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# Assessment of Gene Action for Grain Micronutrient Content, Yield and Yield Contributing Traits in Rice (Oryza sativa L.)

Ashutosh Kumar<sup>1\*</sup>, Avinash Kumar<sup>1</sup>, N. K. Singh<sup>1</sup>, Rajesh Kumar<sup>1</sup>, Nilanjaya<sup>1</sup>, S. K. Singh<sup>1</sup>, Mithilesh Kumar Singh<sup>1</sup>, Aman Tigga<sup>1</sup> and Banshidhar<sup>1</sup>

<sup>1</sup>Department of Plant Breeding and Genetics, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, 848125, Bihar, India.

#### Authors' contributions

This work was carried out in collaboration among all authors. Author Ashutosh Kumar designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors Avinash Kumar, NKS, RK, Nilanjaya, SKS, MKS and AT managed the analyses of the study. Author Banshidhar managed the literature searches. All authors read and approved the final manuscript.

#### Article Information

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#### ABSTRACT

Biofortification of food crops using conventional breeding or biotechnological approach is gaining momentum to alleviate micronutrient malnutrition. Rice is a nice choice for biofortification of grain iron and zinc content as this is a cheap and chief staple food for millions of peoples world-wide particularly the poor. In present study, generation mean analysis was done to estimate the nature and magnitude of gene effects for grain iron and zinc content in rice cross Khusisoi-RI-Sareku × IR 91175-27-1-3-1-3. Scaling test and Joint scaling test indicated the influence of epistasis on the expression of yield, its component traits and grain Fe and Zn content and inadequacy of additive-dominance model to explain the variation in different generations. Dominance [h] gene effect was of higher magnitude as compared to additive [d] gene effect for both grain iron and grain zinc content. Additive × additive, additive × dominance and dominance × dominance component was significant for both grain Fe and Zn content, whereas dominance × dominance component was predominant

\*Corresponding author: E-mail: mrbreeder786@gmail.com;

for both grain Fe and Zn content. Dominance [h] gene effect and dominance × dominance interaction acted in opposite directions, indicating duplicate type of gene action controlling the expression of both grain Fe and grain Zn content which could be a bottleneck to exploit heterosis. Heterosis breeding and recombination breeding with postponement of selection till later generations, could be effective in improving both grain Fe and grain Zn content in rice.

Keywords: Rice; generation mean analysis; biofortification; scaling test; gene action.

#### 1. INTRODUCTION

Rice (Oryza sativa L.) is the world's most important staple food crop for more than half of the world's population and supply more than 50% of the calories consumed by the entire human population [1]. Average daily intake of rice provides 20-80 per cent of dietary energy and 12-17 per cent dietary proteins for Asians. India is the second largest producer and consumer of rice in the world. The leading rice producing countries are China, India, and Indonesia which together account for over 50% the world's total production of [2]. About 90 percent of global rice production is contributed by developing countries. But, unfortunately, about 870 million people are chronic undernourishment. sufferina from globally [3] and vast majority of them are from developing countries. In developing countries. iron and zinc deficiencies are reported to be the sixth and fifth highest health risk factor. respectively, causing high mortality rates [4,5]. Therefore, to overcome these nutritional deficiencies is the need of hour. The amount of mineral nutrients in rice grain is a key determinant of its nutritive value. On an average, brown rice comprises of 90% endosperm, 6-7% bran and 2-3% embryo by weight [6]. Recent Xmicro-fluorescence investigations ray demonstrated that the concentrations of zinc (Zn), iron (Fe), and potassium (K) decrease in the order as: bran > hulls > whole grain > brown rice > polished rice [7,8]. Dehulling and polishing of rice depletes the very element that is deficient in the diets of many of its consumers. Therefore, it is important to retain/increase the grain Fe and grain Zn content. This can be achieved by understanding the nature of gene action involved for Fe and Zn accumulation in grain. Biofortification could be accomplished by breeding or following good agronomic practices (GAP). Assessment of the amount of genetic variability for grain Fe and grain Zn in rice germplasm is the initial step towards improving rice genotypes for improved grain Fe and grain Zn content [9].

The information on the nature of the gene action could be helpful in deciding the choice of appropriate breeding and selection method for improvement of trait. This follows partitioning of genetic variance into additive, dominance and epistatic components. The reliability of selection in segregating population and genetic gain largely depends upon the extent of genetic variability that is attributable to additive genetic variance and additive× additive epistasis.

The Generation Mean Analysis (GMA) is a biometrical tool that enables estimation of the different components of genetic variance and also the presence or absence of epistasis. Scaling test [10] indicates mere the presence/absence of different types of epistatic interactions for the trait of interest in the particular cross whereas, the Joint Scaling test [11] detect as well as estimate different types of epistatic interactions. Estimates like genetic advance, heritability, heterosis and inbreeding depression can also be estimated from the material used in GMA. The estimates obtained from this biometrical tool are statistically robust because the analysis is based on first order statistics. Therefore, the study of genetics of grain yield and guality traits is imperative to formulate a breeding programme directed to improve yield and grain nutritional guality of rice.

Keeping in view the above mentioned facts and figures, the present investigation was undertaken to determine the nature and magnitude of gene action for grain yield and Fe and Zn content in rice genotypes using GMA.

## 2. MATERIALS AND METHODS

Two rice genotypes namely, Khusisoi-RI-Sareku and IR91175-27-1-3-1-3 were procured from Harvest Plus Rice Project at Department of Plant Breeding and Genetics, RPCAU, Pusa, Samastipur, Bihar. Khusisoi-RI-sareku had high grain Fe and Zn content while, IR91175-27-1-3-1-3 had low grain Fe and Zn content. Khusisoi-RI-Sareku (P<sub>1</sub>) and IR91175-27-1-3-1-3 (P<sub>2</sub>) were crossed to generate F<sub>1</sub> during *kharif*, 2017. F<sub>1</sub> was selfed to generate F<sub>2</sub> and backcrossed to both the P<sub>1</sub> and P<sub>2</sub> to generate B<sub>1</sub> and B<sub>2</sub> during *kharif*, 2018. These six basic generations (P<sub>1</sub>, P<sub>2</sub>, F<sub>1</sub>, F<sub>2</sub>, B<sub>1</sub> and B<sub>2</sub>) were raised in a randomized block design with three replications during *kharif*, 2019. The sample size used in each replication for data recording was 10 plants in P<sub>1</sub>, P<sub>2</sub> and F<sub>1</sub>; 20 plants in B<sub>1</sub> and B<sub>2</sub> and 70 plants in F<sub>2</sub>. The estimation of micronutrients from brown rice by XRF (X-Ray Fluorescence Spectrometry) [12] was carried out at HarvestPlus Division, ICRISAT, Hyderabad.

#### 2.1 Generation Mean Analysis

The generation mean analysis was performed according to Hayman (1958) and Jinks and Jones (1958) for the estimation of genetic components of variation, epistasis model and gene effects in two steps (i) testing for epistasis to determine the presence or absence of non-allelic interaction and (ii) estimation of gene effects, variances and the type of epistasis involved.

## 2.2 Scaling Test

Scaling test for A, B, C and D scales as suggested by Mather [10] was applied to test the adequacy of simple additive–dominance model. Utilizing the means of different generations, the values of A, B, C and D scales were constructed using the following formulae.

 $A = 2B_1 - P_1 - F_1; B = 2B_2 - P_2 - F_1; C = 4F_2 - F_1$  $2F_1 - P_1 - P_2$ ; D =  $2F_2 - B_1 - B_2$ ; where, P<sub>1</sub>, P<sub>2</sub>,  $F_1$ ,  $F_2$ ,  $B_1$  and  $B_2$  are the means of parent 1, parent 2, F1, F2 and backcross generations B1 and B2, respectively. Utilizing the variance of different generations, the variances of A, B, C and D scales were computed as follows: VA =  $4VB_1$  +  $VP_1 + VF_1; VB = 4VB_2 + VP_2 + VF_1; VC = 16VF_2 +$  $4VF_1 + VP_1 + VP_2$ ; VD =  $4VF_2 + VB_1 + VB_2$ ; where,  $VP_1$ ,  $VP_2$ ,  $VF_1$ ,  $VF_2$ ,  $VB_1$  and  $VB_2$  are the variances of means of the P1, P2, F1, F2, B1 and B<sub>2</sub> generations, respectively. To test the significance of the scales, the 'Student's t' values for each of these quantities were calculated as follows: t(A) = A/SE(A); t(B) = B/SE(B); t(C) =C/SE(C); t (D) = D/SE(D); where standard error (SE) is the square root of respective variance e.g., SE(A) = (VA)1/2. The significance of the scales were tested using 't' test.

#### 2.3 Joint Scaling Test

Joint scaling test [11] was conducted which combines several scaling test into one and tests

the adequacy of additive–dominance model using a  $\chi^2$  test.

#### 2.4 Estimation of Gene Effects Using Six Generation Means

The generation means were analysed by the method suggested by [13] to provide information on the inheritance of various traits. The generation means were used to estimate the six genetic parameters viz., m, d, h, i, j and l of digenic interaction model representing mean, additive genetic effect, dominance genetic effect, additive × additive gene interaction effect, additive × dominance interaction effect and dominance x dominance gene effects, respectively assuming that no linkage and no higher order gene interaction exists.

#### 3. RESULTS AND DISCUSSION

To elucidate the nature of gene action for yield traits along with grain Fe and Zn content GMA was carried out using the data recorded from six basic generations of the cross Khusisoi-RI-Sareku × IR91175-27-1-3-1-3. The analysis of variance for individual trait was carried out for all the fourteen traits (Table 1). The mean sum of squares revealed significant differences for all the traits in all the generations indicating considerable variability in the experimental material. The values of scaling tests and estimates of six parameters viz., m, d, h, i, j and l for different traits are presented in Table 4 and Table 5. Information on these parameters (genetic architecture of trait) is essential for proper selection of breeding methodology. The mean effects were highly significant for all traits. Epistatic gene effects were found significant for all the traits. Mean performance of F1 was superior to both the parents for the traits viz., panicles per plant, panicle length, flag leaf area, chlorophyll content, harvest index, grain Fe content, grain Zn content and grain yield per plant. For rest of the traits, F1 mean performance of F<sub>1</sub> was midway between the parental values with inclination towards better parent. For most of the traits the mean performance of  $F_1$  was superior to that in F<sub>2</sub> generation (Table 3). The grain Fe content in the experimental material varied from 11.30 ppm to 26.80 ppm with a mean value of 16.26 ppm and grain Zn content varied from 17.10 ppm to 29.30 ppm with a mean value of 22.58 ppm (Table 2). The scaling test showed that all the scales i.e. A, B, C and D were significant for plant height, panicles per plant, grains per panicle, harvest index, grain Fe

content and grain Zn content, that indicated presence of epistasis in expression of these traits. Further, Joint Scaling test was performed to fit the data to three parameter model to estimate mean [m], additive gene effect [d] and dominant gene effect [h] and to validate adequacy of additive-dominance model [13].  $\chi^2$ test was conducted to test the goodness of fit of this model. For all the traits studied, the  $\chi^2$  values were found significant indicating the presence of digenic non-allelic interaction indicating that the data does not fit into additive-dominance model. Therefore, the data was further subjected to six parameter model [13]. Digenic non-allelic interaction model with six parameters namely, m, d, h, i, j and / [13] revealed that the epistatic interaction model was adequate to explain the gene action in the traits like panicles per plant,

panicle length, flag leaf area, grains per panicle, canopy temperature, chlorophyll content, test weight, harvest index, grain Fe content, grain Zn content and grain yield per plant (Table 5). The same sign of [h] and [l] indicated the involvement of complementary type of gene interaction in expression of panicles per plant, flag leaf area and grains per panicle. Complementary type of epistasis tends to enhance the heterotic effect as the magnitude of [I] adds to the main effect [h] as opposed to the case in duplicate type of epistasis. Under such circumstances, selection in advanced segregating generation would be more effective. Dominance [h] and dominance × dominance [1] gene effects displayed opposite signs for the traits viz., days to 50 % flowering, days to maturity, plant height, panicle length.

Table 1. Analysis of variance for fourteen traits in rice cross Khusisoi-RI-Sareku × IR91175-27-1-3-1-3

SI.	Traits	Ме	an Sum of Squares	
No.		Replication (df = 2)	Treatment (df = 5)	Error (df =10)
1	Days to 50% flowering	5.28	416.67**	10.45
2	Days to maturity	2.82	188.66**	6.64
3	Plant height (cm)	51.43	21124.47**	96.85
4	Panicles per plant	4.93	84.60**	3.75
5	Panicle length (cm)	11.04	207.94**	5.67
6	Flag leaf area (cm <sup>2</sup> )	22.97	292.27**	11.22
7	Grains per panicle	11.78	1002.38**	90.12
8	Canopy temperature ( <sup>0</sup> C)	5.78	33.62**	2.76
9	Chlorophyll content (SPAD)	1.63	184.41**	14.69
10	Test weight (g)	29.12	223.27**	14.35
11	Harvest index (%)	4.80	222.02**	2.52
12	Grain Fe content (ppm)	4.68	157.20**	9.89
13	Grain Zn Content (ppm)	7.21	75.20**	4.32
14	Grain yield per plant (g)	32.50	360.78**	26.05

Table 2. Descriptive statistics for fourteen traits in rice cross Khusisoi-RI-Sareku × IR91175-27-1-3-1-3

SI. No.	Traits	Ra	nge	Mean	SE (±)	SD	C.V.
		Lowest	Highest				
1	Days to 50% flowering	79.00	96.00	90.44	0.32	6.67	7.37
2	Days to maturity	115.00	129.00	123.33	0.28	6.48	5.25
3	Plant height (cm)	96.00	180.00	133.81	0.69	18.64	13.93
4	Panicles per plant	6.00	18.00	11.94	0.29	1.89	15.84
5	Panicle length (cm)	20.00	36.00	25.88	0.32	3.23	12.48
6	Flag leaf area (cm <sup>2</sup> )	20.31	39.21	28.22	0.33	3.82	13.55
7	Grains per panicle	112.00	167.00	131.28	0.50	10.03	7.64
8	Canopy temperature ( <sup>0</sup> C)	25.60	33.90	28.89	0.18	1.77	6.13
9	Chlorophyll content (SPAD)	32.60	47.10	40.21	0.28	3.87	9.63
10	Test weight (g)	13.21	35.91	20.78	0.33	3.41	16.41
11	Harvest index (%)	36.54	49.45	44.64	0.23	2.61	5.86
12	Grain Fe content (ppm)	11.30	26.80	16.26	0.26	3.41	20.96
13	Grain Zn Content (ppm)	17.10	29.30	22.58	0.22	2.31	10.25
14	Grain yield per plant (g)	20.03	46.73	30.30	0.37	5.48	18.10

SI. No.	Traits	P	P2	F <sub>1</sub>	F <sub>2</sub>	B <sub>1</sub>	<b>B</b> <sub>2</sub>	Mean
1	Days to 50% flowering	93.80±0.18	85.57±0.27	91.70±0.36	86.79±0.28	93.33±0.37	91.46±0.43	90.44±0.32
2	Days to maturity	126.76±0.22	119.77±0.18	123.60±0.46	122.0±0.22	124.95±0.27	122.92±0.31	123.33±0.28
3	Plant height (cm)	168.60±0.92	104.86±0.67	130.0±0.90	135.39±0.39	143.73±0.72	120.30±0.55	133.81±0.69
4	Panicles per plant	13.28±0.40	10.26±0.25	14.40±0.37	10.96±0.16	12.33±0.30	10.38±0.25	11.94±0.29
5	Panicle length (cm)	27.36±0.25	21.87±0.24	27.43±0.47	28.13±0.20	25.40±0.36	25.08±0.38	25.88±0.32
6	Flag leaf area (cm <sup>2</sup> )	29.25±0.35	26.64±0.19	33.33±0.41	26.73±0.27	27.72±0.41	25.63±0.37	28.22±0.33
7	Grains per panicle	129.7±0.54	126.5±0.45	139.83±0.59	132.48±0.36	130.43±0.49	128.76±0.55	131.28±0.50
8	Canopy temperature ( <sup>0</sup> C)	27.22±0.12	30.37±0.13	29.04±0.29	28.79±0.13	28.60±0.18	29.32±0.21	28.89±0.18
9	Chlorophyll content (SPAD)	40.31±0.19	35.27±0.15	43.36±0.42	39.17±0.30	42.37±0.37	40.75±0.25	40.21±0.28
10	Test weight (g)	21.31±20	19.79±0.14	20.82±0.38	21.63±0.31	20.77±0.50	20.38±0.44	20.78±0.33
11	Harvest index (%)	45.53±0.26	43.48±0.14	47.66±0.28	45.07±0.20	43.09±0.25	42.98±00.26	44.64±0.23
12	Grain Fe content (ppm)	16.85±0.17	13.16±0.21	17.93±0.36	17.63±0.27	14.96±0.19	17.03±0.38	16.26±0.26
13	Grain Zn Content (ppm)	24.23±0.16	21.07±0.14	24.71±0.29	22.78±.15	21.65±.28	21.02±.30	22.58±0.22
14	Grain yield per plant (g)	31.07±0.25	26.24±0.26	34.32±0.38	28.60±0.33	32.40±0.45	29.17±0.54	30.30±00.37

Table 3. Mean performance of six basic generations in rice cross Khusisoi-RI-Sareku × IR91175-27-1-3-1-3

Table 4. Scaling and Joint Scaling test for fourteen traits in rice cross Khusisoi-RI-Sareku × IR91175-27-1-3-1-3

Traits		Sca	ling test			Joint Scalimg te	st	Chi-square	Epistasis
	Α	В	C	D	m	d	h	value	
Days to 50% flowering	1.17± 0.83	3.66**± 0.97	-17.59**± 1.37	-11.21**± 0.80	90.42**± 0.16	3.16**± 0.16	-0.06± 0.35	232.5**	Present
Days to maturity	-0.47± 0.75	2.47**±0.810	-5.73**± 1.33	-3.86**± 0.61	123.18**± 0.13	3.35**± 0.13	-0.13± 0.35	50.61**	Present
Plant height (cm)	-11.13**± 1.92	5.73**± 1.58	8.11**± 2.66	6.75**± 1.20	136.56**± 0.561	29.74**± 0.48	-5.26**± 0.98	85.29**	Present
Panicles per plant	-3.02**± 0.81	-3.91**± 0.67	-8.49**± 1.09	-0.78**± 0.51	10.99**± 0.21	1.38**± 0.20	1.25**± 0.40	66.42**	Present
Panicle length (cm)	-4.00**± 0.89	0.86± 0.93	8.45**± 1.26	5.79**± 0.65	24.79**± 0.17	2.49**±0.17	3.95**±0.38	115.12**	Present
Flag leaf area (cm <sup>2</sup> )	-7.14**± .98	-8.71**± 0.88	-15.62± 1.43	0.20± 0.78	27.18**±0.18	1.01**± 0.18	2.39**± 0.40	179.53**	Present
Grains per panicle	-8.67**± 1.27	-8.80**± 1.33	-5.92**± 1.99	5.77**± 1.03	127.10**± 0.32	1.36**± 0.32	10.03**±0.63	76.74**	Present
Canopy temperature ( <sup>0</sup> C)	0.94*± 0.37	-0.77± 0.52	-0.49± 0.81	-0.33± 0.38	28.79**± 0.08	-1.49**± 0.08	0.24± 0.22	11.61**	Present
Chlorophyll content (SPAD)	1.06± 0.87	2.86**± 0.67	-5.62**± 1.47	-4.77**± 0.70	37.79**± 0.12	2.41**± 0.11	5.93**± 0.32	54.84**	Present
Test weight (g)	0.60± 1.08	0.15± 0.97	3.78**± 1.46	2.11**± 0.90	20.59**± 0.12	0.77**± 0.12	0.61± 0.33	11.42**	Present
Harvest index (%)	-7.07**± 0.77	-5.18**± 0.68	5.05**± 0.73	8.65**± 0.48	44.75**± 0.14	1.30**± 0.14	3.87**± 0.28	343.27**	Present
Grain Fe content (ppm)	-4.86**± 0.55	2.97**± 0.86	4.64**± 1.33	3.27**± 0.69	14.99**± 0.13	1.33**± 0.13	2.07**± 0.31	155.75**	Present
Grain Zn Content (ppm)	-5.63**± 0.65	-3.72**± 0.69	-3.58**± 0.88	2.88**± 0.51	22.42**± 0.11	1.48**± 0.11	0.76**± 0.25	90.14**	Present
Grain yield per plant (g)	-1.22± 1.02	-2.21± 1.19	-12.19**±1.58	-4.38**± 0.97	28.64**± 0.17	2.78**± 0.18	4.27**± 0.39	59.63**	Present

Table 5. Estimates of six parameters (m, d, h, i,	j & l) for fourteen traits in rice cross	Khusisoi-RI-Sareku × IR91175-27-1-3-1-3
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SI. No.	Traits	m	d	h	i	j	I	Epistasis
1	Days to 50% flowering	86.79**± 0.28	1.86**±0.56	23.44**±1.64	22.42**±1.59	-1.25**±0.38	-27.25**±2.64	Duplicate
2	Days to maturity	122.00**± 0.22	2.03**± 0.41	8.07**± 1.31	7.73**± 1.22	-1.47**± 0.44	-9.74**± 2.11	Duplicate
3	Plant height (cm)	135.39**± 0.39	23.43**± 0.91	-20.25**± 2.64	-13.51**± 2.42	-8.43**± 1.07	18.91**± 4.50	Duplicate
4	Panicles per plant	10.97**± 0.17	1.95**± 0.39	4.20**± 1.11	1.57± 1.02	0.44± 0.45	5.36**± 1.80	Complementary
5	Panicle length (cm)	28.14**± 0.19	0.32± 0.52	-8.77**± 1.39	-11.59**± 1.31	-2.43**± 0.55	14.72**± 2.44	Duplicate
6	Flag leaf area (cm <sup>2</sup> )	26.73**± 0.27	2.09**± 0.55	5.14**± 1.63	-0.24± 1.56	0.78± 0.59	16.09**± 2.64	Complementary
7	Grains per panicle	132.48**± 0.36	1.66**± 0.74	0.19± 2.18	-11.54**± 2.07	0.06± 0.82	29.01**± 3.56	Complementary
8	Canopy temperature ( <sup>0</sup> C)	28.79**± 0.13	-0.72**± 0.23	0.91± 0.52	0.66± 0.76	0.86**± 0.25	-0.83± 1.35	-
9	Chlorophyll content (SPAD)	39.17**± 0.30	1.62**± 0.44	15.11**± 1.54	9.54**± 1.48	-0.89± 0.46	-13.47**± 2.30	Duplicate
10	Test weight (g)	21.63**± 6.31	0.38± 0.66	-3.97*± 1.45	-4.24*± 1.08	-0.38± 0.67	4.69± 3.03	-
11	Harvest index (%)	45.07**± 0.20	0.12± 0.36	-4.98**± 1.13	-8.14**± 1.09	-0.91**± 0.32	20.33**± 1.79	Duplicate
12	Grain Fe content (ppm)	17.63**± 0.27	-2.09**± 0.42	-3.61**± 1.44	-6.53**± 1.38	-3.92**±0.44	8.43**± 2.15	Duplicate
13	Grain Zn Content (ppm)	22.79**± 0.15	0.63± 0.41	-3.72**± 1.07	-5.77**± 1.03	-0.95**± 0.33	15.13**± 1.86	Duplicate
14	Grain yield per plant (g)	28.60**± 0.33	3.23**± 0.72	14.11**± 2.01	8.76**± 1.96	0.49± 0.74	-5.33± 3.27	-

chlorophyll content, harvest index, grain Fe content and grain Zn content that indicated presence of duplicate epistasis in expression of these traits. Hence, it could be concluded that these traits are governed by non additive gene action. This conclusion is also supported by the result that the performance of F<sub>1</sub> dissipated in F<sub>2</sub> generation for most of the traits studied. The preponderance of non additive gene action for the traits under study indicated that improvement of these traits could be brought about by resorting to heterosis breeding. The traits that have preponderance of both additive and nonadditive gene action could be exploited through recombination breeding by hybridization followed by selection at later generations. Sobita Devi et al. [14] and Verma et al. [15] reported the predominance of additive gene action for plant height, number of productive tillers and days to 50% flowering in rice. Selection is the reliable breeding method for improving varieties for the traits with preponderance of additive gene action. If the dominance is high, the selection has to be postponed to later generation. Heterosis breeding is not desirable in case of epistasis but it would be possible to isolate segregants as good as that of  $F_1$  in the subsequent generations. More reliance should be placed on selection between families and lines for the traits with relatively high non-allelic interactions.

## 4. CONCLUSION

It is concluded from the present study that dominance (h) gene effect along with dominance × dominance (l) interaction played significant role in the expression of both grain Fe and Zn content. Predominance of duplicate type of epistasis was evident from opposite sign of [h] and [I] for both the grain Fe and grain Zn content. Opposite sign of [h] and [I] tend to weaken or cancel the effect of each other in hybrid combination and hinders the progress under selection and therefore, selection should be deferred to advance segregating generation until dominance effects are dissipated.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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