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GENETICS OF EMBRYO SIZE AND ITS RELATIONSHIP WITH SEED AND SEEDLING VIGOUR IN RICE (*ORYZA SATIVA* L.)

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ABSTRACT

Study of two ecogeographic groups of rice indicated larger embryo weight and length in japonicas than in indicas. Genetic variation is higher for embryo weight and embryo/endosperm weight and moderate for embryo length. Embryo weight correlated strongly with embryo length, kernel breadth and kernel thickness in two ecogeographic groups. Embryo length can be used to evaluate breeding materials for embryo size. In indicas, embryo weight and length correlated with seed and seedling vigour as measured by response to ageing stress, shoot length, root length and seedling dry matter. Both additive and nonadditive gene effects with preponderance of the former governed the inheritance of embryo length. Average dominance over all the loci across all parents was within the range of incomplete dominance. Combining ability also confirmed preponderance of additive gene effects in the inheritance of embryo size. Heritability estimates in narrow sense were very high.

Key words: *Oryza sativa* rice, embryo size, genetic variation, seed vigour, inheritance.

Earlier studies in rice suggest genetic variation for embryo size [1–2]. However, there is very limited information on the nature of variation [3–4] and its relationship with seed yield [3] and quality [3–5]. A preliminary study on inheritance of embryo length in two crosses between long and short embryo parents revealed continuous variation in F_2 indicating polygenic nature of inheritance [5]. Detailed analysis of its inheritance in rice has not been investigated [6]. Therefore, the present study has been carried out to determine (i) the extent of genetic variation for embryo size and its relationship with other seed characters in two ecogeographic groups, (ii) the relationship of embryo size with seed and seedling vigour, and (iii) the inheritance of embryo size (length) in indica rice.

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MATERIALS AND METHODS

Two sets of experiments were conducted. The first experiment aimed at determination of genetic variation for embryo size in both indica and japonica cultivars while its relationship with seed vigour was analysed only in indicas. The second experiment was conducted to determine the inheritance of embryo length and combining ability of the parents and crosses for embryo length only in cultivars of indica rice. The details of both the experiments are given below.

GENETIC VARIATION IN EMBRYO SIZE AND ITS RELATIONSHIP WITH SEED AND VIGOUR TRAITS

Seed lots of indica (n=67) and japonica (n=38) cultivars representing 29 countries obtained from the International Rice Germplasm Centre and the International Rice Testing Programme were grown in randomized block design with three replications. Seeds of all the cultivars were germinated in the petri dishes at 29°C under continuous light. Three-day-old seedlings were transferred to 3.8-litre pots with well mixed soil and fertilizer. N:P:K were applied in ratio of 5:5:2 g per pot. Three plants per pot were maintained till maturity.

For determination of embryo size, freshly harvested seeds were oven dried to a constant moisture content of 14%. Embryo weights were carefully taken for 50 embryos per replication from manually dehulled seed samples, to a nearest point of 0.1 mg on a Mettler Balance. For embryo length determination, 10 well developed seed intact embryos per replication were obtained from dehulled seed samples. The WILD digital length measuring unit (Model MMS 235) with WILD stereo microscope (Model M 54, made in Switzerland) measured the length of individual embryo to a nearest point of one hundredth of a millimeter. Weight of embryo and embryo-endosperm weight ratio (EER) were calculated on the basis of their fresh weight.

For seed and seedling vigour, fresh lots of 50 seeds from phytotron were treated with dry heat at 50°C for 4 days to eliminate any residual dormancy. Germination counts were made on the 2nd, 5th and 7th day. Seeds developing atleast 2 mm long radicles were considered as germinated. Rate of germination was calculated as percentage seeds germinated in 2 days to those germinated in 7 days. For vigour determination through accelerated ageing of seeds (at 43°C and 100% R.H.) for 4 days [6], germination counts on 5th and 7th day were considered.

For root and shoot length and seedling dry matter determinations, 25 seeds per replication were germinated in rolled paper towels at 25°C and 79% R.H. in a germinator. Ten normal seedlings were randomly taken from each towel on the 14th day, and their root and shoot length measured. The seedlings were dried at 85°C for 12 h and seedling dry matter recorded.

INHERITANCE OF EMBRYO LENGTH

Six indica rice cultivars, viz., Nep Non Tre, Sugdasi, BR 10-1-87, Palman-46, Intan Gawri and UPR 231-28-1-2 of wide range of embryo length were crossed in a diallel without reciprocals. Seeds from hand pollination of the parents and F_1 hybrids in each replication were obtained for the diallel experiment. Embryo length of 20 seeds per replication of parents and hybrids was measured as described earlier. For the study of F_2 population, each parent and hybrid was grown in phytotron with three replications to get the parental and F_2 seeds. Fifty seeds of hybrids and 20 seeds of parents per replication was used to determine embryo length.

STATISTICAL ANALYSIS

Statistical analysis were done as per standard procedures. Genetic analysis was done following the methods of [7–8]. In addition, heterogeneity and joint regression coefficient tests were carried out following Allard [9] to determine the adequacy of model with respect to nonallelic interactions. Combining ability analysis of parents and crosses was done by Griffing's Method 2, Model I [10].

RESULTS AND DISCUSSION

GENETIC VARIATION AND RELATIONSHIP WITH SEED CHARACTERS

Analysis of variance indicated highly significant varietal differences for embryo weight, embryo length and other related seed traits in both the ecogeographic groups. Data on means, range and standard deviation were compared for two groups (Table 1). Wide range of variation for embryo length and weight was observed in two groups. Mean embryo weight and length are larger in japonica as compared to the indica group.

Estimates of GCV (Table 1) were very high for embryo weight and embryo-endosperm ratio (EER) and moderate for embryo length. These estimates are slightly higher in japonicas as compared to indicas. Broadsense heritability estimates indicated that embryo weight, length and EER are highly heritable and the prospects of improving the expression of embryo weight and length by genetic manipulation are high.

Close estimates of GCV and PCV and high heritability of embryo weight, length and EER indicate that differences within each varietal group were mostly genetic in nature. However, more information will be required to confirm these findings including estimates of heritability in narrowsense based on genetic analysis involving segregating generations.

Embryo weight showed significantly positive correlation in indicas with embryo length (r = 0.79), kernel thickness (r = 0.61), endosperm weight (r = 0.58), 100-seed weight (r = 0.56) and kernel breadth (r = 0.52). It was also strongly correlated with embryo length (r = 0.88), kernel breadth (r = 0.52), kernel thickness (r = 0.49) and endosperm weight (r = 0.35) in japonicas. Similar relationships have also been reported between embryo weight and endosperm weight in rice [3, 4, 11]. However, Sasahara et al. [1] reported correlation between embryo weight and endosperm weight in japonicas only, while the correlation in indicas was nonsignificant. Our results suggest that embryo length can be used as reliable index to select for larger embryo size in the evaluation of indica germplasms and breeding materials. Determination of embryo length is also easier and more precise than recording embryo weight.

Table 1. Mean, standard deviation, range, genotypic (GCV) and phenotypic coefficients of variability (PCV) and heritability (h^2) for embryo related traits in rice

Parameter		Embryo length (mm)	Embryo weight (g)	100-seed weight (g)	Endo-sperm weight (g)	Embryo or endo-sperm weight (g)
			Indica			
Mean		1.89	0.026	23.2	9.31	0.003
SD		0.17	0.009	5.3	2.11	0.091
Range	min.	1.52	0.013	5.3	1.85	0.002
	max.	2.28	0.048	37.0	14.97	0.011
GCV		9.0	—	23.6	—	38.7
PCV		9.4	—	24.1	—	40.9
h^2		91.8	—	94.9	—	89.4
			Japonica			
Mean		2.10	0.036	26.0	10.60	0.003
SD		0.25	0.010	3.6	1.64	0.000
Range	min.	1.78	0.020	13.3	5.25	0.002
	max.	3.35	0.076	33.4	14.01	0.009
GCV		11.5	—	27.2	—	36.1
PCV		11.7	—	27.3	—	36.6
h^2		96.2	—	98.5	—	98.7

RELATIONSHIP OF EMBRYO SIZE WITH SEED AND SEEDLING VIGOUR

Analysis of variance revealed highly significant differences among indica cultivars for germination percentage (mean square 25.6), germination rate (1654.4), vigour (890.9), shoot length (1361.4), root length (4211.5) and seedling dry matter (1.28). Correlation coefficients of embryo size (i.e. weight and length) with seed and seedling vigour parameters (Table 2) indicated very strong and positive correlation with seed vigour (under accelerated ageing seed), (r = 0.43 and 0.42), seedling dry matter (r = 0.50 and 0.73), shoot length (r = 0.38 and 0.48) and root length (only embryo weight r = 0.25). Germination and rate of seedling emergence under nonstress conditions were not affected by embryo weight. Total germination was positively correlated with embryo length. Such effects of embryo and endosperm size on seedling growth is due to large embryo having more embryonic cells to initiate growth [12]. A larger endosperm would ensure greater energy supply for germination and subsequent growth of seedlings [13, 14]. The study thus clearly indicates that embryo length can be used to select for improved seed and seedling vigour in indica rice.

Table 2. Correlation coefficients of embryo weight and length with parameters of seed and seedling vigour in indica rices

Trait	Seed vigour			Seedling vigour		
	germination	germination rate	vigour (AAT)	shoot length	root length	seedling dry matter
Embryo weight	0.06	0.12	0.43^{**}	0.38^{**}	0.25^{**}	0.50^{**}
Embryo length	0.25^{*}	0.22	0.42^{**}	0.48^{*}	0.18	0.73^{**}

*,**Significant at P = 0.05 and 0.01 levels, respectively.

INHERITANCE OF EMBRYO LENGTH

Performance of parents and hybrids. Preliminary study on inheritance of embryo length have shown polygenic nature [5]. Analysis of variance showed highly significant differences among the genotypes. The mean embryo length of parents and their arrays (Table 3) showed the presence of undirectional dominance of short embryo over large embryo size. The close correspondence between the parental means and the array mean in F_1 (r = 0.93) and F_2 diallels (r = 0.89) suggests a high prepotency of the parents in transmitting embryo length to their offspring (Table 3).

GRAPHIC ANALYSIS OF (Wr, Vr)

Analysis of embryo length in the present material indicated that t^2-value is nonsignificant confirming that overall assumptions underlying diallel are fulfilled and, therefore, adequacy of the additive dominance model. Also (Wr, Vr) regression for both F_1 and F_2 (Fig. 1) deviated significantly from zero, but nonsignificantly from unit slope, implying the absence of nonallelic interactions (Table 4). However, it has been pointed out that with additive x additive interaction (i) alone or with dominance x dominance interaction (l) alone, the array points scatter along the line of unit slope when p=q=0.5. The line of unit slope, therefore, is not an unequivocal indication of the absence of nonallelic interaction.

The estimates of wr, vr graph of embryo length for each of the replication were used and the variation among (Wr + Vr) as well as among (WR–Vr) partitioned into those due to arrays and due to replications [9]. The presence of significant variation for (WR + Vr) among arrays in both F_1 and F_2 of diallel crosses (Table 5) indicates significant nonadditive genetic variation. The nonsignificance of (Wr–Vr) differences among the arrays, on the other hand, indicates that the nonadditive gene action is solely due to dominance effects of genes, distributed independently among the parental lines. Therefore, it indicates a satisfactory fit to an additive dominance model. The following conclusions could be drawn from these observations: (i) the regression line intercepts the Wr axis above the origin (in F_2 of diallel), thus suggesting that average dominance was incomplete. In F_1 of diallel cross, slight over dominance (a = 0.0019) might be due to differences in genetic constitution of F_1 and F_2 populations which might respond differently to the particular environment, (ii) the position of array points on the graph showed wide range of diversity among parents. In F_1 of diallel cross, the parent var. BR 10-1-87 had maximum number of genes in recessive state and in Palman 46 most of the genes were dominant. Parent Vars. Nep Non Tre and Sugdasi with longer embryos and UPR 231-28-1-2 with the shortest embryo parent had some what equal frequencies of dominant and recessive alleles. In F_2 of the diallel cross, the parental order of dominance is changed for var. BR 10-1-87 due to different materials (F_2 as against F_1) used.

Estimates of genetic parameters (Table 6) indicate that D, h^2, F and E in the F_1 of diallel cross and D, H_1, H_2, h^2 and F in the F_2 diallel cross were highly significant. It indicated the importance of only additive gene action in the inheritance of embryo length from F_1 of diallel cross and additive and nonadditive gene action both from F_2 of diallel cross. However, the larger magnitude of D as compared to H_1 in the latter suggests greater importance of

Table 3. Mean, array-parent offspring covariance (Wr) and variance (Vr) for embryo length in F_1 and F_2 generations of 6 x 6 diallel crosses of indica rice cultivars

Parent	Parent		Mean embryo length, mm hybrids[a]						Generations of diallel cross					
	F_1	F_2							F_1			F_2		
			1	2	3	4	5	6	Array	Wr	Vr	Array	Wr	Vr
Nep Non Tre (1)	2.04	2.01	—	2.07	1.79	1.81	2.01	1.75	1.84	0.019	0.019	1.91	0.020	0.037
Sugdasi (2)	2.03	2.14	2.03	—	1.87	1.71	1.88	1.83	1.86	0.017	0.019	1.92	0.036	0.051
BR 10-1 (3)	1.81	1.69	1.74	1.81	—	1.59	1.65	1.50	1.70	0.021	0.023	1.68	0.007	0.022
Palman 46 (4)	1.74	1.76	1.80	1.94	1.64	—	1.71	1.66	1.74	0.008	0.009	1.71	0.015	0.029
Intan Gawri (5)	1.72	1.49	1.76	1.64	1.61	1.61	—	1.70	1.65	0.019	0.021	1.74	0.008	0.020
UPR 231-28-2 (6)	1.57	1.45	1.71	1.71	1.60	1.71	1.56	—	1.64	0.014	0.015	1.65	0.012	0.026

F_1: P = 1.82, F_2 = 1.77, r (Parent, array mean) = 0.93, Vp = 0.034, Vr = 0.000

F_2: P = 1.75, F_2 = 1.73, r (Parent, array mean) = 0.89, Vp = 0.077, Vr = 0.000

a = F_1 (left diagonal), F_2 (right diagonal hybrids).

additive gene action. The high and significant F value indicates gene assymmetry in both the diallels, which was corroborated by the $H_2/4H_1$ ratio. Negative value of F in F_1 of diallel cross indicated that recessive alleles are more frequent than the dominant alleles irrespective of whether the dominant alleles increase or decrease embryo length. The average dominance $(H_1/D)^{1/2}$ was within the range of incomplete dominance and was in agreement with the conclusion drawn from the graphic analysis in the F_2. Moderately high (0.65) for the ratio $| F/2 | / [D (H_1-H_2)^{1/2}]$ in F_1 of diallel cross implied inconsistency of dominance at some of those loci. This agrees well with the performance of F_1 hybrids which showed that the dominance level varied over the loci but not all of them. However, this estimate from F_2 of diallel cross was near unity (0.98) and indicated near consistency of dominance over the loci.

Table 4. Test for joint regression of Wr, Vr for embryo length

Source	b $\pm$ SE (b)	t^2 for	
		b = 0	b = 1
Joint regression for three replications	$1.1^{**} \pm 0.1$ $(1.1 \pm 0.01)^d$	15.3^{**} $(15.0)^{**}$	-1.5^{NS} $(-1.0)^{NS}$
Regressions:			
Replication I	$0.6^{*} \pm 0.1$ (1.0 ± 0.1)	3.8^{*} (11.3^{**})	2.4^{NS} $(0.5)^{NS}$
Replication II	$1.0^{*} \pm 0.2$ $(1.2^{**} \pm 0.1)$	4.4^{*} (13.4^{**})	0.0^{NS} (2.2^{NS})
Replication III	$0.9^{**} \pm 0.1$ $(0.9^{**} \pm 0.1)$	8.1^{**} 11.2^{**}	0.0^{NS} (1.1^{NS})

**Significant at P = 0.01 level.

^{d}Figures in parentheses relate to diallel of crosses in F_2 generations.

Table 5. Heterogeneity test for (Wr + Vr) and (Wr – Vr) estimates for embryo length in F_1 and F_2 of diallel crosses in indica rice

Source	d.f.	Mean squares	
		F_1	F_2
Wr + Vr (replicate)	12	0.00008	0.00004
Wr + Vr (array)	5	0.00038^{*}	0.00158^{**}
Wr – Vr (replicate)	12	0.00007	0.00002
Wr – Vr (array)	5	0.00007^{NS}	0.00001^{NS}

**,*Significant at P = 0.01 and P = 0.05 levels.

Failure of independent assortment of the genes often associated with small diallels may result in the overestimation of average dominance in Wr/Vr analysis [15] as was the case with F_1 of diallel cross. However, in F_2 of diallel cross, the level of average dominance in the graphic analysis was well within the range of incomplete dominance and partial dominance as indicated by the variance component analysis thus excluding the possibility of overestimation in the former.

COMBINING ABILITY ANALYSIS

The mean squares due to gca and sca for F_1 and F_2 of diallel crosses were highly significant (Table 7). The ratio involving variance components $2S^2$ gca/($2S^2$ gca + S^2 sca) showed that the additive effects of genes were more important than nonadditive effects and thus performance of a cross can be predicted to a considerable extent on the basis of gca effects. In both the diallels, results indicate that high gca x high gca cross (Nep Non Tre x Sugdasi) gives the highest per se performance with moderate to low though positive sca effect for embryo length. The results of combining ability analysis are in general good agreement with the conclusions drawn from the variance components in Hayman's method by showing the considerable importance of additive gene action in the inheritance of embryo length.

Table 6. Estimates of genetic parameters for embryo length in F_1 and F_2 of diallel crosses of indica rice

Parameter	F_1	F_2
D	0.03^{**}	0.07^{**}
F	-0.01^{**}	0.06^{**}
H_1	0.00	0.04^{**}
H_2	0.00	0.04^{**}
h^2	-1.37^{**}	111.40^{**}
E	0.006^{**}	0.001
$(H_1/D)^{1/2}$	0.34	0.84
$(H_2/4H_1)$	0.04	0.20
$\lvert F/2 \rvert / [D(H_1-H_2)^{1/2}]$	0.65	0.98

Among the parents with longer embryo, varieties Nep Non Tre and Sugdasi had the highest gca effects, indicating that these parents possess more genes increasing embryo length (Table 8). It was further observed that the hybrids with the var. Nep Non Tre had embryos either shorter or at par with the longer embryo parent (Table 3). The gca effect of variety Nep Non Tre was higher because its cross with the high gca parent, Sugdasi gives the best per se performance while crosses with the other parents having low and/or negative effects resulted into moderate to low length of embryo. Thus the average performance of this parent to give longer embryos was still highest as compared to other parents. UPR 231-28-1-2 on the other hand had the maximum negative gca effect for this trait. On the basis of gca effects of parents and mean performance of hybrids, the crosses Nep Non Tre x Sugdasi, Nep Non Tre x Intan Gawri, Nep Non Tre x Palman 46 and Sugdasi x Palman 46 were promising. In general, the prospects of recovering recombinants with long embryos are high in view of the high narrow sense heritability (F_1: 0.76, F_2: 0.86) of embryo length.

Table 7. Combining ability analysis of embryo length in F_1 and F_2 of diallel crosses of indica rice

Source	d.f.	Mean square	
		F_1	F_2
Gca	5	0.082^{**}	0.113^{**}
Sca	15	0.006^{**}	0.004^{**}
Error	40	0.002	0.000
S^2 gca		0.010	0.014
S^2 sca		0.004	0.004
$2S^2$ gca/$2S^2$ gca + S^2 sca		0.823	0.878
h^2n		0.764	0.866
h^2b		0.928	0.986

**Significant at P = 0.01 level.

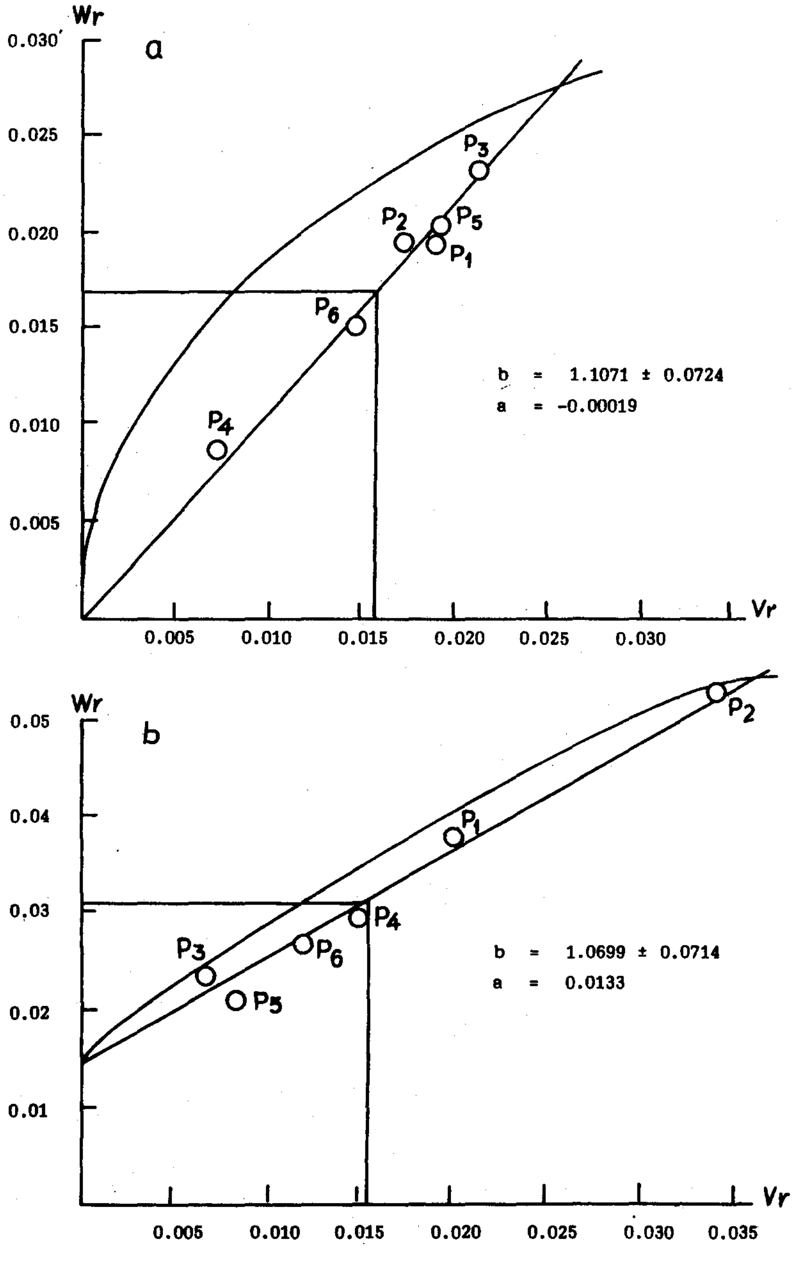

Fig. 1. (Wr/ Vr) graph for embryo length in a 6 X 6 diallel of rice, P_1 = Nep Non Tre, P_2 = Sugdasi, P_3 = BR 10-1-87, P_4 = Palman 46, P_5 = Intan Gawri, P_6 = UPR 231-28-1-2, a = F_1 diallel, b = F_2 diallel.

Table 8. General combining ability (gca) and specific combining ability (sca) effects in F_1 (upper diagonal) and F_2 (lower diagonal) generations of diallel crosses in indica rice

Parent	Gca		Sca					
	F_1	F_2	1	2	3	4	5	6
Nep Non Tre (1)	0.13	0.12	—	0.04	- 0.06	- 0.00	0.10	- 0.05
Sugdasi (2)	0.12	0.16	0.02	—	0.03	- 0.13	- 0.01	0.05
BR 10-1-87 (3)	- 0.06	- 0.04	- 0.06	- 0.04	—	- 0.08	- 0.07	- 0.11
Palman 46 (4)	- 0.06	0.01	- 0.06	0.04	- 0.06	—	0.00	0.05
Intan Gawri (5)	- 0.01	- 0.12	0.03	- 0.13	0.04	- 0.01	—	0.04
UPR 231-28-2 (6)	- 0.11	- 0.12	- 0.02	- 0.06	0.02	0.09	0.09	—
SE (gi)	0.01	0.01						
Se (sij)	0.04	0.02						
SE (gi–gj)	0.02	0.01						

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