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Genetic analysis of tolerance for phosphorous deficiency in rice (*Oryza sativa* L.)

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Abstract The inheritance of phosphorous (P) – deficiency tolerance in rice was investigated by a seven-parent diallel. The parent materials involved were four P-efficient (IR20, IR54, IR28, and Mahsuri), one moderately P-efficient (TN1), and two P-inefficient (IR31406-333-1 and IR34686-179-1-2-1), genotypes. Relative tillering ability (RTA) under P-deficient and P-supplemented soil conditions was the parameter used in determining the tolerance level of the different genotypes. Diallel graph analysis revealed that tolerant parents have an excess of recessive genes, while moderate and susceptible parents possess more dominant genes. Genetic-component analysis suggested that both additive and dominance gene effects are involved in the inheritance of P-deficiency tolerance in rice. The trait exhibited over doiminance as confirmed by the graphical analysis. Narrow-sense heritability of the trait was moderate (0.50) and environmental effects were low. Both the general combining ability (GCA) and the specific combining ability (SCA) were significant, but GCA was more prevalent than SCA. Tolerant parents exhibited a high GCA whereas susceptibles have a very poor GCA, suggesting that tolerant parents were mostly enriched in additive genes and susceptible parents in non-additive genes. Crosses involving two high general combiners showed low SCA effects whereas crosses between poor general combiners manifested highly-significant SCA values.

Key words Genetics · Rice · Phosphorous efficiency · Diallel analysis

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Introduction

For plant breeding purposes, phosphorous (P) deficiency signifies the non-availability of added or soil *P* caused by soil chemical characteristics such as acidity. Both acid and alkali soils are *P* deficient. *P* deficiency is perhaps the most important factor that limits rice yields on many soils.

Rice, one of the most important cereal crops, is moderately sensitive to *P* deficiency. Significant differences have been reported to exist among rice varieties for tolerance to *P* deficiency (IRRI 1971, 1976; Katyal et al. 1975; Ikehashi and Ponnamperuma 1978; Fageria and Barbosa-Filho 1982; Gopalkrishna Pillai et al. 1984; Senanayake 1984; Fageria et al. 1988). Gunawardena and Wijeratne (1978) surmised two causative factors to account for such differences: namely, the plant's ability to absorb *P* from the soil and its ability to utilize absorbed *P* may both influence P-deficiency tolerance. Majumder et al. (1989) grew diallel crosses of rice on P-deficient soil and observed a heterotic response in some crosses for both grain yield and some morpho-physiological characters. The heterotic effect was reported to be a quantitative trait. However, no specific information on the genetics of P-deficiency tolerance in rice has been available to-date.

In rice, tiller number correlates with yield in both P-adequate and P-deficient soils (Gunawardena 1979). Similarly, tillering ability has been considered as the best marker of P-deficiency tolerance in rice (Hung 1985). IRRI formulated a scale for the screening of P-deficiency-tolerant rice cultivars based on relative tillering ability (IRTP 1988).

An understanding of the genetic nature of P-deficiency tolerance in rice will help rice breeders to design effective breeding programs for developing improved tolerant varieties. Information on the genetics of P-deficiency tolerance is fragmentary. Gunawardena and Wijeratne (1978), after screening rice cultivars under low *P* and supplemened *P* field conditions, hypothesized the

presence of two independent genes for tolerance to low-P stress. The nature of inheritance of low-P tolerance in rice is also not clear from the findings of Majumder et al. (1989). The present study, therefore, was undertaken to examine the inheritance of P-deficiency tolerance in rice by a seven-parent diallel analysis.

Materials and methods

Assessment of parents for P-deficiency tolerance

The performance of seven randomly-selected rice genotypes, previously rated in various P-deficiency tolerance tests at IRRI, were re-evaluated under greenhouse (soil culture) and field conditions for relative tillering ability (RTA) under P-deficient and P-fertilized (50 kg/ha P) conditions. The varieties involved were IR20, IR54, IR28, Mahsuri, TN1, IR31406-333-1, and IR34686-179-1-2-1. The RTA of a genotype was calculated as:

$$\text{Relative tillering ability} = \frac{\text{Number of tillers under P-deficient conditions}}{\text{Number of tillers under P-supplemented conditions}} \times 100$$

Greenhouse experiment. Four pre-germinated seeds of each rice cultivar were grown in 5-l plastic pots containing either P-deficient ($P = 0.5$ mg/l) or P-supplemented ($P = 10$ mg/l) nutrient solution (Yoshida et al. 1976) in a completely randomized design with two replications. At the maximum tillering stage, the tiller number of all plants of each pot was recorded.

Field experiments. All field experiments of this study were carried out in a farmer's field at Pangil, Laguna, the Philippines. For the past 20 years this site has been used by the Soil and Water Sciences Division of IRRI for their experiments on *P* deficiency. The soil is acidic (pH = 4.9) with a total *P* concentration of about 700 mg/kg and available *P* of about 2 mg/kg. Previous experiments have shown that addition of 50 kg/ha of *P* corrects the deficiency. Large plots in this field are maintained without any addition of *P* fertilizers. The soil has no other known stresses.

Twenty-four-day-old seedlings of the seven rice varieties were transplanted in both P-deficient and P-supplemented (50 kg P/ha) plots at Pangil. For the field experiment, the seedlings were raised in the IRRI greenhouse using the same type of soil. Urea was applied as both basal and as top dressing 3 weeks after transplanting at the rate of 30 kg N/ha in each application. Standard crop management practices were followed. The experiment was conducted in a randomized complete block design (RCBD) with four replications for both P-deficient and P-supplemented plots. Each experimental unit comprised of three 5-m rows. The spacing between rows was 30 cm and within rows 20 cm. Tiller number of ten randomly-chosen hills of the midle row of each experimental unit was recorded at maximum tillering.

Genetics study

The genetics of tolerance for *P* deficiency was investigated using a seven-parent diallel (excluding reciprocals). The parents involved were four tolerant varieties (IR20, IR54, IR28, and Mahsuri), one moderately-efficient (TN1), and two susceptible varieties (IR31406-333-1 and IR34686-179-1-2-1).

To determine the mode of inheritance of tolerance for *P* deficiency by diallel analysis, 29-day-old seedlings (raised as in the previous experiment) of the 21 F_1 progenies and their parents were transplanted on both P-deficient and P-supplemented plots at Pangil. A RCBD with four replications was used. Planting and crop management practicles were the same as in the previous field experiment. Each experimental unit comprised a single row of 14 plants. Excluding two plants from both ends of each row, the tiller number of the remaining ten plants was recorded individually at the maximum tillering stage.

Statistical analysis

Genetic components of variance were calculated following the Hayman approach (Hayman 1954, 1957; Jinks 1954). The diallel analysis of Hayman (1954) was used to compute the array variance (*Vr*) and parent-array progeny covariance (*Wr*). The calculated *Wr* values were regressed on the *Vr* values and the relationship was plotted to make a *Wr-Vr* graph. The heritability estimates were computed following the formulae of Mather and Jinks (1982). Griffing's model II (random effects for genotypes) method 2 (parents + F_1s) was applied to analyze the general combining ability (GCA) of parents and the specific combining ability (SCA) of the crosses (Griffing 1956).

Results and discussion

Performance of parents for P-deficiency tolerance

In the field experiment, the concentration of available *P* in P-deficient plots was 2.4 mg/kg. Estimates of RTA of parent varieties used in the study under greenhouse and field conditions are given in Table 1. Under P-fertilized conditions, available *P* in the field experiment increased by twofold to 4.7 mg/kg; however, it was still below the critical limit for *P* deficiency, which is about 5 mg/kg. RTA was found to be associated with P-deficiency tolerance based on the calculated relative performance. The findings confirmed the previous rating of these varieties for P-deficiency tolerance. Tolerant parents had a significantly higher degree of tillering than sensitive parents. Varieties IR20, IR28, IR54, and Mahsuri were adjudged tolerant. TN1 was moderately tolerant, and IR34686-179-1-2-1 and IR31406-333-1 were susceptible.

Diallel graph analysis

Inheritance of P-deficiency tolerance based on the RTA of parents and F_1s was studied in a 7 × 7 diallel cross.

Table 1 Relative tillering ability (RTA) of different rice varieties expressed as the percentage of performance under P-deficient conditions compared with P-supplemented conditions in greenhouse and field tests

Variety	Type	RTA (%)	
		Greenhouse	Field
1. IR20	Tolerant	50.9	90.5
2. IR54	Tolerant	50.4	90.8
3. IR28	Tolerant	50.6	92.4
4. Mahsuri	Tolerant	54.1	98.6
5. TN1	Moderate	39.2	71.4
6. IR31406-333-1	Susceptible	34.4	81.1
7. IR34686-179-1-2-1	Susceptible	31.1	79.1
CD (0.5)		15.4	14.4
CV (%)		14.7	7.1

Analysis of variance among the genotypes (parents and F_1s) showed significant genetic differences among parents and hybrids. However, there were no significant differences between the parents and hybrids (Table 2). This initial result suggested that hybrids were comparable to their parents in response to *P* deficiency. The mean performance of parents and F_1s of the 7 × 7 diallel cross for *P* deficiency is presented in Table 3. The validity of the additive-dominance model for RTA was satisfied as a uniformity test ($t^2 = 0.001$) was found not to be significant. Therefore, there existed homogeneity of array variance (*Vr*) and parent-array progeny covariance (*Wr*) values, which clearly indicated the absence of any non-allelic interactions. The regression coefficient for RTA ($b = 0.786$) was significantly different from zero (Fig. 1) but its deviation from unity was not significant. This validated the assumptions for the additive-dominance model showing absence of epistasis or the non-random distribution of genes among parents; thus the data set fully satisfied the simple additive-dominance model. These results, therefore, permitted further estimation of various genetic components of P-deficiency tolerance.

The regression graph of *Wr* and *Vr* in the diallel was established to assess the genetic relationship among homozygous parents (Fig. 1). Hayman (1954) and Jinks (1954) have shown that when there are only two alleles at each locus, and provided the genes are distributed independently in the parents, the non-additive genetic variance is found in the form of dominance variance only and the linear regression of *Wr* and *Vr* has a unit slope. The position of the pairs of points *Vr*/*Wr*, relative to the regression line of unit slope through the mean *Vr*/*Wr*, indicates the nature of the dominance. The line through the origin indicates complete dominance: the greater the intercept with the abcissa, the greater the tendency to overdominance. The graph for *Vr*-*Wr* produced a regression line with $b = 0.786 \pm 0.270$ (Fig. 1) which intercepted the *Wr* axis below the point of origin, indicating overdominance as its average degree of dominance. The position of parental arrays on the graph will indicate if it has more dominant genes (when close to the origin) or more recessive genes (when far from the origin). IR20 and IR28 are located at the upper end of the regression line, thus exhibiting an excess of recessive genes, possessing rarely, if any, dominant genes for P-deficiency tolerance. Susceptible IR34686-179-1-2-1 and IR31406-333-1 and moderately-tolerant TN1 were at the lower end of the regression line, indicating that they were completely dominant. Mahsuri occupied the middle region of the graph but was closer to the origin, indicating an almost similar proportion of dominant and recessive genes though with more dominant genes. The scattering of the parental array points in the regression graph showed their pronounced genotypic dissimilarity from each other.

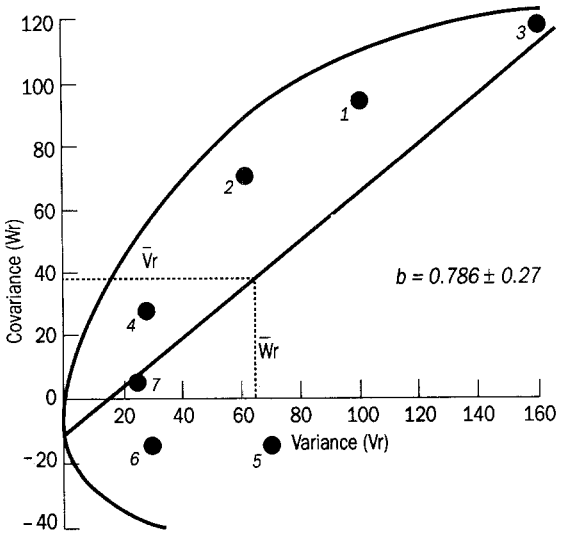

Fig. 1 Variance and covariance (*Vr*, *Wr*) regression graph of the 7 × 7 diallel cross for relative tillering ability (Array: *1*, IR20; *2*, IR54; *3*, IR28; *4*, Mahsuri; *5*, TN1; *6*, IR31406-333-1; and *7*, IR34686-179-1-2-1)

Table 2 Analysis of variance in a 7 × 7 diallel for relative tillering ability

SV	*df*	Mean square	*F* value
Replication	3	124.17	2.84*
Genotype	27	300.43	6.88**
Parents	6	356.32	8.16**
F_1s	20	296.71	6.79**
Parents vs F_1	1	39.30	$< 1^{ns}$
Error	81	43.68	

CV = 7.8%

*, ** Significant at $P < 0.05$ and $P < 0.01$; ns not significant

Table 3 Average relative tillering ability of the 7 × 7 diallel

Male parent	Female parent						
	1	2	3	4	5	6	7
1. IR20	95.1	96.0	95.2	94.0	76.4	78.7	77.1
2. IR54		93.2	96.4	98.0	81.6	83.2	84.0
3. IR28			93.1	85.0	62.7	72.9	80.5
4. Mahsuri				91.3	86.3	85.6	89.2
5. TN1					72.9	84.3	81.3
6. IR31406-333-1						76.5	84.4
7. IR34686-179-1-2-1							78.3

CD (0.5) = 25.14
CV (%) = 7.8

Genetic components of P-deficiency tolerance

Estimates of the genetic components of variation and proportional values are presented in Table 4. Variances due to additive (*D*) and dominance (H_1 and H_2) gene effects were significant for RTA. This indicated that

Table 4 Estimates of genetic parameters for relative tillering ability in a 7 × 7 diallel

Genetic parameter		Estimate ± *SE*
(D) Additive effect		78.16 ± 24.39*
(H) Dominance effect		
H_1		149.08 ± 58.69*
H_2		132.25 ± 51.72*
h^2		0.15 ± 34.73^{ns}
(F) Gene distribution		7.87 ± 58.48^{ns}
(E) Environmental effect		10.92 ± 8.61^{ns}
		Proportional value
(H_1/D)	Mean degree of dominance	1.38
$(H_2/4H_1)$	Proportion of genes with + or − effects in parents	0.22
$(K_D/K_R)^a$	Proportion of dominant and recessive genes in the parents	1.08
[r between $(Wr + Vr)$ and Yr]	Direction of dominance	0.71
(h^2/H_2)	Number of gene groups that exhibit dominance	0.001
(h_{ns})	Heritability (narrow sense)	0.50

* Significant at $P < 0.05$; ^{ns} not significant
^{a} $K_D/K_R = [(4DH_1)^{1/2}\,1/2F]/[(4DH_1)^{1/2} - 1/2F]$

P-deficiency tolerance is governed by both additive and dominant components. Environmental effects were negligible. In the F_1 overdominance was revealed. The non-significance of gene distribution (F) indicated a symmetrical distribution of genes: there are equal frequencies of dominant and recessive allels in the parents irrespective of their increasing or decreasing effects. This gene symmetry was confirmed and the ratio estimate was equal $(H_2/4H_1 = 0.22)$ indicating equal mean allelic frequencies at loci affecting P-deficiency tolerance. Moreover, this ratio implied an equal proportion of positive and negative genes in the parents. The proportion of dominant to recessive genes in the parents $(K_D/K_r = 1.08)$ was also equal. The mean degree of dominance $[(H_1/D)^{1/2} = 1.38]$ at each locus indicated overdominance. These genetic parameters and proportional values confirmed the graphical analysis shown in Fig. 1. No distinct group of genes exhibiting dominance could be detected $(h^2/H_2 = 0.001)$. The value for narrow-sense heritability was moderate (0.5). This confirmed that both additive and dominance gene effects govern the inheritance of P-deficiency tolerance in rice.

Table 5 Analysis of variance for combining ability of RTA

Sources of variation	Degrees of freedom	Mean squares	*F* value	GCA-SCA variance ratio
GCA	6	192.88	17.66**	
SCA	21	41.46	3.80**	4.65
Error	81	10.92		

** Significant at $P < 0.01$

The results revealed that the early generation selection for P-deficiency tolerance may be ineffective. The significant presence of dominance gene effects, however, suggests that selection should be relatively mild or else be deferred until these effects are fixed in a homozygous state.

Combining-ability analysis

Combining ability should be examined when the objective is the development of superior genotypes. An analysis of variance for combining ability is presented in Table 5. Although both GCA and SCA variances for RTA were highly significant, the mean squares for GCA was almost five-times larger in magnitude than the mean squares for SCA. It can be stated that P-deficiency tolerance in rice is controlled more by additive than by non-additive (dominance) genes.

Estimates of GCA and SCA effects for RTA are presented in Table 6. Tolerant parents IR54, Mahsuri, and IR20 were found to be good general combiners; IR28 was average, and susceptible parents IR31406-333-1 and IR34686-179-1-2-1 were very poor. The moderately-tolerant parent TN1 was the poorest general combiner. However, crosses between poor general combiners (e.g., TN1 × IR31406-333-1) produced progenies with the highest SCA. A combination of high × average GCA parents also produced progenies with significantly-high SCA values. Moreover, no cross involving high × high general combiners produced progenies with a high SCA. This suggests that the additive gene effects contributed by both tolerant parents of the cross do not bring any increase in the expression of RTA in the F_1, whereas dominance gene effects from sensitive parents abruptly enhance the expression of the trait in the F_1 possibly as a result of some non-allelic interaction. It also further shows that the tolerant parents mostly carry

Table 6 General combining ability (diagonal) and specific combining ability effects (off diagonal) of RTA (*SE* (diagonal) = 1.02 *SE* (off diagonal) = 2.97)

*,** Significant at $P < 0.05$ and $P < 0.01$, respectively

Parents	1	2	3	4	5	6	7
1. IR20	3.28**	2.67	7.03*	1.19	−5.0	−5.36	−8.14**
2. IR54		5.29**	6.26	3.23	−1.8	−2.85	−3.27
3. IR28			0.09	−4.63	−15.51**	−7.93**	−1.55
4. Mahsuri				4.74**	3.43	0.12	2.45
5. TN1					−6.63**	10.16**	5.96*
6. IR31406-333-1						−3.98**	6.37*
7. IR34686-179-1-2-1							−2.78**

additive genes whereas the sensitive parents possess non-additive genes.

From the results it does not seem possible to draw any general conclusion about the combining ability of individual parents. Usually the GCA value of a certain parent allows for the SCA estimation of that parent. But, in the present study, the best general combiners, IR54 and Mahsuri, have no significant desirable SCA values and the third best general combiner, IR20, had only one significant SCA value (Table 6). TN1, the poorest general combiner, produced two high SCA values out of six crosses, while IR31406-333-1 and IR34686-179-1-2-1, the other two poor general combiners, produced two high positive SCA values each. This may be an indication that GCA is not a reliable basis for the estimation of SCA for RTA. The reliability may increase when more parents are studied in a diallel, such that each parent is checked in a large number of combinations. However, it is probable for parents with a high GCA to have a positive desirable SCA more frequently than parents with a poor GCA. Parents with a low GCA may render highly-positive SCA values. Nevertheless, parents with the highest RTA were the best general combiners and parents with the lowest mean value for RTA were the poorest general combiners.

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