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Research Note

Gene action and heterosis studies for grain Fe and Zn content in rice (*Oryza sativa* L.)

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Abstract
Development of rice genotypes possessing high yielding potential; with increased grain iron content is the need of the hour to tackle hidden hunger. Considering the above said fact, genetical analysis was conducted in six improved cultivars along with 15 potential crosses thereof. Analysis of variance for combining ability revealed significant differences in GCA and SCA variances for grain yield as well as for iron and zinc content, indicating the possibility of realization of development of iron fortified varieties with higher yielding potential. The preponderance of additive gene action for these two important characters *i.e.*, iron and zinc content indicate that selection will be highly rewarding for genetic improvement pertaining to these two traits. The genotype Gouri was found to be very good combiner for both grain yield (0.93) and iron content of grain (2.04). Similarly, genotype Sebati was found as a very good combiner for both grain yield (0.13) and zinc content of grain (0.65). Some of the superior crosses identified concerning both grain yield and grain iron content were Birupa / Pradeep, Sebati / Gouri, Sebati / Pratap, Gouri / Pradeep and Pratap / Pradeep. Hence, these specific crosses can be used in further breeding programme for the improvement of both grain yield as well as grain iron content. The crosses viz., Bhanja / Pratap and Bhanja / Sebati were found to be superior crosses for both grain yield and grain zinc content.

Keywords: Rice, Fe, Zn , combining ability, heterosis , gene action

Rice grain typically contain very little iron (5-6 mg/kg), which is insufficient to fulfil the recommended daily intake of 10-15 mg/kg (Borrill *et al.,* 2014). The age and gender of an individual determine the recommended daily amount (RDA) for iron. Infants need 0.27 mg of iron per day, whereas men and women between the ages of 19 and 50 require 8.0 mg and 18 mg per day, respectively. Thus, in the food chain, rice that has been biofortified with iron is necessary. In the wild species of rice, O.nivara, Fe concentration in brown rice has been reported to range from 6 ppm to 72 ppm , while in *O.rufipogon* Zn concentration has been observed to be 67 ppm (Neelamraju *et al.,* 2012). Iron biofortification in rice can be accomplished by genetic engineering or traditional breeding. Conventional breeding entails locating and choosing a parental line that carries desired characteristics and hybridising them to identify progenies that possess favorable nutritional and agronomic characteristics.

Heterosis is typically investigated for features that contribute to grain yield (Bhatti *et al.,* 2015; Thorat *et al.,* 2017; Anusha *et al.,* 2021; Vennela *et al.,* 2022). However, in order to develop biofortified genotypes without compromising yield potential, a significant reorientation of the plant breeding technique is now needed. One of the most crucial methods for determining the breeding value of parents or hybrids is the general combining ability (GCA) and specific combining ability (SCA). Therefore, the goal of the current study is to evaluate, by half diallel analysis, the combining ability of six prospective rice genotypes.

Six rice varieties *viz*., Bhanja, Sebati, Birupa, Gouri, Pratap and Pradeep, which are characterised by high grain yield and iron content were crossed in half diallel fashion. The 15 hybrids generated were raised along with six parents were raised in a randomized block design with two replications at EB-I section of the Department of Genetics and Plant Breeding, College of Agriculture, OUAT, Bhubaneswar during *Kharif* 2022. The paddy seeds were sown in the petri dishes and 10 days old seedlings were transplanted at a spacing of 20 cm x 15 cm between the rows and plants. All intercultural operations and plant protection measures were practised to raise a good crop stand. Observations were recorded on ten randomly selected plants without border effect for eight traits *viz*., days to maturity, plant height, tleer per plant, panicle length, 100-grain weight, grain yield per plant (g), Fe (ppm) and Zn (ppm) content. Seeds from all the entries were dehusked gently using a palm dehusker and the red rice was used for estimation of Fe and Zn concentration using Atomic Absorption spectrophotometer (AAS, Varian) at National Rice Research Institute, Cuttack, India and the concentration was expressed in parts per million (ppm). A minimum of two replications from each of the cultivars and wild accessions were analyzed for the two micronutrients. The mean of the two replicates was taken for data analysis. The Analysis of variance was worked out for all the studied traits. The mean performances of hybrids and paternal lines were compared using the Least Signifcant Diference (LSD) method at a 5% level of significance. The general combining ability of parents and specifc combining ability of hybrids were determined following Grifng's method 2 model 1 (Griffing, 1956). The relative heterosis (%), standard heterosis and heterobeltiosis (%) for the characters were estimated using the standard approach (Turner, 1953). All the statistical analysis were carried out using OPSTAT software.

In this present investigation, 15 crosses produced from six parents through half diallel manner were evaluated for the study of gene action. Observations were recorded for eight characters in the parents as well as the 15 crosses. ANOVA for the eight characters revealed the existence of significant differences among the parents, crosses, parent vs. crosses for all the traits (**Table 1**). The mean of the parental genotype and its crosses for eight traits revealed substantial variation among the parents and hybrids for all the traits studied (**Table 2**) The mean Zn content ranged from 19.96 ppm (Pradeep) to 28.85 ppm (Bhanja) among parents, whereas for the crosses it ranged from 21.90 ppm (Birupa × Gouri) to 26.77 ppm (Bhanja × Sebati) with a general mean of 24.17 ppm. The mean of Fe content ranged from 46.03 ppm (Bhanja) to 62.82 ppm (Pradeep) with a general mean of 53.35 ppm for parental genotypes, whereas for crosses it ranged from 49.94 ppm (Bhanja × Birupa) to 56.95 ppm (Gouri × Pradeep) with a general mean of 53.76 ppm.

Table 1. Mean performance of parents and crosses for eight characters in rice

Characters	Parent (P)		Crosses (F_1)		F_1-P_1	% increase over parental mean
	Mean	Range	Mean	Range		
Days to maturity	130.32	126.00-132.00	133.67	128.32-138.50	3.35	2.57
Plant height	90.92	77.50-120.00	101.01	98.03-103.28	10.09	11.09
Tillers per plant	13.82	11.40-16.50	15.52	13.60-17.15	1.70	12.30
Panicle length	23.79	22.81-25.28	28.19	23.75-30.35	4.40	18.49
Grain yield per plant	33.59	31.21-34.15	40.31	34.13-43.18	6.72	20.00
100 Grain weight	2.43	2.150-2.80	2.96	2.66-3.15	0.53	9.46
Zn	23.18	19.96 – 28.85	24.17	21.90 – 26.77	0.99	4.27
Fe	53.35	46.03 – 62.82	53.76	49.94 – 56.95	0.41	0.76

Table 2. Analysis of variance of parents and the crosses (F_1) in a six parent half diallel crosses for eight characters in rice

Characters	Parent (5)	Crosses (14)	Parent Vs. Crosses (1)	Error (20)
Days to maturity	36.95**	4.34	101.01**	6.87
Plant height	450.80**	5.48	858.91**	9.84
Tillers/plant	9.70**	2.43	24.85**	1.44
Panicle length	1.64	6.12**	166.32**	1.08
Grain yield/plant	17.52**	11.07**	270.59**	3.87
100 grain weight	33.10**	3.91**	824.99**	0.92
Zn	21.40**	4.34**	8.49**	0.90
Fe	86.04**	11.11*	1.42	4.62

*and** indicate significance at 5% and 1% levels probability respectively

ANOVA for combining ability revealed significant differences in GCA and SCA variances for all the eight studied character, indicating the possibility of realization of development of iron fortified varieties with higher yielding potential (**Table 3**). This also fosters the importance of both additive and non-additive gene effects in the material under study and exploitation of both additive and non-additive gene effects would be more desirable for the improvement of the traits. The estimates variance components (**Table 4**) due to GCA and SCA effects and their ratio showed that SCA variance was higher than GCA variance for days to maturity, plant height, tiller number per plant, panicle length and grain yield, while for iron and zinc content, the GCA variance was found to be higher than the SCA variance. The preponderance of additive gene action for these two important characters i.e., for both grain iron and zinc indicates the role of judicious selection for the development of iron and zinc fortified genotypes. Similar findings on general combining ability effect have been reported by Lingaiah *et al*., 2021; Pradhan *et al*., 2006, Hijam and Sarkar 2016, and Kirubha *et al*., 2019.

The selection of suitable parents for hybridization is one of the most important steps in a breeding programme to get best combination in F_1 hybrids. Hence the GCA effect that determines the average performance of a parent in crosses was determined for each of the parents (**Table 5**).The genotype Gouri was found to be very good combiner for both grain yield (0.93) and iron content of grain (2.04). Similarly, genotype Sebati was found as a very good combiner for both grain yield (0.13) and zinc content of grain (0.65). Hence, it is apparent that, while breeding for development of bio-fortified varieties, these two genotypes namely, Gouri and Sebati could be given preference for exploitation as parents. The results are in agreement with Lingaiah *et al*., 2021; Pradhan *et al*., 2006 and Kirubha *et al*., 2019.

Table 3 Analysis of variance of combining ability effects of 6 × 6 half diallel crosses for eight characters in rice

Source	Days to maturity	Plant height	Tillers per plant	Panicle length	100 grain weight	Grain yield per plant	Zn	Fe
GCA	12.54*	116.45**	3.97**	2.23**	6.55**	3.72*	48.84**	12.24**
SCA	7.37*	67.52**	2.26**	7.93**	32.66**	15.86**	3.29	1.79**
Error	3.43	4.92	0.72	0.54	0.46	1.93	2.31	0.45

*and** indicate significance at 5% and 1% levels of probability respectively

Table 4 Components of variance for eight characters in 6 × 6 half diallel crosses in rice

	Days to maturity	Plant height	Tillers per plant	Panicle length	100 grain weight	Grain yield per plant	Zn	Fe
σ^2g	1.138	13.93	0.40	0.21	0.76	0.22	1.47	5.81
σ^2s	3.93	62.60	1.54	7.39	32.20	13.93	1.34	0.98
σ^2e	3.43	4.92	0.72	0.54	0.46	1.93	0.45	2.31
σ^2g/ σ^2s	0.28	0.22	0.26	0.02	0.024	0.01	1.09	5.92
PF	0.36	0.30	0.34	0.05	0.04	0.03	0.68	0.92
$\sigma^2a = 2\,\sigma^2g$	2.27	27.87	0.81	0.42	1.52	0.44	2.94	11.63
$\sigma^2D = \sigma^2s$	3.93	62.60	1.5	7.39	32.20	13.93	1.34	0.98

σ^2g = variance due to GCA, σ^2s = variance due to SCA, σ^2e = variance due to environment, σ^2a = additive variance, σ^2D = Dominance variance, PF (Predictability factor) = 2 σ^2g / σ^2g + σ^2s

Table 5. Estimates of general combining ability effects of six rice parents for eight characters

	Days to maturity	Plant height	Tillers per plant	Panicle length	100 grain weight	Grain yield per plant	Zn	Fe
Bhanja	-0.22	-0.75	0.47	-0.80**	-0.94**	-0.92	2.02**	-2.36**
Birupa	-2.19**	-1.15	-0.88**	-0.21	0.25	0.56	-1.12**	0.45
Sebati	1.59*	-2.93**	-0.89**	-0.01	0.62**	0.13	0.65**	-2.64**
Gouri	0.28	-1.46	0.44	0.04	1.10**	0.93	-0.46*	2.04**
Pratap	-0.05	-1.33	0.65*	0.17	0.18	-0.28	0.17	-1.02*
Pradeep	0.59	7.63**	0.19	0.81**	-1.22**	-0.42	-1.26**	3.53**

*and** indicate significance at 5% and 1% levels of probability respectively

The usefulness of a particular cross in exploiting heterosis is judged by specific combing ability effects (**Table 6 and 7(a, b, c**) and is an important criterion for the evaluation of hybrids. For breeding early duration genotypes, negative estimates for days to maturity are eminent components. The cross Gouri x Pradeep exhibited highest negative SCA and relative heterosis for days to maturity. Similarly, for plant height, a due consideration is usually given to the negative estimates of both *sca* and heterosis. The cross Gouri x Pratap showed highest negative estimate

Table 6. Estimates of specific combining ability effects in a 6 × 6 half diallel crosses for eight traits in rice

	Days to maturity	Plant height (cm)	Tillers per plant	Panicle length	100 grain weight (g)	Grain yield per plant (g)	Zn (ppm)	Fe (ppm)
Bhanja × Birupa	2.29	6.36**	0.27	-2.16**	2.78**	-3.22*	0.35	-1.79
× Sebati	-2.39	7.36**	1.63*	0.33	4.56**	0.70	0.20	2.57
× Gouri	-1.96	3.14	0.79	2.48**	1.81**	1.74	0.67	2.43
× Pratap	-0.99	4.18	0.83	0.55	5.33**	1.73	-0.94	2.44
× Pradeep	-0.23	-1.76	1.44	2.95**	6.93**	2.95*	-2.10**	0.10
Birupa × Sebati	-2.13	6.87**	1.74*	2.94**	1.23	2.57	-1.04	-0.07
× Gouri	-1.06	2.90	-0.99	3.18**	2.17**	0.79	-0.39	0.15
× Pratap	0.90	4.47*	0.44	0.96	2.30**	2.36	0.31	-0.17
× Pradeep	1.82	-3.93	2.24**	-0.78	2.73**	2.45	2.50**	-0.75
Sebati × Gouri	0.74	6.57**	0.81	0.59	-0.58	3.72**	1.62*	1.37
× Pratap	-1.88	9.07**	-0.80	1.46*	1.32*	5.61**	0.56	0.28
× Pradeep	-2.34	-0.31	-0.08	1.76*	4.204**	2.34	-0.22	-1.02
Gouri × Pratap	-2.17	6.09**	-1.08	1.95**	3.07**	-2.45	0.13	-1.26
× Pradeep	-3.81 *	-6.29**	-0.26	0.20	3.25**	-0.66	1.03	-2.27
Pratap × Pradeep	-1.47	-1.84	0.31	2.42**	0.89	3.40*	1.57*	-0.25
SE (ii)	**1.35**	**1.62**	**0.62**	**0.53**	**0.49**	**1.01**	**0.49**	**1.11**

*and** indicate significance at 5% and 1% levels of probability respectively

Table 7a. Estimates of heterobeltiosis (HB) and relative heterosis (RH) for days to maturity, plant height and tillers per plant in 6 × 6 half diallel crosses in rice

Crosses	Days to maturity		Plant height		Tillers per plant	
	Heterobeltiosis	Relative heterosis	Heterobeltiosis	Relative heterosis	Heterobeltiosis	Relative heterosis
Bhanja × Birupa	-1.00	1.49	17.26**	17.60**	10.37	19.68*
× Sebati	-5.94**	-3.86*	17.05**	23.81**	20.37*	29.48**
× Gouri	-4.86*	-3.62	11.32**	12.59**	2.76	12.42
× Pratap	-2.97	-2.42	15.23**	16.91**	3.03	13.33
× Pradeep	-3.00	-1.92	-14.29**	-0.46	26.10**	26.57**
Birupa × Sebati	-7.17**	-2.78	15.35**	22.35**	29.31*	30.43**
× Gouri	-5.65**	-2.05	10.60**	11.54**	-16.56*	-1.81
× Pratap	-3.01	-0.03	14.45**	16.45**	-7.58	9.32
× Pradeep	-2.93	0.59	-16.44**	-3.18	21.95*	32.68**
Sebati × Gouri	-3.31	-2.43	12.72**	20.50**	-5.52	10.39
× Pratap	-5.44**	-3.88*	21.85**	27.11**	-15.15	-0.36
× Pradeep	-5.31*	-4.28*	-14.90**	3.58	4.78	13.10
Gouri × Pratap	-4.89*	-4.19*	13.99**	16.95**	-8.79	-8.23
× Pradeep	-5.62**	-5.45**	-18.65**	-6.42*	-5.46	3.08
Pratap × Pradeep	-3.79	-3.25	-14.85**	0.11	-1.82	7.64

Table 7b. Estimates of heterobeltiosis (HB) and relative heterosis (RH) for panicle length, 100-grain weight and grain yield per plant in 6 × 6 half diallel crosses in rice

Crosses	Panicle length		100 grain weight		Grain yield per plant	
	Heterobeltiosis	Relative heterosis	Heterobeltiosis	Relative heterosis	Heterobeltiosis	Relative heterosis
Bhanja × Birupa	-2.78	-0.36	33.18**	60.96**	-8.44	-3.98
× Sebati	13.28**	13.54**	36.84**	68.29**	15.59	24.44**
× Gouri	23.28**	24.43**	19.17**	50.05**	5.20	13.51
× Pratap	13.77**	14.65**	51.55**	79.75**	18.10*	23.52**
× Pradeep	18.28**	23.25**	105.70**	113.63**	22.62*	28.81**
Birupa × Sebati	21.37**	24.11**	27.48**	30.32**	17.59*	32.27**
× Gouri	22.60**	26.80**	25.65**	32.37**	7.14	10.40
× Pratap	14.00**	15.97**	36.20**	39.38**	15.20	26.09**
× Pradeep	5.81	7.62	31.71**	54.28**	14.99	26.38**
Sebati × Gouri	17.99**	19.37**	15.76**	19.36**	16.07*	34.02**
× Pratap	20.97**	21.62**	27.55**	33.36**	52.18**	56.86**
× Pradeep	16.69**	21.32**	34.05**	59.91**	37.78**	41.38**
Gouri × Pratap	23.31**	25.40**	29.09**	38.99**	-7.52	4.00
× Pradeep	10.76*	16.45**	23.98**	51.59**	-1.61	11.09
Pratap × Pradeep	20.06**	24.18**	28.75**	47.89**	39.45**	40.09**

Table 7c. Estimates of heterobeltiosis (HB) and relative heterosis (RH) for iron and zinc content in 6 × 6 half diallel crosses in rice

Crosses	Zn		Fe	
	Heterobeltiosis	Relative heterosis	Heterobeltiosis	Relative heterosis
Bhanja × Birupa	-12.83**	1.35	-10.63*	-1.99
× Sebati	-7.19*	0.12	9.41	10.33*
× Gouri	-9.46**	3.92	-3.07	7.69
× Pratap	-12.83**	-3.77	3.16	8.53*
× Pradeep	-21.88**	-7.65*	-12.58**	0.90
Birupa × Sebati	-9.16*	-1.44	-8.04	0.09
× Gouri	2.26	3.82	-2.13	-0.71
× Pratap	-0.66	5.27	-5.33	-1.09
× Pradeep	15.50**	17.81**	-9.45*	-4.15
Sebati × Gouri	4.30	11.59**	-5.39	4.33
× Pratap	2.66	5.26	-1.61	2.70
× Pradeep	-6.46	3.34	-14.81**	-2.36
Gouri × Pratap	1.35	5.87	-7.16	-1.66
× Pradeep	8.24	12.05**	-9.34*	-5.35
Pratap × Pradeep	4.06	12.35**	-11.01**	-1.83

of for plant height with respect to sca, relative heterosis and heterobeltiosis. Some prominent crosses exhibiting positive significant sca for tillers per plant include Birupa × Pradeep and Birupa × Sebati. These two crosses also showed higher relative heterosis for the trait concerned. Hence, these two crosses need careful consideration for genetic improvement in tillers per plant. Heterosis and sca estimated in positive direction is highly essential for breeding traits like panicle length, 100-seed weight, grain yield, grain iron and zinc content. The crosses including Birupa × Gouri and Bhanja × Pradeep exhibited superior performance in terms of both sca and heterosis for panicle length. The cross Bhanja × Pradeep showed highest positive significant sca effect followed by Bhanja × Pratap and Sebati × Pradeep for 100-grain weight. These crosses also performed good while the evaluation

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was based on heterosis estimation, there by indicating their potential for improvement of the trait concerned. Some of the superior crosses identified concerning both grain yield and grain iron content were Birupa x Pradeep, Sebati x Gouri, Sebati x Pratap, and Pratap x Pradeep. Hence, these crosses can be used in further breeding programme for the improvement of both grain yield as well as grain iron content. The crosses namely Bhanja x Pratap and Bhanja x Sebati were found to be superior for both grain yield and grain zinc content. Therefore, the segregating generations from these crosses needs to be handled with great care to identify lines with higher yield and better nutritional value.

Significant variation was found among the parents and crosses pertaining to grain iron and zinc content as indicated by combining ability variance analysis. Involvement of additive gene action in the governance of Fe and Zn content as indicated by combining ability analysis suggests the importance of selection for these traits in the breeding for bio-fortified rice varieties. The genotype Gouri was identified to be a good combiner for grain yield and grain iron content. Similarly, the genotype Sebati identified as an excellent combiner for grain yield and zinc content. In terms of grain yield and grain iron content, the crosses Birupa x Pradeep, Sebati x Gouri, Sebati x Pratap, Gouri x Pradeep, and Pratap x Pradeep were identified to be superior, while the crosses Bhanja x Pratap and Bhanja x Sebati were superior in terms of grain zinc content and grain yield. These crosses could be exploited for selection of transgressive segregants in later generations.

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