Frontiers in Crop Improvement, Vol. 7 (2) : 106-111 (2019)
Print ISSN : 2393-8234, Online ISSN : 2454-6011

ASTHA FOUNDATION
Meerut (U.P.) INDIA

GENE EFFECTS AND HETEROSIS FOR GRAIN IRON AND ZINC DENSITY IN PEARL MILLET (*Pennisetum glaucum* (L.) R. BR.)

V.Y. Pawar[1*], N.S. Kute[2], V.R. Awari[2], N.M. Magar[1], H.T. Patil[1], G.P. Deshmukh[2] and S.P. Singh[3]
[1]*AICRP on Pearl Millet, College of Agriculture, Dhule-424001 Maharashtra*
[2]*Mahatma Phule Krushi Vidyapeeth, Rahuri-413722 Maharashtra*
[3]*CSAUAT–Agriculture Research Station, Kalai, Aligarh, U.P.*
E-mail : *vypawar2gene@gmail.com*

ABSTRACT

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is a major warm-season cereal, grown primarily for grain production in the arid and semi-arid tropical regions of Asia and Africa. Micronutrient malnutrition, especially the paucity of iron (Fe) and zinc (Zn) is posing a big threat to the world affecting nearly 25% of worldwide population. Improving Fe and Zn densities of staple crops by breeding offers a cost-effective and sustainable solution to reducing micronutrient malnutrition in resource poor communities. In the present study eleven inbred lines and their half diallel crosses were used to study the nature of gene action and heterosis for these micronutrients. The analysis of variance of diallel progenies exhibited significant genotypic differences. The general combining ability (GCA) effects of parents and specific combining ability (SCA) effects of hybrids showed significant differences for both of the micronutrients. However, the predictability ratio PR: 2 ^{2}gca/(2 ^{2}gca + ^{2}sca)(PR for Fe=1.29, Zn=1.36) was around unity both for Fe and Zn densities, implying preponderance of additive gene action. Considering the performance per se and GCA effects together, the high Fe/Zn parents S-12/30060 and S-12/30088 was identified as the best combiner for further breeding programs. Further, highly significant positive correlation between mid-parent values and hybrid per se performance (Fe: r = 0.80, Zn: r = 0.85, P = 0.01), and no correlation between mid-parent values and mid-parent heterosis (Fe: r = 0.12, Zn: r = 0.05, P = 0.01) confirmed again the predominant role of additive gene action for these micronutrients. High Fe and Zn levels in both of the parental lines would be required to increase the probability of breeding high Fe and Zn hybrids and there would be little opportunity, if any, to exploit heterosis for these mineral micronutrients in pearl millet.

Key words : *Combining ability, grain iron and zinc density, gene action, heterosis, pearl millet.*

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is one of the most important stable food crops in Asia and Africa. It is a highly cross-pollinated crop with more than 85% out crossing. This floral system provides for open-pollinated varieties (OPVs) and hybrids as the two broad cultivar options. It is the cheapest source of Fe and Zn micronutrients as compared to other cereals and vegetables. Cultivated pearl millet has higher levels of both micronutrients, especially Fe content, than other major cereals such as rice, wheat, maize and sorghum (1). Micronutrient malnutrition resulting from the dietary deficiency of minerals such as iron (Fe) and zinc (Zn) has recently been recognized as a serious human health problem, especially in the developing countries (2). A recent study has shown that pearl millet accounts for a major share of Fe and Zn intake in some of the region of pearl millet growing areas in India and it is the cheapest source of Fe and Zn as compared to other cereals (3). It is a major source of mineral micronutrients in several African countries as well. Results of a preliminary study have shown large variability for grain Fe and Zn among breeding lines and populations as well as within the populations (4). An understanding of the nature of gene action and heterosis would be a significant input into designing effective breeding strategies for the development of OPVs and hybrids. There is little information available on the nature of gene action and heterosis for grain Fe and Zn densities in pearl millet. The objective of the research reported in this paper was to fill this gap and examine its implications in breeding pearl millet cultivars with high levels of grain Fe and Zn densities.

MATERIALS AND METHODS

The experimental material for the present study comprised of 11 inbred lines obtained from Bajra Research Scheme, College of Agriculture, Dhule and International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Telangana. These inbreds were crossed in diallel fashion to obtain F_1's excluding reciprocals during Summer 2013. Sufficient quantity of seed for 55 cross combinations was obtained. These 55 crosses along with eleven inbreds were used to study stability during Kharif-2013, at Bajra Research Scheme, College of Agriculture, Dhule. The 66 genotypes were raised in a randomized block design with three replications and two rows (of 3.0m length) plots per replication. Standard agronomic practices were followed for raising and maintenance of plants.

Representative soil samples were collected from each plot at 15 cm depth before sowing and at harvest of crop. The collected soil samples were air dried in shade

Table-1 : The parents of diallel crosses in pearl millet.

Sr. No.	Code	Inbred/parent	Source/ Origin	Fe density (mg/kg)	Zn density (mg/kg)
1.	P_1	RHRBI 138	MPKV	60.21	41.38
2.	P_2	RHRBI 458	MPKV	69.57	47.66
3.	P_3	DHLBI 967	MPKV	37.14	28.81
4.	P_4	DHLBI 731	MPKV	48.17	31.25
5.	P_5	ICMB 98222	ICRISAT	91.24	57.46
6.	P_6	S-12/30069	ICRISAT	54.38	37.78
7.	P_7	S-12/30109	ICRISAT	41.51	35.00
8.	P_8	S-12/30071	ICRISAT	34.62	31.67
9.	P_9	S-12/30060	ICRISAT	115.24	62.41
10.	P_{10}	S-12/30074	ICRISAT	79.34	54.34
11.	P_{11}	S-12/30088	ICRISAT	97.26	56.60

Table-2 : Analysis of variance of parents, hybrids and combining ability for grain Fe and Zn densities during season *Kharif*-2013 at Dhule.

	df	Grain Fe (mg/kg)	Grain Zn (mg/kg)
Analysis of variance of parents and hybrids			
Treatments	65	1264.38**	298.67**
Parents	10	2139.81**	441.31**
Hybrids	54	1124.75**	277.73**
Parents Vs. Hybrids	1	49.89**	2.63*
Error	130	5.29	4.34
Analysis of variance for combining ability			
gca	10	2217.32**	532.72**
sca	55	94.94**	20.80**
Error	130	1.76	1.45
^{2}gca		170.43	40.87
^{2}sca		93.18	19.35
^{2}gca/ ^{2}sca		1.83	2.11
2 ^{2}gca/(^{2}gca+ ^{2}sca)		1.29	1.36

*, ** Significant at 0. 05 and 0.01 probability levels, respectively

on paper sheet, gently ground in wooden mortar and pestle, mixed and sieved through 2 mm plastic sieve. The processed soil samples were used for determination of Fe and Zn on Automic Absorption Spectrophotometer at Micronutrient laboratory, MPKV, Rahuri by DTPA (Diethylene triamine penta acetic acid) extraction method (5). The data on available soil Fe and Zn content (mg/ kg) diethylene triamine pentaacetic acid (DTPA) in the experimental fields were average 5.65 mg/kg for Fe and 0.74 mg/kg for Zn for the field used during the 2013-*Kharif* season. Representative grain samples from the middle of the ear head of the plants from net plot area were collected at harvest stage. The grains were manually cleaned and care was taken to avoid any contamination of the grains with dust particles and any other extraneous matter. The samples were dried in an oven at 70^{0}C, ground to fine powder using sample mill consisting of hard plastic and used for micronutrient analysis. A known quantity (0.2 g) of grain samples powder were digested following wet digestion of the dried grain sample material with triacid mixture HNO_3:$HClO_4$:H_2SO_4 in the ratio of 9:3:1 and this acid extract was used for determination of Fe and Zn on Automic Absorption Spectrophotometer (5). The estimates of general combining ability (GCA), specific combining ability (SCA) and reciprocals effects were obtained following Griffing's method-II model-I (fixed model) (6), which included one set of F_1s excluding reciprocals, leading to [p(p-1)/2] hybrids. Significance of GCA, SCA and reciprocal effects was determined by at test (6). Estimate of variances due to general combining ability (^{2}gca) and specific combining ability (^{2}sca) were derived to get estimates of predictability ratio (PR):

Table-3 : Average grain Fe density (mg/kg) of parents and hybrids, and general combing ability (GCA) and specific combining ability (SCA) of diallel during *Kharif*-2013 in pearl millet, Dhule.

Parents/ Hybrids	1	2	3	4	5	6	7	8	9	10	11
1.	60.2 (1.77**)	70.91**	48.32	39.98**	73.51	67.12**	54.35*	51.67**	91.56*	81.29**	67.84**
2.	0.77	69.6 (3.24**)	67.84**	64.53**	86.33**	50.73**	42.29**	60.41**	60.41**	82.64**	82.31
3.	-5.89**	12.16**	37.1 (-12.68**)	48.78**	54.67**	38.64**	42.38	31.64*	76.1	69.89**	64.04
4.	-16.82**	6.26**	6.44**	48.2 (-10.10**)	72.15	58.58**	39.85**	37.29*	58.74**	79.56**	54.39**
5.	-6.30**	5.06**	-10.68**	4.22**	91.2 (12.91**)	48.33**	71.49**	67.89**	100.21	96.84**	95.39
6.	12.28**	-5.58**	-1.75	15.61**	7.76**	54.4 (-12.05**)	36.48**	49.87**	55.28**	51.29**	59.71**
7.	1.76	-11.77**	4.25**	-0.87	7.76**	-2.29	41.5 (-14.30**)	39.2	65.94**	52.34**	68.17
8.	7.45**	6.50**	-6.35**	-3.28**	4.31**	11.25**	2.84*	34.6 (-14.45**)	51.64**	74.53**	60.18**
9.	1.36	-9.10**	6.44**	-13.51**	4.96**	-15.01**	-2.1	-16.25**	115.2 (17.22**)	102.61**	96.28**
10.	1.36	1.24	4.41**	11.50**	5.77**	-14.82**	-11.52**	10.82**	7.23**	79.3 (13.04**)	101.30**
11.	12.16**	-1.46	-3.80**	-16.03**	1.96	-8.76**	1.95	-5.89**	-1.46	7.74**	97.3 (15.40**)

Diagonal Fe density of parents, diagonal parentheses GCA effects of parents, above diagonal Fe densities of crosses, below diagonal. SCA effects of crosses.

*, ** Significant at 0. 05 and 0.01 probability levels, respectively.

The correlation coefficient between mean performance per se of parents and GCA effects Fe densities, r = 0.95; P=0.01

2 ^{2}gca/ (2 ^{2}gca+ ^{2}sca) (Baker 1978). Estimation of relative heterosis as per (7).

RESULTS AND DISCUSSION

Analysis of variance of diallel set in *Kharif*-2013, Dhule environment revealed significant mean sum of squares due to treatments and its sub division (parents, hybrids and parents vs. hybrids) for both micronutrients (Table 2). The analysis of variance further revealed that the parents vs. hybrids differed significantly for Fe and Zn indicating sufficient variability among parents and hybrids. The Fe density varied from 31.64 to 105.37 mg/kg and Zn density from 25.0 to 64.69 mg/kg (Tables-3 and 4). The correlation of mid-parental values and hybrid per se performance for Fe (r = 0.80; P = 0.01) and Zn (r = 0.85; P = 0.01), indicating high levels of consistency of the rankings of parents for environment.

Combining ability analysis : Combining ability analysis indicated highly significant mean squares due to gca and sca for both micronutrients revealing importance of both additive and non additive gene effects controlling these two micronutrients. Significant mean sum of square due to gca and sca were also reported by (4, 8) for Fe and Zn density.

However, the magnitude of GCA mean squares was much higher than SCA mean squares for both Fe and Zn densities, implying the predominance of additive gene action for these traits. (9) suggested that the ratio of combining ability variance components (2 ^{2}gca/(2 ^{2}gca+ ^{2}sca)) termed as predictability ratio provides a measure of the predictability of the performance of hybrids and its progenies. The closer this ratio to unity, the greater the predictability based on GCA alone. Based on combined analysis, predictability ratio was 1.29 for Fe and 1.36 for Zn, implying preponderance of additive gene action and indicating that hybrid performance can be predicted based on GCA alone. Assessing the contribution of individual lines to hybrid performance was accomplished by comparing the GCA effects among the parents (Tables-3 and 4). GCA effects were highly significant (P=0.01) for all the parents for both Fe and Zn densities. The high Fe and Zn parents were the best general combiners having positive significant GCA effects and the parents with medium and low grain Fe and Zn densities had significant negative GCA effects.

Out of eleven parents, six inbreds *viz*., RHRB 138, RHRB 458, ICMB 98222, S-12/30060, S-12/30074 and S-12/30088 registered significant and positive estimates of gca effect for both micronutrients and grouped as good general combiners. While remaining inbreds *viz*., DHLBI 967, DHLBI 731, S-12/30069, S-12/30109 and

Table-4 : Average grain Zn density (mg/kg) of parents and hybrids, and general combing ability (GCA) and specific combining ability (SCA) of diallel during *Kharif*-2013 in pearl millet, Dhule.

Parents/ Hybrids	1	2	3	4	5	6	7	8	9	10	11
1.	41.4 (0.99**)	49.07**	39.51**	31.27**	51.38	45.72**	39.61	41.37**	49.66	54.32**	47.22**
2.	1.53	47.7 (2.26**)	47.22**	42.31	53.53	40.41	33.62**	43.74**	43.74**	46.37**	54.53
3.	1.66	8.11**	28.8 (-7.43**)	34.88**	37**	27.11**	30.17	28.39	50.14**	42.36	40.45
4.	-7.28**	2.50*	4.76**	31.3 (-6.73**)	48.53**	39.28**	25**	32.61	41.29**	52.08**	34.22**
5.	-1.02	-0.13	-6.97**	3.85**	57.5 (7.12**)	44.37*	49.07	48.66**	62.31	63.24**	51.00**
6.	5.34**	-1.23	-4.84**	6.63**	4.37**	37.8 (-4.91**)	28.63**	36.02	43.11	42.09	45.38
7.	1.04	-6.22**	0.02	-5.85**	4.37**	-4.04**	35 (-6.71**)	30.5	45.25*	43.64	48.77*
8.	-3.51**	2.87*	-2.79*	0.73	2.93**	2.31*	-1.41	31.7 (-5.67**)	41**	51.82**	40.28**
9.	1.48	0.25	5.40**	-4.15**	3.02**	-4.16**	-0.22	-5.50**	62.4 (7.89**)	64.69**	57.05
10.	1.48	-7.73**	-2.05	6.97**	4.28**	-4.84**	-1.49	5.65**	4.96**	54.3 (7.55**)	60.37**
11.	8.11**	2.33*	-2.06	-8.99**	-6.06**	0.34	5.54**	-3.99**	-0.78	2.87*	56.6 (5.65**)

Diagonal Fe density of parents, diagonal parentheses GCA effects of parents, above diagonal Fe densities of crosses, below diagonal SCA effects of crosses.

*, ** Significant at 0. 05 and 0.01 probability levels, respectively.

The correlation coefficient between mean performance per se of parents and GCA effects Zn densities, r = 0.97; P\0.01.

S-12/30071 depicted negative significant gca effect and identified as poor combiners for Fe and Zn density. Similar parallelism in pearl millet has been observed by (4, 8).

The correlation coefficient between mean performance per se of parents and GCA effects was highly significant and positive for both Fe (r = 0.95; P = 0.01) and Zn densities (r = 0.97; P = 0.01), indicating that selection of lines with high Fe and/or Zn levels would be highly effective in selecting for high GCA. Considering the performance per se and GCA effects together, the high Fe/Zn parent 'S-12/30060' was identified as the best combiner for further breeding programs. The SCA effects in each parental combination are shown in Tables-3 and 4 for Fe and Zn, respectively. Forty two hybrids for Fe and forty six hybrids for Zn had significant SCA effects, indicating presence of non-additive effects. Significant positive SCA effects were observed in 23 hybrids for Fe and 20 hybrids for Zn, of which four hybrids for Fe and three hybrid for Zn had 'S-12/30060' in their parentage.

Estimates of heterosis : Highly significant positive correlation between the mid-parental values and hybrid

Fig.-1(a) : Relationship between mid-parent values and hybrid performance per se for Fe densities (mg/kg) in pearl millet.

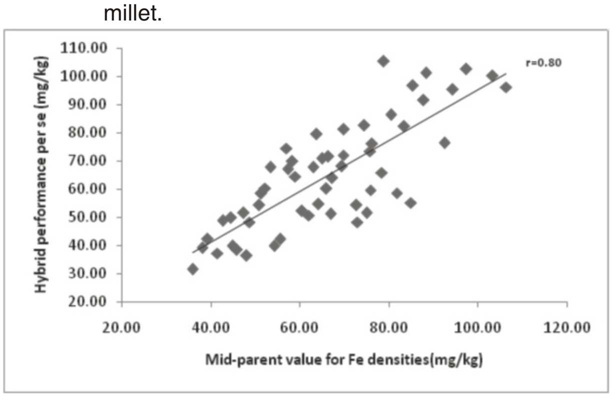


Fig.-1(b) : Relationship between mid-parent values and hybrid performance per se for Zn densities (mg/kg) in pearl millet.

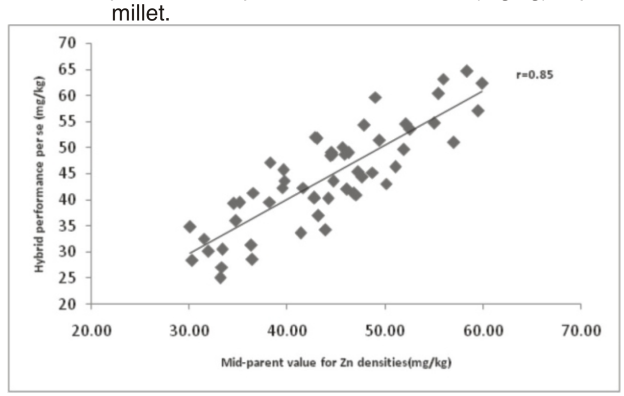


Fig.-2(a) : Relationship between mid-parent values and mid-parent heterosis for Fe density in pearl millet.

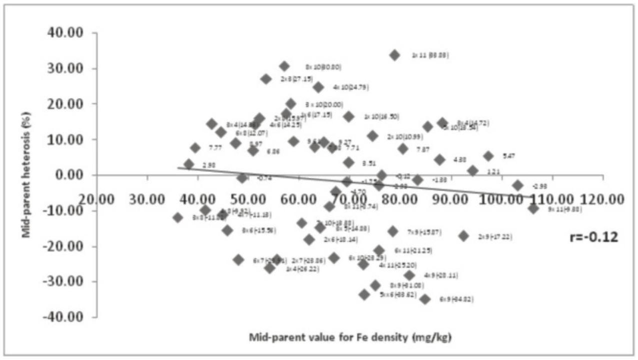
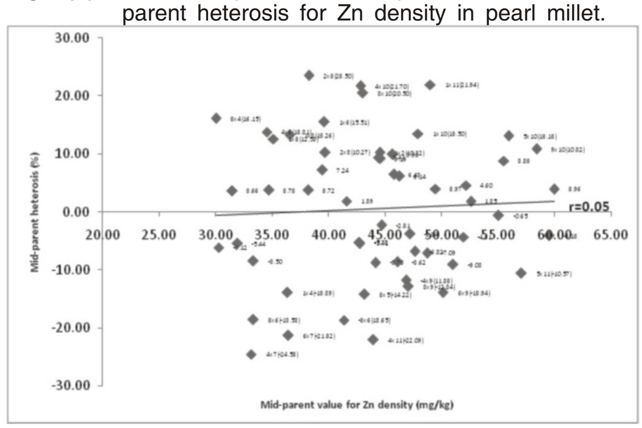


Fig.-2(b) : Relationship between mid-parent values and mid-parent heterosis for Zn density in pearl millet.



performance per se (r = 0.80; P\0.01 for Fe and r = 0.5; P = 0.01 for Zn) (Fig.-1a. and b), and no correlation between mid-parent values and mid-parent heterosis (r = 0.12 for Fe and r=0.05 for Zn) (Figs.-2a and b), provided additional indications of the predominant role of additive gene action for these traits. Some of the earlier studies have also reported the greater importance of additive gene action (GCA effects) for grain Fe and Zn densities in maize (11, 11, 12) and in pearl millet (4, 8).

The level of heterosis varied widely among hybrids both for Fe (-34.82 to 33.83 %) (Fig.-2a) and for Zn (-24.53 to 23.50%) (Fig.-2b). Overall, average heterosis of the hybrids was negative for both Fe (-2.03%) and positive Zn (0.70%). Among the hybrids, 44 hybrids showed significant mid-parent heterosis for Fe and 35 hybrids for Zn, of which 23 were in positive direction for Fe (4.33–33.83%) and 19 for Zn (6.48–23.50%). Highest positive significant mid-parent heterosis was observed in a cross 'RHRBI 138 x S-12/30088 (1 x 11)' for Fe, and 'RHRBI 458 x DHLBI 967 (2 x3)' for Zn. Hybrids with significant mid-parent heterosis are shown in Figs. 2 and 3 for Fe and Zn, respectively, and the significant heterotic hybrids were clearly distinct from others as they were away from regression line, suggesting parents of the hybrids with significant positive mid-parent heterosis could be used as potential parents in breeding. Of the five hybrids having high grain Fe, three hybrids were derived from Low x high and one hybrid from a medium x low and one from medium x low cross combination (Table 3). Similarly for Zn, of the four hybrids having high Zn, three hybrids had one high parents and one hybrid from a medium x low parents cross (Table-4). None of the hybrids outperformed significantly the parents that had high levels of Fe and Zn, indicating that there would be little opportunity, if any, to exploit heterosis for these micronutrients. In general, higher micronutrient levels in both parental lines would be required to breed hybrids with elevated levels of grain Fe and Zn densities.

To conclude, high predictability ratios (2 ^{2}gca/ (2 ^{2}gca+ ^{2}sca)) indicated that the expression of grain Fe and Zn densities in pearl millet is governed predominantly by additive gene effects, suggesting high effectiveness of progeny selection in pedigree selection or population breeding to develop lines and populations with increased levels of grain Fe and Zn densities. The higher additive genetic variance also prompts for recurrent selection method to improve the levels of grain Fe and Zn densities. The highly significant and positive correlation between GCA and performance per se of lines suggests that the performance per se of the genotypes could be a good indicator of its ability to transmit grain Fe and Zn densities to its hybrids and progenies; and genetically superior parents could be identified by evaluation of their Fe and Zn densities. Barring a few exceptions with one parent, none of the hybrids significantly outperformed the parents having high levels of Fe and Zn, indicating that there would be little opportunity, if any, toexploit heterosis for these micronutrients in pearl millet, and to breed high Fe and Zn hybrids would require incorporating these traits into both parental lines.

ACKNOWLEDGMENTS

This research was conducted as a part of a Ph.D. thesis of the senior author submitted to Mahata Phule krishi Vidyapeeth, Rahuri-413 722, Maharashtra, India. The authors gratefully acknowledge Dr. H.T. Patil, Associate Professor (Breeding), ICAR-All India Coordinated Research Project on Pearl Millet, Bajra Research Scheme, College of Agriculture, Dhule for growing the crop in experimental station and providing the grain samples. The authors express sincere thanks to Dr. K.N. Rai, Ex-Principal Scientist (Pearl Millet) and Director, Harvest Plus Progamme, ICRISAT, Patancheru, Telangana for providing the inbreds used in the study.

REFERENCES

1. Dwivedi S.L., Sahrawat K.L., Rai K.N., Blair M.W., Andersson M.S. and Pfeiffer W. (2012). Nutritionally

enhanced staple food crops. *Plant Breeding Reviews, 36:* 169–291.

2. WHO (2002). Reducing risks and promoting healthy life. The World Health Report. *World Health Organization*, Geneva, pp. 168. http://www.who.int/whr/2002/en/
3. Parthasarathy Rao P., Birthal P.S., Reddy B.V.S., Rai K.N. and Ramesh S. (2006). Diagnostics of sorghum and pearl millet grains based nutrition in India. *Int Sorghum Millets Newsl., 47:* 93–96.
4. Velu G., Rai K.N., Muralidharan V., Longvah T. and Crossa J. (2011). Gene effects and heterosis for grain iron and zinc densityin pearl millet (*Pennisetum glaucum* (L.) R. Br). *Euphytica, 180:* 251–259.
5. Lindsay W.L. and Norvell W.A. (1978). Development of DTPA soil test for zinc, iron, manganese and copper. *Soil Science Society of America Journa, 42:* 421-428.
6. Griffing B. (1956). Concept of general and specific combining ability in relation to diallel crossing system. *Australian J. Bio. Sci., 9:* 484-493.
7. Fonesca S. and Patterson F.L. (1968). Hybrid vigour in a seven parent diallel crosses in common winter wheat (*T. aestivum*). *Crop Sci., 8:* 85-88.
8. Rai K.N., Govindaraj M. and Rao A.S. (2012). Genetic enhancement of grain iron and zinc content in pearl millet. *Quality Assurance and Safety of Crops and Foods, 4:* 119-125.
9. Baker R.J. (1978). Issues in diallel analysis. *Crop Sci., 18:* 533–536.
10. Gorsline G.W., Thomas W.I. and Baker D.E. (1964). Inheritance of P, K, Mg, Cu, B, Zn, Mn, Al and Fe concentrations by corn (*Zea mays* L.) leaves and grain. *Crop Sci., 4:* 207-210.
11. Arnold J.M., Bauman L.F. (1976). Inheritance and interrelationships among maize kernel traits and elemental contents. *Crop Sci., 16:* 439–440.
12. Long J.K., Banziger M. and Smith M.E. (2004). Diallel analysis of grain iron and zinc density in southern African-adapted maize inbreds. *Crop Sci., 44:* 2019–2026.

Received : July-2019; Revised : July-2019; Accepted : July-2019