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Gene effects and heterosis for grain iron and zinc concentration in sorghum [*Sorghum bicolor* (L.) Moench]

A. Ashok Kumar[a,*], Belum V.S. Reddy[a], B. Ramaiah[a], K.L. Sahrawat[a], Wolfgang H. Pfeiffer[b]

[a] *International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502324, India*
[b] *HarvestPlus, International Center for Tropical Agriculture (CIAT), A.A. 6713, Cali, Colombia*

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ABSTRACT

The aim of this study was to understand the inheritance of grain iron (Fe) and zinc (Zn) concentrations in sorghum [*Sorghum bicolor* (L.) Moench] and to assess the possibility of exploiting heterosis to improve these micronutrients. Three sets of full diallel crosses were made, one set using five parents contrasting for both grain Fe and Zn concentrations; the second set using six parents contrasting only for Fe, and the third set with four parents contrasting only for Zn. The crosses and parents were evaluated in replicated trials for two years. The results indicated that both additive and non-additive gene action play a role in conditioning grain Fe and Zn concentration in sorghum. However, non-additive gene action is predominant in conditioning grain Fe; and additive gene action in conditioning grain Zn. Some of the crosses showed significant heterosis for grain Fe concentration without yield penalty and some crosses showed targeted grain Zn concentration (40 mg kg^{-1}) coupled with higher grain yields. The results also showed that it is possible to improve grain Fe concentration through exploiting heterosis, but there would be little opportunity if any for improving grain Zn through heterosis breeding. To develop hybrids with high grain Fe and Zn concentration in sorghum both parents needs to be improved for these micronutrients. Combining higher grain Fe and Zn with high yield is feasible.

1. Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is the fifth most important cereal staple crop in the sub-tropical and semi-arid regions of Africa and Asia (Kresovich et al., 2005; Reddy et al., 2011). It is the second cheapest source of energy and micronutrients (after pearl millet) with a vast majority of the population in Africa and central India depend on sorghum for their dietary energy and micronutrient requirements (Parthasarathy Rao et al., 2006). Micronutrient malnutrition, primarily the result of diets deficient in bio-available vitamins and minerals, can cause blindness and anemia (even death) in more than half of the world's population, especially among women of reproductive age, pregnant and lactating women and pre-school children (Underwood, 2000; Sharma, 2003; Welch and Graham, 2004). Efforts are being made to provide fortified foods to these vulnerable groups. Biofortification, where possible, is the most cost-effective and sustainable solution for tackling micronutrient deficiencies in developing countries of arid-tropical and sub-tropical regions as the intake of micronutrients is on a continuous basis with no additional cost to the consumer. Widespread interest is being shown in the biofortification of sorghum by increasing mineral micronutrients (especially Fe and Zn) in grains (Pfeiffer and McClafferty, 2007; Zhao, 2008; Ashok Kumar et al., 2009).

The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) conducts research on sorghum biofortification to enhance the grain Fe and Zn concentrations. Preliminary studies by Reddy et al. (2005) indicated limited variability for grain Fe and Zn concentrations in sorghum hybrid parents, advanced breeding lines and germplasm accessions. Large genetic variability for grain Fe and Zn concentrations however, was reported in sorghum core germplasm accessions and hybrid parents and commercial hybrids (Ashok Kumar et al., 2009, 2012). Significant positive correlation was observed between grain Fe and Zn concentrations (Reddy et al., 2010; Ashok Kumar et al., 2011a). In a field study conducted at ICRISAT-Patancheru, India to enhance grain Fe and Zn under balanced nutrient application, nitrogen (N), phosphorus (P), potassium (K) along with sulfur (S), boron (B), iron (Fe) and zinc (Zn) were applied to the soil. However, these did not increase grain Fe and Zn concentrations, probably because the inherent availability of these nutrients in the soil was not limiting (Ashok Kumar et al., 2010). Therefore, genetic enhancement for grain Fe and Zn concentrations

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* Corresponding author. Tel.: +91 40 30713348; fax: +91 40 30713074/75.
E-mail addresses: a.ashokkumar@cgiar.org (A. Ashok Kumar), b.reddy@cgiar.org (B.V.S. Reddy), b.ramaiah@cgiar.org (B. Ramaiah), k.sahrawat@cgiar.org (K.L. Sahrawat), w.pfeiffer@cgiar.org (W.H. Pfeiffer).

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is of critical importance. An understanding of the nature and magnitude of gene action and the possibility of exploitation of heterosis for Fe and Zn helps in developing an effective breeding program to improve the grain Fe and Zn concentrations. The present study was made with three sets of full diallel crosses to understand the genetics of grain Fe and Zn concentrations, and to examine the extent of heterosis for these traits along with grain yield in order to develop cultivars possessing high grain Fe and Zn concentrations along with high grain yield.

2. Materials and methods

2.1. Experimental material and field trials

The parents used in the crossing program were chosen from our earlier studies on screening sorghum core germplasm accessions and hybrid parents (Ashok Kumar et al., 2009, 2012). During the 2006 postrainy season, three sets of full diallel crosses (sets I, II and III) were made using 15 parents at ICRISAT. Set I was with five parents contrasting for grain Fe and Zn concentrations, set II with six parents contrasting for grain Fe concentration only, and set III with four parents, contrasting only for grain Zn concentration. From set I, a total of 19 F_1s were made using five parents (including reciprocals, one cross was missed) and evaluated along with five parents and one control. Set II included 28 crosses (including reciprocals, two crosses missed), 6 parents and one control, and set III included 12 crosses, four parents and a control. All these sets were evaluated in three different randomized complete block design (RCBD) trials with three replications on deep black soils (Vertisols) during two seasons (2007 and 2009) under high fertility conditions (N80:P40:K0) at the ICRISAT farm in Patancheru (altitude 545 m above mean sea level, latitude 17.53° N and longitude 78.27° E), Andhra Pradesh, India. The soils at the experimental site had pH ranging from 6.8 to 7.9. The soils were moderate in organic carbon (C), and adequate in extractable phosphorus (P), zinc (Zn) and iron (Fe); they were high in extractable potassium (K). The experimental site soils had a very low electrical conductivity, indicating no salt problem.

For the trials, we applied recommended rates of NPK (80:40:0 kg/ha), but no additional micronutrients were added. Half the N and all P was applied as basal as diammonium phosphate (DAP), and the rest half of N was top dressed as urea at 35 days after sowing. The minimum temperature during the crop growing period ranged from 13 to 22 °C, and maximum temperature ranged from 28 to 38 °C. The experimental site received a total rainfall of 95 mm during the crop period in 2007, and 145 mm in 2009. Therefore, four to five irrigations were given to the crop as required during the cropping season. Care was taken to raise a healthy crop, and to obtain clean grain for analysis. In all the three trials, each genotype was grown in two-row plots of 2 m length with an inter-row spacing of 75 cm. Prior to flowering, 2–3 panicles were bagged with Kraft paper bags in each replication to avoid pollen contamination; and to harvest pure seed for grain Fe and Zn analysis. The selfed panicles were harvested at maturity and the grain was carefully hand threshed avoiding any contact with metal containers to avoid contamination. The remaining panicles in the plot were harvested and threshed to obtain per plot grain yield. Grain yield (g) from selfed and open-pollinated panicles was combined in each plot and extrapolated to get grain yield in Mg ha^{-1}.

2.2. Laboratory analysis

The cleaned grain samples were collected in cloth bags for micronutrient analysis in the Charles Renard Analytical Laboratory (CRAL) at ICRISAT-Patancheru, India. The Fe and Zn concentrations in the samples were estimated by the Association of Analytical Communities (AOAC) Official Methods as described below by using the atomic absorption spectrophotometric method (AOAC Official Method 965.09). The test samples were powdered to pass through a No. 40 sieve. The known amount of the test sample was taken and charred in a silica crucible and the powder was then heated in a muffle furnace at 550 °C for 8–10 h or until the test portion turned ash gray (AOAC Official Method 923.03). The ash residue was dissolved in 2 ml of HCl and made up to a known volume. The measurements were made using the Flame AAS-Varian Spectra AA 220, Version 2.10, with the operating parameter set as per the AOAC Official Method Table 965.09. A set of standard solutions of Fe/Zn (Sima, USA) within the analysis range was run before and after each set of 10 test solutions. An external quality control sample was run along with each set of analysis. The Fe/Zn concentration in test samples were obtained from their respective calibration curve and expressed as mg/1000 g of the test sample. Samples were analyzed in duplicate (two independent analyses), and the analysis was repeated if results differed by more than 10%.

2.3. Statistical analysis

Estimates of general combining ability (GCA), specific combining ability (SCA) and reciprocals effects were obtained following Griffing's method 3 model 1 (fixed model) (Griffing, 1956), which included one set of F_1s and reciprocals, leading to $[p(p-1)]$ hybrids. Data were analyzed with the DIALLEL-SAS05 program (Zhang et al., 2005). Significance of GCA, SCA and reciprocal effects was determined by a t-test (Griffing, 1956). Estimate of variances due to GCA (σ^2gca) and SCA (σ^2sca) were derived to obtain estimates of predictability ratio (PR): $2\sigma^2\text{gca}/(2\sigma^2\text{gca}/\sigma^2\text{sca})$ (Baker, 1978). The percentages of heterosis in F_1 over the mid-parent (MP) and better parent (BP) were calculated using standard formulae. For each cross combination, relative mid-parent heterosis (MPH) and better-parent heterosis (BPH) were calculated as $\text{MPH} = 100 \times (F_1 - \text{MP})/\text{MP}$ and $\text{BPH} = 100 \times (F_1 - \text{BP})/\text{BP}$ respectively. The significance of the heterosis value was identified by the t-test using the error variance of the experiment. The significant superiority of hybrid mean was tested by comparing it with the mean performance of the control variety ICSR 40, which possesses high Fe and Zn concentrations. The mean performance of hybrids one $\text{CD}_{0.05}$ above the control variety was used as a criterion to test their significance.

3. Results

3.1. Combining ability analysis

The results of pooled analysis of variance and mean sum of squares (ANOVA) of set I (crosses from parents contrasting for both Fe and Zn concentrations) over two environments and combining ability analysis results are presented hereunder (Tables 1 and 2). Although three sets of diallel crosses were made and evaluated in replicated trials, to avoid repetition of results, however, results from set II (crosses from parents contrasting for Fe only) and set III (crosses from parents contrasting for Zn only) on mean performance and heterosis for Fe (set II) and Zn (set III) and grain yield are presented and discussed, to validate the results from set I. ANOVA of set I indicated that there were significant differences ($P < 0.01$) between the parents for grain Fe and Zn concentrations and grain yield (Table 1). The Parent × Year (P × Y) interaction was significant for grain Fe and Zn and grain yield, however there was significant positive correlation between parental values in the two test environments for Fe ($r = 0.88$; $P < 0.01$) and Zn ($r = 0.84$; $P < 0.01$). Averaged over the two environments, the Fe

Table 1
Pooled analysis of variance (ANOVA) and mean sum of squares of sorghum parents and crosses (set I) for grain Fe and Zn concentrations and grain yield across 2007 and 2009 postrainy seasons at ICRISAT – Patancheru, India.

Source	df	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Grain yield (Mg ha^{-1})[b]
Parents				
Year (Y)	1	497.00**	981.00**	2.42**
Reps with in Y	4 (2)	75.93	26.14	0.19
Parents (P)	4	130.0*	181.00**	23.41**
P × Y	4	38.36**	51.58**	1.22**
Error	14 (7)[a]	28.36	27.41	0.15
Crosses (F_1)				
Year (Y)	1	409.00**	1007.00**	21.02**
Reps with in Y	4 (2)[a]	37.3	36.1	0.15
Crosses (C)	18	125.33**	183.56**	10.24**
General combining ability (GCA)	4	307.00**	474.00**	19.00**
Specific combining ability (SCA)	10	113.93**	54.00**	5.18**
Reciprocal	10	196.00**	112.20**	7.567**
Cross × Y	17	44.00**	57.19**	3.67**
GCA × Y	4	85.17**	83.94**	0.71**
SCA × Y	10	36.85**	29.33**	2.14**
Reciprocal × Y	10	46.86**	47.56*	0.95**
Error	57	18.02	22.54	1.04
σ^2gca		30.3	47	1.54
σ^2sca		110.3	50	1.58
$2\sigma^2\text{gca}/(2\sigma^2\text{gca}+\sigma^2\text{sca})$		0.35	0.65	0.66

* Significant at 0.05 probability level.

** Significant at 0.01 probability level.

[a] Values in parentheses indicate individual environment degree of freedom.

[b] Mg (Mega grams) = 1000 kg.

concentration of parents in set I varied from 30.4 to 45.0 mg kg^{-1} and Zn concentration from 22.8 to 36.2 mg kg^{-1} and the grain yield from 2.82 to 4.56 Mg ha^{-1}. Highly significant differences were also observed for years, crosses and crosses × year (C × Y) interactions for grain Fe and Zn concentrations and grain yield (Table 1). The reciprocal effects among the hybrids were significant for both Fe and Zn concentrations and grain yield. The GCA and SCA effects were highly significant for all three traits indicating that both additive and non-additive gene actions involved in controlling these traits. However, the magnitude of GCA mean squares was higher than the SCA mean squares. The C × Y interaction was significant, thus total sum of squares was partitioned into GCA × Y, SCA × Y and reciprocals × Y interaction effects. The GCA × Y, SCA × Y and reciprocals × Y interaction effects were found to be significant.

Table 2
GCA and SCA effects of parents and crosses (set 1) respectively for grain Fe and Zn concentrations in sorghum across 2007 and 2009 postrainy seasons at ICRISAT – Patancheru, India.

Parent/cross	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Grain yield (Mg ha^{-1})[a]
Parents			
IS 13205	6.45**	7.01**	−0.36**
IS 23464	−1.20**	0.62	0.01
IS 518	−2.67**	−3.34**	−0.48**
ICSR 40	−3.27**	−5.68**	−0.83**
SPV 1359	0.7	1.40**	1.66**
Direct crosses			
IS 13205 × IS 23464	−0.53	−0.22	1.11**
IS 13205 × IS 518	1.90*	0.36	0.84**
IS 13205 × ICSR 40	3.65**	0.54	0.45**
IS 13205 × SPV 1359	−3.09**	−0.34	−0.85**
IS 23464 × IS 518	4.30**	2.37**	1.17**
IS 23464 × ICSR 40	−4.43**	−3.78**	−0.38*
IS 23464 × SPV 1359	2.32**	1.65*	0.28
Missing	–	–	–
IS 518 × SPV 1359	−1.26	−1.36	0.70**
ICSR 40 × SPV 1359	2.96**	2.85**	1.09**
Reciprocal crosses			
IS 23464 × IS 13205	3.55**	6.97**	−0.2
IS 518 × IS 13205	1.78	1.92	−0.1
ICSR 40 × IS 13205	1.78	3.86**	−0.70**
SPV 1359 × IS 13205	9.21**	7.82**	−2.26**
IS 518 × IS 23464	1.06	0.67	0.08
ICSR 40 × IS 23464	−9.51**	−6.43**	−1.77**
SPV 1359 × IS 23464	−1.6	−1.53	−0.17
ICSR 40 × IS 518	−16.95**	−10.34**	−2.87**
SPV 1359 × IS 518	0.2	−0.36	−0.33
SPV 1359 × ICSR 40	2.09*	0.99	−1.33**

* Significant at 0.05 probability level.

** Significant at 0.01 probability level

[a] Mg (Mega grams) = 1000 kg.

Based on a combined analysis of the results over the two environments for set I, the predictability ratio was 0.35 for Fe, 0.65 for Zn and 0.66 for grain yield (Table 1), and it was of the similar order for Fe in set II (Fe 0.21) and for Zn in set III (Zn 0.66). The GCA and SCA effects of parents and crosses and reciprocal crosses (set I) were assessed for grain Fe and Zn concentrations. The GCA was highly significant for parent IS 13205 for both Fe and Zn and GCA effect of parent SPV 1359 was significant for Zn and grain yield (Table 2). Similarly, in set II, ICSB 52 had higher mean Fe concentration and highly significant GCA and SPV 1359 had higher grain yield and highly significant GCA for grain yield. In Set III, IS 2248 showed higher mean Zn concentration and highly significant GCA; IS 20843 showed higher mean Zn concentration and grain yield and highly significant GCA effects for both traits. Further PVK 801 had higher mean grain yield and highly significant GCA in set III (data not shown) which is a popular rainy season sorghum cultivar in India.

The parents with high Fe and Zn concentration were the best general combiners having positive significant GCA effects compared to those with low mean values for Fe and Zn, which had significant negative GCA effects (Table 2). Though correlation coefficient between mean performance of parents and GCA effects was highly significant and positive for Zn concentrations ($r = 0.95$; $P < 0.01$), the correlation was non-significant in the case of Fe. Considering the mean performance and GCA effect together, the high Fe/Zn parent IS 13205 was identified as the best combiner. Among the others, ICSB 52 was best combiner for Fe (set II), IS 2248 and IS 20843 were best combiners for Zn concentrations (set III).

The results on SCA effects for Fe and Zn in each parental combination (set I) was studied and five hybrids for Fe and three hybrids for Zn showed significant SCA effects, indicating the presence of non-additive effects (Table 2). Significant positive SCA effects were observed in five hybrids for Fe and three hybrids for Zn, of which two hybrids for Fe and a hybrid for Zn had IS 13205 in their parentage. Reciprocal differences were also noticed among the crosses.

3.2. *Performance per se of crosses and parents*

The concentration of Fe ranged from 29.35 to 47.76 mg kg^{-1} in the crosses, from 30.42 to 45.02 mg kg^{-1} in the parents and 37.32 mg kg^{-1} in the control, ICSR 40. The Fe concentrations observed in these crosses were higher than that observed in sorghum commercial hybrids grown in India (Ashok Kumar et al., 2010). The mean performance of crosses for Zn was 27.93 mg kg^{-1}. The Zn ranged from 21.46 to 42.45 mg kg^{-1} in the crosses, again higher than the Zn in commercial hybrids grown in India; from 22.81 to 36.16 mg kg^{-1} among parents and 25.18 mg Zn kg^{-1} in the control ICSR 40, indicating the variability present in set I for Fe and Zn concentration. The grain yield ranged from 3.29 to 8.35 Mg ha^{-1} in the crosses compared to 2.35 to 4.56 Mg ha^{-1} of the parents, indicating high levels of heterosis for grain yield in the crosses. The control ICSR 40 showed a grain yield of 3.94 Mg ha^{-1} (Table 3). As hybrids are the cultivar choice in sorghum, the grain yield levels observed in these crosses were quite interesting and provide a hope that it is possible to deliver biofortified sorghum cultivars with high grain yield. The mean performance of set II crosses for Fe contents was 36.70 mg kg^{-1}. Fe contents ranged from 28.73 to 50.17 mg kg^{-1} in the crosses which is 13% higher than that was reported earlier (Ashok Kumar et al., 2010), from 28.87 to 41.29 mg kg^{-1} in the parents and 38.32 mg kg^{-1} in the control, ICSR 40 indicating the variability present in set II for Fe concentration. The grain yield ranged from 1.75 to 6.24 Mg ha^{-1} in the crosses, and from 0.87 to 4.97 Mg ha^{-1} in the parents and 2.49 Mg ha^{-1} in the control ICSR 40 (Table 4). The mean performance of set III crosses for Zn concentration was 36.9 mg kg^{-1}. Zn ranged from 23.43 to 55.46 mg kg^{-1} in the crosses which is significantly higher than our targeted 40 mg kg^{-1} in sorghum; from 29.72 to 45.70 mg kg^{-1} in parents and 31.23 mg kg^{-1} in the control ICSR 40 indicating the variability present in set III for Zn concentration. The grain yield ranged from 3.77 to 7.61 Mg ha^{-1} in the crosses, from 2.74 to 6.04 Mg ha^{-1} in the parents and 3.30 Mg ha^{-1} in the control ICSR 40 (Table 5) indicating the superiority of the crosses. In the sorghum biofortification work under the HarvestPlus, it was targeted to develop sorghum lines with 60 mg kg^{-1} Fe and 40 mg kg^{-1} Zn; and indeed some of the crosses generated in this experimentation showed grain Zn concentrations greater than the targeted 40 mg kg^{-1}, and selected hybrids showed Fe concentration up to 50 mg kg^{-1} with significantly higher yield.

The commercial sorghum cultivars (66) currently being cultivated by the farmers in India was assessed to identify high Fe and Zn concentrations in adapted backgrounds. The mean grain Fe concentration in them ranged Fe from 30 to 44 mg kg^{-1} and grain Zn from 15 to 33 mg kg^{-1} (Ashok Kumar et al., 2012).

3.3. *Estimate of heterosis*

Heterosis for grain yield and component traits is well established in sorghum (Quinby and Karper, 1946; Niehaus and Pickett, 1966; Kirby and Atkins, 1968a,b; Blum et al., 1977, 1990; Ashok Kumar et al., 2011b). Therefore exploitation of heterosis for enhancing grain Fe and Zn concentration is of major interest. In this study, the means of reciprocal and direct crosses were used to calculate heterosis over the mid-parent, better parent and standard control in all the three sets. Significant positive correlation between the mid-parental values and hybrid performance *per se* ($r = 0.54$; $P < 0.05$ for Fe and $r = 0.73$; $P < 0.01$ for Zn) (Fig. 1), and no correlation between mid-parent values and mid-parent heterosis ($r = -0.12$ for Fe and $r = -0.36$ for Zn) was observed in set I crosses. In set II crosses, highly significant positive correlation between the mid-parental values and hybrid performance *per se* for Fe ($r = 0.78$; $P < 0.01$ for Fe) was observed (Fig. 2) and highly significant positive correlation between the mid-parental values and hybrid performance *per se* for Zn ($r = 0.95$; $P < 0.01$ for Zn) was observed in set III (Fig. 3). This indicates the importance of additive gene action and need to improve both the parents for developing hybrids with high Fe and Zn concentration.

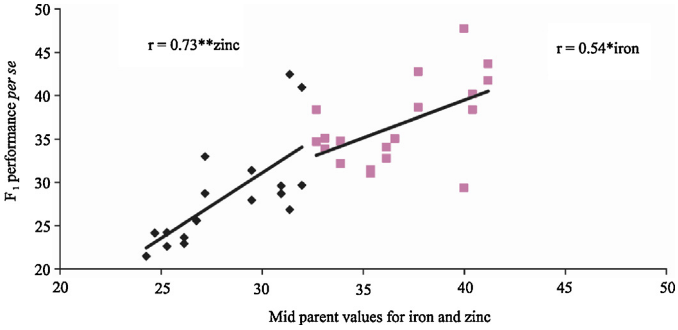

Fig. 1. Relationship between mid-parent values and F_1 performance *per se* for grain Fe (in solid square) and Zn (in solid diamond) concentrations (mg kg^{-1}) in sorghum crosses (set I).

In set I crosses (Table 3), mid-parent heterosis varied widely from −27 to 19% with an average of −0.14% for Fe; from −15 to 35% with an average −0.21% for Zn; and from −11 to 123% with an average 85% for grain yield. One cross IS 13205 × SPV 1359 showed 47.76 mg kg^{-1} Fe concentration and 42.45 mg kg^{-1} Zn concentration; and it was heterotic for Fe (by 19%) and significantly heterotic (by 35%) for Zn over the mid-parent. The same cross was also 69% highly ($P < 0.01$) significantly heterotic over the standard control. But its reciprocal cross SPV 1359 × IS 13205 with 29.35 mg kg^{-1} Fe concentration and 26.82 mg kg^{-1} Zn concentration showed highly ($P < 0.01$) significantly −27% negative heterosis over the better parent and −35% negative heterosis over the mid-parent for Fe; and for Zn significantly ($P < 0.05$) negative effect (−26%) over the better parent indicating the possible role of cytoplasm which needs to be further investigated. Significantly positive mid-parent heterosis was observed in all the crosses except three IS 13205 × SPV 1359, IS 13205 × ICSR 40 and IS 23464 × ICSR 40 and all these crosses showed significantly positive heterosis over better parent for grain yield barring the cross ICSR 40 × SPV 1359.

In set II crosses (Table 4), the mid-parent heterosis varied widely from −9 to 43%, with an average 11% for Fe concentration. Significantly positive mid-parent heterosis was observed in six crosses for Fe and better parent heterosis in five crosses ($P < 0.05$). Among these, five crosses had ICSB 52 as female parent which had the highest Fe concentration among the parents used in this set. ICSB 52 is a popular seed parent developed by ICRISAT which is widely used by public and private sector sorghum researchers in India for hybrids development. The mid-parent heterosis for grain yield varied from −30 to 86%, with an average of 27% for grain yield in this set. Seven crosses involving the parents SPV 1359, IS 10305, IS 13211 and ICSR 93031 had significantly positive mid-parent heterosis ranging from 39 to 85%. The cross IS 13211 × IS 10305 had 37 mg kg^{-1} Fe concentration with 85% mid-parent heterosis for grain yield. These results suggest that there is some scope for exploitation of heterosis for improving grain Fe concentration. In set III crosses (Table 5), the mid-parent heterosis varied widely from −26.5 to 29% with an average −2% for Zn concentration. However, no significant

Table 3
Heterosis and mean performance for grain Fe and Zn concentrations and grain yield in sorghum crosses (set I) across 2007 and 2009 postrainy seasons at ICRISAT – Patancheru, India.

Cross/parent	Fe (mg kg^{-1})				Zn (mg kg^{-1})				Grain yield (Mg ha^{-1})[a]			
	Mean of cross/parent	Mid-parent heterosis	Better parent heterosis	Superiority over standard control	Mean of cross/parent	Mid-parent heterosis	Better parent heterosis	Superiority over standard control	Mean of cross/parent	Mid-parent heterosis	Better parent heterosis	Superiority over standard control
IS 13205 × SPV 1359	47.76	19.44	6.09	27.97*	42.45	35.34*	17.39	68.59**	3.29	−10.84	−27.85	−16.5
IS 13205 × ICSR 40	43.67	6.05	−3	17.02	31.36	6.36	−13.27	24.54	3.65	21.46	14.42	−7.36
IS 13205 × IS 23464	42.76	13.36	−5.02	14.58	40.94	28.06	13.22	62.59**	5.64	96.52**	93.15**	43.15
ICSR 40 × IS 13205	41.76	1.41	−7.24	11.9	27.93	−5.27	−22.76*	10.92	5.05	68.05*	58.31*	28.17
IS 13205 × IS 518	40.17	−0.58	−10.77	7.64	29.57	−4.43	−18.22	17.43	4.99	93.04**	76.95**	26.65
IS 23464 × IS 13205	38.65	2.47	−14.15	3.56	29.65	−7.26	−18	17.75	6.04	110.45**	106.85**	53.3*
IS 518 × IS 13205	38.38	−5.01	−14.75	2.84	28.68	−7.3	−20.69	13.9	5.2	101.16**	84.4**	31.98
SPV 1359 × IS 23464	38.38	17.42	9.81	2.84	32.96	21.29	18.65	30.9	7.22	93.05**	58.33**	83.25**
IS 23464 × IS 518	35.11	6.06	−1.9	−5.92	25.6	−4.3	−7.85	1.67	5.87	122.77**	101.03**	48.98*
ICSR 40 × IS 518	35.04	−4.17	−6.16	−6.11	21.46	−11.56	−16.56	−14.77	5.8	109.39**	81.82**	47.21*
ICSR 40 × IS 23464	34.77	2.63	−6.88	−6.83	24.19	−4.37	−12.92	−3.93	5.66	85.27**	77.43**	43.65
IS 23464 × SPV 1359	34.7	6.16	−0.72	−7.02	28.7	5.61	3.31	13.98	6.87	83.69**	50.66*	74.37**
ICSR 40 × SPV 1359	34.05	−5.8	−8.81	−8.76	24.11	−2.35	−9.26	−4.25	5.81	49.94*	27.41	47.46*
IS 518 × IS 23464	33.85	2.25	−5.42	−9.3	25.54	−4.52	−8.06	1.43	5.72	117.08**	95.89**	45.18*
SPV 1359 × ICSR 40	32.78	−9.31	−12.21	−12.17	24.16	−2.15	−9.07	−4.05	8.35	115.48**	83.11**	111.93**
IS 23464 × ICSR 40	32.15	−5.11	−13.9	−13.85	22.6	−10.65	−18.65	−10.25	4.26	39.44	33.54	8.12
IS 518 × SPV 1359	31.45	−11.08	−12.13	−15.73	22.91	−12.37	−13.77	−9.02	6.65	92.47**	45.83**	68.78**
SPV 1359 × IS 518	31.06	−12.19	−13.22	−16.77	23.62	−9.66	−11.1	−6.2	7.31	111.58**	60.31**	85.53**
SPV 1359 × IS 13205	29.35	−26.6*	−34.81**	−21.36	26.82	−14.49	−25.83*	6.51	7.8	111.38**	71.05**	97.97**
IS 13205	45.02				36.16				2.82			
IS 23464	30.42				27.78				2.92			
IS 518	35.79				25.72				2.35			
ICSR 40	37.34				22.81				3.19			
SPV 1359	34.95				26.57				4.56			
ICSR 40 (control)	37.32				25.18				3.94			
Mean	36.5	−0.14	−8.17	−1.87	27.93	−0.21	−9.1	11.47	5.24			
CD (5%)	7.83				8.07				1.44			

* Significant at 0.05 probability level.

** Significant at 0.01 probability level.

[a] Mg (Mega grams) = 1000 kg.

Table 4

Heterosis and mean performance for grain Fe concentrations and grain yield in sorghum crosses and parents (set II) across 2007 and 2009 postrainy seasons at ICRISAT – Patancheru, India.

Cross/parent	Fe ($mg\,kg^{-1}$)				Grain yield ($Mg\,ha^{-1}$)[a]			
	Mean of cross/parent	Mid parent heterosis	Better parent heterosis	Superiority over standard control	Mean of cross/parent	Mid parent heterosis	Better parent heterosis	Superiority over standard control
ICSB 52 × SPV 1359	50.17	43.02[**]	21.51[**]	30.92[**]	4.17	−4.58	−16.10	67.47[**]
ICSB 52 × ICSR 93031	48.33	33.90[**]	17.05[*]	26.12[*]	3.26	−2.83	−13.53	30.92
ICSB 52 × IS 13211	48.10	26.56[**]	16.49[*]	25.52[**]	2.02	−29.62	−46.42[**]	−18.88
ICSB 52 × IS 2263	47.69	25.30[**]	15.50[*]	24.45[*]	3.37	−16.06	−20.89	35.34
ICSR 93031 × IS 2263	45.41	38.17[**]	30.38[**]	18.50	3.42	−5.00	−19.72	37.35
ICSB 52 × IS 10305	45.30	26.20[**]	9.71	18.22	3.30	42.24	−12.47	32.53
IS 13211 × ICSB 52	42.77	12.54	3.58	11.61	3.69	28.57	−2.12	48.19[*]
ICSR 93031 × ICSB 52	41.96	16.25	1.62	9.50	3.25	−3.13	−13.79	30.52
IS 10305 × ICSB 52	38.97	8.57	−5.62	1.70	3.19	37.50	−15.38	28.11
IS 13211 × IS 2263	38.41	10.45	10.28	0.23	3.18	2.09	−25.35[*]	27.71
IS 13211 × IS 10305	37.42	14.75	7.78	−2.35	2.63	85.21*	33.50	5.62
ICSR 93031 × IS 13211	36.58	11.49	5.36	−4.54	3.26	32.79	10.88	30.92
IS 13211 × SPV 1359	36.27	14.07	4.46	−5.35	3.33	−4.03	−33.00[**]	33.73
IS 2263 × IS 13211	36.18	4.04	3.88	−5.58	3.21	3.05	−24.65[*]	28.92
SPV 1359 × ICSB 52	35.77	1.97	−13.37	−6.65	4.61	5.49	−7.24	85.14[**]
IS 13211 × ICSR 93031	35.24	7.41	1.50	−8.04	4.41	79.63[**]	50.00[**]	77.11[**]
ICSR 93031 × SPV 1359	34.58	15.71	11.91	−9.76	5.06	27.94	1.81	103.21[**]
SPV 1359 × ICSR 93031	33.88	13.37	9.64	−11.59	6.24	57.77[**]	25.55[**]	150.6[**]
IS 2263 × IS 10305	33.56	2.74	−3.65	−12.42	4.78	86.35[**]	12.21	91.97[**]
IS 2263 × SPV 1359	33.28	4.49	−4.45	−13.15	4.93	6.83	−0.80	97.99[**]
IS 2263 × ICSR 93031	32.71	−0.47	−6.09	−14.64	4.57	26.94	7.28	83.53[**]
SPV 1359 × IS 2263	31.39	−1.44	−9.88	−18.08	5.74	24.38	15.49	130.52[**]
IS 10305 × ICSR 93031	30.64	−0.20	−0.84	−20.04	2.29	20.21	−22.11	−8.03
IS 10305 × IS 13211	30.44	−6.65	−12.33	−20.56	1.75	23.24	−11.17	−29.72
SPV 1359 × IS 10305	30.44	2.54	−0.20	−20.56	5.05	72.95[**]	1.61	102.81[**]
SPV 1359 × IS 13211	29.97	−5.74	−13.68	−21.79	4.84	39.48[*]	−2.62	94.38[**]
IS 10305 × IS 2263	29.70	−9.08	−14.73	−22.49	3.66	42.69	−14.08	46.99[*]
IS 10305 × SPV 1359	28.73	−3.22	−5.80	−25.03	5.17	77.05[**]	4.02	107.63[**]
IS 2263	34.83				4.26			
IS 13211	34.72				1.97			
IS 10305	30.50				0.87			
ICSB 52	41.29				3.77			
ICSR 93031	30.90				2.94			
SPV 1359	28.87				4.97			
ICSR 40 (contro)	38.32				2.49			
Mean	36.70	10.95	2.86	−2.71	3.73			
CD (5%)	5.90				0.94			

[*] Significant at 0.05 probability level.

[**] Significant at 0.01 probability level.

[a] Mg (Mega grams) = 1000 kg.

mid-parent heterosis or better parent heterosis was observed for grain Zn indicating that there is little or no possibility for exploitation of heterosis for improving grain Zn in sorghum. The mid-parent heterosis for grain yield varied from −20 to 120%, with an average of 47% for grain yield. Eight crosses had significantly positive mid-parent heterosis ranging from 33 to 120%. The cross ICSB 56 × IS 2248 showed 40 mg kg^{-1} Zn concentration with 56% significant mid-parent heterosis for grain yield.

4. Discussion

Sorghum is one of the cheapest sources of energy and micronutrients (Parthasarathy Rao et al., 2006); and in most countries it is consumed directly by whole grain milling or by processing in to various products after removing the outer pericarp. The sorghum grain can be divided in to three major components—pericarp, embryo and endosperm. Endosperm is the largest component accounting for 81–85% of the dry weight of grain which consists of the aleurone layer and the peripheral, corneous, intermediate and floury portions. The aleurone layer contains large amounts of minerals, water-soluble vitamins, autolytic enzymes, oil and protein bodies (Chavan and Patil, 2010; U.S. Grains Council, http://www.agmrc.org/media/cms/Sorghum_Handbook_B5FE1C2B5DBCF.pdf verified on 8 November 2012). In seeds of related cereals wheat, corn and barley, Zn is preferentially accumulated in the husk, aleurone layers or embryo (Lin et al., 2005; Ozturk et al., 2006; Liu et al., 2007; Hansen et al., 2009; Persson et al., 2009; Cakmak et al., 2010a,b; Lombi et al., 2011; Stomph et al., 2011), which might be associated with high levels of Zn-binding compounds, such as proteins and phytate. As it is difficult to fractionate aleurone layer from each sample, as a standard practice the sorghum grain Zn and Fe concentrations are assessed on the basis of whole grain which helps in identifying lines with high grain Fe and Zn. Sorghum is mainly consumed as *Roti* in Asia and as *Tô*, *Injera* and *Kisra* in many countries in Africa. The grains are processed to remove the pericarp to make these preparations keeping the endosperm (including aleurone layer) and germ portion intact which harbor the minerals, vitamins, proteins and oils (ICRISAT, 1982). Therefore any improvement in Fe and Zn concentration through sorghum biofortification will have direct bearing on improving the nutritional status of major sorghum eating populations in Asia and Africa.

To understand the genetic control of grain Fe and Zn concentration in sorghum, three sets of diallel crosses were developed and evaluated along with their parents over two years in this study. The ANOVA from set I crosses indicated that there were significant differences ($P < 0.01$) between the parents for grain Fe and Zn concentrations and grain yield, indicating the suitability of this material for our study. The P × Y interaction was significant for Fe and Zn concentrations indicating environmental influence on expression of grain Fe and Zn. However there was significant positive correlation between parental values in the two test environments for Fe ($r = 0.88$; $P < 0.01$) and Zn ($r = 0.84$; $P < 0.01$) indicating the consistency in their relative performance for grain Fe and Zn contents. ANOVA also indicated highly significant differences for Years, Crosses and Crosses × Year interactions for grain Fe and Zn concentrations and grain yield. The reciprocal effects among the hybrids were significant for both Fe and Zn concentrations and grain yield indicating the possible role of cytoplasmic factors in conditioning grain Fe and Zn concentrations in sorghum, which needs to be further investigated. Gene effects for grain yield and its component traits were extensively studied in sorghum (Haussmann et al., 2000; Iyanar and Gopalan, 2006; Mahdy et al., 2011), so our focus was mainly on grain Fe and Zn concentration. Secondly, the parents we used in this study do not represent maximum variability

Table 5
Heterosis and mean performance for grain Zn concentrations and grain yield in sorghum crosses and parents (set III) across 2007 and 2009 postrainy seasons at ICRISAT – Patancheru, India.

Cross/parent	Zn (mg kg^{-1})				Grain yield (Mg ha^{-1})[a]			
	Mean of cross/parent	Mid parent heterosis	Better parent heterosis	Superiority over standard control	Mean of cross/parent	Mid parent heterosis	Better parent heterosis	Superiority over standard control
IS 2248 × IS 20843	55.46	29.20	21.36	77.59**	4.75	27.18	18.45	43.94*
IS 20843 × IS 2248	50.10	16.72	9.63	60.42**	4.33	15.93	7.98	31.21
IS 2248 × PVK 801	45.53	14.22	−0.37	45.79**	3.77	−20.63	−37.58**	14.24
PVK 801 × IS 2248	42.23	5.95	−7.59	35.22*	5.32	12.00	−11.92	61.21**
ICSB 56 × IS 2248	40.30	6.87	−11.82	29.04	4.83	55.81*	39.60*	46.36*
IS 2248 × ICSB 56	37.98	0.72	−16.89	21.61	5.05	62.90**	45.95**	53.03*
IS 20843 × PVK 801	31.70	−14.52	−21.05	1.50	7.61	51.44**	25.99**	130.61**
ICSB 56 × IS 20843	31.44	−10.00	−21.69	0.67	6.62	96.15**	65.09**	100.61**
PVK 801 × IS 20843	30.28	−18.35	−24.58	−3.04	6.69	33.13*	10.76	102.73**
IS 20843 × ICSB 56	29.73	−14.90	−25.95	−4.80	7.41	119.56**	84.79**	124.55**
PVK 801 × ICSB 56	26.57	−16.63	−21.90	−14.92	6.62	50.80**	9.60	100.61**
ICSB 56 × PVK 801	23.43	−26.48	−31.13	−24.98	6.90	57.18**	14.24	109.09**
IS 2248	45.70				3.46			
IS 20843	40.15				4.01			
PVK 801	34.02				6.04			
ICSB 56	29.72				2.74			
ICSR 40 (control)	31.23				3.30			
Mean	36.90				5.26			
CD (5%)	11.44				1.13			

[*] Significant at 0.05 probability level.
[**] Significant at 0.01 probability level.
[a] Mg (Mega grams) = 1000 kg.

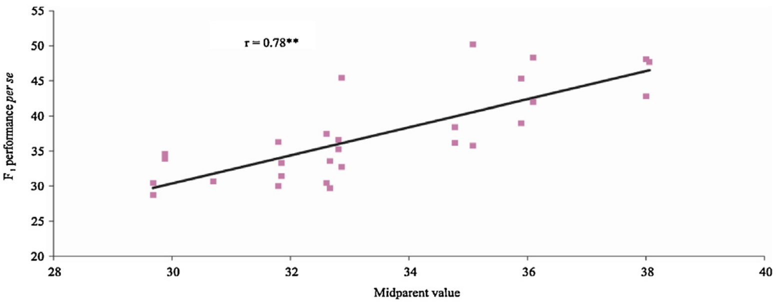

Fig. 2. Relationship between mid-parent values and F_1 performance *per se* for grain Fe concentration (mg kg^{-1}) in sorghum crosses (set II).

for grain yield as they were selected for their diversity for grain Fe and Zn, rather than for grain yield. The GCA and SCA effects were highly significant indicating that both additive and non-additive gene effects controlling these two micronutrients and grain yield. However, the magnitude of GCA mean squares was higher than the SCA mean squares for both Fe and Zn and grain yield, implying the predominance of additive gene action in conditioning these traits. This means, we need to improve both the parental lines for these traits so as to get desired hybrids. It is well established in sorghum that one should develop high yielding parental lines to derive heterotic hybrids (Beil and Atkins, 1967; Kirby and Atkins, 1968a,b; Iyanar and Gopalan, 2006). Upon partitioning the sum of squares into GCA × Y, SCA × Y and reciprocals × Y interaction effects, all these effects were found to be significant, indicating that Fe and Zn and grain yield were sensitive to the environmental conditions, and that data from additional seasons or environments would provide more precise estimates of GCA, SCA and reciprocal effects.

Baker (1978) suggested that the ratio of combining ability variance components [$2\sigma^2$gca/($2\sigma^2$gca + σ^2sca)] termed as predictability ratio, provides a measure of the predictability of the performance of hybrids and its progenies. The closer this ratio is to unity, the greater the predictability based on GCA alone. Based on a combined analysis over the two environments the predictability ratio was 0.35 for Fe and 0.65 for Zn in set I, 0.21 for Fe in set II and 0.66 for Zn in set III, implying the preponderance of non-additive gene action for grain Fe and additive gene action for grain Zn. These results indicate that hybrid performance can be predicted based on the GCA alone for Zn, but based on both GCA and SCA in the case of Fe. The GCA and SCA effects of parents and crosses and reciprocal crosses (set I) indicate that the GCA was highly significant for parent IS 13205 for both Fe and Zn and GCA effect for parent SPV 1359 was significant for Zn and grain yield. Similarly, in set II, ICSB 52 had higher mean Fe concentration and highly significant GCA and SPV 1359 had higher mean grain yield and highly significant GCA. In set III, IS 2248 and IS 20843 had higher mean Zn concentrations and highly significant GCA. The high Fe and Zn parents were the best general combiners having positive significant GCA effects than those with low mean values for Fe and Zn, which had significant negative GCA effects. Similar results were reported in other crops like pearl millet (Velu et al., 2011).

The correlation coefficient between mean performance *per se* of parents and GCA effects was highly significant and positive for Zn concentrations ($r = 0.95$; $P < 0.01$), indicating that the selection of lines with high Zn levels would be highly effective in selecting for high GCA. However, this correlation was non-significant in the case of Fe. Considering mean performance and GCA effects together, the high Fe/Zn parent IS 13205 was identified as the best combiner for both the Fe and Zn for future breeding programs and SPV 1359 as the best combiner for Zn and grain yield. IS 13205 is a *durra-caudatum* landrace of Spanish origin. Similarly, based on mean performance and GCA effects together, ICSB 52 was found to be the best combiner for Fe, and IS 2248 and IS 20843 were best combiners for Zn concentrations. The results on SCA effects for Fe and Zn in each parental combination showed that hybrids for Fe and hybrids for Zn had significant SCA effects, indicating the presence of non-additive effects. Significant positive SCA effects were observed in five hybrids for Fe and three hybrids for Zn, of which two hybrids for Fe and a hybrid for Zn had IS 13205 in their parentage. Reciprocal differences were noticed among the crosses, indicating the possible role of cytoplasm, which needs further investigation in future.

From the heterosis studies, significant positive correlation between mid-parental values and hybrid performance ($r = 0.54$;

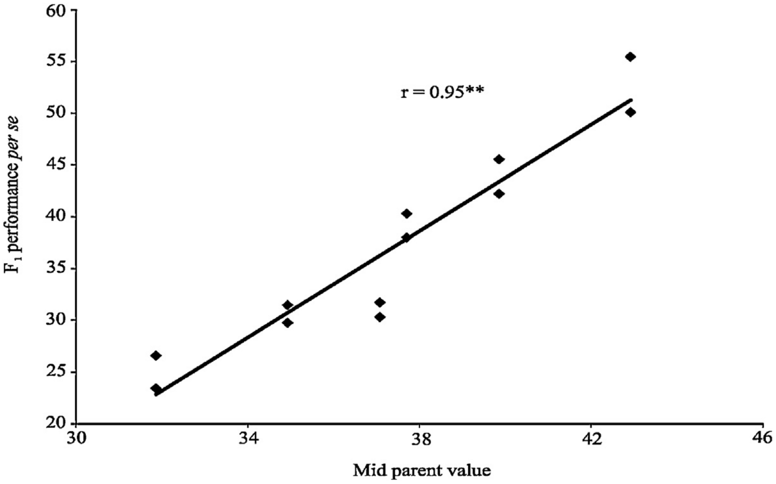

Fig. 3. Relationship between mid-parent values and F_1 performance *per se* for grain Zn concentration (mg kg^{-1}) in sorghum crosses (set III).

$P < 0.05$ for Fe and $r = 0.73$; $P < 0.01$ for Zn), and no correlation between mid-parent values and mid-parent heterosis ($r = -0.12$ for Fe and $r = -0.36$ for Zn) in set I crosses provided additional indications of the predominant role of additive gene action for these traits. In set II crosses, significant positive correlation between mid-parental values and hybrid performance for Fe ($r = 0.78$; $P < 0.01$) and in set III crosses, highly significant positive correlation between the mid-parental values and hybrid performance for Zn ($r = 0.95$; $P < 0.01$) substantiate the predominant role of additive gene action in governing grain Zn concentration and both additive and non-additive gene action contributing to grain Fe concentrations. Some of the earlier studies have reported the greater importance of additive gene action (GCA effects) for grain Fe and Zn concentrations in maize (Gorsline et al., 1964; Arnold and Bauman, 1976; Long et al., 2004), rice (Gregorio, 2002) and pearl millet (Velu et al., 2011). However, in the case of sorghum, additive gene action is predominant in governing grain Zn concentration and both additive and non-additive gene action contribute to grain Fe concentration as indicated by the present study.

Upon computing the mid-parent, better parent heterosis, set I crosses showed reciprocal differences for mid-parent, better parent heterosis for grain Fe and Zn concentrations. High positive heterosis was observed in crosses when the female parent possessed higher mean value for Fe and Zn, indicating the importance of the female parent in contributing to these micronutrients. Significantly positive mid-parent heterosis was observed in six crosses for Fe and better parent heterosis in five crosses ($P < 0.05$) in set II without significant deviations in grain yield. Among these, five crosses had ICSB 52 as female parent, which has the highest concentration of Fe among the parents used in this set. Incidentally, ICSB 52 is a popular female parent used in the development of large number of commercial sorghum hybrids. This shows that it is feasible to produce hybrids with higher grain Fe concentration without yield penalty provided that at least one of the parents used in deriving the hybrid has high Fe concentration. In set III crosses, mid-parent heterosis varied widely. However, no significant mid-parent heterosis or better parent heterosis was observed for grain Zn concentrations, further indicating the importance of additive gene action for grain Zn. However, two crosses, ICSB 56 × IS 2248 and its reciprocal cross showed significantly higher grain yields with better parent heterosis up to 46% and mid-parent heterosis up to 63% and the grain Zn concentration close to 40 mg kg^{-1} which is highly desirable. In our sorghum improvement program we are targeting 60 mg kg^{-1} Fe and 40 mg kg^{-1} Zn along with high grain yields and some of the hybrids in this experimentation are close to achieving this.

5. Conclusions

This study indicated that the expression of grain Zn concentrations in sorghum is governed predominantly by additive gene effects, suggesting the high effectiveness of progeny selection in pedigree selection or population breeding to develop lines with increased levels of grain Zn concentrations while the grain Fe concentrations is governed predominantly by non-additive gene effects in combination with additive gene effects, suggesting scope for heterosis breeding in addition to progeny selection to develop lines with increased levels of grain Fe concentrations. The performance of the crosses can be predicted based on GCA for grain Zn but information on both GCA and SCA required for Fe. There is scope of exploitation of heterosis for improving the grain Fe content. Some of the crosses developed in the study significantly outperformed parents for Fe and Zn concentration with no yield penalty indicating that it is possible to develop high grain Fe and Zn cultivars in high yielding backgrounds.

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