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RESPONSE OF DIFFERENT RICE GENOTYPES TO PHOSPHORUS DEFICIENCY

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ABSTRACT

Phosphorus (P) deficiency limits plant production in many soils worldwide even when P is added. It has long been known that genotypic differences for P deficiency tolerance exist in rice. The main objective of this study was to investigate plant and soil P interactions in P deficient conditions. Using pots in a preliminary experiment, a 25/75% subsoil/sand mix was determined as a P deficient to be used for screening 30 rice genotypes (*Oryzasativa* L.). During June2011, a box experiment when watered with Yoshida's nutrient solution (YNS) either with (YNS+P) or without P (YNS-P) was designed using a randomised complete block designto assess whether rice genotypes differ in extracting P added in liquid form. Results indicated that P treatment x genotype interaction was significant on shoot dry weight (SDW). On average, YNS-P treatment significantly reduced the SDW for genotypes compared to that of plants grown in YNS+P treatment. Rice genotypes from the*aus* subgroup grown in -P treatment accumulated significantly more SDW than *indica* and *japonica* genotypes. In -P treatment, the genotypes that accumulated higher SDW relative to the others were Black Gora, Rayada, Kasalath, Azucena, IAC 25, Dom Sufid, Aux1Wild type, FR 13A and especially Sadu Chowhich can be used in breeding program.

INTRODUCTION

Riceis the staple food for nearly one half of the ever growing world's population. P deficiency limits plant production in many soils worldwide even when P is added. P is important for plant growth being a key factor in crop production worldwide (20). Almost all over the world, optimum crop production relies upon chemical fertilizers especially N and P (7). Unlike nitrogen, P is a problematic nutrient because when applied to soil it is readily bound to soil particles and becomes immobile (19). The ability of an individual or species in acquiring resources determines its adaptation and productivity in a given environment (1). Gill *et al*. (9) described P efficient plants to have large root systems, low shoot growth rate and high P influx. P efficiency may be as a result of differences between plants in P uptake efficiency from the soil, or differential P-utilization ability of plants for more biomass production per unit of P taken up, or both of these traits (6). The advantages of genotypes with improved phosphorus use efficiency (PUE) are not only the plant's ability to grow well under P deficient condition with low inputs, but also include high grain P content, which may improve nutritional value for humans and promote vigorous-growing of seedlings when seeds are used in the next season (5). The physiological PUE is defined as the total production of plant biomass obtained per unit of P absorbed (2). Since PUE is an important breeding objective (17), breeding strategies must allow selecting for this objective. In order to improve

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PUE in crops, it is important to explore genetic variation for all the traits associated with PUE. Inter- and intra-specific variations for these traits are known to exist and are shown to be under genetic and physiological control, but modified by the plant-environment interactions. Some plant species have adopted strategies to solubilize P in the rhizosphere. However, genotypes are different in one or more strategies and hence show differential P uptake from P limiting conditions (8; 10). These strategies developed by plants are biochemical and physiological functions to overcome low available P and adapt to P stress (14). PUE has received much attention in terms of research work due to challenges that face the sustainable production of P fertilizer industry as the reserves of rock phosphate are being run out. Plant breeders screen and evaluate multiple varieties looking for P-efficient genotypes as an alternative strategy to changes in management that increase P fertilizer use efficiency (12, 18, 21). Against this background, this study was intended to screen different rice genotypes in order to evaluate genotypic differences in P uptake and PUE under P deficient soil. Therefore the objective of this study was to investigate whether rice genotypes respond differently to liquid P treatment in P limiting soil.

MATERIAL AND METHODS

Determination of plant available and total P in soil

Available P in the soil was estimated by using acetic acid extraction as described by Allen (3). Five g, in four replicates, of each four air-dried soils were weighed into 250 ml conical flasks then 150 ml of extractant (2.5% v/v acetic acid) was added. The soil samples and blank flasks were shaken for two hours on a rotary shaker, and then allowed to settle overnight. The clear supernatant was then filtered through Whatman No. 40 paper into centrifuge tubes and the first 5–10 ml of filtrate was rejected. P was determined in the remaining filtrate of all samples. P concentration was measured by colorimetric analysis using the automated spectrophotometric flow injection analyser (FIA). P content was calculated by multiplying soil dry weight with P concentration. Results are demonstrated in Table 1. The available P in soil can be classified based on Bray P1 test, as defined by Marx *et al.* (15): < 20 mg kg^{-1} as low, 20-40 mg kg^{-1} as medium, 40 – 100 mg kg^{-1} as high and > 100 mg kg^{-1} as excessive. Accordingly, the soil test result for available P in Insch subsoil used in this experiment is 12.24 mg kg^{-1} indicating that it is low. As for total P in soil, it was determined using procedure described by Allen et al., (4) which can be used for measuring total P in soil or plant materialand will be explained in the following section.

Table 1: Plant available and total P in the sand and subsoil used in the experiment. Mean of 4 replicates ± standard deviation

	Available P (µg g^{-1})	Total P (µg g^{-1})
Sand	1.95 ± 0.08	12.2 ± 5.9
Subsoil	12.24 ± 0.63	814 ± 59

Determination of total phosphorus and Nitrogen (N) in plant

To determine total P and N in plant, the procedure described by Allen et al. (4) was used. The shoots were ground in stainless steel ball mill (Retsch MM200), after being oven dried at 70°C for 48 hours. 4.5 ml of digest reagent (2.8 ml of concentrated sulphuric acid, 0.08 g of lithium sulphate and 2.33 ml hydrogen peroxide) was added to approximately 0.2 g of oven dried, finely ground sample of each plant and heated to 360°C for two hours to allow digestion. After this time 1 ml of hydrogen peroxide was again added and further

digested for an hour. To determine total P and N in shoot, the diluted digest then underwent FIA. Based on the concentration of each element present in the shoot, total each element in shoot was determined by multiplying element concentration (mg g^{-1}) in shoot with shoot dry weight (g). P use efficiency was calculated by dividing shoot dry weight (g) by total P in shoot (mg).

Rice genotype selection

In a preliminary experiment (data not shown), only one genotype (Azucena) was chosen to be grown. A total of 30 different rice genotypes were used which were mostly obtained from the International Rice Research Institute. Twenty of the genotypes belong to the Oryza SNP set (16): Akihikari, Aswina, Azucena, Bala, Black Gora, CT 9993, Cypress, Dom Sufid, Dular, FR 13A, IAC 165, IAC 25, IR 64, KinandangPatong, Labelle, Lemont, M 202, Minghui 63, Moroberekan, N22, Nipponbare, Rayada, Sadu Cho, Sanhuangzhan No 2, Swarna, Tainung 67 and Zhenshan 97. This Oryza SNP panel was selected because they have received extensive genetic (16) and phenotypic (11) studies. Two genotypes are mutants of the Aux1 gene which is known to affect root growth (Aux1Mutant 1 and Aux1Mutant 2) while the genotype called Aux1 Wild type is genotype Zhonghua 11 in which genotype the mutants were made.

Preparation of rice seeds for germination:

Seed of rice cultivars were surface sterilised in 1% sodium hypochlorite for two minutes then washed under running tap water before being soaked in a beaker filled with tap water for 5 minutes. The seeds were placed on wet filter paper in a Petri dish, which was sealed with Para film (Pechiney Plastic Packaging, Chicago) then kept in an incubator at a temperature of 30 ºC for two days.

Experimental design and growing the plants

A box experiment was conducted in the glasshouse of the Cruickshank Building during June and continued to July 2011 at the School of Biological Sciences, University of Aberdeen, UK. A total of 30 rice genotypeswere evaluated for their growth response in a mixture of 25 % P-limited (814 µg g^{-1}dw) Insch subsoil uniformly added to 75 % blast sand (P content = 12.2 µg g^{-1}dw). The experiment was with two treatments, Yoshida's nutrient solution (22) either with P (YNS+P) or without P (YNS-P). The experiment was conducted using a randomised complete block design (RCBD) with three replicate blocks (boxes) for each treatment, with two plants of each genotype in each box arranged in two randomised sub-blocks. At the bottom of each box (53 x 33 cm at the top, 49 x 27 cm at the bottom and 39 cm depth), five drainage holes of five mm diameter were introduced then a non-woven fabric (Teram, UK) sheet was placed inside. The Insch subsoil and sand were thoroughly mixed and distributed among clear 60 litre plastic boxes. A total of six boxes were prepared. A plastic sheet (52 x 32 cm length x width) was place on the soil surface; the plastic sheets had 60 perforations (2 cm diameter) for sowing plants maintaining a 5 x 5 cm distance. A black/white plastic sheet was wrapped around the box to prevent heat gain and light entry. Before sowing, each box was saturated with eight litres of suitable YNS either -P or +P (pH 5.5). Seeds were surface sterilised in diluted bleach (1% Na hypochlorite) before being germinated at 30^{o}C for two days. Two pre-germinated, uniform and healthy seedlings for each genotype were sown in each hole on the 10th June 2011. Each genotype was represented twice in each box. At the second leaf stage, the seedlings were thinned to one per hole. Each box was watered with four litres of suitable YNS, three times a week for the first

two weeks and five litres three times a week for another two weeks. In the final week, four litres of nutrient solution a day were supplied until harvested on day 35 so that each plant was supplied with 1.5 litres of suitable YNS. To minimize the accumulation of nutrients in the growth medium, each box was watered with six litres of deionised water once a week. Plants were grown in a glasshouse with natural light and dark hours. The average day/night temperature was 28/24^{o}C and the relative humidity ranged from 55 to 70%. Weeds were controlled by hand weeding. Plant height was monitored on weekly basis. After 35 days the plants were harvested and the shoot samples were oven-dried for two days at 70^{o}C to constant weight and the SDW was measured. Before analysis, each box was treated as two randomised replicate blocks and the mean for each genotype per box was calculated. The resulting data (one value for each genotype in a box) were treated as a randomised complete block with three replicates. The effect of block on traits was assessed by analysis of variance and data were checked for normality and log transformed when needed.

RESULTS AND DISCUSSION

A preliminary pot experiment (data not shown) was conducted to determine a suitable medium for larger screens of rice genotypes in response to P treatments. The 25/75% subsoil/sand mix was selected to be used as a growth medium in the main experiment under study because this treatment appeared to be the most severely low P treatment that still supported some continued slow growth over the five week period. The main experiment was conducted in large storage boxesto investigate the response of 30 rice genotypes. The plants were sown in the box maintaining five centimetres between each other in order to minimize the box size that can accommodate a large number of genotypes. There is one limitation with this is that the more the plants grow the more the competition will be. To minimize both the competition among plants and the need for a large box and in the meantime to allow the genetic variations to be expressed, the duration of the experiment conducted here was only five weeks. Nonetheless, it is highly likely that above and below ground competition will be operating in these experiments. Below ground may not be unwelcome since it may emphasise the relative ability of genotypes to access the growth limiting P. Above ground competition is not welcome and it would be useful to verify some of these genotype differences detected here in larger pots where above ground competition could be minimised.

Figure (1) shows representative boxes of two treatments (YNS-P and YNS+P) as they display different shoot growth. Plants in YNS+P treatment have long, wide and healthy leaves with high number of tillers while the growth of those in YNS-P treatment is somewhat stunted with thin stems and reduced number of tillers, while the leaves are shorter and narrower than those of plants in the YNS+P treatment. The mean trait data are reported in Table 2 along with the results of the ANOVA. All traits showed significant ($p<0.001$) genotype by treatment interaction. In addition, for all traits, genotype and treatment significantly affected values at *P* value less than 0.001. Overall, a remarkable proportion of the variation was explained by the terms in the ANOVA (92.2 – 95%). For shoot length, in both YNS-P and YNS+P treatments genotype explains about 87% of variation indicating that phenotypic variation is for the most part driven by the genotypic variation.

Examining a bar chart for SDW in both YNS-P and +P treatments (Figure 2) shows that all genotypes had more SDW in YNS+P treatment than

their YNS-P treatment grown counterparts. +P plants had 2.48 times higher biomass than -P plants. On average the SDW for genotypes grown in the YNS-P treatment reduced by 60% compared to that of plants grown in YNS+P treatment (Table 2). This is clearly indicative of low P availability in this treatment. However, the significant differences in SDW between rice genotypes in the low P treatment are indicative of genotypic variations for P acquisition from conditions lacking of P.

When SDW in -P was plotted against +P treatment (Figure 3) the genotypes clearly categorized themselves into two groups; group one are those genotypes below the line which are relatively good at growing at low P; group two are those above and are relatively good at growing under high P. This gives the impression that the genotypes below the line (category one) could be classified as P tolerant and above (category two) as P responsive. These genotypic variations derived from differences between genotypes in their ability in exploring P deficient soil and utilizing the absorbed P to accumulate more biomass. Such genotypic variation can be utilized in breeding programs for sustainable production. Evidence presented here indicates that rice genotypes interact strongly with P treatment for almost all growth parameters and P uptake indicating that they differ significantly in their ability for P uptake and growth under low P. From a practical perspective, the most desirable genotypes are those that grow well under P limiting conditions and/or responded well to P addition.

In the YNS-P treatment, Dom Sufid was the best genotype, and it accumulated 3.2 fold higher SDW than Aux1Mutant1, the least P efficient genotype. Thesignificant P treatment by genotype interaction on SDW and all growth parameters measured means that by using recombinant breeding, there is an opportunity to develop genotypes with more P efficiency (13). Therefore, genotypes Dom Sufid, M 202, IAC 25, Azucena, Sadu Cho, Dular and FR 13A were superior in terms of high SDW accumulation and P uptake. These genotypes can be used in breeding programs and should attract more research attention to find more about mechanisms behind their superiority for P uptake and PUE.

With the addition of P (in YNS+P treatment), subgroupswere statistically different for SDW (F=19.71, P<0.001, R^2=20.18) where genotypes of the *aus* had 1.4 and 1.5 fold SDW compared to *indica* and *japonica* types respectively (Table 2). This pattern of *aus* superiority in accumulation SDW was also observed in YNS-P treatment where subgroup effect was significant (F=13.54, *P*<0.001, R^2=14.41) and the *aus* genotypes accumulated 1.22 and 1.24 fold more SDW than *indica* and *japonica* genotypes respectively. This means that *aus* genotypes had a slightly better P uptake in -P treatmentand, on average, for the genotypes used in this study *aus* types possibly can be categorized as more tolerant to P deficiency than either *indicas* or *japonicas*.In YNS-P treatment, within each subgroup, some genotypes were superior in accumulating shoot biomass to the others. Of all genotypes used in the study five were outstanding genotypes with a SDW of approximately 1.3 fold more than the average: one was *aus* (Black Gora), two *tropical japonicas* (Azucena and IAC 25), one *temperatejaponicas* (M 202) and one *aromatic* (Dom Sufid). In contrast, the five genotypes with a reduced SDW of about 1.5 fold less than the average are: two *indicas* (Sanhuangzhan No 2 and Swarna), two *temperate japonicas* (Akihikari and Tainung 67) and one *tropical japonica* (Labelle).

The genotypes in the five top and six bottom of the list of SDW in YNS-P treatment were subjected to shoot P analysis (Table 3). There were highly significant genotypic difference for P concentration, total P uptake and PUE. When PUE is plotted against SDW, there is a trend for the high mass plants to have high P efficiency and *vice versa* (Figure 4). The two genotypes IAC 25 and Azucena, which belong to *Japonica*subgroup, discriminate themselves as having high shoot mass and high P efficiency than the rest while Labelle has a low mass but also a high P efficiency.

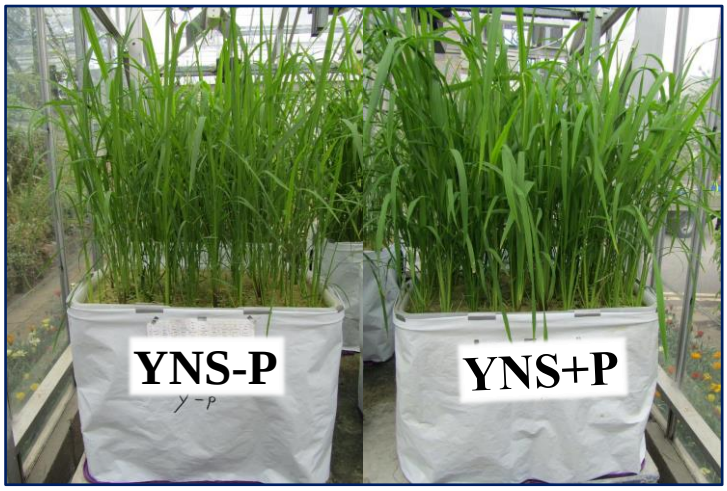

Figure 1: Rice grown in subsoil/sand mix with YNS-P (left) and +P (right).

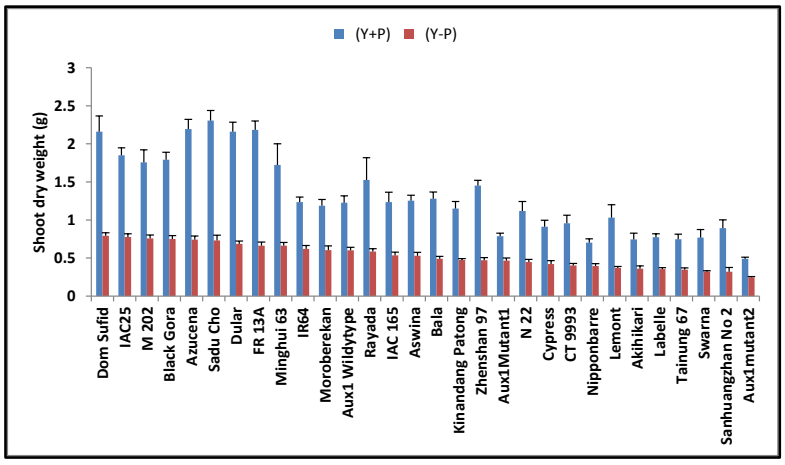

Figure 2: Shoot dry weight of rice genotypes grown in a mixture of 25/75% subsoil/sand mix either with -P (red) or +P (blue). n = 6, Bar = s.e.

Table 2: Genotype subgroup (IND, *indica*; TEJ, *temperate japonica*; TRJ, *tropical japonica*), country of origin, analysis of variance and average parameters of shoot length, number of tillers, SDW and relative SDW (SDW in YNS-P/SDW in YNS+P treatment) for 30 rice genotypes grown in a 25/75% subsoil/sand mix with either YNS-P or YNS+P. Mean= 6

			Shoot length day 35 (cm)		Number of tillers		SDW — (mg)		Relative SDW	
Genotype	Origin	Type	Y-P	Y+P	Y-P	Y+P	Y-P	Y+P	(Y-P/Y+P)	Subgroup
Black Gora	India	-	77.3	84.4	0.5	2.5	751	1792	0.36	*aus*
Dular	India	Lowland	71.8	86.9	0.67	2.83	684	2160	0.39	*aus*
FR 13A	India	-	72.8	94.5	0	2	662	2184	0.42	*aus*
N22	India	Upland	63.9	75.5	0.67	2.17	446	1118	0.40	*aus*
Rayada	Bangladesh	-	59.5	74.5	2.33	5.2	584	1805	0.39	*aus*
aus mean			69.1	83.2	0.8	2.9	625	1812	0.39	
Aswina	Bangladesh	-	66.4	87.4	0.17	2.17	528	1253	0.44	IND
Bala	Eastern India	Lowland	54.3	68.3	0.67	2.83	490	1278	0.40	IND
IR64	Philippines	Lowland	45.8	51.5	2.33	6.17	621	1336	0.46	IND
KinandangPatong	Malaysia	Upland	61.4	80.2	0	1.67	474	1150	0.38	IND
Minghui 63	China	-	49.7	61.8	2	5.83	662	1723	0.40	IND
Sadu Cho	Korea	-	67.6	88.3	2	5.17	732	2305	0.41	IND
Sanhuangzhan No 2	-	-	40.5	52	1.67	3.67	321	891	0.37	IND
Swarna	India	-	33.1	41.8	2	4	321	768	0.44	IND
Zhenshan 97	China	-	35	58.9	2	3.67	471	1452	0.43	IND
indica mean			50.4	65.6	1.4	3.9	513	1340	0.41	
Akihikari	Japan	Lowland	54.9	64	1	2.67	359	744	0.40	TEJ
Nipponbare	Japan	Lowland	54.4	65.9	0.67	2.83	395	701	0.46	TEJ
Taimung 67	Taiwan	-	50.6	62.7	0.17	2	345	747	0.44	TEJ
M 202	United sates	-	60	69.7	1.5	3.17	757	1756	0.41	TEJ
Azucena	Philippines	Upland	77.3	93.1	0.17	2.17	741	2193	0.49	TRJ
CT 9993	-	-	59.7	76.4	0.17	2.17	402	956	0.42	TRJ
Cypress	United States	-	52.3	64.9	1.17	2.5	419	911	0.46	TRJ
IAC 165	-	-	63.8	79.4	0	1.83	535	1235	0.44	TRJ

Continued Table 2.

IAC 25	**Brazil**	**Upland**	**77.1**	**91.9**	**0.17**	**2.17**	**775**	**1849**	**0.43**	**TRJ**			
Labelle	-	**Lowland**	**62.3**	**71.6**	**0**	**2**	**355**	**772**	**0.42**	**TRJ**			
Lemont	**United States**	-	**47.7**	**65.2**	**0.17**	**2.5**	**369**	**1031**	**0.46**	**TRJ**			
Moroberekan	**West Africa**	**Upland**	**71.1**	**88**	**0**	**1.17**	**602**	**1186**	**0.43**	**TRJ**			
***Japonica* mean**			**60.9**	**74.4**	**0.43**	**2.27**	**505**	**1173**	**0.44**				
Aux1 Wild type	-	-	**59.2**	**68.7**	**1.83**	**3**	**600**	**1226**	**0.34**	-			
Aux1Mutant 1	-	-	**54.4**	**66.3**	**1.17**	**2.5**	**466**	**787**	**0.37**	-			
Aux1Mutant 2	-	-	**44.8**	**57.3**	**0.17**	**2.17**	**245**	**489**	**0.41**	-			
Dom Sufid	**Iran**	-	**75.7**	**92.5**	**1.17**	**3**	**793**	**2160**	**0.48**	***aromatic****			
Mean			**58.8**	**72.8**	**0.88**	**2.9**	**530**	**1329**	**0.42**				
#ANOVA			**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***			
T (1)			**788.57**	**0.000**	**1124.25**	**0.000**	**1122.61**	**0.000**					
G (29)			**90.08**	**0.000**	**32.85**	**0.000**	**27.46**	**0.000**	**0.93**	**0.570**			
TxG (29)			**2.71**	**0.000**	**3.86**	**0.000**	**9.17**	**0.000**					
R^2			**95.03%**		**92.25%**		**92.23%**		**0.00%**				

 # ANOVA output and R^2; T, YNS treatment; G, genotype; degrees of freedom between brackets.
*** The *aromatic* referrers to (Group V) subpopulation classifications {source Zhao *et al.* (23)}.**
The factors and interactions in bold are significant.

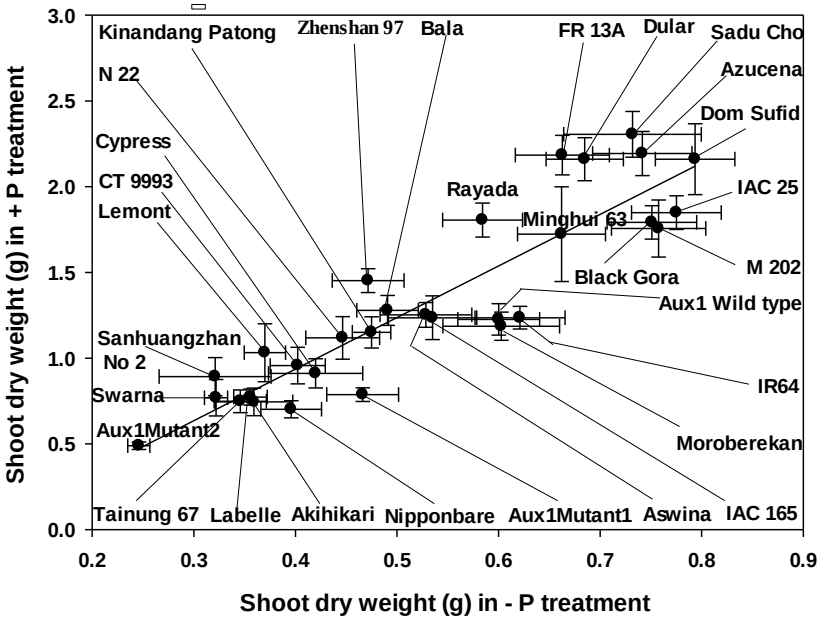

Figure 3: Scatter plot for SDW in -P versus +P YNS treatment. Bars are standard errors.

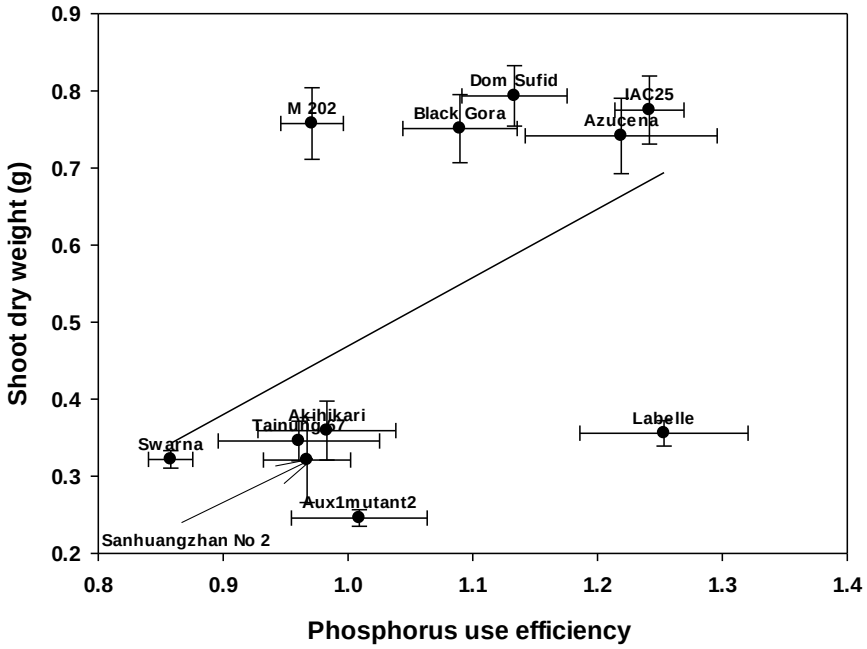

Figure 4: Scatter plot for SDW in YNS-P treatment versus PUE (g SDW/mg P in shoot). Bars are standard errors.

Table 3:ANOVA output and average of P element status in shoot of eleven rice genotypes grown in a 25/75% subsoil/sand mix with YNS-P treatment. Mean of 6 replicates

Genotype	SDW (mg)	Relative SDW	Shoot P concentration (mg g^{-1})	Total P in shoot (µg)	P use efficiency (PUE)
Akihikari	359	0.40	1.03	377	0.98
Aux1Mutant 2	246	0.41	1.01	247	1.01
Azucena	742	0.49	0.84	636	1.22
Black Gora	751	0.36	0.93	704	1.09
Dom Sufid	794	0.48	0.89	703	1.13
IAC 25	775	0.43	0.81	626	1.24
Labelle	356	0.42	0.81	287	1.25
M 202	758	0.41	1.03	782	0.97
Sanhuangzhan No 2	321	0.37	1.04	329	0.97
Swarna	322	0.44	1.17	376	0.86
Tainung 67	346	0.44	1.06	366	0.96
Mean	524	0.42	0.97	494	1.06
ANOVA					
F	37.66	1.10	6.21	15.15	7.12
P	0.000	0.381	0.000	0.000	0.000
R^2	84.94	1.47	44.48	68.52	48.49

Total P in shoot = element concentration in shoot (mg g^{-1}) x SDW (g).
Relative SDW = SDW in YNS-P/SDW in YNS+P.
PUE = SDW (g)/P in shoot (mg). *P* value in bold is significant.

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استجابة تراكيب وراثية مختلفة من الرز إلى نقص الفوسفور

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الملخص

تتأثر انتاجية نباتات المحاصيل في كل انحاء العالم بشكل كبير في نقص الفوسفور حتى وإن أُستُخدِمت الأسمدة الفوسفاتية. من المعلوم انه يوجد هنالك اختلافات وراثية بين اصناف الرز في تحمل نقص الفوسفور في التربة. يهدف هذا البحث الى دراسة التاثير المتبادل بين النبات وفوسفور التربة في وسط من تربة تفتقر للمستوى المثالي منالفوسفور الكافي لنمو النباتات. أجريت تجربة أولية في اصص لتحديد خليط من الرمل وتحت التربة (تربة جُمِعَت من عُمق دون 20سم) بنسبة 25/75% ليكون وسط زراعي يفتقر للفسفور ثم أُستخدم هذا الخليط في غربلة 30 صنفاً من الرز (*Oryzasativa* L.). في شهر مايس من عام 2011 صممت تجربة بتصميم القطاعات العشوائية الكاملة استخدمت مستويان من محلول يوشيدا المغذي (اما مع او بدون عنصر الفوسفور) وذلك لاختبار فيما إذا يوجد هنالك إختلاف بين اصناف الرز في امتصاص الفوسفور من التربة بعد ان أضيف بشكل سائل. لقد دلت النتائج انه يوجد هناك تأثيرمعنوي متبادل بين معاملة الفوسفور والاصناف في الوزن الجاف للمجموع الخضري (SDW)، إذان معاملة المغذي بدون الفوسفور (YNS-P) خفضت الوزن الجاف للمجموع الخضري (SDW) بشكل معنوي مقارنةً بمعاملة المغذي مع الفوسفور (YNS+P).كذلك تشير النتائج إلى أن الأصناف ألتي تعود لتحت المجموعة *aus* المزروعة في المعاملة التي خلت من إضافة الفوسفور(P-)أعطت SDW أكثر وبشكل معنوي من قريناتها التي تعود لتحت المجاميع **indica** و**japonica** المزروعة في المعاملة نفسها.أن الاصناف التي تفوقت على قريناتها في معاملة (P-) هي **FR 13A, Black Gora, Rayada, Kasalath, Azucena, IAC 25, Dom Sufid, Aux1Wild type** وخصوصاً **Sadu Cho** التي يمكن الاستفادة منها في برامج التربية.

جزء من اطروحة دكتوراه للباحث الأول.

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