization efficiency (PUE) is defined as the amount of total biomass or yield that is produced per unit P uptake (Hammond *et al*., 2009) which is determined by two factors at an early stage of the plant; (a) the efficiency with which it is utilized in metabolism and growth, (b) the duration of its presence in living parts where it contributes to these processes. Based on it, a concept was formulated by Berendse and Aerts (1987) (Berendse and Aerts *et al*., 1987)

PUE_t = (Biomass production per unit P per unit time) * (P residence time)

Where, PUE_t indicates P utilization efficiency based on biomass, P residence time implies the time that a unit of P remains in living parts of the plant. This simple concept manifests P utilization efficiency into different aspects which encompass physiological, structural, and developmental traits as they determine tissue-level use of P, allocation, and reallocation of P among different plant parts with different functions and efficiencies.

So far, several reports highlighted the importance of PUE and biomass partitioning with P allocation patterns under both hydroponic and soil conditions (Irfan *et al*., 2019; Dissanayaka *et al*., 2018; Adem *et al*., 2020; Anandan *et al*., 2022). However, this study goes further by illustrating the true nature and/or contribution of selected traits by path co-efficient analysis, which to the author's knowledge, is the very first case study under low P hydroponic conditions at the seedling stage of rice genotypes. Path co-efficient analysis partitions correlation coefficient into direct and indirect effects through other attributes to select traits useful for improving crop performance under stress conditions. In this study, 18 rice genotypes were utilised selected from 65 popular rice genotypes and 3 checks through initial soil-based screening (as per our earlier study; (Bhatta *et al*., 2021 one more) which were subjected to hydroponic study to identify potential traits and decipher the relationship, specifically between PUE and its associated traits with tissue-specific biomass through path co-efficient analysis giving our study a new dimension simultaneously drawing the attention of researchers to explore and enhance their understanding in this direction. So, based on this hypothesis, this study was conducted to find out (i) the potential low P-associated traits and (ii) to understand the relationship between P utilization efficiency and its attributes with tissue-specific biomass.

MATERIALS AND METHODS

Plant materials

Seeds of 18 rice genotypes were utilised screened from 65 popular rice (*Oryza sativa* L.) genotypes developed for Odisha region and 3 checks (Dular, Kasalath, and IC459373) (Supplementary Table 1) were obtained from Regional Research and Technology Transfer Station (RRTTS), Coastal zone, Bhubaneswar; Odisha University of Agriculture and Technology (OUAT); and ICAR-National Rice Research Institute (NRRI), Cuttack, Odisha, India for this study.

HYDROPONIC STUDY

Uniform size seeds of 18 selected genotypes were handpicked and subjected to heat treatment in hot air oven for 45 hr at 50° C to break seed dormancy. Further, seeds were surface sterilized with 75% ethanol (Merck, India) and 2.5% sodium hypochlorite (NaClO) (Merck, India) for 1 min and 20 min respectively followed by thorough washing with sterile distilled water for several minutes to remove any traces of sterilizing agent. From each genotype, two seeds were sown on Styrofoam fixed with mesh placed in a plastic tray with 10 litres of tap water for three days in dark conditions for germination. Subsequently, one set of Styrofoam with uniform germination and healthy seedlings was transferred to tray with full strength of Yoshida solution (10 l/tub) (Yoshida *et al*., 1976) as control, whereas another set was placed in Yoshida solution containing 0.5 ppm (deficient P) of $NaH_2PO_4.H_2O$ (Merck, India). The study was conducted with three replications for 35 days. On alternate day, the nutrient solution pH was maintained between 4.5 and 4.55, and to compensate for the loss in the solution distilled water was added. The nutrient solution was renewed once a week with a fresh solution in order to ensure a continuous supply of nutrients keeping pH in the same range.

Sample collection and parameters measurement

The SPAD (SPAD-502, Konica Minolta, Tokyo, Japan) value was measured to assess the chlorophyll content, on the fourth leaf from the bottom on the penultimate day of the experiment. Subsequently, in order to measure the morphological and physiological traits, plant samples were carefully detached from Styrofoam on the 36th day of the experiment. Ten plants were utilised to observe physio-morphological traits such as shoot length (cm), maximum root length (cm), number of tillers per plant, number of leaves per plant, number of roots, dry weight of total leaf, stem, shoot and root (g). Additionally, some other root traits such as total root length (cm), root volume (cm^3), root surface area (cm^2), and the average root diameter (mm) were measured from three plants per biological replicate by utilizing WinRHIZO™ (Régent Instruments Inc. 2013). The relative value of all traits except internal P remobilisation (IPR) of shoot, root, and total plant was calculated as the ratio of the trait from P deficit level to normal P level multiplied with 100. Coherently, the ratio between root length and shoot length (RL: SL) and root dry weight and shoot dry weight (RDW: SDW) were calculated for a better understanding of the role of root architecture under low P conditions.

Plant phosphorus assay and evaluating P-efficiency attributes

Each plant part viz. leaf, stem, and root were separated and kept at 60 °C in a hot air oven for 5-6 days for complete drying to determine the dry weight of respective plant parts. Post dry weight estimation, the samples were finely ground and about 300 mg and 90 mg of shoot and root samples respectively were used for total P quantification following phospho-molybdo vanadate colorimetric method. The digestion of plant samples was carried out in a digestion chamber, where digestion tubes containing shoot and root samples in ternary acid mixture (conc. HNO_3+ conc. H_2SO_4 + conc. $HClO_4$; 5:1:2) (Merck, India) kept for 1:45 to 2:15 hr at 150 to 170 °C to ensure P quantification for shoot and root. Subsequently, the digest P concentrations were determined using Systronics (Gujarat, India) UV Spectrophotometer at 420 nm, and total shoot and root P concentrations were measured on an mg/g dry weight basis.

Following this, several P efficiency-related parameters were determined to understand the relationship between tissue-specific biomass and PUE including its attributes such as P uptake (PU) of shoot (SPU), root (RPU) and total plant (TpPU) (Zhang *et al*., 2007), IPR of shoot (SIPR), root (RIPR) and total plant (TpIPR) (Maillard *et al*., 2015), PUE of shoot (SPUE) and root (RPUE) (Gunes *et al*., 2006), root efficiency ratio (RER) (Jones *et al*., 1989) using the following formulas.

- Phosphorus uptake (PU)

  PU (mg $plant^{-1}$) = P concentration (mg g^{-1}) X dry weight (g $plant^{-1}$)

- Internal phosphorus remobilisation (IPR)

$$\text{IPR (\%)} = \frac{\text{P uptake under control} - \text{P uptake under deficient condition} \times 100}{\text{P uptake under control}}$$

- Phosphorus utilization efficiency (PUE)

$$\text{PUE} = \frac{\text{P concentration in control} - \text{P concentration in deficient condition}}{\text{P applied in control} - \text{P applied in deficient condition}} \times 100$$

- Root efficiency ratio (RER)

$$\text{RER (mg P in shoot g}^{-1}\text{ RDW)} = \frac{\text{Shoot P uptake (mg plant}^{-1}\text{)}}{\text{RDW (g plant}^{-1}\text{)}}$$

Statistical analysis

The data collected were undergone various statistical analyses to identify key traits *vis a vis* to discover linkage among tissue-specific biomass and PUE and its attributes. Analysis of variance (ANOVA) was executed to understand the significant variation of various traits within 18 genotypes simultaneously to exhibit the effect of genotypes, P levels and their interaction (G * P) in hydroponics using OPSTAT software (Sheoran *et al*., 1998). Concurrently, to display the relationship among selected traits, Pearson correlation and linear regression among a few selected characters were carried out by using SPSS *v*20 software (SPSS Inc., Chicago, IL) and MS Excel respectively. Interestingly to examine the direct and indirect effect of traits (independent variables) over stem dry weight (dependent variable) path analysis was studied using OPSTAT software (Sheoran *et al*., 1998). Based on our earlier report (Bhatta *et al*., 2021), stem dry weight exhibited highest heritability under low P concentration implying it as the prominent quantitative parameter to be considered while evaluating low P tolerant rice genotypes. Further, it is being strengthened by the present finding where relative stem dry weight exhibited significant variation ($P \leq 0.01$) among 18 genotypes reflected from analysis of variance (ANOVA) prompting to consider stem dry weight as a dependent variable.

RESULTS AND DISCUSSION

Degree of Variation in Physio-Morphological Traits under Hydroponic Study

ANOVA was conducted to decipher the variability among different physio-morphological traits, and simultaneously to exhibit the effect of genotypes, phosphorus (P) levels and their interaction (G x P) for the traits selected (Table 1). Among various observed traits, morphological trait relative root number, physiological traits such as relative SPAD, relative stem dry weight, relative shoot P concentration, relative SPU, shoot IPR, TpIPR, relative SPUE and relative root diameter (Table 1) displayed significant variation ($P \leq 0.01$) among 18 genotypes. These outcomes suggest utilising these relative measured traits aid in selecting P deficient tolerant genotypes.

Table 1. ANOVA for the relative value of traits of rice genotypes grown under hydroponics condition

Traits	Degree of Freedom	Treatment MSS
Relative SL	17	0.01^{ns}
Relative tiller no.	17	0.02^{ns}
Relative leaf no.	17	0.009^{ns}
Relative root no.	17	0.04^{**}
Relative RL	17	0.04^{ns}
Relative SPAD	17	0.01^{**}
Relative total leaf dry wt	17	0.05^{ns}
Relative stem dry wt	17	0.10^{**}
Relative SDW	17	0.05^{ns}
Relative RDW	17	0.18^{ns}
Relative shoot P conc.	17	0.001^{**}
Relative root P conc.	17	0.001^{ns}
Relative SPU	17	0.001^{**}
Relative RPU	17	0.002^{ns}
SIPR	17	11.73^{**}
RIPR	17	23.38^{ns}
TpIPR	17	11.27^{**}
Relative SPUE	17	0.003^{**}
Relative RPUE	17	0.003^{ns}
Relative total root length	17	0.09^{ns}
Relative surface area	17	0.18^{ns}
Relative root volume	17	0.47^{ns}
Relative root diameter	17	0.06^{**}

P-values shown as "ns" (non-significant); ** - significant at $P \leq 0.01$. SL – shoot length; RL – root length; SDW – shoot dry weight; RDW – root dry weight; shoot P conc – shoot P concentration; root P conc – root P concentration; SPU – shoot phosphorus uptake; RPU – root phosphorus uptake; SIPR – shoot internal phosphorus remobilisation; RIPR – root internal phosphorus remobilisation; TpIPR – total plant internal phosphorus remobilisation; SPUE – shoot phosphorus utilization efficiency; RPUE – root phosphorus utilization efficiency

Besides the interaction effect (G x P) was studied for six traits and they were found to be significant (Table 2) ($P \leq 0.01$) on all characters except root efficiency ratio (RER) under hydroponic condition. The studied parameters such as RL: SL and RDW: SDW, SPU, RPU and total plant P uptake (TpPU) were significantly influenced by all the factors providing a deeper insight into their genetic basis and adaptability under low P environment making them as most determinant characters to be exploited in breeding programme for selection of low P tolerant genotypes. However, the trait RER was only significantly influenced by P levels making it a different, yet promising parameter to be studied under low P conditions.

The ANOVA (Table 1) unravels the relative value of root number, SPAD, stem dry weight, shoot P conc, SPU, SIPR, TpIPR, SPUE and root diameter as the potential low P related traits as it is quite evident from their significant variation exist across genotypes. Besides these traits, RL: SL and RDW: SDW, RPU, SPU and TpPU were significantly influenced by genotypes, P levels and their interaction (G x P) (Table 2) providing a deeper insight into their genetic basis and adaptability under low P environment making them as most determinant characters to be exploited in breeding programme for selection of low P tolerant rice genotypes. However, RER was only largely influenced by P levels, pointing its sensitivity towards it and making it an adaptive trait under P deficit domain. Further our findings suggest the modification in root architectures such as enhancement in ratio between RL:SL and RDW:SDW, relative value of root number, root diameter supporting the theory of greater photosynthates transportation from shoot to root under P deprivation in order to discover the larger volume of soil to excavate greater nutrients including P through diffusion and/or by contact exchange (Marschner, 1995; Lambers *et al*., 2010; Reddy *et al*., 2020; Panda *et al*., 2021a). Meanwhile, the impact of P levels on RER indicating the possibility of amplification in expression of certain P transporter genes facilitating this particular trait for better P uptake (Anandan *et al*., 2021).

Relationship between Traits Measured under Hydroponic Experiment

The inter-relationship among various traits were analysed by correlation matrix and displayed their significance (Table 3) which can be utilised for the selection of P efficient genotypes under low phosphorus conditions. Among various physiological traits, a very strong positive correlation was detected between the relative value of SDW and total leaf dry weight; the relative value of root volume and root surface area (> 0.920, $P < 0.01$). A nearly one-to-one positive correlation was exhibited between TpIPR and SIPR (0.990, $P < 0.01$); relative value of SPUE and SPU with shoot P concentration and the relative value of RPUE with root P concentration (0.999, $P < 0.01$). Similarly, a greater positive association is quite evident among the relative value of stem dry weight and RDW with SL; relative value of root surface area with total root length (≥ 0.840, $P < 0.01$) and relative value of SPU with total leaf dry weight (> 0.80, $P < 0.01$). Including these, morphological traits such as relative value of root number positively manifest with SL and leaf number (0.736, $P < 0.01$) in conjunction with relative value of SPU with SDW, RPU with root P concentration and RPUE (> 0.710, $P < 0.01$).

Contrastingly, a one-to-one negative association was observed between SIPR and relative SPU; RIPR with relative RPU (-1.000, $P < 0.01$) followed by TpIPR and relative SPU (-0.990, $P < 0.01$). Coherently, the negative trend is continuing among different traits such as SIPR with relative value of total leaf dry weight and shoot P concentration, TpIPR with relative total leaf dry weight, relative SPUE with SIPR and TpIPR (> -0.80, $P < 0.01$). Interestingly, SIPR and RIPR had negatively associated with relative value of leaf number (> -0.730, $P < 0.01$) and SDW (> -0.740, $P < 0.01$).

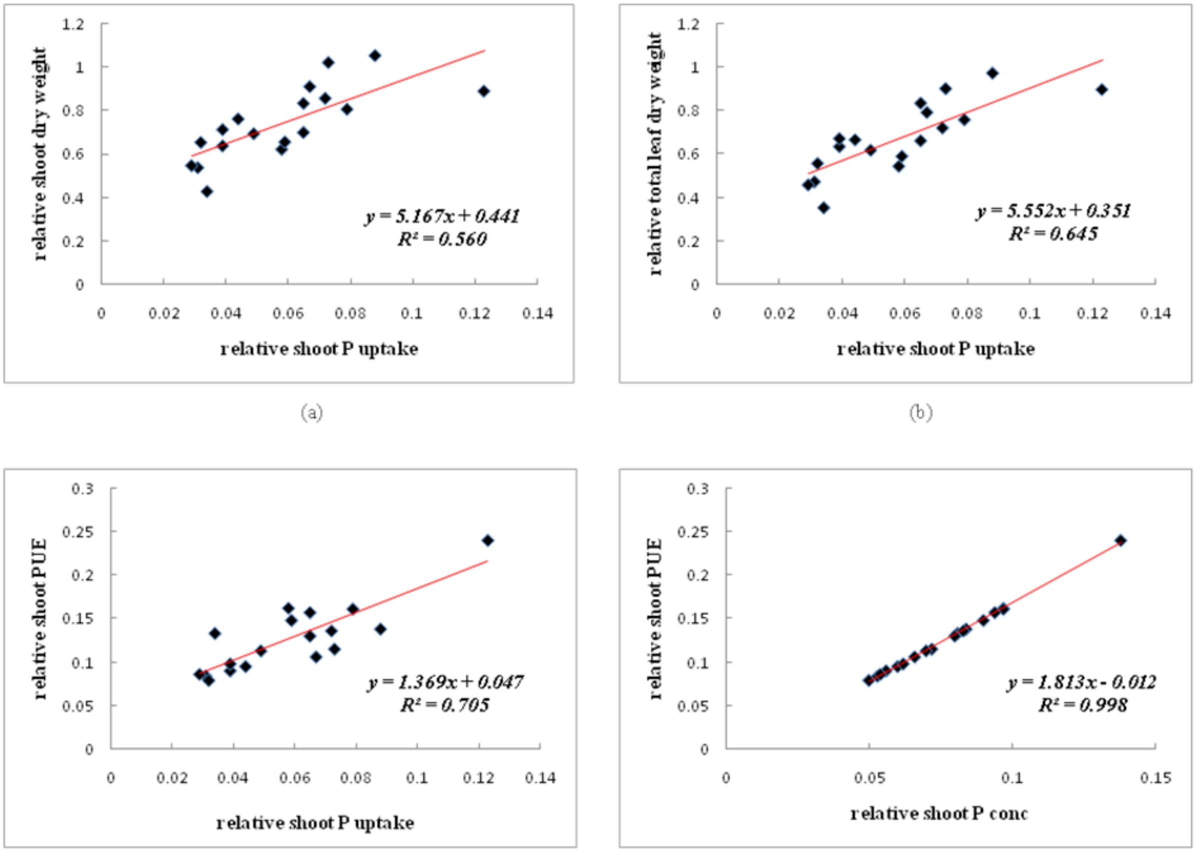
To decipher the nature of relationship among relative values of selected traits, a correlation analysis (Table 3) has been conducted with major focus on tissue-specific biomass and PUE and its related characteristics; the spotlight of our study. The total leaf, stem, shoot and root dry weight were positively associated with SL, leaf number and root number. Interestingly, root number was strongly positively associated with SL and leaf number (Panda *et al.*, 2021a). This positive trend is continuing among SDW with total leaf and stem dry weight followed by RDW with stem and shoot dry weight; stem dry weight with total leaf dry weight (Panda *et al.*, 2021a; Panda *et al.*, 2021b; Anandan *et al.*, 2022). Surprisingly, RL does not furnish any kind of correlation with either morphological traits or biomass-based traits which implies the importance of root number rather RL in the excavation of P from the surface layer of soil again supporting the fact of being presence of most P on top layer of soil (Panda *et al.*, 2021a; Anandan *et al.*, 2022). The greater mining of P along with other nutrients not only supports biomass accumulation in above described plant parts but also maintains overall plant architecture by enhancing morphological plasticity (Panda *et al.*, 2021b; Anandan *et al.*, 2020). While elaborating the role of root architecture in enhancing P uptake, a positive nexus is visible among root volume and root surface area followed by root surface area with total root length and root diameter with root volume and root volume with total root length and root number (Anandan *et al.*, 2021; Anandan *et al.*, 2022). Amidst these, root surface area displayed a positive correlation with total leaf dry weight and SDW. This positive interlinking among root architectural traits pointing their proliferation under low P stress for increased P absorption from a greater volume of soil to support plant growth and biomass again supporting and strengthening our findings (Bates and Lynch, 2001; Anandan *et al.*, 2021; Anandan *et al.*, 2022).

Table 2. Analysis of variance for the relative value of traits under hydroponics condition of phosphorus (P) and genotype (G) interaction effect

Traits	Degree of freedom	G MSS	P MSS	G*P MSS
RL:SL	17	0.01**	0.20**	0.003*
RDW:SDW	17	0.003**	0.23**	0.002**
RER	17	74.28^{ns}	64,725.5**	73.87^{ns}
SPU	17	0.08**	13.10**	0.08**
RPU	17	0.002**	0.22**	0.002**
TpPU	17	0.10**	16.73**	0.10**

P–values shown as “ns” (non-significant); ** - significant at $P \leq 0.01$; * - significant at $P \leq 0.05$. RL:SL - ratio between root length and shoot length; RDW:SDW - ratio between root dry weight and shoot dry weight; SPU – shoot phosphorus uptake; RPU – root phosphorus uptake; TpPU – total plant phosphorus uptake

Furthermore, SPU was positively correlated with shoot P concentration preceded by total leaf dry weight and SDW reflecting the dependency of SPU upon shoot P concentration and its dry weight. Also, a linear and positive relationship between SPU with total leaf dry weight (R^2 = 64.5%) and SDW (R^2 = 56.0%) is visible from regression analysis (Fig. 1) further supporting our findings. But the positive relation with total leaf dry weight signifies the contribution of total leaf dry weight towards shoot biomass increment. Subsequently, SPU is positively associated with physio-morphological traits such as leaf number, SPAD and tiller number indicating the prime role of P in maintaining above-ground structure and final yield under P deficit conditions. Again, RPU displayed a positive association with root P concentration and RDW in a similar fashion like SPU directing the PU of a particular tissue relies upon P concentration and dry weight of the respective tissue. Besides these, SIPR exhibited a perfect negative correlation with SPU followed by shoot P concentration, total leaf dry weight, shoot dry weight; physio-morphological traits such as leaf number, SPAD, tiller number. Nevertheless, SIPR showed a lesser but significant negative correlation (-0.522, $P < 0.05$) with the root number. Moreover, RIPR displayed a one-to-one negative correlation with RPU followed by root P concentration, but it throws back a moderate but significant negative correlation with SL (-0.550, $P < 0.05$). This could be due to two possible mechanisms; at an early stage of plant, the amount of senescing tissues are much smaller than amounts of metabolically active growing tissue, such that the optimal P benefit from remobilisation is quite negligible, possibly only a fraction of total P required (Kikuzawa and Lechowicz, 2006). Under such circumstances, P acquisition by root by far the most reliable source and root architecture need to be enhanced for better P uptake from P rich region of soil which supports our findings here. It also indicates that the P uptake by root and shoot tissue is sufficient for plant



(a) (b)

(c) (d)

Fig. 1. Linear regression between relative shoot P uptake with (a) relative shoot dry weight; (b) relative total leaf dry weight; (d) relative shoot phosphorus utilization efficiency and (c) relative shoot P conc. with relative shoot phosphorus utilization efficiency

growth leading to better performance under low P environment without relying on internal P remobilisation at early growth stage of plant. Secondly, membrane lipid remodelling, a P deficit induced mechanism (Pant *et al.*, 2015), is regulated by a well-known transcription factor, MYB (myeloblastosis) family factor PHR1 (PHOSPHATE STARVATION RESPONSE 1) (Nilsson *et al.*, 2007). PHR1 by combining with microRNA399 (miR399) and PHO2 (PHOSPHATE 2), an E2 ubiquitin-conjugase, constitutes a systemic signalling pathway that communicates shoot P status to root (Pant *et al.*, 2008). Contrastingly, P starvation stimulates the loss of PHR1 protein which further impoverished the decrease of phospholipids and the accumulation of MGDG (Monogalactosyl diacylglycerol) and SQDG (Sulphoquinovosyl diacylglycerol) in shoots and roots (Nilsson *et al.*, 2007). Additionally, TpIPR positively correlated with SIPR specifying that majority of IPR occurs from matured leaves and stem (Irfan *et al.*, 2020). Nonetheless, TpIPR negatively correlated with SPU preceded by total leaf dry weight, shoot P concentration, SDW, leaf number, tiller number, SPAD; while it displayed a moderate but significant negative correlation (-0.528, $P < 0.05$) with root number in a parallel fashion like SIPR suggesting a strong bonding between them which reflects from their strong positive association. Subsequently, SPUE had

Table 3. Pearson correlation matrix of the relative traits measured in hydroponic system

Traits	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1																						
2	0.291																					
3	0.628**	0.624**																				
4	0.736**	0.298	0.736**																			
5	0.294	-0.137	0.131	0.07																		
6	0.178	0.623**	0.661**	0.539*	-0.444																	
7	0.614**	0.434	0.668**	0.604**	0.164	0.529*																
8	0.844**	0.488*	0.753**	0.768**	0.259	0.43	0.64															
9	0.754**	0.477*	0.754**	0.714**	0.201	0.526*	0.964**	0.814**														
10	0.875**	0.128	0.551*	0.675**	0.489*	0.109	0.481*	0.756**	0.628**													
11	-0.181	0.602**	0.493*	0.215	-0.11	0.695**	0.399	0.066	0.297	-0.153												
12	0.028	0.418	0.015	-0.125	0.081	0.048	0.236	-0.014	0.164	-0.06	0.361											
13	0.316	0.666**	0.742**	0.522*	0.085	0.711**	0.805**	0.478*	0.749**	0.257	0.840**	0.375										
14	0.550*	0.35	0.283	0.263	0.423	-0.03	0.357	0.399	0.406	0.577*	0.1	0.712**	0.375									
15	-0.316	-0.666**	-0.742**	-0.522*	-0.085	-0.711**	-0.805**	-0.478*	-0.749**	-0.257	-0.840**	-0.375	-1.000**	-0.375								
16	-0.550*	-0.35	-0.283	-0.263	-0.423	0.03	-0.357	-0.399	-0.406	-0.577*	-0.11	-0.712**	-0.375	-1.000**	0.375							
17	-0.379	-0.681**	-0.732**	-0.528*	-0.126	-0.666**	-0.806**	-0.514*	-0.763**	-0.323	-0.798**	-0.452	-0.990**	-0.495*	0.990**	0.495*						
18	-0.18	0.601**	0.489*	0.204	-0.115	0.687**	0.396	0.057	0.293	-0.156	0.999**	0.376	0.841**	0.126	-0.841**	-0.126	-0.802**					
19	0.02	0.413	0.004	-0.139	0.078	0.041	0.222	-0.027	0.15	-0.059	0.361	0.999**	0.367	0.714**	-0.367	-0.714**	-0.445	0.376				
20	0.3	0.518*	0.345	0.341	0.062	0.353	0.559*	0.479*	0.553*	0.122	0.374	0.357	0.578*	0.317	-0.578*	-0.317	-0.588*	0.367	0.344			
21	0.419	0.329	0.303	0.520*	-0.046	0.368	0.619**	0.506*	0.616**	0.284	0.244	0.124	0.514*	0.235	-0.514*	-0.235	-0.517*	0.237	0.116	0.872**		
22	0.459	0.16	0.263	0.608**	-0.111	0.354	0.570*	0.464	0.574*	0.391	0.138	-0.06	0.406	0.159	-0.406	-0.159	-0.403	0.13	-0.063	0.629**	0.926**	
23	0.31	-0.17	-0.003	0.301	0.004	0.074	0.232	0.209	0.236	0.452	-0.136	-0.323	0.022	0.004	-0.022	-0.004	-0.023	-0.141	-0.318	-0.058	0.37	0.644**

Traits 1 – relative shoot length; 2 - relative tiller number; 3 – relative leaf number; 4 – relative root number; 5 – relative root length; 6 – relative SPAD; 7 – relative total leaf dry weight; 8 – relative stem dry weight; 9 – relative shoot dry weight; 10 – relative root dry weight; 11 - relative shoot P conc; 12 – relative root P conc; 13 - relative shoot P uptake; 14 - relative root P uptake; 15 - shoot internal P remobilisation; 16 - root internal P remobilisation; 17 - total plant internal P remobilisation; 18 - relative shoot P utilization efficiency; 19 - relative root P utilization efficiency; 20 - relative total root length; 21 - relative root surface area; 22 - relative root volume; 23 - relative root diameter. ** - correlation is significant at $P \leq 0.01$. * - correlation is significant at $P \leq 0.05$.

nearly one-to-one positive correlation with shoot P concentration followed by SPU again intensifying the fact that SPUE would bank upon respective tissue P availability. This finding is further strengthened by a linear and positive relationship between relative value of SPUE with shoot P concentration (R^2 = 99.8%) and SPU (R^2 = 70.5%) which is showcased in regression analysis (Fig. 1). Likewise, RPUE furnishes a nearly one-to-one positive relation with root P concentration followed by RPU again strengthening the theory of PUE of a particular tissue being dependent on respective tissue P availability. Yet, it strongly negatively correlates with RIPR highlighting the hypothesis that at an early stage of plant, roots are quite young and metabolically active which makes them a stronger sink for already absorbed P (Martinez *et al*., 2005).

Regression Analysis Revealed Relationship between Specific Characters

A simple linear regression analysis (Fig. 1) has been carried out to exhibit the relationship between specific traits. The parameters studied here displayed a linear and positive relation with each other. The relative value of SDW, total leaf dry weight, SPUE displayed 56.0%, 64.5%, and 70.5% variation in SPU respectively; whereas relative SPUE showed 99.8% variation in shoot P concentration.

Determination of Direct and Indirect Sources of Correlations among Observed Traits

The simple correlation coefficient estimation would not provide a deeper insight into the contribution of traits towards relative stem dry weight (dependent variable for this study); rather partitioning of correlation matrix into direct and indirect effects through path analysis displayed the true nature of independent variable towards the dependent variable. Path analysis showcases the direct effects and their indirect effects through other attributes by apportioning the correlation for a better understanding of cause and effect. Path co-efficient analysis displayed (Table 4) a high positive direct effect by the relative value of root P concentration (2.949), shoot P concentration (2.555), SDW (2.325) and SL (1.139); whereas, a moderate positive direct effect has been delivered by the relative root surface area (0.897), relative SPU (0.691), relative SPAD (0.346), SIPR (0.319), relative RL (0.310) on relative stem dry weight. Similarly, a high negative direct effect has been exhibited by the relative value of total leaf dry weight (-2.818), RPUE (-2.61), SPUE (-2.526), and RPU (-1.223). However moderate to negligible negative direct effect has been possessed by TpIPR (-0.634), relative value of number of roots (-0.583), RIPR (-0.566), total root length (-0.561), number of tillers (-0.209), root volume (-0.192), number of leaves (-0.161), root diameter (-0.08), RDW (-0.049) on relative stem dry weight.

Further explaining, the highest positive direct effect on relative stem dry weight by relative root P concentration is mainly attributed to a high positive indirect effect by relative value of RPUE (2.946) followed by RPU (2.1) and number of tillers (1.222). Similarly, relative shoot P concentration delivers high positive indirect effect majorly through relative value of SPUE (2.553), SPU (2.14) and SPAD (1.771). Again, relative SDW had positive indirect effect via relative value of total leaf dry weight (2.24), number of leaves (1.752) and SL (1.751). In addition to these, relative SL expressed positive indirect effect primarily through relative value of RDW (0.997), SDW (0.858) and number of roots (0.838). On the other hand, relative total leaf dry weight possessed higher negative indirect effect on relative stem dry weight mainly through relative value of SDW (-2.715), SPU (-2.271) and number of leaves (-1.878). Similarly, relative RPUE recorded high negative indirect effect *via* relative value of root P concentration (-2.606), RPU (-1.866) and number of tillers (-1.075). Moreover, relative SPUE displayed significant negative indirect effect through relative value of shoot P concentration (-2.524), SPU (-2.122) and SPAD (-1.734). Then relative RPU provided an indirect negative outlook on relative stem dry weight essentially through relative value of RPUE (-0.874), root P concentration (-0.871) and RDW (-0.704).

Albeit, correlation analysis establishes the nature of the relationship among selected traits, yet it could not provide a transparent picture of the importance of each individual trait in determining relative stem dry weight (dependent variable in this case). Preferably, dissemination of correlation matrix into direct and indirect effects via path analysis allows the estimates of contribution of each trait towards dependent variable. Path analysis provides a precise way of discovering the direct and indirect sources of correlations with certain amount of residual effect. The residual effect predicts how best the causal factors describe the variability of the dependent variable such as relative stem dry weight here. In

Table 4. Path co-efficient analysis displaying direct and indirect effects of different traits of rice cultivars on relative stem dry weight

Traits	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1	**1.139**	-0.061	-0.101	-0.429	0.091	0.061	-1.727	1.751	-0.042	-0.469	0.078	0.218	-0.671	-0.101	0.311	0.24	0.453	-0.054	-0.168	0.375	-0.088	-0.025	0.844**
2	0.332	**-0.029**	-0.101	-0.174	-0.042	0.216	-1.225	1.11	-0.006	1.543	1.222	0.458	-0.432	-0.212	0.198	0.432	-1.52	-1.075	-0.291	0.295	-0.031	0.014	0.488*
3	0.716	-0.13	**-0.161**	-0.429	0.041	0.228	-1.878	1.752	-0.027	1.258	0.027	0.513	-0.346	-0.236	0.159	0.463	-1.232	-0.011	-0.193	0.27	-0.05	0	0.753**
4	0.838	-0.062	-0.119	**-0.583**	0.022	0.187	-1.701	1.659	-0.033	0.544	-0.382	0.363	-0.323	-0.167	0.149	0.335	-0.517	0.356	-0.192	0.466	-0.117	-0.024	0.768**
5	0.336	0.029	-0.021	-0.041	**0.31**	-0.153	-0.462	0.468	-0.024	-0.281	0.236	0.061	-0.515	-0.027	0.239	0.08	0.29	-0.202	-0.035	-0.042	0.021	0	0.259
6	0.202	-0.13	-0.107	-0.315	-0.137	**0.346**	-1.492	1.226	-0.005	1.771	0.138	0.491	0.034	-0.227	-0.017	0.423	-1.734	-0.11	-0.198	0.331	-0.068	-0.006	0.43
7	0.698	-0.091	-0.108	-0.352	0.051	0.183	**-2.818**	2.24	-0.023	1.009	0.689	0.557	-0.438	-0.257	0.202	0.511	-1	0.583	-0.314	0.556	-0.11	-0.019	0.640**
8	0.858	-0.1	-0.122	-0.416	0.062	0.182	-2.715	**2.325**	-0.03	0.75	0.474	0.518	-0.497	-0.239	0.23	0.484	-0.738	-0.394	-0.31	0.553	-0.111	-0.19	0.814**
9	0.997	-0.027	-0.089	-0.394	0.151	0.038	-1.356	1.459	**-0.049**	-0.399	-0.178	0.179	-0.704	-0.082	0.327	0.205	0.393	0.15	-0.069	0.254	-0.075	-0.036	0.756**
10	-0.209	-0.126	-0.079	-0.124	-0.034	0.24	-1.113	0.683	0.008	**2.555**	1.058	0.579	-0.14	-0.267	0.063	0.505	-2.524	-0.951	-0.21	0.217	-0.026	0.011	0.066
11	0.03	-0.087	-0.001	0.076	0.025	0.016	-0.658	0.373	0.003	0.917	**2.949**	0.254	-0.871	-0.118	0.402	0.284	-0.941	-2.606	-0.197	0.105	0.012	0.026	-0.014
12	0.359	-0.139	-0.12	-0.306	0.027	0.246	-2.271	1.743	-0.013	2.14	1.085	**0.691**	-0.461	-0.319	0.212	0.627	-2.122	-0.957	-0.325	0.462	-0.078	-0.002	0.478*
13	0.625	-0.074	-0.046	-0.154	0.131	-0.01	-1.008	0.944	-0.028	0.292	2.1	0.26	**-1.223**	-0.12	0.566	0.315	-0.329	-1.866	-0.18	0.213	-0.031	0	0.399
14	-0.359	0.139	0.12	0.305	-0.027	-0.246	2.268	-1.741	0.012	-2.141	-1.09	-0.691	0.461	**0.319**	-0.212	-0.627	2.123	0.962	0.324	-0.46	0.078	0.002	-0.478*
15	-0.627	0.073	0.046	0.153	-0.131	0.01	1.006	-0.943	0.028	-0.284	-2.097	-0.259	1.223	0.12	**-0.566**	-0.314	0.321	1.863	0.178	-0.21	0.031	0	-0.399
16	-0.432	0.143	0.118	0.308	-0.039	-0.231	2.271	-1.774	0.016	-2.035	-1.32	-0.684	0.608	0.316	-0.28	**-0.634**	2.025	1.166	0.33	-0.463	0.078	0.002	-0.514*
17	-0.204	-0.126	-0.079	-0.119	-0.036	0.237	-1.116	0.68	0.008	2.553	1.099	0.58	-0.159	-0.268	0.072	0.508	**-2.526**	-0.989	-0.205	0.21	-0.025	0.011	0.057
18	0.024	-0.086	-0.001	0.08	0.024	0.015	-0.63	0.351	0.003	0.931	2.946	0.253	-0.874	-0.118	0.404	0.283	-0.957	**-2.61**	-0.194	0.104	0.012	0.026	-0.027
19	0.342	-0.108	-0.056	-0.199	0.019	0.122	-1.576	1.286	-0.006	0.956	1.037	0.4	-0.393	-0.184	0.18	0.373	-0.921	-0.901	**-0.561**	0.782	-0.121	0.005	0.479*
20	0.477	-0.069	-0.049	-0.303	-0.014	0.128	-1.746	1.433	-0.014	0.617	0.347	0.356	-0.29	-0.164	0.133	0.328	-0.592	-0.304	-0.49	**0.897**	-0.178	-0.03	0.506*
21	0.522	-0.034	-0.042	-0.354	-0.034	0.122	-1.608	1.335	-0.019	0.343	-0.191	0.282	-0.197	-0.13	0.09	0.256	-0.324	0.163	-0.354	0.83	**-0.192**	-0.052	0.464
22	0.354	0.035	0.001	-0.175	0.001	0.025	-0.652	0.55	-0.022	-0.362	-0.951	0.015	-0.004	-0.007	0.002	0.014	0.357	0.832	0.032	0.331	-0.124	**-0.08**	0.209

Residual effect = 0.000. Diagonal and bold values represent direct effects. Traits 1 – relative shoot length; 2 – relative tiller number; 3 – relative leaf number; 4 – relative root number; 5 – relative root length; 6 – relative SPAD;
7 – relative total leaf dry weight; 8- relative shoot dry weight; 9 – relative root dry weight; 10 – relative shoot P conc.; 11 –relative root P conc.; 12 – relative shoot P uptake; 13 – relative root P uptake; 14 –shoot internal P remobilisation;
15 – root internal P remobilisation; 16 - total plant internal P remobilisation; 17 – relative shoot P utilization efficiency; 18 – relative root P utilization efficiency; 19 – relative total root length; 20 – relative root surface area;
21 – relative root volume; 22 –relative root diameter; 23 –relative stem dry weight (dependent variable)

this case, the residual effect is 0.00 indicating the characters selected narrating 100% variability towards relative stem dry weight. The findings from path co-efficient analysis here (Table 4) stated that relative value of root P concentration, shoot P concentration, SDW and SL have high positive direct effect comparing to other traits on relative stem dry weight under low P solution culture experiment which brings novelty to this study. Including these, other traits such as relative value of root surface area, SPU, SPAD, SIPR and RL provide a moderate positive direct effect on relative stem dry weight. Opposite to these, relative value of total leaf dry weight, RPUE, SPUE, RPU impart high direct negative effect on relative stem dry weight. Among the positive direct effect bearing traits, relative root P concentration possess a negative correlation but the highest positive direct effect on relative stem dry weight. Under these circumstances, a restricted simultaneous selection model needs to be followed; in other words, restrictions need to be imposed to nullify the undesirable effects in order to make use of direct effect (Rashid *et al.*, 2010). But, relative shoot dry weight and relative SL carries both significant positive correlations and high positive direct effect on relative stem dry weight making these the most important traits to be used as selection criterion which would be helpful for the screening and further improvement in low P tolerant rice genotypes.

Contrary to it, relative shoot P concentration has non-significant positive correlation with a high positive direct effect on relative stem dry weight suggesting it to be promising character need to be considered while selecting low P tolerant rice genotypes. The relative SDW reflects a high positive indirect effect mainly through relative value of total leaf dry weight, number of leaves and SL. Including these, relative SL displays high indirect effect through key traits such as relative value of RDW, SDW and number of roots. The indirect effect suggested by key intermediary characters sought them to be utilised in facilitating in selection of tolerant rice genotypes under P deficient environment. Further explaining, the traits revealing the moderate positive direct effect on relative stem dry weight might be used as surrogate traits to identify low P tolerant rice genotypes. However, traits describing the high negative direct effect on dependent variable need to be studied further.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the support extended by the Director of NRRI to conduct this experiment, and special thanks go to the administrative staff of our institute who supported the conduct of this experiment in official terms. The research was supported by Dr. D. K. Bastia by providing elite rice varieties suitable for Odisha conditions to conduct this experiment.

Conflict of interest

The authors declare that they have no conflict of interest.

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