Original Research Article

Assessment of gene action for grain micronutrientcontent, yield and yield contributing traits in rice (*Oryza sativa* L.)

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 a 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 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 at later generations, could be effective in improving both grain Fe and Zn content.

Introduction

Rice (*Oryza sativa* L. 2n = 24) belongs to the family of Poaceae. It is the world's most important staple food crop for more than half the world's population and together they directly supply more than 50% of all calories consumed by the entire human population [1]. India is the second largest producer and consumer of rice in the world. The leading producers of this cereal are China, India, and Indonesia which together account for over 50% of the world's total production [2]. Average daily intake of rice provides 20-80 per cent of dietary energy and 12-17 per cent dietary proteins for Asians [3]. Total rice production is increased to 751.9 million tonnes worldwide [4] and among that 90 percent is produced and consumed in developing countries. But unfortunately, about 870 million people are suffering from chronic undernourishment globally [5] and vast majority of them are from developing countries where rice is closely associated with food security and political stability. In developing countries, iron and zinc deficiencies are reported to be the sixth and fifth highest health risk factor respectively [6 & 7] causing a high mortality rates. So, overcoming these nutritional deficiencies is need of hour. The amount of mineral nutrients in rice grain is a key determinant of its nutritive value. Brown rice comprises 90% endosperm, 6–7% bran and 2–3% embryo on average by weight [8]. Recent X-ray micro-fluorescence investigations demonstrated that the concentrations of Zn, Fe, and potassium (K) decrease in the order: bran > hulls > whole grain > brown rice and polished rice [9], [10]. Dehulling and polishing of rice removes bran from the grain, polishing rice depletes the very element that is deficient in the diets of many of its consumers. Therefore, it is important to increase the grain Fe and Zn content and this can only be achieved by understanding of the genetics of gene action responsible for iron and zinc accumulation in grain. Biofortification could be accomplished genetically through plant breeding and agronomically through Zn fertilization. Identification of the amount of genetic variability for Zn concentration in the germplasm is the initial step, then improving rice Zn concentration [11].

To achieve genetic improvement of yield and quality traits, it is imperative to have knowledge about the nature of gene interactions for different characters. The information on the nature of the gene action could be helpful in predicting the effectiveness of selection in a population. The major thrust area for genetic improvement would lie in identifying desirable parents for hybridization programme. This would depend to a large extent on the knowledge of gene actions controlling various characters. A distinct knowledge about type of gene effect, its magnitude and composition of genetic variance are essential to achieve genetic improvement of yield and quality traits like mineral fortification. The efficient partitioning of genetic variance into additive, dominance and epistasis helps in formulating an effective and sound breeding programme. The reliability of the estimates and genetic gains of selection in segregating population largely depend upon the genetic divergence largely related to additive epistasis.

The generation mean analysis has been considered to be one of the best methods for estimating the different components of genetic variance and presence or absence of epistasis. Scaling test [12] at the most, indicates types of the epistatic interaction effects in the crosses; whereas, joint scaling test [13] estimates the main gene effects and also indicates the epistatic interaction. However, generation mean analysis provides estimates of main gene effects and interactions effects, which are of more value for understanding the performance of the parents in crosses and potential of the crosses to be used either for heterosis exploitation or pedigree selection. Generation mean analysis also provides information about the types of epistasis. Some other useful estimates like genetic advance, heritability, heterosis and inbreeding depression can also be estimated from the material used in generation mean analysis. The estimates obtained by this technique are statistically robust because the analysis of this technique is based on first order statistics. Therefore, the study of genetics of yield and quality traits is important to formulate a breeding programme to improve yield while maintaining the quality of rice.

Keeping in view the above mentioned facts, present investigation was undertaken to determine the nature and magnitude of gene action for iron and zinc accumulation in rice genotypes using generation mean analysis.

MATERIALS AND METHODS

The parents used for crossing were Khusisoi-RI-sareku and IR91175-27-1-3-1-3. In present study Khusisoi-RI-sareku was used as female parent having high grain Fe and high Zn content and IR91175-27-1-3-1-3 was used as male parent having low grain Fe and low Zn content. Parents for making cross were procured from Harvest Plus Rice Project at Department of Plant Breeding and Genetics, RPCAU, Pusa, Samastipur, Bihar.

Six generations namely P_1, P_2, F_1, F_2, B_1 and B_2 of the four crosses were raised in a randomized block design with three replications during kharif season 2019-20. For P_1, P_2, F_1 observations were recorded on ten plants, for $B_1$and B_2 observations were recorded on twenty plants where as for F_2 observations were recorded from 70 plants in each entry in each replication for different characters.

Crossing programme

The crosses were made in *kharif* 2017 at Department of Plant Breeding and Genetics, RPCAU, Pusa, Samastipur, Bihar between Khusisoi-RI-sareku (P_1) and IR91175-27-1-3-1-3 (P_2) to raise the F_1. In the next season F_1 were backcrossed with recurrent parent (P_1) and donor parent (P_2) to generate B_1 and B_2 population and also fresh F_1's were developed. In *kharif* 2019, Six generations, namely, P_1, P_2, F_1, F_2, B_1 and B_2 were raised in a complete randomized block design with three replications. Observations were recorded on fourteen traits on 10 selected plants from parental generation and F_1's, 20 plants each from B_1 and B_2 generations and 70 plants from each F_2, from each replication. The estimation of micronutrients from brown rice [14] by XRF (X-Ray Fluorescence Spectrometry) was carried out at Harvest Plus Division, ICRISAT, Hyderabad.

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) estimationof gene effects, variances and the type of epistasis involved.

Scaling test

Scaling test for A, B, C and D scales as suggested by Mather [12] 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 = 2B1 − P1 − F1; B = 2B2 − P2 − F1;C =4F2 − 2F1 − P1 − P2; D = 2F2 − B1 − B2; where, P1,P2, F1, F2, B1 and B2 are the means of parent 1, parent 2,F1, F2 and backcross generations B1 and B2, respectively. Utilizing the variance of different generations, the variancesof A, B, C and D scales were computed as follows: VA =4VB1 + VP1 + VF1;VB = 4VB2 + VP2 + VF1;VC =16VF2 + 4VF1 + VP1 + VP2;VD = 4VF2 + VB1 + VB2;where, VP1, VP2, VF1, VF2, VB1 and VB2 are the variances of means of the P1, P2, F1, F2, B1 and B2 generations, respectively. To test the significance ofthe scales, the 'Student's *t*' values for each of these quantitieswere 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 was evaluated using calculated *P* values for respective calculated '*t*'values.

Joint scaling test

Joint scaling test [13] was conducted which combines several scaling test into one and tests the adequacy of additive–dominance model using a $\chi 2$ test.

Estimation of gene effects using six generation means

The generation means were analysed by the method suggested by [15] 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 × dominance gene effects, respectively assuming that no linkage and no higher order gene interaction exists.

Results and discussion

To elucidate the nature of gene action for yield traits along with grain Fe and Zn content generation mean analysis was carried out using the data recorded from six generations of the cross Khusisoi-RI-Sareku and IR91175-27-1-3-1-3. The analysis of variance for individual character was carried out for all the fourteen traits (Table 1). The mean sum of squares revealed significant difference among all generations in all the crosses indicating considerable variability in the experimental material. Significant differences in all the characters in all the generations suggesting that lot of diversity exist and there is ample scope for selection of promising line from the present gene pool for yield, it's component traits along with grain Fe and Zn content. The mean performancesof the six generation materials P_1, P_2, F_1, F_2, $B_1$and B_2 for 14 quantitative traits are presented in table 3.The values of individual scaling tests and estimates of gene effects *viz*., *m, d, h, i, j* and *l* for different characters in this cross were estimated (tables 4 and 5). Information on these aspects in genetic architecture of the various traits is essential for proper selection of parents and breeding methodology. The mean effects were highly significant for all traits. Epistatic gene effects were found significant for all the traits mean performance of F_1 was superior than both the parents for the traits *viz*., effective tillers per plant, panicle length, flag leaf area, SPAD, harvest index, grain Fe, grain Zn content and grain yield per plant. For rest other traits F_1 mean performance was midway between the parental values with inclination towards better parent. For most of the traits F_1 performance was found better than F_2 also. Grain Fe content among all generation materials varied from 11.30 to 26.80 ppm with a mean value of 16.26 and grain Zn content varied from 17.10 to 29.30 ppm with a mean value of 22.58 ppm The scaling test [2] showed all A, B, C and D scales were significant for plant height, effective tillers per plant, grains per panicle, harvest index, grain Fe content and grain Zn content, indicating presence of epistasis. All the other traits related to yield in this study were significant in either one of the scales or in combination representing the existence of non-interactions between the genes involved. Further, jointscaling test was adapted to fit the data to three parameter model to estimate mean (*m*), additive gene effects (*d*) and dominant gene effects (*h*) and to evaluate adequacy of simple additive–dominance model [13]. Chi square test was conducted to evaluate the goodness of fit of this model. For all the traits studied chi square values were found significant indicating the presence of digenic non-allelic interaction for all these traits, indicating the data does not fit into simple additive–dominance model. The role of epistatic interactions was identified by lack of goodness of fit into three parameter model and the data was further subjected to six parameter model [15]. Digenic nonallelic interaction model with six parameters namely *m*, *d*, *h*, *i*, *j* and *l* [15] revealed that the epistatic interaction model was found adequate to explain the gene action in the traits like effective tillers per plant, panicle length, flag leaf area, grains per panicle, canopy temperature, SPAD, 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 effective tillers per plant, flag leaf area and grains per panicle. Complementary type of epistasis tends to enhance the heterotic effect as the magnitude of [l] adds to the main effect [h] as opposed to the case in duplicate type of epistasis. Under such circumstances selection would be more effective. Dominance(*h*) and dominance × dominance (*l*) gene effects displayed opposite signs for the traits *viz*., days to 50 % flowering, days to maturity, plant height, panicle length, chlorophyll content, harvest index, grain Fe content and grain Zn content indicating duplicate epistasis. Hence, it is concluded that these charactersare governed by non additive gene action; it is also evident from the superior performance of F_1's than advanced lines for most of the traits studied. The predominance of non additive gene action for these characters under study indicated that improvement of these characters could be possible through heterosis breeding. To obtain better genotypes through recombination breeding, hybridization followed by selection at later generations is suggested for exploiting dominance gene action. Sobita Devi *et al.* [16] and Verma *et al.* [17] 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 characters with predominant 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 filial generations. More reliance should be placed on selection between families and lines for the traits with relatively high non-allelic interactions.

Conclusion

It may be concluded dominance (h) gene effect along with dominance × dominance (l) interaction is playing significant role in the expression of both grain Fe and Zn content. Predominance of duplicate type of epistasis as evidenced from opposite sign of [h] and [l] was noticed for the expression of both grain Fe and Zn content in the rice cross Khusisoi-RI-Sareku × IR 91175-27-1-3-1-3. Duplicate type of epistasis tends to weaken or cancel the effect of each other in hybrid combination and hinders the progress made under selection and therefore, selection have to be deferred till later generations of segregation where dominance effects are dissipated.

Table 1. Analysis of variance for different characters in cross KHUSISOI-RI-SAREKU × IR91175-27-1-3-1-3

Sl. No.	Characters	Mean Sum of Squares		
		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	Plantheight (cm)	51.43	21124.47**	96.85
4	Effective Tillers per Plant (No.)	4.93	84.60**	3.75
5	Panicle length (cm)	11.04	207.94**	5.67
6	Flag leaf area (cm^2)	22.97	292.27**	11.22
7	Grains per Panicle (No.)	11.78	1002.38**	90.12
8	Canopy Temperature (^0C)	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 Iron Content (ppm)	4.68	157.20**	9.89
13	Grain Zinc Content (ppm)	7.21	75.20**	4.32
14	Grain yield per plant (g)	32.5	360.78**	26.05

Table 2.Range, Mean, Standard error, Standard deviation and Coefficient of variation for yield components in rice for Cross KHUSISOI-RI-SAREKU × IR91175-27-1-3-1-3

Sl. No.	Characters	Range		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	Plantheight (cm)	96.00	180.00	133.81	0.69	18.64	13.93
4	Effective Tillers per Plant (No.)	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^2)	20.31	39.21	28.22	0.33	3.82	13.55
7	Grains per Panicle (No.)	112.00	167.00	131.28	0.50	10.03	7.64
8	Canopy Temperature (^{0}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 Iron Content (ppm)	11.30	26.80	16.26	0.26	3.41	20.96
13	Grain Zinc 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

Table 3.Mean performance along with respective standard error of different generations in cross KHUSISOI-RI-SAREKU × IR91175-27-1-3-1-3

		P_1	P_2	F_1	F_2	B_1	B_2	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	Plantheight (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	Effective Tillers per Plant (No.)	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^2)	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 (No.)	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 (^{0}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 Iron 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 Zinc 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 4. Different scales for yield components in cross KHUSISOI-RI-SAREKU × IR91175-27-1-3-1-3

	Scaling test				Joint scalimg test			Chi-sqare value	Epistais
	A	B	C	D	m	d	h		
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
Plantheight (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
Effective Tillers per Plant (No.)	-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^2)	-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 (No.)	-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 (^{0}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 Iron 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 Zinc 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.Estimation of component of generation means for different characters in cross KHUSISOI-RI-SAREKU × IR91175-27-1-3-1-3as suggested by Hayman 1958.

		m	d	h	i	j	l	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	Plantheight (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	Effective Tillers per Plant (No.)	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^2)	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 (No.)	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 (^{0}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 Iron 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 Zinc 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	----------

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