

**GENE EFFECTS AND HETEROSIS FOR GRAIN Fe AND Zn CONTENT IN  
BARNYARD MILLET (*Echinochloa frumentacea* (Roxb.) Link)**

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Barnyard millet (*Echinochloa frumentacea*) is an unexplored nutri-rich crop that thrives  
well in harsh environments and supports many small farmers in Southern and Eastern  
Asia. Although it has rich sources of micronutrients, the genetic studies are very limited  
which further impedes in its genetic improvement. Therefore, we attempted to assess  
the genetic diversity for Fe and Zn content in 40 barnyard millet germplasm and to  
evaluate the combining ability and heterosis in sixteen F<sub>1</sub> cross combinations through  
line × tester model. The Mahalanobis D<sup>2</sup> analysis grouped the 40 genotypes into nine  
different clusters. Cluster III and I were the largest groups containing 22 and 6  
genotypes, respectively and the rest of seven clusters were the lowest group containing  
one or two genotypes. Positive correlation was observed between Fe and Zn content  
though both had a non-significant association with grain yield. This indicate that there  
would not be any compromise on increase or decrease of grain yield while breeding for  
varieties high in micronutrient content. Combining ability analysis revealed that lines,  
testers, and their interaction components are significant. The predictability ratio

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indicated the predominance of additive variance for Fe and Zn content and non-additive variance in the inheritance of yield components. Genotypes, ACM 331, ACM 333, ACM 335 and MA 10 exhibited positive *gca* effects for Fe and Zn content and grain yield. Two cross combinations, ACM 331 × ACM 335 and ACM 331 × MA 10 involved one or both the parents with good *gca* effects exhibited, high mean, positive mid-parent heterosis and *sca* effects for Fe, Zn content and yield components. Thus, the present investigation provided a significant understanding of the gene action and the possibility of utilizing the selected parents and cross combination for exploiting micronutrient traits in barnyard millet crop.

**Keywords:** Barnyard millet, correlation, combining ability, GCA, SCA, gene action, heterosis, micronutrients, Line × Tester analysis

## INTRODUCTION

Barnyard millet [*Echinochloa frumentacea* (Roxb.) Link], one of the important small millets used as food and fodder in the semi-arid tropics of India and in several African countries and as bird feeds in United States (SOOD *et al.*, 2015). It is a self-pollinated, annual, C<sub>4</sub> grass that belongs to the family *Poaceae*. In Japan, *Echinochloa esculenta* also known as Japanese barnyard millet, a temperate species is cultivated mainly for food and fodder in Japan, Korea and China (YABUNO, 1987). *E. frumentacea* also known as Indian barnyard millet, a tropical species mainly cultivated from hilly region of Himalayas to rain fed regions of Deccan plateau of Southern India (SOOD *et al.*, 2015). Though it is cultivated in Madhya Pradesh, Uttar Pradesh, Tamil Nadu, Andhra Pradesh, Karnataka, Maharashtra and Bihar, the Uttarkhand and South Tamil Nadu are major barnyard millet producing states in India (IIMR, 2018). In recent years, the area under barnyard millet follows an increasing trend in India with an substantial increase in yield and farmers income (MICHAELRAJ and SHANMUGAM, 2013). This could be attributed not only due to its wider adaptability to vagaries of climatic conditions but also to the change in consumer preference for food rich in various nutrients than that of the other major cereal crops like rice and wheat. According to World Health Organization (WHO) (2012), the two micronutrients iron and zinc are recognized to be limiting for the women of reproductive age, lactating women and pre-school children especially in developing countries.

The grains of barnyard millet are rich in protein (8.08 to 11.84%), crude fibre (9.8 to 30%), calcium (98.7 to 147.0 mg/100g), iron (20 to 46.20 ppm), and zinc content (36.4 to 40.2 ppm) (SALEH *et al.*, 2013; CHANDEL *et al.*, 2014) which is adjudged to be as a good source of essential nutrients for malnourished people, living in developing countries. The dietary fibre of its grains well suit for diabetic patients (UGARE *et al.*, 2014) and antioxidants property also helps against cancer in human cells (KIM *et al.*, 2011).

To date, reports on characterization of agronomical traits in barnyard millet are satisfactorily adequate, but little is known about the genetics and breeding behavior of micronutrient content. Hence, exploiting of barnyard millet for increased nutritive value in addition to yield is foremost importance to the breeders. The line × tester, a potential mating design that has been widely adopted to assess the combining ability of inbred parents in order to establish their potential to develop superior hybrids for grain quality traits (PARMAR *et al.*, 2013) and to assess gene action and heterosis for micronutrients like Zn and Fe content (GOVINDARAJ *et*

*al.*, 2013). The correlation analysis help the plant breeder to know how the improvement of one character will bring simultaneous improvement for other traits in the subsequent segregating population (KOHNAKI *et al.*, 2013). Up to date, no studies were conducted on heterosis and gene effects for Fe and Zn content in this crop. Therefore the present experiment was conducted with the following objectives: (i) to assess the genetic diversity for Fe and Zn content and grain yield components (ii) to estimate combining ability and nature of gene action for Fe and Zn content and grain yield components (iii) to determine the association between the Fe and Zn content and grain yield components.

## MATERIAL AND METHODS

### *Plant genetic materials*

The seed materials of 40 barnyard millet genotypes were obtained from Department of Plant Breeding and Genetics, Agricultural College and Research Institute, Madurai, Department of Millets, Tamil Nadu Agricultural University, Coimbatore, All India Coordinated Small Millets Improvement Project, Bengaluru and International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) Patancheru, Hyderabad.

### *Experiment plot and design*

Field experiments were conducted at Agricultural College and Research Institute, Madurai, which is geographically located at 9° 54' N latitude and 78° 54' E longitude at an elevation of 147 m above mean sea level. The 40 barnyard millet genotypes were planted in a randomized complete block design (RCBD) with two replications during *kharif*, 2015. Each genotype was sown in 3m length, spaced at 30 cm, between rows and 15 cm within row. The standard agronomic practices and crop protection measures were followed during the entire crop period. The diverse genotypes (four female and four male) from clusters having high and low mean value for Fe and Zn content were chosen for 4×4, line × tester mating design (KEMPTHORNE, 1957) during summer, 2016. The F<sub>1</sub>s of sixteen crosses along with their parents were replicated twice and evaluated during *kharif* 2016.

### *Measurement of morphological traits*

The measurement of quantitative traits (morphological traits) was followed as per the International Board for Plant Genetic Resources (IBPGR), barnyard millet descriptor, 1983. At maturity, the following observations were recorded on five randomly selected plants per genotype per replication for days to maturity, plant height (cm), flag leaf length (cm), flag leaf breadth (cm), ear head length (cm), ear head breadth (cm), number of racemes per inflorescence, single ear head weight (g), number of productive tillers, thousand-grain weight (g), fodder yield per plant (g) and grain yield per plant (g).

### *Estimation of Fe and Zn content*

The Fe and Zn contents for each genotype were determined through ICP-OES (inductively coupled plasma atomic emission spectroscopy). The panicle of main tillers of F<sub>1</sub>s and parents in each replication were harvested separately and sun-dried to the moisture level of 14%, followed by powdering through a house-hold wet grinder. The solution for Fe and Zn

estimation was prepared according to Triple acid (Nitric acid + Sulfuric acid + Perchloric acid) extract method (PIPER, 1966), where 1gm of the sample was subjected to acid digestion. The solution was filtered and readings were taken using ICP-OES and value represented in mg/100g.

#### Statistical analysis

Fourteen quantitative traits of 40 barnyard millet germplasm were statistically analyzed using Mahalanobis  $D^2$  statistic as per RAO (1952) method. The Pearson's product-moment correlation (TAYLOR, 1990) was carried out for all quantitative traits. The  $F_1$  data were subjected to analysis of variance as per the line  $\times$  tester method described by SINGH and CHOUDHARY (1985). Variances attributed to general combining ability (GCA) and specific combining ability (SCA) were derived to estimate the predictability ratio (BAKER, 1978). Mid-parent heterosis (MPH) and better parent heterosis (BPH) were estimated as the percentage deviation of the  $F_1$  mean from the mid-parent (MP) and better parent (BP) values, respectively (HALLAUER and MIRANDA, 1981).

## RESULTS

#### Genetic diversity of germplasm

Analysis of variance indicated highly significant differences among genotypes for all 14 quantitative traits (micronutrients and agronomic traits), suggesting the occurrence of a considerable amount of variability among the germplasm studied. The relative contribution of individual plant traits towards divergence is depicted in Figure 1.

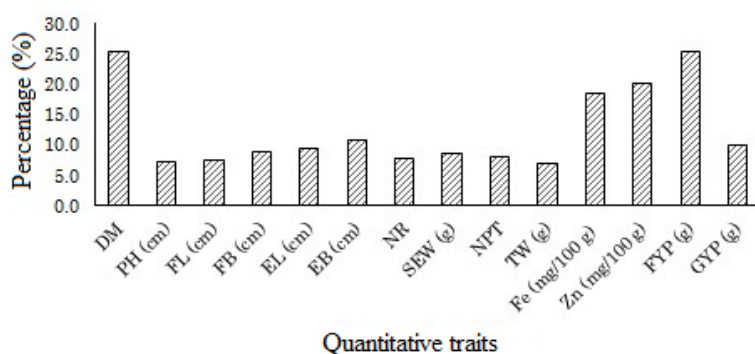


Figure 1. Relative contributions of the 14 quantitative traits of 40 barnyard millet genotypes to the total divergence. DM=Days to maturity, PH=Plant height, FL=Flag leaf length, FB=Flag leaf breadth, EL=Ear head length, EB=Ear head breadth, NR=Number of racemes per inflorescence, SEW=Single ear head weight, NPT=Number of productive tillers per plant, TW=Thousand grain weight, Fe=Iron, Zn=Zinc, FYP=Fodder yield per plant, GYP=Grain yield per plant.

The traits, days to maturity (25.4%) and fodder yield per plant (25.4%) showed maximum contribution towards genetic divergence followed by Zn content (20.0%), Fe content (18.5%), ear head breadth (10.7%) and grain yield per plant (10.0%). The  $D^2$  analysis of 40

barnyard millet germplasm resulted in nine clusters (Figure 2) among which, cluster III was the largest comprising of twenty-two genotypes followed by cluster I with six genotypes. The remaining clusters II, IV, V, VI, VII and IX with two and clusters VIII and IX with one genotype each respectively.

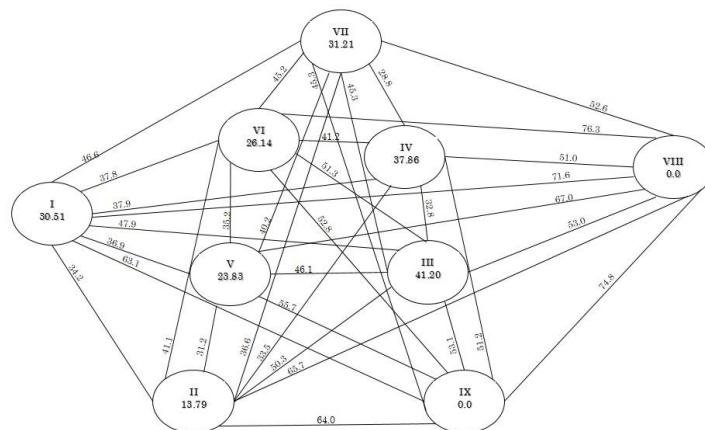


Figure 2. Cluster diagram of 40 barnyard millet genotypes based on  $D^2$  analysis. Note: The cluster diagram presented here was not exactly to the scale

Table 1. Cluster mean for 14 quantitative traits of 40 barnyard millet genotypes

	DM	PH	FL	FB	EL	EB	NR	SEW	NPT	TW	FYP	Fe	Zn	GYP
Cluster I	96.75	159.95	25.17	3.49	25.06	6.59	52.83	14.73	5.92	3.70	65.40	6.64	2.12	34.66
Cluster II	103.75	160.72	25.08	3.22	22.28	3.29	42.25	11.82	9.25	3.20	87.05	6.34	2.36	30.08
Cluster III	95.16	137.54	25.45	3.14	21.28	5.03	49.73	12.34	5.80	3.09	50.66	9.70	3.60	28.10
Cluster IV	93.50	111.47	20.84	2.83	19.45	4.55	50.25	6.46	6.50	3.21	42.48	10.21	3.15	20.97
Cluster V	95.25	121.63	18.33	2.66	18.03	4.05	33.75	12.19	3.75	3.19	36.90	2.25	1.12	24.37
Cluster VI	83.00	140.29	23.72	2.95	22.50	3.74	51.50	11.41	5.25	3.49	65.26	4.12	0.91	29.99
Cluster VII	91.00	144.99	22.04	2.98	21.75	4.35	44.00	12.13	4.25	2.95	34.77	11.60	3.42	17.80
Cluster VIII	91.50	111.57	24.63	3.00	26.50	14.22	41.50	9.80	3.50	2.40	36.66	9.57	3.08	24.32
Cluster IX	77.50	99.66	21.02	2.20	12.55	4.51	22.00	5.93	4.50	3.38	37.23	13.43	3.28	13.98

DM=Days to maturity, PH=Plant height, FL=Flag leaf length, FB=Flag leaf breadth, EL=Ear head length, EB=Ear head breadth, NR=Number of raceme per inflorescence, SEW=Single ear head weight, NPT=Number of productive tillers per plant, TW=Thousand grain weight, FYP=Fodder yield per plant, Fe=Iron; Zn=Zinc, GYP=Grain yield per plant.

The maximum inter-cluster distance  $D^2$  value was observed between cluster VI and VIII (76.35) followed by cluster I and VIII (71.63) and the least inter-cluster distance was observed between cluster IV and cluster VII (28.79). Intra-cluster  $D^2$  values ranged from 0.00 (cluster VIII & IX) to 41.20 (cluster III). The results obtained from the data on cluster means

(Table 1) from the different traits revealed that cluster I had the highest mean value for number of racemes per inflorescence (52.83), single ear head weight (14.73 g), thousand grain weight (3.70 g) and grain yield per plant (34.66 g). The genotypes with high productive tillers (11.42) and grain yield per pant (30.08 g) were found in cluster II. The cluster III had the moderate Fe and Zn content, where the genotypes with maximum of Fe content (16.40 mg/100g) and Zn content (4.90 mg/100g) were grouped. The clusters IV, VII and IX with one genotype each recorded high Fe content (10.21 to 13.43 mg/100 g) and Zn content (3.20 to 3.42 mg/100 g) content, but grain yield components were low. In cluster V and VI, the genotypes were found early maturing (83 days) and low in Fe and Zn content (2.25 & 0.91 mg/100 g). Similarly the cluster IX with one genotype was found with early maturity (77.50 days) and low in grain yield (13.90 g).

### Correlation

According to bivariate correlation coefficient method, the Pearson correlation coefficients ( $r$ ) classified the coefficient value  $r < 0.35$  as weak,  $r = 0.37$  to  $0.67$  as moderate and  $r = 0.68$  to  $1.00$  as strong. In general, among most of the traits, significant positive correlation was observed at  $p$ -value of  $0.05$  (Figure 3).

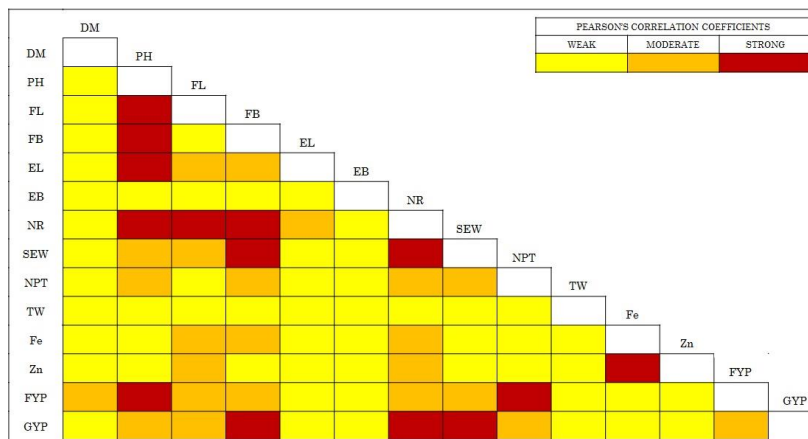


Figure 3. Pearson's product-moment correlation among 14 quantitative traits measured in 40 barnyard millet genotypes. DM=Days to maturity, PH=Plant height, FL=Flag leaf length, FB=Flag leaf breadth, EL=Ear head length, EB=Ear head breadth, NR=Number of racemes per inflorescence, SEW=Single ear head weight, NPT=Number of productive tillers per plant, TW=Thousand grain weight, Fe=Iron, Zn=Zinc, FYP=Fodder yield per plant, GYP=Grain yield per plant. Significant at  $*P = 0.05$ ,  $r < 0.35$  (weak, light yellow),  $r = 0.37$  to  $0.67$  (moderate, dark yellow) and  $r = 0.68$  to  $1.00$  (strong, red)

Among the pair of traits, single ear head weight had the highest correlation coefficient value with grain yield ( $r=0.954$ ) followed by Fe content with Zn content ( $r=0.893$ ). The number of racemes per inflorescence and flag leaf breadth subsequently showed a high correlation

coefficient value with yield ( $r=0.829$  &  $r=0.689$ ). A strong correlation in plant height was observed with majority of the traits; viz., flag leaf length ( $r=0.768$ ), flag leaf breadth ( $r=0.726$ ), ear head length ( $r=0.682$ ), number of racemes per inflorescence ( $r=0.749$ ), number of productive tillers per plant ( $r=0.662$ ) and fodder yield per plant ( $r=0.782$ ). The flag leaf and flag leaf breadth were found to have moderate association with Fe ( $r=0.491$  &  $r=0.563$ ) and Zn content ( $r=0.418$  &  $r=0.520$ ). The grain yield per plant has a moderate association with plant height ( $r=0.567$ ), flag leaf length ( $r=0.529$ ), number of productive tillers ( $r=0.538$ ), and fodder yield per plant ( $r=0.581$ ).

#### *Gene effect and combining ability analysis*

Based on  $D^2$  analysis, ACM 295 and ACM 331 in cluster II, IEC 370 and IEC 747 from cluster V and VIII respectively are chosen as female parents (Lines) and ACM 333, ACM 335, MA 10 and GECH 10 from cluster III as male parents (Testers). These lines and testers were selected to estimate the combining ability and to understand the gene action for the improvement of Fe and Zn content with high yield. The features of promising genotypes selected across the clusters were provided in Table 2.

*Table 2. Details of promising genotypes selected against gene action study for micronutrient traits*

S.No	Parents	GYP (g)	Fe (mg/100g)	Zn (mg/100g)	IC	IS	IFC
Lines							
1	ACM 296	31.76	5.13	1.84	Green	Pyramidal	Compact
2	ACM 331	31.78	8.38	3.45	Purple	Cylindrical	Compact
3	IEC 370	25.30	9.73	3.13	Purple	Globose	Open
4	IEC 747	40.16	2.29	1.50	Purple	Cylindrical	Compact
Testers							
1	ACM 335	46.45	17.40	7.63	Green	Pyramidal	Intermediate
2	GECH 10	30.98	13.34	5.06	Green	Pyramidal	Compact
3	ACM 333	43.45	15.83	7.14	Green	Pyramidal	Compact
4	MA 10	46.68	16.65	9.73	Green	Pyramidal	Compact

GYP=Grain yield per plant, Fe=Iron, Zn=Zinc, IC=Inflorescence colour, IS=Inflorescence shape, IFC=Inflorescence compactness

The analysis of variance for quantitative traits revealed that the parents and their hybrids involved in the present study differed significantly at the probability level of 5% for all traits, except for flag leaf breadth in parents and thousand-grain weight in both parents and hybrids (Table 3). For Fe and Zn content, parents and hybrid showed significant, while line  $\times$  tester interaction showed non-significant values. The variance due to SCA was higher than that of GCA for almost all of the yield and yield components, whereas for Fe and Zn content the magnitude of GCA was higher than SCA. Similarly, the predictability ratio, a measure of the relative importance of GCA and SCA, was less than one for all the yield and yield components, whereas above one was observed for Fe and Zn content. Proportional contribution to the total variance by the lines, testers, and line  $\times$  tester interaction revealed that, except days to maturity, flag leaf length and ear head breadth, all the other remaining traits shared by the lines and testers, of which testers contributed more variability for Fe and Zn content.

Table 3. Analysis of variance for combining ability and related statistics for 14 quantitative traits in barnyard millet

Source of variation	Mean square														
	df	DM	PH	FL	FB	EL	EB	NR	SEW	NPT	TW	FYP	Fe	Zn	GYP
Replication	1	0.4	5.9	0.1	0.1	0.5	0.0	0.13	0.2	0.1	0.0	25.90	0.0013	0.1	2.03.6
Crosses	15	113.3*	1334.1*	63.2*	1.75	64.5 *	3.8*	329.7*	58.1*	9.3*	0.4	773.7*	8.68*	1.5*	216.3*
Lines	3	250.6*	3799.8*	99.3*	3.6*	116.5*	1.5	1182.0*	172.3*	29.2*	1.2	2006.2*	26.57*	0.9	612.1*
Testers	3	85.1*	910.1*	81.4*	2.8*	104.8*	0.6	104.2*	18.0*	7.5*	0.8	277.7*	16.0*	6.5*	79.1*
Line × Tester	9	76.9*	653.6*	45.1*	0.7	33.7*	5.7*	120.7*	33.3*	3.2*	0.1	528.0*	0.26	0.1	130.1*
Error	31	0.37	11.0	0.43	0.04	0.27	0.02	1.7	0.76	1.1	0.0	20.2	0.18	0.1	6.4
Genetic components															
GCA variance		1.89	35.45	0.94	0.05	1.60	-0.09	10.88	1.28	0.31	0.02	12.78	0.43	0.08	4.49
SCA variance		38.28	325.14	22.53	0.35	16.70	2.84	59.57	16.30	1.06	0.06	260.88	0.04	0.02	61.87
Predictability ratio		0.09	0.18	0.08	0.22	0.16	-0.07	0.27	0.14	0.37	0.40	0.09	0.96	0.89	0.13
% contribution of lines		44.23	56.96	31.42	41.68	36.14	7.69	71.70	59.33	62.9	50.40	51.87	61.25	12.36	56.59
% contribution of testers		15.03	13.64	25.67	32.97	32.50	2.90	6.32	6.21	16.2	32.12	7.18	36.93	83.77	7.32
% contribution of line × testers		40.75	29.39	42.91	25.35	31.37	89.42	21.98	34.46	20.8	17.48	40.96	1.82	3.87	36.10

Significant at \*P = 0.05. DM=Days to maturity, PH=Plant height, FL=Flag leaf length, FB=Flag leaf breadth, EL=Ear head length, EB=Ear head breadth, NR=Number of raceme per inflorescence, SEW=Single ear head weight, NPT=Number of productive tillers per plant, TW=Thousand grain weight, FYP=Fodder yield per plant, Fe=Iron, Zn=Zinc, GYP=Grain yield per plant. Predictability ratio: [2 GCA variance/(2 GCA variance+ SCA variance)]

#### General combining ability and specific combining ability effects

Estimates of *gca* effects of parents was not consistent for all the yield components and micronutrient traits studied. The female parent, ACM 296 was found to have negative *gca* effect for Fe and Zn content and yield components (Table 4). In contrast, ACM 331 was exhibited positive *gca* effect for Fe and Zn content and yield components while desirable negative *gca* effect for days to maturity. The female, IEc 370 showed negative *gca* effect for days to maturity and yield components while positive *gca* effect for Fe and Zn content. The genotype IEc 747, showed almost negative *gca* effect for both Fe and Zn content and yield components. Among the male parents, ACM 335, a high micronutrient content genotype exhibited a positive *gca* effect for Fe and Zn content and a negative *gca* effect for yield and yield components. The tester, GECH 10, exhibited a negative *gca* effect for Fe and Zn content and yield components. The genotype, ACM 333 recorded positive *gca* effect for Fe and Zn content and almost for all the yield and yield components. The parent MA 10, a micronutrient-rich genotype exhibited positive *gca* effect for Fe and Zn content while, negative *gca* effect for earliness and yield.

Considering the estimates of *sca* effect of 16 cross combinations, the crosses ACM 296 × GECH 10, ACM 331 × ACM 335, ACM 331 × MA 10 and IEc 747 × GECH 10 were showed positive *sca* effect for most of the grain yield components. The *per se* performance and specific combining ability effects of 16 cross combination for yield components and micronutrients were given in Table 5. Crosses with positive *sca* effect for Fe and Zn content were displayed by ACM 331 × ACM 335, ACM 331 × MA 10, IEc 370 × ACM 335 and IEc 370 × GECH 10. The best combiners based on *per se* performance and positive *sca* for both micronutrient and grain yield components were shown by the crosses ACM 331 × ACM 335 and ACM 331 × MA 10.



Table 4 (a). Performance per se of the lines and testers and their general combining ability (GCA) effects for 13 quantitative traits

Parents	DM		PH		FL		FB		EL		EB		NR	
	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA
Lines														
ACM 296	109.00	3.05**	166.93	6.95**	25.63	1.25**	3.15	0.25**	23.24	1.55**	3.35	-0.26**	41.50	0.81
ACM 331	104.00	5.86**	169.93	23.82**	26.01	3.75**	3.45	0.67**	23.81	3.34**	3.64	0.01	46.50	16.44**
IEc 370	92.00	-6.77**	115.54	-2.42**	24.98	-0.35*	2.75	0.01	26.71	0.57**	15.97	0.60**	39.00	-5.19**
IEc 747	93.50	-2.14**	113.06	-	28.35**	-4.64**	3.30	-0.93**	19.30	-5.46**	4.46	-0.35**	35.00	-
Testers														
ACM 335	92.50	3.17**	151.94	-7.17**	29.67	-3.93**	4.44	-0.15*	22.38	-2.25**	4.71	-0.36**	57.00	-1.06*
GECH 10	83.00	-1.64**	113.85	-	24.09	-0.28	3.33	-0.73**	19.04	-3.58**	4.71	0.23**	50.00	-3.19**
ACM 333	94.50	2.23**	148.78	11.85**	30.59	3.85**	4.35	0.71**	20.10	4.41**	4.70	0.16**	54.50	5.19**
MA 10	96.00	-3.77**	143.75	5.98**	30.60	0.36*	4.50	0.16*	23.24	1.41**	4.75	-0.02	58.50	-0.94

Significant at \*P = 0.05 and \*\*P = 0.01. DM=Days to maturity, PH=Plant height (cm), FL=Flag leaf length (cm), FB=Flag leaf breadth (cm), EL=Ear head length (cm), EB=Ear head breadth (cm), NR=Number of raceme per inflorescence.

Table 4 (b). Performance per se of the lines and testers and their general combining ability (GCA) effects for 13 quantitative traits

Parents	SEW		NPT		FYP		Fe		Zn		GYP	
	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA
Lines												
ACM 296	12.70	-1.11**	10.00	-1.28**	111.96	-3.42**	5.13	-0.34**	1.84	-0.45**	31.76	-1.90
ACM 331	13.35	6.83**	12.50	2.84**	123.18	22.84**	8.38	1.24**	3.45	0.31**	31.78	12.85**
IEc 370	9.60	-3.33**	3.50	-0.78	53.62	-5.79**	9.73	1.54**	3.13	0.23**	25.30	-4.82**
IEc 747	17.35	-2.38**	3.50	-0.78	56.22	-13.62**	2.29	-2.44**	1.50	-0.09	40.16	-6.13**
Testers												
ACM 335	19.73	-1.18**	8.50	-0.16	89.04	-2.11*	17.40	0.84**	7.63	0.12	46.45	-2.00*
GECH 10	12.25	-0.64	8.00	-1.28**	43.37	0.85	13.34	-2.10**	5.06	-1.28**	30.98	-1.18
ACM 333	18.31	2.20**	8.00	0.97*	88.33	7.63**	15.83	0.42**	7.14	0.37**	43.45	4.69**
MA 10	20.32	-0.38	7.00	0.47	88.36	-6.38**	16.65	0.84**	9.73	0.80**	46.68	-1.52

Significant at \*P = 0.05 and \*\*P = 0.01. SEW=Single ear head weight (g), NPT=Number of productive tillers per plant, TW=Thousand grain weight (g), FYP=Fodder yield per plant (g), Fe=Iron (mg/100g), Zn=Zinc (mg/100g), GYP=Grain yield per plant (g).

Table 5. *Per se* performance and specific combining ability effects of 16 cross combination for yield components and micronutrients

S.No	Cross	FB		NR		SEW		Fe		Zn		GYP	
		Mean	sca	Mean	sca	Mean	sca	Mean	sca	Mean	sca	Mean	sca
1	ACM 296 × ACM 335	4.1	0.15	46.5	-2.94**	17.2	1.49*	11.84	-0.12	5.2	-0.03	36.6	4.11*
2	ACM 296 × GECH 10	3.5	-0.02	49.1	12.69**	12.0	4.80**	8.0	-0.28**	3.6	0.03	32.8	7.53**
3	ACM 296 × ACM 333	4.1	0.59**	53.5	-1.19	18.3	-1.43*	10.22	0.24*	4.3	-0.21**	40.9	-2.76
4	ACM 296 × MA 10	4.6	-0.71**	55.0	-8.56**	19.6	-4.86**	10.6	-0.08	4.8	0.22**	42.1	-8.89**
5	ACM 331 × ACM 335	4.7	0.80**	53.5	6.44**	18.3	4.40**	14.1	0.31**	6.2	0.03	39.1	7.84**
6	ACM 331 × GECH 10	3.3	-0.64**	47.0	-12.94*	12.2	-5.92**	10.5	-0.41**	4.1	-0.53**	34.6	-12.40**
7	ACM 331 × ACM 333	4.5	-0.28*	58.0	-1.31	20.7	-1.63*	12.7	0.16	5.6	0.10	47.8	-2.75
8	ACM 331 × MA 10	4.7	0.12	63.0	7.81**	22.5	3.15**	13.9	0.56**	5.9	0.37**	50.5	7.31**
9	IEc 370 × ACM 335	2.8	-0.52**	38.5	-3.94**	13.1	-1.24	13.8	0.25*	5.9	0.10	25.2	-3.75
10	IEc 370 × GECH 10	2.5	0.16	41.5	0.19	10.7	-0.39	10.6	0.49**	4.8	0.54**	26.8	-0.17
11	IEc 370 × ACM 333	3.3	-0.32*	42.0	3.31**	12.1	1.27	11.7	-0.08	4.9	-0.24**	32.7	5.48**
12	IEc 370 × MA 10	3.6	0.68**	40.0	0.44	10.6	0.36	11.9	-0.66**	4.8	-0.40**	29.5	-1.56
13	IEc 747 × ACM 335	4.1	-0.43**	40.7	0.44	17.2	-4.65**	10.2	-0.06	4.1	-0.10	32.0	-8.20**
14	IEc 747 × GECH 10	3.5	0.50**	40.5	0.06	14.4	1.51*	7.0	0.20*	2.5	-0.04	34.1	5.05**
15	IEc 747 × ACM 333	3.9	0.01	41.5	-0.81	18.5*	1.78*	8.2	-0.33**	3.8	0.33**	29.0	0.02
16	IEc 747 × MA 10	3.7	-0.09	52.0	0.31	20.5	1.35*	9.44	0.19	3.4	-0.19**	39.9	3.13

Significant at \*P = 0.05 and \*\*P = 0.01. FB=Flag leaf breadth, NR=Number of raceme per inflorescence, SEW=Single ear head weight, Fe=Iron, Zn=Zinc, GYP=Grain yield per plant.

### Heterosis

The estimate of magnitude of mid-parent heterosis varied from -31.90% (ACM 296 × GECH 10) for Fe content to 28.27% (ACM 331 × MA 10) for single-ear head weight. The better parent heterosis varied from -59.46 (IEc.747 × GECH 10) for Fe content to 8.43% (ACM 296 × MA 10) for ear head length (data not shown). Mid-parent and better parent heterosis exhibited significant positive direction for yield and yield components in most of the cross combinations. However, for Fe and Zn content, though mid-parent heterosis was significant in positive or negative direction in most of the crosses, the negative direction was almost observed for better

parent heterosis in all the cross combinations. The percentage of mid-parent and better parent heterosis for micronutrients and grain yield were depicted in Figure 4.

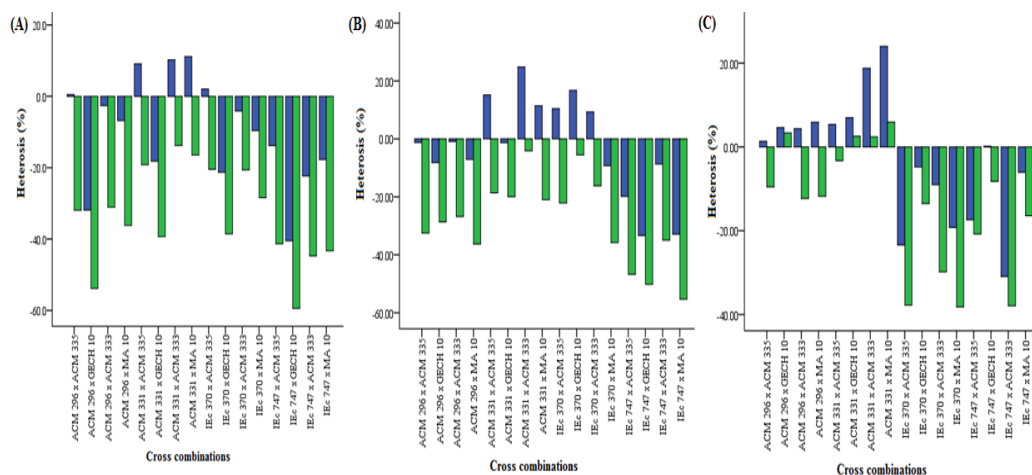


Figure 4. The pictorial representation of the manifestation of heterosis for Fe (A), Zn (B) and grain yield per plant (C) in respective cross combinations over mid-parent (blue) and better parent (green) values

## DISCUSSION

Forty germplasm of diverse origin were used in diversity studies for Fe and Zn content and yield components. A wide range of variation was observed for the traits days to maturity, fodder yield per plant, Zn content, Fe content and grain yield per plant which contributed above 90% of the total divergence. Previously, SOOD *et al.*, (2014), RENGANATHAN *et al.*, (2017) and MANIMEKALAI *et al.*, (2018) also observed wide range of variation for agro-morphological and micronutrient traits in barnyard millet. The cluster diagram further depicted the genetic diversity in an easy understandable manner where cluster III followed by cluster IV and VII showed maximum intra-cluster distance that showed high divergence than the other clusters (Figure 2). All the clusters showed considerable differences in the mean value for different quantitative traits under the present study (Table 1). However, none of the clusters contained the genotypes that are desirable for all the agronomical as well as micronutrient traits which could be directly selected and utilized (Table 1). For instance, the clusters, I and II hold the genotypes that were high in grain yield and yield components but, moderate/low for Fe and Zn content. In contrast, the genotypes from cluster III had high values for Fe and Zn content but moderate/low for grain yield and yield components. A similar pattern was also observed for the clusters of VII, VIII, and

IX. Therefore, the selection of parents in the appropriate clusters will give a satisfactory result when the trait of interest is present in higher mean value in different clusters or also within the same cluster when intra-cluster distance is high (MEDHABATI *et al.*, 2012; DEVI 2016). In our study, to improve the Fe and Zn content along with grain yield, we selected the contrast genotypes from the cluster II (ACM 331), cluster III (ACM 296, ACM 333, ACM 335, GECH 10, MA 10), cluster V (IEc 747) and cluster VIII (IEc 370) that varied for grain yield (30.98-46.68 g/plant) and micronutrient traits (Fe, 13.34-17.4 mg/100g; Zn, 5.06-9.73 mg/100g).

Association analysis, on the other hand revealed the high association (Figure 3) among single earhead weight, number of racemes per inflorescence and flag leaf breadth with grain yield. It indicates that plants produce more grain yield via increased number of spikelets per panicle as the area of flag leaf area is larger. The similar increase of grain yield and number of spikelets per panicle via flag leaf area was also reported previously in rice (YUE *et al.*, 2006) and wheat (HAQ *et al.*, 2010; IFTIKHAR *et al.*, 2012). In contrast, the grain yield had a non-significant but positive association with Fe and Zn content, indicating that there would be no consequence of grain yield losses while improving for micronutrients. This supports the findings of CHAKRABORTHI *et al.*, (2009), GUPTA *et al.*, (2009) and NAGESH *et al.*, (2012) in maize, pearl millet and rice respectively. The moderate association of flag leaf length and flag leaf breadth with Fe and Zn content indicates that flag leaf played a significant role not only in photosynthesis and grain yield but also in the translocation of Fe and Zn to the grain (SPEROTTO *et al.*, 2013; WU *et al.*, 2010).

To develop the genotype with high genetic potential for both micronutrient and yield, it requires information on the mode of inheritance, GCA and SCA components in selecting the good parents and crosses (CAMDZIJA *et al.*, 2018). In this study, combining ability analysis revealed sufficient variation for all the traits among lines (ACM 296, ACM 331, IEc 370, IEc 747) and testers (ACM 333, ACM 335, GECH 10, MA 10) except, flag leaf breadth and thousand-grain weight, indicating that the expression of genes differed in performance over parental material. Significant parents  $\times$  hybrids and lines  $\times$  testers mean sum squares indicated the presence of non-allelic interactions. In contrast, for Fe and Zn content, parents and hybrid showed significant, while line  $\times$  tester interaction showed non-significant variance due to higher GCA of parents. Although both GCA and SCA variances were significant for all traits studied, the GCA was predominant with a predictability ratio of  $\geq 0.96$  for Fe content and  $\geq 0.89$  for the Zn content and for yield and yield components it was  $< 0.50$ . This showed that the both Fe and Zn content were largely under additive genetic control (VELU *et al.*, 2011; GOVINDARAJ *et al.*, 2013), while yield and yield components were under the control of non-additive interaction (ADILAKSHMI and UPENDRA, 2014; RENGANATHAN *et al.*, 2015; NANDANIYA *et al.*, 2016). Therefore, the greater importance of additive gene action in their expression indicated a very good prospect for the exploitation of additive genetic variation for micronutrient traits in barnyard millet. However, incorporating Fe and Zn in both the parents are highly required to achieve hybrid Fe/Zn through mid-parents heterosis than the better parents heterosis as SCA is lesser in magnitude for these traits. A large additive gene action for micronutrient content was also observed in maize (LONG *et al.*, 2004; CHEN *et al.*, 2007) and pearl millet (VELU *et al.*, 2011; GOVINDARAJ *et al.*, 2013). In contrast, non-additive gene action for yield and its components in barnyard millet suggested their exploitation *via* heterosis breeding as previously reported in pearl

millet and rice (ADILAKSHMI and UPENDRA, 2014; RENGANATHAN *et al.*, 2015; NANDANIYA *et al.*, 2016).

From the general combining ability of parents (Table 4), it was perceived that in the most cases the best general combiners for grain yield and its components were often related to poor combiners for one or both micronutrients (Fe & Zn) and *vice versa*. It indicates that these parents did not transmit both yield components and micronutrient traits simultaneously in the desirable direction. An exception to this was ACM 331, a line parent and ACM 333 and MA 10, the tester parent's that were recorded as good general combiners for both Fe and Zn content along with yield components simultaneously. It is well-known that, high *per se* performance of parents for a particular trait will not always return high *gca* parent for that trait *vice versa* and even in other cross combination (GOVINDARAJ *et al.*, 2013). However this fact was true for yield and yield components but Fe and Zn content, the high *per se* and high *gca* effect of parents were always present. These results provided the opportunity for a breeder to effectively choose the parents with the best-combining ability for micronutrient content based on maximum *per se* performance itself. A similar association between *per se* performance of the parental lines and their *gca* were observed for Fe and Zn density in pearl millet (GOVINDARAJ *et al.*, 2013).

Considering the *sca* effect, most of the cross combinations expressed positive *sca* effects that involved one good general combiner as the parent for yield and yield components, which indicated the predominance of non-additive gene action (Table 5). In contrast, for Fe and Zn content, negative *sca* effects in most of the cross combinations were present, except, ACM 331 × ACM 333; ACM 331 × ACM 335 and ACM 331 × MA 10 even that their parental combination with positive *gca* effect (H × H) implied that their additive × additive gene effects and complementary gene action exists. This similar additive type of gene action controlling both the Fe and Zn content in the grain was earlier reported in maize and pearl millet (CHEN *et al.*, 2007; CHAKRABORTI *et al.*, 2009; GUPTA *et al.*, 2009; VELU *et al.*, 2011; GOVINDARAJ *et al.*, 2013; KANATTI *et al.*, 2014). Thus, desirable *sca* effects for both micronutrients and grain yield could be obtained by using these cross combinations, ACM 331 × ACM 335 and ACM 331 × MA 10. Further, it could also be worthwhile to attempt biparental matings in the segregating generation among these selected crosses to generate greater recombinations for selection of high yielding genotype with increased micronutrients

The exploitable traits of hybrids will depend on the mean performance of cross combination, *percent* heterosis over mid-parent and/or higher parent (SHASHIKUMAR *et al.*, 2011). Heterosis over mid-parent and better parent (Figure 4) for grain yield and yield components was significant in positive direction revealed that the performance of cross combination was more towards the best-combining parent for most of the yield components and in the negative direction for days to maturity (DEORAJ *et al.*, 2007; VENKATESAN *et al.*, 2008; RENGANATHAN *et al.*, 2015). For grain yield and its components, most of the cross combinations transgressed their parents (better parent) which implied their non-additive gene action or heterosis. Of them, the cross combinations ACM 296 × GECH 10, ACM 331 × ACM 333, ACM 331 × ACM 335 and ACM 331 × MA 10 were identified as the best heterotic crosses for high grain yield with two or more component traits. On the contrary, for Fe and Zn content, except, ACM 331 × ACM 335, ACM 331 × ACM 333 and ACM 331 × MA 10 crosses, all the other crosses showed significant mid-parent heterosis in the negative direction, suggesting that their

additive nature of gene action control the both Fe and Zn content in majority of the crosses. In addition, the crosses with high micronutrient content involved mostly both parental lines having high levels of these micronutrients, implying that breeding hybrids with high Fe and Zn content would entail genetic improvement of both parental lines for high Fe and Zn content. In earlier studies, VELU *et al.* (2011) and GOVINDARAJ *et al.* (2013) also reported similar results in pearl millet. Similarly, there was not a single cross combination that transgressed any one of the parental lines for both micronutrients, implying that there was no better parent heterosis. These results also support the fact that the physiological processes determining Fe and Zn content in grains were mainly under additive gene control as proven in maize by LONG *et al.* (2004) and pearl millet by CHEN *et al.* (2007), VELU *et al.* (2011) and GOVINDARAJ *et al.* (2013). Thus, to exploit both non-additive (grain yield) and additive (micronutrients) controlling traits, multiple or reciprocal recurrent selection or biparental schemes rather hybrid production could be adopted in the genetic improvement of both the traits simultaneously in barnyard millet. The best cross combinations for improvement of grain yield and micronutrients was given in Table 6.

Table 6. Top ranking crosses for specific combining ability effect, heterosis and per se performance of yield components and micronutrients traits

S. No	Character	Predominant gene action, GCA/SCA variance	Crosses	GCA of parents	Value of SCA effect	Per se performance	Heterosis (MPH)	Heterosis (BPH)
1	Days to maturity	Non additive	ACM 296 × GECH 10	High × Low	-10.30 **	86.0	-10.88 **	-21.10 **
			ACM 331 × ACM 335	High × High	-3.92 **	100.0	1.78 *	-3.80 **
			ACM 331 × ACM 333	Low × High	-4.98 **	98.0	-1.26	-5.77 **
			IEc 370 × ACM 335	Low × High	-4.80 **	86.0	-6.23 **	-6.49 **
			IEc 747 × ACM 333	low × High	-4.48 **	90.0	-1.32 *	-4.23 **
2	Iron (Fe)	Additive	ACM 331 × ACM 335	High × High	0.31**	14.1*	9.08**	-19.2**
			ACM 331 × ACM 333	High × Low	0.16	12.7*	10.21**	-13.76**
			ACM 331 × MA 10	High × High	0.56**	13.9*	11.15**	-16.46**
3	Zinc (Zn)	Additive	ACM 331 × ACM 335	High × High	0.03	6.2*	15.21**	-18.61**
			ACM 331 × ACM 333	High × Low	0.10	5.6*	24.83**	-4.09**
			ACM 331 × MA 10	High × High	0.37**	5.9*	11.41**	-21.02**
4	Grain yield per plant	Non additive	ACM 296 × GECH 10	Low × Low	7.53 **	39.8	26.96 **	25.40 **
			ACM 331 × ACM 335	High × Low	7.84 **	54.0	38.22**	16.38 **
			ACM 331 × MA 10	High × Low	7.31 **	54.0	37.68 **	15.70 **
			IEc 370 × ACM 333	Low × High	5.48 **	35.7	18.44 **	-6.29

Significant at \*P = 0.05 and \*\*P = 0.01. MPH=Mid-parent heterosis, BPH=Better parent heterosis

### CONCLUSIONS

The present study found that there was a considerable amount of diversity for grain yield (7.64-47.68 g/plant) and Fe (1.05-16.41 mg/100g) and Zn (0.45-6.85 mg/100g) content in the barnyard millet germplasm. Combining ability analysis revealed the additive type of gene action in governing the Fe and Zn content and non-additive type of gene action in governing yield and yield components. Therefore, the best cross combination such as, ACM 331 × ACM 335, ACM 331 × ACM 333 and ACM 331 × MA 10 could be selected for biparental mating approach to generate the maximum variability followed by selection of genotype with higher grain yield and increased micronutrients. Besides, strong association between Fe and Zn content suggested their possibility of simultaneous improvement of both as a part of a breeding of barnyard millet for improved micronutrient content.

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**EFEKAT GENA I HETEROZIS ZA SADRŽAJ Fe I Zn U ZRNU PROSA**  
**(*Echinochloa frumentacea* (Roxb.) Link)**

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Izvod

Japanski proso (*Echinochloa frumentacea*) je neistražena kultura bogata hranljivim sastojcima koja dobro uspeva u nepovoljnim sredinama i značajna je za mnoge male poljoprivrednike u južnoj i istočnoj Aziji. Iako ima bogate izvore mikronutrijenata, genetske studije su malobrojne, što dodatno ometa njegovo genetsko poboljšanje. Zbog toga smo pokušali da procenimo genetski diverzitet za sadržaj Fe i Zn kod 40 uzoraka prosa i da procijenimo kombinacionu sposobnost i heterozis u šesnaest F1 kombinacija ukrštanjem pomoću linija  $\times$  tester modela. Mahalanobis  $D^2$  analiza grupisala je 40 genotipova u devet različitih klastera. Klasteri III i I bili su najveće grupe koje su sadržale 22 i 6 genotipova, a ostalih sedam klastera sadržali su samo po jedan ili dva genotipa. Primećena je pozitivna korelacija između sadržaja Fe i Zn, mada su oba nutrijenta imala značajnu povezanost sa prinosom zrna. Ovo ukazuje da ne bi trebalo biti kompromisa za povećanje ili smanjenje prinosa zrna tokom oplemenjivanja za stvaranje sortata sa visokim sadržajem mikronutrijenata. Analiza kombinacione sposobnosti otkrila je da su linije, tester i komponente njihove interakciju značajni. Koeficijent predvidljivosti ukazivao je na prevladavanje aditivne varijanse za sadržaj Fe i Zn i neaditivne varijanse u nasleđivanju komponenata prinosa. Genotipovi, ACM 331, ACM 333, ACM 335 i MA 10 pokazali su pozitivne *gca* efekte na sadržaj Fe i Zn i prinos zrna. Dve kombinacije ukrštanja, ACM 331  $\times$  ACM 335 i ACM 331  $\times$  MA 10 uključivale su jednog ili oba roditelja sa dobrim *gca* efektima, pokazale su visoku srednju, pozitivni heterozis srednjeg roditelja i uticaj *sca* na sadržaje Fe, Zn i prinos. Dakle, ovo istraživanje je omogućilo značajno razumevanje delovanja gena i mogućnosti

korišćenja odabranih roditelja i kombinacija ukrštanja za iskorišćavanje mikrohranjivih sastojaka iz useva prosa.

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