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Diallel analysis of submergence tolerance in rice, *Oryza sativa* L.

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Summary. The genetics of submergence tolerance in rice was studied in a 10×10 half-diallel cross set involving 10 lowland rice varieties, four of which were tolerant ('FR13A', 'FR43B', 'Kurkaruppan', and 'Goda Heenati') and the remaining six were nontolerant ('RD19', 'IR42', 'IR17494-32-1', 'IR19672-24-3', 'Jagannath', and 'CR1009'). Estimates of genetic parameters following Hayman's method showed significant additive and nonadditive gene action and the latter appeared to be solely due to dominance. Narrow sense heritability (0.70) indicated that additive gene effects were more important in the inheritance of the trait. Tolerance was dominant over nontolerance and the average dominance was within the range of incomplete dominance. Dominant alleles were more concentrated in the three tolerant parents, 'FR13A', 'Kurkaruppan', and 'FR43B' in that order. Wr/Vr graphic analysis suggested the involvement of both major and minor genes. Combining ability analysis by Griffing's method also indicated significance of both additive and nonadditive effects, and the former appeared to be more important than the latter. The hybrids involving 'FR13A' with 'RD19', 'IR42', and 'IR17494-32-1', and those of 'Kurkaruppan' with RD19' and 'CR1009' appeared to be promising for incorporating an adequate level of tolerance to submergence into lowland rice cultivars.

Key words: Combining ability – Narrow sense heritability – Additive gene effects – Dominant alleles

Introduction

Submergence tolerance is one of the important objectives in rice varietal improvement. Lowland submergence-prone areas constitute more than 20% of the total rice area in South and Southeast Asia and are exposed to the hazards of annual monsoon flooding. The existence of varietal differences for submergence tolerance in rice has been reported by a number of workers (Ramiah and Rao 1953; Alim et al. 1962; Datta and Banerjee 1972). Systematic screening of the world rice collection at the International Rice Research Institute (IRRI) has resulted in the identification of a few traditional, flood-tolerant rice cultivars such as 'FR13A' and 'FR43B' from Orissa (India) and 'Kurkaruppan' and 'Goda Heenati' from Sri Lanka as donors for submergence tolerance (IRRI 1978).

The genetics of submergence tolerance in rice has not been studied. In a preliminary study Mohanty et al. (1982) reported that tolerance was dominant over nontolerance, and that both major and minor genes were involved in the inheritance of the trait. The experiment described here was designed to study the nature and magnitude of genetic variation of submergence tolerance in 10 rice varieties of diverse origin by the diallel cross methods of Hayman (1954) and Griffing (1956 b).

Materials and methods

Eight rice varieties and two breeding lines (Table 1) were crossed in all possible combinations without reciprocals to obtain 45 F_1 hybrids.

The 10×10 half-diallel population was grown in pots and studied for submergence tolerance in the greenhouse submergence tank, IRRI Department of Plant Physiology during October–November 1981. The screening method of Vergara and

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Table 1. Rice cultivars and breeding lines used in the study

Parent no.	Cultivar or breeding line	IRRI accession no.	Origin	Reaction to submergence
1	'FR13A'	6,144	India	Tolerant
2	'FR43B'	6,143	India	Tolerant
3	'Kurkaruppan'	15,449	Sri Lanka	Tolerant
4	'Goda Heenati'	15,419	Sri Lanka	Tolerant
5	'RD19'	39,174	Thailand	Nontolerant
6	'IR42'	36,959	Philippines	Nontolerant
7	'IR17494-32-1'	–	IRRI	Nontolerant
8	'IR19672-24-3'	–	IRRI	Nontolerant
9	'Jagannath'	12,887	India	Nontolerant
10	'CR1009'	39,253	India	Nontolerant

Table 2. Analysis of variance for submergence tolerance in a 10× 10 diallel cross in rice

Source	Batch A		Batch B		Batch (A+B)	
	DF	MS	DF	MS	DF	MS
Replicate	1	4.4000	1	7.6455	3	4.0167
Genotype	54	6.0032**	54	12.6075**	54	16.5668**
Parents (P)	9	8.1444**	9	14.4458**	9	19.6312**
F_1	44	5.4259**	44	12.0793**	44	15.6114**
P vs F_1	1	12.1338**	1	19.3032**	1	31.0228**
Error	54	0.7981	54	0.8445	162	1.2289

** Significant at the 1% level

Table 3. Mean phenotypic (in a 1–9 scale), array parent-offspring covariance (Wr) and variance (Vr) for submergence tolerance in a 10× 10 diallel and in the 9×9 subdiallel (omitting array No. 4) cross in rice

Parent[a]	Mean phenotypic score										10× 10 diallel			9×9 subdiallel		
	1	2	3	4	5	6	7	8	9	10	Array mean	Vr	Wr	Array mean	Vr	Wr
1	2.25	2.25	2.50	1.88	1.75	2.63	2.38	2.13	2.63	3.13	2.35	0.1590	0.1142	2.41	0.1476	0.1292
2		1.88	2.75	2.63	3.13	3.63	2.50	3.00	2.75	3.50	2.80	0.2889	1.0615	2.82	0.3207	1.1947
3			3.13	2.25	2.00	2.50	2.50	3.50	3.38	2.13	2.66	0.2675	−0.2564	2.71	0.2773	−0.2876
4				5.25	5.75	7.50	8.25	4.88	7.75	6.75	5.29	5.5800	4.0943	–	–	–
5					7.50	6.25	5.38	4.63	5.58	3.88	4.61	3.5849	3.4143	4.49	3.8533	3.8280
6						8.50	7.50	6.25	7.75	7.38	5.99	5.0120	4.1648	5.82	5.3207	4.6830
7							5.00	5.25	6.25	4.88	4.99	4.2203	3.5068	4.63	3.2695	3.9395
8								6.13	4.75	5.50	4.60	1.8083	2.5795	4.57	2.0239	2.9017
9									6.00	6.13	5.32	3.5632	3.4472	5.06	3.1918	3.8737
10										6.75	5.00	3.1771	2.8802	4.81	3.1489	3.2369

(10× 10): P = 5.24, F_1 = 4.26, r(parent, array mean) = 0.883, V_p = 4.91, V_r = 1.6369
(9×9): P = 5.24, F_1 = 4.01, r(parent, array mean) = 0.929, V_p = 5.5213, V_r = 1.4316

[a] For identification of parents, see Table 1

Mazaredo (1975) with minor modifications was followed. The experiment was laid out in a randomized complete block design with four replications, but because of space limitations in the submergence tank the experiment was completed in two succesive batches, A and B, each having two replicates.

Ten pregerminated seeds of each entry were sown in separate clay pots (12 cm diameter, 12 cm high) filled with 700 g of Maahas clay containing 0.8 g of ammonium sulphate, 0.5 g of solophos, and 0.4 g of muriate of potash. When the seedlings were 10 days old, the pots were submerged for eight days in the submergence tank with water depth of 30 cm above the soil level in the pots. Water temperature was kept constant at 40°C and light intensity was 400 lux at the pot level. At the end of the treatment period the pots were taken out and placed outside the submergence chamber for recovery of the plants. On the 10th day of the recovery period a survival count was made and submergence tolerance was scored on a 1–9 scale, based on survival percentage and growth vigor of the recovered plants (Mohanty et al. 1982): the lower the score, the higher the tolerance.

Statistical analysis

Phenotypic scores were used in statistical analysis. Analysis of variance was computed separately for A and B batches and their combination, (A + B), to have a general test of significance; the test showed similar trends in all three analyses (Table 2). For diallel analysis the two batches were combined as one experiment with four replications and the analysis was done following Hayman (1954). Validity of assumptions underlying diallel analysis was tested following Allard (1956) and Mather and Jinks (1982).

Combining ability analysis was carried out following Model I and Model II of Griffing (1956b). Following Baker (1978), the variance ratio $2S^2gca/2S^2gca + S^2sca$ was computed from expected components of mean squares assuming a fixed model, to assess the relative importance of additive and nonadditive gene effects.

Results

Performance of the parents and the F_1s

Analysis of variance showed highly significant differences among the parents (P), the F_1 and P versus F_1 (Table 2). Mean scores for the parents and their arrays showed ‘FR13A’, FR43B’, and ‘Kurkaruppan’ to be the top tolerant parents in that order, thus confirming earlier published reports (Table 3). Contrary to earlier findings, however, ‘Goda Heenati’ appeared to possess only a moderate level of tolerance. Among the remaining entries, ‘IR17494-32-1’ was the most tolerant line while ‘IR42’ was the least tolerant.

Significant comparison for P versus F_1 and the mean score of the parents (5.24) and the F_1s (4.26) showed the presence of unidirectional dominance. However, F_1 performance differed greatly from cross to cross, depending on the level of tolerance of the parents used in the cross. The close correspondence between the parental means and their array means (r = 0.880) suggets a high prepotency of the parents in transmitting submergence tolerance to their offspring (Table 3).

Graphic analysis of (Wr, Vr)

A regression graph of parent-offspring covariance (Wr) and variance (Vr) provides a useful means of assessing genetic relationships among the parents. As Jinks (1954) and Hayman (1954) have shown, in the absence of epistasis and with independent distribution of genes among the parents, the linear regression of Wr/Vr has a unit slope and the Wr, Vr array points would remain along the regression line, Wr = a + bVr and within an area delimited by the parabola, $Wr^2 = Vp.Vr$ where Vp is the variance of the parental means. Further, magnitude and sign of the intercept cut off by the regression line show the level of dominance.

The adequacy of the additive-dominance (no epistasis) model of gene action, which is distributed independently in the parents, was tested by analysis of variance of (Wr + Vr) and (Wr – Vr) and joint regression analysis of Wr/Vr (Tables 4 and 5). The first test showing heterogeneity of (Wr + Vr) and homogeneity of (Wr – Vr) over arrays suggested the existence of a significant nonadditive gene action due to dominance gene effects, thereby proving the validity of the additive-dominance model. The second test showed significant joint regression and nonsignificant heterogeneity of regression, suggesting consistency of regression over the replication; but the joint regression coefficient, b = 0.762 ± 0.088 was significantly different from zero as well as from unity, thus indicating that nonadditive gene action could possibly be attributed to both dominance and epistasis (Fig. 1). Under these circumstances, the genetic interpretation of the Wr/Vr graph would be misleading.

Table 4. Heterogeneity test for (Wr + Vr) and (Wr – Vr) estimates for submergence tolerance in rice

Source	DF	MS
Wr + Vr (array)	9	70.0729**
Wr + Vr (replicate)	30	13.3868
Wr–Vr (array)	9	1.9461 ^{ns}
Wr–Vr (replicate)	30	1.0050

** Significant at the 1% level; ns = not significant

Table 5. Joint regression analysis for (Wr, Vr) values

Source	DF	MS
Joint regression	1	140.6808**
Heterogeneity of regression	3	0.7366 ^{ns}
Remainder	32	0.6282

** Significant at the 1% level; ns = not significant

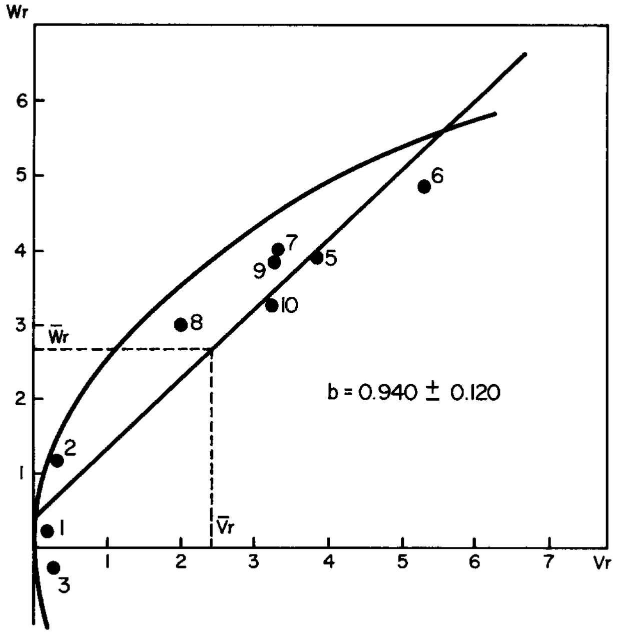

Fig. 1. (Wr, Vr) Regression graph for submergence tolerance in a 10×10 diallel in rice

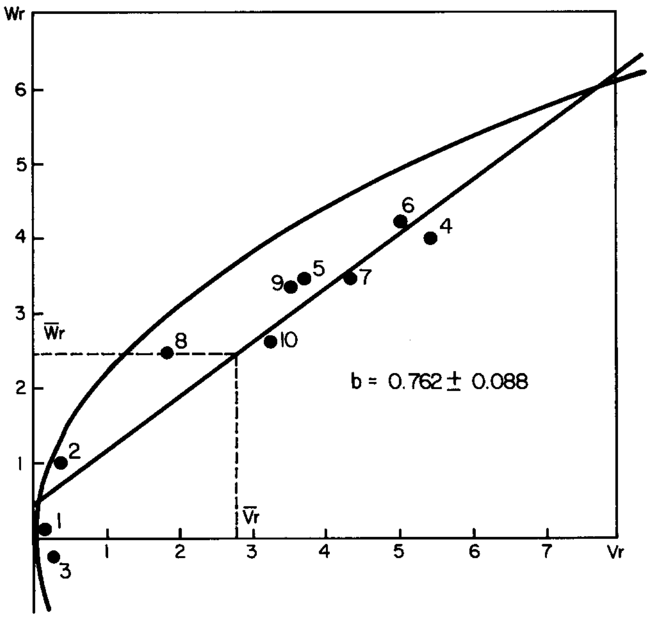

Fig. 2. (Wr, Vr) Regression graph for submergence tolerance in the 9×9 sub-diallel in rice

Following Hayman (1954), the data were reanalyzed by omitting array(s) having extreme (Wr−Vr) values, with the objective of reconstituting noninteracting sub-diallel and the exercise led to identification of 'Goda Heenati' as the only incriminating parent. Analysis of the 9×9 subdiallel showed a regression coefficient, b = 0.940 ± 0.120 which was significantly different from zero but not from unity, thus indicating the absence of epistasis (Fig. 2). There was good correspondence between the two graphs with respect to the point at which the regression line intercepted the Wr-axis and the distribution of the array points along the regression line, thus suggesting that the bias due to the interacting array in the 10×10 diallel was probably not of a high order. However, the following conclusions could be drawn from the 9×9 subdiallel: (i) the regression line cut the Wr-axis above the origin, thus suggesting that the average dominance was within the range of incomplete dominance; (ii) the position of the array points on the graph showed that the dominant alleles were concentrated in the three tolerant parents, e.g. 'FR13A', 'Kurkaruppan', and 'FR43B' in that order, and 'IR42', the most non-tolerant line, had the highest concentration of the recessive alleles followed by parents 5, 7, 9, 10 and 8; (iii) the striking discontinuity between the array points of the tolerant group versus the nontolerant

Table 6. Estimates of genetic parameters for submergence tolerance in rice

Genetic parameters	Estimates	
	10×10 subdiallel	9×9 subdiallel
D	4.5879±0.5142	5.0720±0.4379
H_1	5.0781±1.0945	3.4088±0.9665
H_2	3.8770±0.9302	2.9546±0.8309
h^2	31.1240±0.6226	5.8662±0.5566
F	−0.6946±1.1863	−0.1003±1.0216
E	0.3072±0.1550	0.4493±0.1385
$(H_1/D)^{1/2}$	1.0521	0.8198
$(H_2/4\,H_1)$	0.1909	0.2167
h^2/H_2	8.03	1.98
Heritability:		
Broad sense	0.91	0.89
Narrow sense	0.72	0.70
$1/2F/(D(H_1-H^2))^{1/2}$	0.15	0.02

Table 7. Combining ability analysis for submergence tolerance in rice

Source	10×10 diallel		9×9 subdiallel	
	DF	MS	DF	MS
gca	9	18.2048**	8	15.3160**
sca	45	1.3291**	36	1.1707**
Error	162	0.3072	132	0.4493
S^2gca $(=1/11 \sum_i g_i^2)$		1.4933		1.3515
S^2sca $(=1/66 \sum_y s_{ij}^2)$		1.0193		0.7214
$2S^2$gca$/(2S^2$gca$+S^2$sca$)$		0.7456		0.7893

** Significant at the 1% level

group suggested the possible involvement of one or more major genes in the inheritance of the trait; (iv) the array points in the nontolerant group were relatively more dispersed, suggesting greater genetic diversity in submergence tolerance among the six parents.

Genetic component of variance

Additive genetic variance (D) and the three components of dominance (H_1, H_2 and h^2) were highly significant, thus indicating the importance of both additive and nonadditive types of gene action in the inheritance of the trait (Table 6). The low magnitude and nonsignificance of the F values suggested the absence of gene asymmetry. This was not corroborated by the ratio estimate $(H_2/4H_1)=0.2167$ which was less than the maximum expected value of 0.25 under equal frequencies of positive and negative alleles; however, the gene asymmetry was of a low order. The average dominance $(H_1/D)^{1/2}$ was within the range of incomlete dominance and was in agreement with the conclusion drawn from the graphic analysis. However, the near zero value for the ratio $\frac{1}{2}F/(D(H_1-H_2))^{1/2}$ indicated an inconsistency of dominance over loci. This corroborated well with the performance of the F_1 hybrids which showed varying levels of dominance – from comlete absence to overdominance (Table 3). The ratio of 1.98 for h^2/H_2 suggested the involvement of two groups of genes having dominance. A high narrow sense heritability value (0.70) showed the greater importance of additive gene action in submergence tolerance. In can be further seen that estimates of the three components of dominance, ratio of h^2/H_2, and the average dominance $(H_1/D)^{1/2}$ were somewhat inflated in the 10×10 diallel, evidently because of epistasis involving 'Goda Heenati'.

Combining ability analysis

The mean squares due to gca and sca effects were highly significant (Table 7). The ratio involving the variance components $2S^2gca/(2S^2gca+S^2sca)=0.79$ showed that additive gene effects of genes were more important than nonadditive effects and thus the performance of a single cross could be predicted to a considerable extent on the

Table 8. General combining ability (g_i) and specific combining ability (s_{ij}) effects in 10×10 diallel (upper figure) and the 9×9 subdiallel (lower figure)

Parent	g_i	s_{ij}								
		2	3	4	5	6	7	8	9	10
1	−1.925** −1.696**	1.315* 1.082	1.575** 1.309*	−1.407** –	−1.164* −1.293*	−1.518** −1.600**	−0.643 −0.555	−0.671 −0.861	−0.758 −0.748	−0.049 −0.111
2	−1.581** −1.389**		1.482** 1.252	−1.008 –	−0.133 −0.225	−0.862 −0.907	−0.862 −0.736	−0.140 −0.293	−0.978 −0.930	−0.018 −0.043
3	−1.591** −1.366**			−1.373* –	−1.248* −1.373*	−1.977** −2.055**	−0.852 −0.759	0.370 0.184	0.034 −0.327	−1.383* −1.441*
4	0.773* –				0.138 –	0.659 –	2.534** –	−0.620 –	1.670** –	0.877 –
5	0.398* 0.486*					−0.216 −0.157	0.034 0.264	−0.494 −0.543	0.170 0.320	−1.622** −1.543**
6	1.625** 1.668**						0.930 1.207*	−0.098 −0.100	0.815 1.014	0.649 0.775
7	0.503* 0.373							0.027 0.195	0.440 0.809	−0.726 −0.430
8	0.280 0.429								−0.838 −0.748	0.120 0.139
9	0.867** 0.816**									0.159 0.377
10	0.658** 0.679*									– –
SE	0.152 0.191				0.511 0.613					

For names of parents, see Table 1

** Significant at the 1% level; * Significant at the 5% level basis of general combining ability effects. The importance of gca effects was also evident from the high correlation between the parental means and the gca effects ($r=0.912$ in 10×10 and 0.951 in 9×9 dialleles). Thus, the combining ability analysis was in good agreement with the conclusions from genetic variance components in Hayman's method in showing the greater importance of additive gene action in the inheritance of the trait.

Among the tolerant parents, 'FR13A', 'Kurkaruppan' and 'FR43B' were good general combiners; 'IR19672-24-3', 'IR17494-32-1', and 'RD19' were more promising among the nontolerant lines (Table 8). It is further seen that the hybrids in the 'FR43B' array, with the exception of 'Goda Heenati' (3×5), showed lower levels of submergence tolerance than the tolerant parent 'FR43B' (Table 3). Among the hybrids involving tolerant/nontolerant crosses 1×5, 1×6, 3×5, 3×6, and 3×10 were highly heterotic and showed significant sca effects for higher tolerance. The cross 5×10 involving the two nontolerant parents 'RD19' and 'CR1009' was highly heterotic, with significant sca effects for higher tolerance. However, the sca effects varied greatly from cross to cross and in most of the crosses the effects were marginal.

Discussion

Hayman's diallel method

The validity of the genetic analysis of quantitative characters by diallel cross methods depends upon the fulfillment of assumptions underlying the method. Baker (1978) critically reviewed the literature on the effects of failing to fulfill various assumptions and concluded that some of the assumptions might be violated with impunity while others, like the additive-dominance (no epistasis) action of genes and noncorrelated distribution of genes among the parents, need careful analysis.

In the present study the first test, following the method of Mather and Jinks (1982), showed the validity of the additive-dominance model; in contrast, the second test suggested a complex gene action involving non-allelic interaction (Tables 4 and 5, Fig. 1). Analysis of the non-interacting 9×9 subdiallel data constituted without the array of 'Goda Heenati' showed that in the original diallel there was an upward bias in the estimates of genetic parameters and ratios with the exception of additive variance (D) and heritability, presumably due to epistasis (Table 6).

From the theoretical considerations of Coughtrey and Mather (1970) on the effects of interactions involving two gene pairs on the Wr/Vr graph, it has been inferred that a line of unit slope is not a completely unequivocal indication of the absence of epistasis. Jinks (1956) in *Nicotiana rustica* for flowering time, and Ganashan and Whittington (1976) in rice for response to daylength, demonstrated the insensitivity of the F_1 Wr/Vr graph to duplicate and complementary types of epistasis, respectively. In the present study, the Wr/Vr graph appeared to be sensitive to epistasis as removal of the offending array ('Goda Heenati') considerably improved the rectilinearity. Further, the analysis of the subdiallel following omission of the above mentioned array showed a striking reduction in the estimates of the three dominance variance components and average dominance by Hayman's method, suggeting elimination of at least a part of the epistasis. Thus, some amount of bias due to epistasis in estimates from the apparently, non-interacting 9×9 subdiallel cannot be ruled out. Hence, the results of the present study involving only F_1 Wr/Vr graphic analysis must be considered in view of these limitations.

Failure of the assumption of independent distribution of genes, often associated with small diallels, may result in the overestimation of average dominance in Wr/Vr analysis (Nasser 1965; Feyt 1976). In the present study, the level of dominance in the graphic analysis was well within the range of incomplete dominance against near complete dominance indicated by the analysis of variance components in the 9×9 subdiallel, thus ruling out the question of overestimation in the former (Fig. 2 and Table 2).

Combining ability analysis

Unlike Hayman's method, the combining ability analysis does not provide any test for epistasis. On the other hand, in the combining ability analysis, additive × additive epistasis forms a specific part of variance due to gca while epistasis of additive × dominance and dominance × dominance types are included in sca (Griffing 1956a). As additive gene action and additive × additive types of epistatic gene action are exploitable in homozygous genotypes, the estimates of gca effects of individual lines are a useful predictor for progeny performance in self-fertilizing species (Baker 1978). High correlation between parental means and gca effects in the present study well attests to that. Hence, it is obvious that the two methods together provide more useful information on the mechanism of inheritance than each alone does.

Breeding implication

The varietal improvement program in lowland rice aims at developing superior homozygous lines combining an adequate level of submergence tolerance with other desirable traits (IRRI 1980). Such recombinants could possibly be realized from some of the tolerant/nontolerant hybrids. On the basis of gca effects and the mean performance of the hybrids (low score denoting higher tolerance), the crosses of 'FR13A' with 'RD19', 'IR42', and 'IR17494-32-1' and of 'Kurkaruppan' with 'RD19' and 'CR1009' were considered promising. In general, the possibility of recovering such desirable recombinants is high in view of high heritability in a narrow sense, which indicates high prepotency of the parents in transmitting the trait.

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