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Genetic control and heterosis for grain iron and zinc contents in sorghum [*Sorghum bicolor* (L.) Moench]

K. Hariprasanna*, V. Agte[1] and J. V. Patil

ICAR-Directorate of Sorghum Research, Rajendranagar, Hyderabad 500 030; [1]Agharkar Research Institute, G.G. Agarkar Road, Pune 411 004

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Abstract

For formulating an appropriate breeding strategy, genetic control of grain iron (Fe) and zinc (Zn) in sorghum was studied in a half-diallel mating design with nine parents. Both additive and non-additive gene actions were important in the genetic control but dominant gene action was predominant in case of Fe. In case of grain Zn additive gene action was more important with a σ^2 gca/σ^2 sca ratio of 0.94. Predictability ratio was 0.14 for grain Fe and 0.65 for Zn. Significant correlation between mean parental performance and GCA effects (r = 0.86 for Zn, r = 0.67 for Fe) indicated that selection of genotypes with high mean Zn and/or Fe levels would be highly effective in selecting for high GCA. The results suggest the scope for heterosis breeding to improve grain Fe, while for improving grain Zn progeny selection in pedigree breeding will be effective. High Fe and Zn levels in both of the parental lines would be required to develop heterotic hybrids with high grain micronutrient contents.

Key words: Biofortification, gene action, grain iron, grain zinc, micronutrients

Introduction

Globally malnutrition is responsible for more deaths than any other cause, accounting for >20 million mortalities annually [1]. Micronutrient malnutrition, often known as hidden hunger, alone afflicts more than one-half of the developing world's population or more than 2 billion people, especially the women and preschool children [2]. Two of the most widespread micronutrient deficiencies are that of iron (Fe) and zinc (Zn), which have recently been recognized as serious human health problems, especially in the developing countries [3] leading to a number of health disorders [4, 5]. Development of micronutrient-dense staple crop cultivars with high Fe and Zn densities by the best traditional breeding practices and modern biotechnology (biofortification) offers a cost-effective and sustainable solution to reduce micronutrient malnutrition among the target populations [6].

Sorghum is the fifth most important cereal staple crop in the sub-tropical and semi-arid regions of Africa and Asia, and is a principal source of energy, protein, vitamins and minerals for millions of the poor people in these regions. The developing countries contribute nearly 70% of the world sorghum production. India ranks first globally in terms of area under sorghum, with an annual production of 5.28 m tonnes from an area of 6.18 m ha [7] spread over both rainy and post-rainy seasons. The states of Maharashtra, Karnataka and Andhra Pradesh (undivided) together account for close to 80% of the all-India production [8] with an annual per capita consumption up to 75.2 kg/year in the major sorghum-producing regions by rural consumers [9]. Considering the high prevalence of micronutrient deficiency among the rural population in the dominant sorghum consumption regions, and superiority of sorghum grains for Fe and Zn contents compared to other most popular staples, Biofortification holds promise to enhance Fe and Zn supply to these sorghum consuming population, and thereby improving the micronutrient status in a sustainable manner [10]. Genetic variability for grain Fe and Zn in sorghum has been reported [10-12], and hence an understanding of the genetic control of grain Fe and Zn content is essential for designing appropriate breeding strategies for the development of open-pollinated varieties and hybrids with micronutrient-dense grains. The present study was conducted to understand the nature of gene

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*Corresponding author's e-mail: hari@sorghum.res.in
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action for grain Fe and Zn contents, and propose an effective breeding strategy for the development of micronutrient-enriched sorghum cultivars.

Materials and methods

Plant material and micronutrient estimation

Genetic control of grain Fe and Zn contents was studied using a half-diallel mating design. Nine parents selected from our earlier studies [10] were crossed in a half-diallel mating design (each parent is mated with every other parent, excluding selfs and reciprocals) during post-rainy season of 2010 to generate 36 crosses. The F_1 hybrids along with parents were evaluated in a randomized complete block design with two replications during rainy season of 2011. The recommended package of practices was adopted to raise a good crop. The crop was harvested at maturity and replicated grain samples (50 g each) from each plot were collected after proper drying for analysis of micronutrients. The grain Fe and Zn contents were analyzed at Agharkar Research Institute, Pune following standard protocols by employing Atomic Absorption Spectrophotometer (AAS-Perkin Elmer 5000AA, USA) as previously reported by us [13].

Statistical analysis

The replication-wise data on grain Fe and Zn content were analyzed statistically for analyses of variance and comparison of means using WINDOSTAT Ver. 7.5 (www.windostat.org). Estimates of general combining ability (GCA), specific combining ability (SCA) and gene action were obtained following Model I, Method 2 of Griffing [14], which included one set of F_1 hybrids and parents. Significance of GCA and SCA effects was determined by a *t*-test. Estimate of variances due to GCA (σ^2 gca) and SCA (σ^2 sca) were derived to obtain estimates of predictability ratio (PR) as PR = $2\sigma^2\text{gca}/(2\sigma^2\text{gca} + \sigma^2\text{sca})$ [15]. The heterosis (%) in F_1 over the mid-parent (MP) and better parent (BP) were calculated using mean values for grain Fe and Zn. For each cross combination, mid-parent heterosis (MPH) and better parent heterosis (BPH) were calculated as MPH = $(F_1 - \text{MP})/\text{MP} \times 100$ and BPH = $(F_1 - \text{BP})/\text{BP} \times 100$, respectively. The significance of the heterosis value was determined by the *t*-test using the error variance of the experiment.

Results and discussion

The analysis of variance showed significant to highly significant differences among the parents for grain Zn and Fe contents (Table 1) indicating that there were significant differences among the parents for these micronutrients. The mean grain Fe content varied from 23.84 to 40.53 mg/kg and Zn content from 15.32 to 22.13 mg/kg (Tables 2 and 3). The crosses also showed highly significant variation for both Fe and Zn as indicated by the significant mean sum of squares. The content of grain Fe ranged from 23.99 to 47.63 mg/kg in the crosses, while Zn content ranged from 14.30 to 29.60 mg/kg (Table 2 and 3). The average grain Fe content in the hybrids (36.54 mg/kg) was 6.78% more than that of parents, while the average grain Zn content of hybrids (20.68 mg/kg) was only marginally higher by 4.55% over the parents.

Table 1. ANOVA for half-diallel cross for grain Fe and Zn

Source of variation	d.f.	Fe	Zn
Replicates	1	376.8***	33.1*
Treatments	44	47.1***	23.3***
Parents	8	51.4**	15.8*
Hybrids	35	45.3***	26.4***
Parent vs.Hybrids	1	77.5*	11.67
Error	44	13.4	7.3
Total	89	34.2	15.5
GCA	8	22.0**	34.2***
SCA	36	23.9***	6.6*
Error	44	6.7	3.7
σ^2 gca (estimate)		1.39	2.77
σ^2 sca (estimate)		17.20	2.95
σ^2 A		2.78	5.54
σ^2 D		17.20	2.95
σ^2 gca/σ^2 sca		0.08	0.94

*$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$

The GCA and SCA effects were significant to highly significant for both grain Zn and Fe (Table 1) indicating that both additive and non-additive gene actions are involved in controlling these traits. In case of grain Fe, GCA and SCA mean squares were almost of equal magnitude, whereas in case of grain Zn the magnitude of GCA mean squares was much higher than the SCA mean squares. Similar result has been reported in case of grain Zn [16]. For grain Zn content additive gene action was more important with a σ^2 gca/σ^2 sca ratio of 0.94, while in case of grain Fe dominant gene action was more predominant with a very low σ^2 gca/σ^2 sca ratio (Table 1). The magnitude of additive variance (σ^2 A) was nearly double that of dominance variance

Table 2. Average grain Fe content (mg/kg) of parents and hybrids, general combining ability (gca) and specific combining ability (sca) of crosses

Parents/ hybrid	EP 95	GGUB 39	EP 5	CSV 18	CSV 20	CSV 23	CSV 216R	EP 114	C 43
EP 95	35.69 (-0.22)	36.49 (0.37)	35.12 (-1.79)	43.05 (6.89**)	30.21 (-7.25**)	30.49 (-5.56**)	39.41 (3.69)	35.76 (-0.52)	36.49 (4.08)
GGUB 39		32.02 (0.26)	42.91 (5.51**)	34.85 (-1.79)	34.56 (-3.38)	34.79 (-1.74)	35.41 (-0.80)	40.7 (3.94)	39.93 (7.04**)
EP 5			31.67 (1.05)	35.35 (-2.08)	42.23 (3.50)	37.22 (-0.10)	36.14 (-0.86)	42.38 (4.83*)	37.72 (4.04)
CSV 18				32.17 (0.30)	47.63 (9.65**)	41.41 (4.85*)	30.24 (-6.01**)	29.30 (-7.49**)	37.91 (4.99*)
CSV 20					37.29 (1.60*)	35.90 (-1.97)	40.81 (3.27)	38.17 (0.08)	34.29 (0.07)
CSV 23						35.16 (0.19)	37.88 (1.74)	37.67 (0.99)	37.21 (4.39)
CSV 216R							40.53 (-0.14)	34.40 (-1.96)	23.99 (-8.51**)
EP 114								39.68 (0.42)	27.63 (-5.41**)
C 43									23.84 (-3.45**)

Diagonal: Fe content of parents, diagonal parenthesis: gca effects of parents, above diagonal: Fe content of crosses, above diagonal parenthesis: sca effects of crosses, parental mean: 34.22 mg/kg, hybrid mean: 36.54 mg/kg, $^*p < 0.05$ and $^{**}p < 0.01$

Table 3. Average grain Zn content (mg/kg) of parents and hybrids, general combining ability (gca) and specific combining ability (sca) of crosses

Parents/ hybrid	EP 95	GGUB 39	EP 5	CSV 18	CSV 20	CSV 23	CSV 216R	EP 114	C 43
EP 95	21.57 (2.02**)	27.58 (4.31**)	23.35 (-1.00)	26.26 (1.68)	21.96 (1.52)	19.98 (0.09)	23.00 (1.08)	21.95 (-0.02)	19.98 (-1.73)
GGUB 39		21.22 (0.75)	26.59 (3.50)	19.93 (-3.38)	15.12 (-4.05*)	17.85 (-0.77)	19.44 (-1.21)	22.13 (1.43)	22.15 (1.72)
EP 5			22.13 (1.84**)	24.68 (0.28)	21.55 (1.30)	18.46 (-1.25)	22.51 (0.78)	19.97 (-1.82)	23.83 (2.31)
CSV 18				21.82 (2.06**)	18.18 (-2.31)	22.29 (2.36)	23.58 (1.62)	19.51 (-2.50)	29.60 (7.86**)
CSV 20					18.95 (-2.08**)	16.58 (0.79)	15.38 (-2.44)	18.15 (0.28)	17.30 (-0.31)
CSV 23						15.32 (-2.63**)	19.60 (2.33)	16.35 (-0.97)	14.30 (-2.76)
CSV 216R							18.44 (-0.60)	21.41 (2.06)	16.60 (-2.49)
EP 114								21.07 (-0.55)	17.30 (-1.83)
C 43									17.48 (-0.82)

Diagonal: Zn content of parents, diagonal parenthesis: gca effects of parents, above diagonal: Zn content of crosses, above diagonal parenthesis: sca effects of crosses, parental mean: 19.78 mg/kg, hybrid mean: 20.68 mg/kg, $^*p < 0.05$ and $^{**}p < 0.01$

Table 4. Heterosis over mid-parent (MP) and better parent (BP) for grain Fe and Zn

Hybrid	Heterosis (%) for Fe over		Heterosis (%) for Zn over	
	MP	BP	MP	BP
EP 95 × GGUB 39	7.79	2.24	28.92*	27.86*
EP 95 × EP 5	4.28	-1.60	6.88	5.54
EP 95 × CSV 18	26.89**	20.62	21.04	20.35
EP 95 × CSV 20	-17.20*	-18.98	8.39	1.81
EP 95 × CSV 23	-13.92	-14.57	8.32	-7.37
EP 95 × CSV 216R	3.42	-2.75	14.99	6.63
EP 95 × EP 114	-5.11	-9.88	2.95	1.76
EP 95 × C 43	22.60*	2.24	2.34	-7.37
GGUB 39 × EP 5	34.77**	34.03**	22.70*	20.18
GGUB 39 × CSV 18	8.60	8.35	-7.38	-8.66
GGUB 39 × CSV 20	-0.26	-7.31	-24.71*	-28.73*
GGUB 39 × CSV 23	3.59	-1.04	-2.29	-15.86
GGUB 39 × CSV 216R	-2.37	-12.62	-1.94	-8.37
GGUB 39 × EP 114	13.54	2.57	4.67	4.31
GGUB 39 × C 43	42.99**	24.72*	14.50	4.41
EP 5 × CSV 18	10.76	9.90	12.32	11.55
EP 5 × CSV 20	22.49*	13.26	4.93	-2.60
EP 5 × CSV 23	11.40	5.87	-1.40	-16.56
EP 5 × CSV 216R	0.12	-10.82	11.00	1.74
EP 5 × EP 114	18.80*	6.80	-7.54	-9.74
EP 5 × C 43	35.93**	19.12	20.35	7.71
CSV 18 × CSV 20	37.16**	27.75**	-10.82	-16.68
CSV 18 × CSV 23	23.02*	17.79	20.03	2.15
CSV 18 × CSV 216R	-16.80	-25.38**	17.15	8.07
CSV 18 × EP 114	-18.44*	-26.16**	-9.02	-10.59
CSV 18 × C 43	35.39**	17.86	50.66**	35.66**
CSV 20 × CSV 23	-0.88	-3.71	-3.24	-12.51
CSV 20 × CSV 216R	4.90	0.70	-17.72	-18.84
CSV 20 × EP 114	-0.81	-3.81	-9.30	-13.86
CSV 20 × C 43	12.21	-8.03	-5.01	-8.71
CSV 23 × CSV 216R	0.11	-6.53	16.13	6.32
CSV 23 × EP 114	0.67	-5.07	-10.14	-22.40
CSV 23 × C 43	26.16*	5.85	-12.79	-18.17
CSV 216R × EP 114	-14.22	-15.11	8.39	1.61
CSV 216R × C 43	-25.45*	-40.80**	-7.55	-9.95
EP 114 × C 43	-13.00	-30.37**	-10.23	-17.89
SEd	3.15	3.62	2.35	2.71
CD (0.05)	6.35	7.30	4.73	5.46
CD (0.01)	8.48	9.75	6.32	7.30
Average Heterosis	6.78		4.55	

*$p < 0.05$ and **$p < 0.01$

(σ^2 D) for grain Zn.

The predictability ratio (PR) provides a measure of the predictability of the performance of hybrids and its progenies [15]. The closer this ratio to unity, the greater the predictability based on GCA alone. Predictability ratio was 0.14 for grain Fe and 0.65 for Zn implying the preponderance of additive gene action for grain Zn content and indicating that hybrid performance can be predicted based on GCA alone. A PR of 0.35 for grain Fe and 0.65 for Zn in sorghum has previously been reported [16].

The GCA and SCA effects of parents and crosses were assessed for grain Fe (Table 2) and Zn (Table 3) contents. The GCA effect was significant and positive for grain Fe in only one parent (CSV 20), while for grain Zn positive significant GCA effects were observed for three parents (EP 95, EP 5 and CSV 18). The correlation coefficient between mean performance *per se* of parents and GCA effects was highly significant and positive in case of grain Zn ($r = 0.863$; $p < 0.002$), and nearly significant in case of Fe ($r = 0.665$; $p = 0.05$), indicating that selection of genotypes with high Zn and/or Fe levels would be highly effective in selecting for high GCA. Significant SCA effects were observed in 13 hybrids for Fe (Table 2) and three hybrids for Zn (Table 3) indicating the presence of non-additive gene effects. Out of 13, seven hybrids had significant positive SCA effects in case of grain Fe, and two out of three hybrids had significant positive SCA effects in case of grain Zn. Considering the performance *per se* and GCA together CSV 20 was considered as the best combiner among the parents used for grain Fe, while CSV 216R and EP 114, though had high mean value were poor combiners. EP 5 was good combiner for grain Zn, but the mean value was only marginally higher compared to other lines like EP 95 and CSV 18 that had significant and positive GCA.

Heterosis over mid-parent ranged from –25.5 to 43% for grain Fe while for grain Zn it ranged from –24.7 to 50.7% (Table 4). Better parent heterosis ranged between –40.8 and 34%, and –28.7 and 35.7% for grain Fe and Zn, respectively. Highly significant positive correlation between the mid-parental values and hybrid performance *per se* ($r = 0.6$, $p < 0.001$), and no correlation between mid-parental values and mid-parent heterosis ($r = 0.22$, $p = 0.20$) for grain Zn provided additional indications of the predominant role of additive gene action for this trait. But in case of grain Fe no correlation was observed between mid-parental values and hybrid performance ($r = 0.01$), and significant negative correlation ($r = -0.59$, $p < 0.001$) was observed between mid-parental values and mid-parental heterosis indicating predominant non-additive gene action unlike previous reports in sorghum [16] and pearl millet [17]. Overall, 11 hybrids exhibited significant positive mid-parental heterosis for grain Fe (Table 4). These results suggest that there is some scope for exploitation of heterosis for improving grain Fe concentration. However, number of hybrids with significant and positive mid-parent and better parent heterosis for grain Zn was very less indicating that there is only limited possibility for exploitation of heterosis for improving grain Zn in sorghum as previously reported [16].

To conclude, grain Fe content in sorghum is governed predominantly by dominant gene action, while grain Zn content is governed predominantly by additive gene effects. The results suggest the scope for heterosis breeding to improve grain Fe, while for improving grain Zn progeny selection in pedigree breeding will be effective. To develop hybrids with high grain Fe and Zn content both parents need to be improved for these micronutrients. Further, high correlation between mean grain micronutrient contents of the parents and GCA suggests that the performance *per se* of the genotypes could be a good indicator of its ability to transmit grain Zn and Fe densities to its hybrids and progenies, and genetically superior parents could be identified by evaluation of their Fe and Zn densities. The higher additive genetic variance also prompts for recurrent selection or population breeding method to develop lines with increased levels of grain Zn contents. Predominant role of non-additive gene effects in combination with additive gene effects, suggests scope for heterosis breeding in addition to progeny selection to develop genotypes with increased levels of grain Fe contents.

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