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Genetic variability, genotype × environment interaction and correlation analysis for grain iron and zinc contents in recombinant inbred line population of pearl millet [*Pennisetum glaucum* (L). R. Br.]

Mahesh D. Mahendrakar[1], Sushil Kumar[2], Ram Baran Singh, Abhishek Rathore, Gopi Potupureddi, P. B. Kavi Kishor[1], Rajeev Gupta, Rakesh K. Srivastava*

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, Hyderabad; [1]Osmania University 500 007, Hyderabad, Telangana State; [2]Centre of Excellence in Biotechnology, Anand Agricultural University, Anand 388 110, Gujarat

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Abstract

Micronutrient malnutrition is one of the major health problems, especially iron (Fe) and zinc (Zn) deficiencies that are widespread coupled with inadequate food supply in the developing world. Pearl millet grains are a good source of Fe and Zn elements making it a potential staple crop for overcoming hidden-hunger and micronutrient deficiencies. Breeding pearl millet with high levels of grain Zn and Fe contents represents a major opportunity to enhance the intake of these minerals for poor and malnourished people. A precise understanding of the genetic variability, correlation of mineral nutrients, genotype × environment (G × E) interaction is important for developing improved lines with high Fe and Zn content. To get fair estimates, we used a bi-parental recombinant inbred lines (RIL) mapping population representing F_2 phenotypic variance. A total of 317 RILs were evaluated for grain iron and zinc content in two seasons, Summer 2016 (E1) and Summer 2017 (E2). The result from the analysis of variance exhibited a large variability for grain Fe and Zn content across the two environments. The G × E for high grain Fe were significant at $P < 0.01$. The mean performance across the two environments data for grain Fe ranged from 22.9 to 154.5 mg kg^{-1} (ppm) and Zn content ranged from 19.3 to 121 mg kg^{-1}. The correlation coefficient for grain Fe and Zn was 0.9, and 0.8 and across the two (E1 and E2) environments. The value of correlation coefficient (0.9) was found to be highly significant at $P < 0.01$ level, that indicated good opportunities for simultaneous genetic improvement of both iron and zinc contents in pearl millet.

Key words: Pearl millet, micronutrients, iron and zinc, recombinant inbred lines (RILs), genotype x environment (GxE) interaction

Introduction

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] (2*n* =2*x* =14) is a prominent tropical C4 small-grained cereal crop belonging to family *Poaceae* and subfamily *Panicoideae*. It is a cross-pollinating crop having a relatively large (1.76 Gb) genome size (Varshney et al. 2017). It is highly resilient to diverse climate conditions and is cultivated in marginal environments of arid and semi-arid tropics of sub-Saharan Africa and Asia (Ramya et al. 2018). It shows a higher degree of tolerance to severe drought, heat stress and high temperature (Anuradha et al. 2017; Govindaraj et al. 2018). Pearl millet grains are the rich source of the several macro- and micro-nutrients (like iron, zinc, phosphorus, and magnesium), high fiber content, α-amylose, metabolizable energy, proteins, essential amino acids, thus ensuring food and nutritional security (Nambiar et al. 2011; Kumar et al. 2018).

Micronutrients deficiency in the human daily diet leads to malnutrition is a serious health problem in the human population globally (WHO, 2002). The deficiencies of the mineral nutrients iron (Fe), zinc (Zn) and vitamin A are more common among the weaker section of societies especially in African and Asian. More than two billion individuals or one in three people are affected by Fe deficiency alone and a number of Zn deficiencies are also reported (FAO 2013). India contributes about one-third to the total

*Corresponding author's e-mail: r.k.srivastava@cgiar.org
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global malnourished and underprivileged human population (Barthakur et al. 2010). Micronutrient deficiency synonymously termed as 'hidden hunger' (Allen 2003) leads to devastating health problems including, poor growth and compromised psychomotor development in children, reduced immunity, fatigue, wasting of muscles, and sterility in adults (Stein, 2010). Deficiency of iron and zinc results in retarded growth, impaired immune system, irritability, weakness, hair loss, morbidity and even death in severe conditions (Kumar et al. 2018).

Biofortification is one of the alternative approaches to overcome the problem of malnutrition and hidden hunger across the world (Kaur et al. 2019). Biofortification can contribute significantly towards reducing the burden of micronutrient deficiencies in the poor and underprivileged people in a highly cost-efficient manner (Meenakshi et al. 2010; Kumar et al. 2016). Hence there is a frequent and compelling requirement to develop cultivars with improved levels of micronutrients using biofortification even in relatively poor soils with target nutrients within the critical range (Kumar et al. 2016).

Breeding for high grain Fe and Zn content needs an adequate range of genetic variability in available germplasm and understanding of the genetic control of grain micronutrient density. A substantial positive association has been reported in previous investigations between the Fe and Zn traits point out common gene pool or genes and metabolic pathway engaged in the expression of the traits. So, the information on phenotypic correlation and their association with each other generate a preliminary idea for simultaneous improvement of the traits. In addition, knowledge of the environmental factors has considered vital in breeding for traits that influenced by several other factors (Rai et al. 2015; Phuke et al. 2017). Limited efforts have been made to identify G × E interaction for grain Fe and Zn content in pearl millet from an immortal bi-parental mapping population representing the phenotypic variance of the entire F_2 population. In the present study, we investigated genetic variability, heritability, and genotype × environment (G × E) interaction for grain iron (Fe) and zinc (Zn) content in recombinant inbred lines (RILs) population.

Materials and methods

Plant population development

A population of 317 RILs developed by single-seed descent (SSD) method was used in the current study (Kumar et al. 2018). The parents of the population were ICMS 8511-S1-17-2-1-1-B-P03 Fe and Zn content (low) (hereafter ICMS) and AIMP 92901-S1-183-2-2-B-08, (high) (hereafter AIMP) differing in grain Fe and Zn content. The present study was based on the advance open-pollinated (OP) RILs in F_{10} and F_{11} stage segregating for grain Fe and Zn content along with their parents. The Fe and Zn contents were recorded for two seasons of summer 2016 and summer 2017.

Field experiment

The field experiment was conducted in an alpha lattice design in Alfisol field at ICRISAT, Patancheru with two replications in two seasons during summer 2016 and summer 2017. The experiment trial was carried out with total 320 entries (317 RILs + 2 parental lines + 1 filler entry) in 16 blocks of 20 plots. Each line was sown in single row of 2 meters length with 60 cm inter-row and 15 cm intra-row spacing to produce bulk of open-pollinated (OP) grain for Fe and Zn analysis.

Mineral analysis

Grains obtained from open-pollinated panicles were analyzed for Fe and Zn content at the Charles Renard Analytical Laboratory, ICRISAT, following the method described by (Wheal et al. 2011). The grain samples were powdered in mill followed by oven-dried at 60°C for 48 hrs before analyzing them for Fe and Zn contents. Ground samples (0.2g) were transferred to 25 ml polypropylene PPT tubes; digestion was initiated by adding 2.0 ml of concentrated nitric acid (HNO_3) and 0.5 ml of 30% hydrogen peroxide (H_20_2). Tubes were vortexed to ensure that the entire sample was wetted and then pre-digested overnight at room temperature. The samples agitated again before placing them into the digestion block and initially heated at 80°C for 1 hr, followed by digesting at 120°C for 2 hrs. After digestion, the volume of the digest was made up to 25 ml using distilled water and the content was agitated for 1 min by vortex mixer. The digests were filtered and Fe and Zn contents were determined using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES).

Statistical analysis

Analysis of Variance (ANOVA)

The Analysis of variance with two seasons data was carried out and error variance using mixed model, where replication and block were considered to fixed effects, while genotype, replication and genotype

interaction with year were random effect and using residual maximum likelihood (ReML) algorithm with a Pearson correlation coefficient were carried out for studied traits; namely Fe and Zn content (mg kg^{-1}) using GenStat (17^{th} edition) statistical software (VSN International 2015.

Heritability and correlation

Broad-sense heritability was estimated using the following formula derived by Falconer (1989)

$$H^2 = \sigma^2 g / (\sigma^2 g + \sigma^2 e/nr)$$

and, pooled broad sense heritability was estimated by the following formula

$$H^2 = \sigma^2 g / \{(\sigma^2 g) + (\sigma^2 g\varepsilon /n\varepsilon + \sigma^2 \varepsilon / (n\varepsilon \times nr))\}$$

where, H^2 is broad sense heritability, $\sigma^2 g$ is genotypic variance; $\sigma^2 g\varepsilon$ is G × E interaction variance, $\sigma^2 \varepsilon$ is residual variance, $n\varepsilon$ is the number of environments, and nr is the number of replications. The correlation coefficient between both traits was calculated using GenStat (17^{th} edition) statistical software (VSN International 2015. The observed value of the correlation coefficient was tested at (n-2) degrees of freedom using 't' table by Fisher and Yates (1938) at 0.05 and 0.01 probability levels.

Genetic advance (GA)

Genetic advance was estimated by using the formula given by Johnson et al. (1955)

$$GA = H^2 k\sigma p$$

where, h^2 = Heritability in broad sense, k = Selection differential which is equal to 2.06 at 5% intensity of selection (Lush et al. 1949), σp = Phenotypic standard deviation

Genetic advance as percent of mean (GAM)

$$GAM = \frac{GA}{\text{Grand mean (X)}} \times 100$$

where, GA = Genetic advance, X = trait mean

Genetic advance as percent mean was categorized into the following levels such as low, moderate and high genetic advance described by Falconer (1989). It is as follows; 0-10% low; 10-20% moderate; and 20% and above high.

Results

Mean performance of the RIL population

The mean performance and the descriptive statistics for Fe and Zn content in open-pollinated grain from RILs with their parents grown in 2016 (E1) and 2017 (E2) was analyzed (Table 1). Fe and Zn content of OP grains ranged from 21.7 to 162.1 mg kg^{-1} and from 20.3 to 127.4 mg kg^{-1} in E1, respectively. While the range of the across the two environments data for grain Fe and Zn content ranged from 22.9 to 154.5 mg kg^{-1} and 19.3 to 121 mg kg^{-1}, respectively. The parental lines were statistically significant for both traits. The mean values for Fe and Zn traits were found to be significant ($P < 0.01$) with means of RILs and both the parents across the two environments. Whereas, high Fe trait was varied between RILs and AIMP 92901- derive-08 and showed significant at $P < 0.01$ level in E2. Both traits were significant at $P < 0.01$ level for both the parents in E1, E2 and across the two environments.

Analysis of variance (ANOVA)

The results from ANOVA showed the presence of large variability in the RIL population under the study for

Table 1. Descriptive statistics of phenotypic values observed in RILs derived from cross AIMP-08 × ICMS 8511, and their parental lines, in two different environments and across the two environments

Trait	Environment	ICMS 8511 (P1)	AIMP 92901 (P2)	RILs	RANGE	P1 vs RILS	P2 vs RILS
		Mean	Mean	Mean			
Fe	2016 (E1)	28±2.26	131.15±3.74	52.83±19.70	21.7-162.1	**	**
	2017 (E2)	34.52±2.70	90.26±13.94	58.13±21.46	24.17-146.9	**	**
	Across	31.26±3.26	110.70±20.44	55.48±2.65	22.93-154.5	**	*
Zn	2016 (E1)	25.95±2.47	101.15±2.89	48.46±15.70	20.3-127.4	**	**
	2017 (E2)	27.32±12.11	72.07±0.12	49.72±15.52	18.33-114.61	**	**
	Across	26.63±0.68	86.61±16.86	49.09±0.62	19.31-121.00	**	**

Note: All the values in parts per million (mg kg^{-1}); *Significant at 5% level of significance; **Significant at 1% level of significance

grain Fe and Zn content across the two environments (Table 2). The coefficient of variance (CV) was 23.5 and 20.1 for Fe and Zn traits respectively. The error variance was found 170.0, 97.5 in Fe and Zn, respectively. The genotypic variance for Fe was 223.5 and 127.6 for Zn and genotypic × environmental (G × E) interaction was 6.4 and 4.2 for grain high Fe and Zn, respectively.

Table 2. Analysis of variance of RILs derived from cross ICMS 8511 × AIMP-08 across the two environments

Source of variation	d.f	Fe content (mg kg^{-1})	Zn content (mg kg^{-1})
Replication (Year)	2	2.6	1.6
Genotype	319	223.5**	127.6**
G × E	319	6.4**	4.2**
Error	578	170.0**	97.5**
CV		23.5	20.1
Heritability%		83.0	82.8

Significant at 1% level of significance

Variance components

A wide range of variation of both traits was detected among the RILs population in E1, E2 and across the seasons (Table 3). The genetic variation (σ^2g) in E1 for both (Fe and Zn) the traits were slightly higher than in E2 environment. Considering the analysis across the two screening environments, the results showed that variances due to genotypes were significant (P < 0.01) for all observed traits. Likewise, variance due to G × E interactions was also significant (P < 0.01) for all the observed traits across two environments.

Heritability, correlation analysis, and genetic advance

The two observed traits were found to be highly heritable across the environments (Table 3). While, as per the scale (>0.60) devised by Robinson et al. (1949) Fe and Zn content in E2 (summer 2017), were found to be less heritable than the E1 (summer 2016) and across the two environments (Table 3).

A significant positive correlation was observed between Fe and Zn content (Table 4). Correlation coefficient was 0.9, 0.86 and 0.9 in E1, E2, and across the environment, respectively, the levels were highly significant at P < 0.01 level. The genetic advance in percentage mean (GAM) of across the environments was recorded lesser (than the individual season (Table 4) and were 21.2% for Fe and 43.1% for Zn. The GAM in E1 and E2 for Fe was 57. 7% and 44.5%, respectively, whereas GAM for Zn in E1 and E2 was 44.5% and 35.6%, respectively.

Table 3. Genotypic variance, G×E interaction, standard error, heritability in broad sense, and Genetic parameters for Fe and Zn traits in ICMS 8511 × AIMP-08 RIL population

Trait	S 2016 (E1)						S 2017 (E2)						Across					
	σ^2g	SE	σ^2p	GA	GAM	H^2	σ^2g	SE	σ^2p	GA	GAM	H^2	σ^2g	SE	σ^2g×E	SE	GA	GAM	H^2
Fe	268.4	26.9	328.1	30.5	57.7	0.8	233.6	29.5	345.2	25.9	44.5	0.67	223.5	23.6	6.4	9	17.7	21.2	83
Zn	177.85	17.2	211.8	25.9	44.5	0.8	114.7	15.4	178.1	17.7	35.6	0.64	127.6	13.55	4.2	5.33	21.2	43.1	82.8

σ^2g: genotypic variance; SE: standard error; H^2: heritability (broad sense); σ^2p: phenotypic variance; σ2g × E: genotypic and environmental interaction, GA: genetic advance; GAM: genetic advance as percent of mean

Discussion

Most of the studies have been conducted for the evaluation and breeding for micro-nutrient (grain Fe and Zn) levels using self-pollinated lines of millets (Govindaraj et al. 2011; Kumar et al. 2018). Here, we have made use of an F_{10} bi-parental RIL mapping population consisting of 317 lines representing a near-total genetic variability of the F_2 population using open-pollinated lines for grain Fe and Zn content analysis. A little information is available on genetic variability for mineral nutrients-traits like Fe and Zn in open-pollinated (OP) grains. The differences in mineral contents of self and open-pollinated grain samples maybe because of the differences in numbers of seed set per panicle, due to dilution effect (Garvin et al. 2006). In

Table 4. Phenotypic correlation coefficient among 2 traits observed in RILs in two environments and pooled over year, Pearson Correlation Matrix

Environments	Traits	Fe content	Zn content
S2016 (E1)	Fe	1	
S2017 (E2)	Fe	1	
Across	Fe	1	
S2016 (E1)	Zn	0.900**	1
S2017 (E2)	Zn	0.865**	1
Across	Zn	0.908**	1

**Significant at 1% level of significance

an earlier study, it was reported that differences among the selfed and open-pollinated seeds for Fe, Zn content were small in magnitude and not always significant, and also there was no xenia effect in pearl millet (Rai et al. 2015). Moreover, the production of OP grains is cheaper than the selfed grains and they can be used for consistent estimation of Fe and Zn content when using a large population and breeding lines, thus improving breeding efficiency for these micronutrients in pearl millet.

Present research investigation revealed that parent lines had significant differences for both the micronutrients Fe and Zn in all environments (Table 1). Mean Fe and Zn were higher (Fe: 52.8 and Zn: 58.5) in summer 2016 followed (Fe: 58.1 and Zn: 49.7) by summer 2017 and across (Fe: 55.5 and Zn: 49.0) the environments. A large variation has been reported for grain Fe and Zn content in pearl millet (Velu et al. 2008). The parents of the used RIL population revealed statistically varying the degree of phenotypes for Fe and Zn traits in this study (Kumar et al. 2018). A broad range of variation for both the traits was assessed between the RILs population (Table 1), which was in accordance with previous studies performed in pearl millet (Kumar et al. 2018). The coefficient of variation (CV) was 23.5 and 20.1 for Fe and Zn traits, respectively (Table 2). The genotypic variance for Fe was 223.5 and 127.6 for Zn. The present findings were found to significant ($P < 0.01$), which showed deviation from the earlier reported values (Anuradha et al. 2018). The genetic variation (σ^2g) for both the traits were significant within RIL population and the trait heritability (H^2) estimated were high as 0.83 (Zn) and 0.83 (Fe) (Table 3). The similar findings were reported in other research experiments in sorghum (Phuke et al. 2017) and pearl millet (Kumar et al. 2016). However, somewhat variable results have also been published for Fe and Zn heritability in pearl millet inbred lines (Govindaraj et al. 2011), it might be possible due to the genotype × environments (G × E) interaction and diversified parents genetic background (Velu et al. 2008).

The estimated trait heritability was found to be high for both the traits studied whereas, genetic advance in percentage mean (GAM) was high. High heritability and high GAM indicates the preponderance of additive gene effect which responds to selection of the line (Table 3). Analogous results of high heritability and GAM was reported in previous studies in pearl millet (Govindaraj et al. 2011). High heritability with moderate GAM was observed across the two environments, which indicates the presence of additive and non-additive gene effect. The correlation between Fe and Zn content was found to be reasonably significant (Table 4). The study of the correlation between grain Fe and Zn content have been reported in several crops which showed similar trends (Pfeiffer and McClafferty, 2007). Phenotypic correlation between grain Fe and Zn content was found to be very strong and significantly positive. This may point to common molecular mechanisms controlling the uptake and metabolism of minerals in grains or common transporters regulating the movement of these minerals in plants (Vreugdenhil et al. 2004; Ghandilyan et al. 2006). Co-segregation of genes for traits under study might be the reason for a strong correlation between the micronutrients in millets. The direction and intensity of correlation suggest good opportunities for simultaneous genetic improvement of micronutrients traits by co-transferring superior alleles into the genetic backgrounds of elite lines (Velu et al. 2008). The crop improvement depends upon the degree of genetic variability exist within the germplasm (Anuradha et al. 2018). The genetic variability, heritability, and genetic advance together help in predicting the phenotypic expression of traits in succeeding generations (Johnson et al. 1955).

The present study on pearl millet RIL population revealed a wide range of variability for both grain micronutrients (Fe and Zn) and the ranges for these traits fall outside the parent values indicating the presence of transgressive segregant lines. Also occurrence of genotype × environment (G × E) interaction for two mineral nutrients pointing out the effect of environment on the expression of these quality traits. Compared to Fe, environments exhibited more influence on grain Zn content. Moreover, high grain

Fe and Zn traits evaluated showed high values of heritability indicating eminence of the RIL population for molecular breeding practices to improve micronutrient levels in pearl millet.

Authors' contribution

Conceptualization of research (RKS); Designingof the experiments (RKS); Contribution ofexperimental materials (RKS, SK, MDM, RG); Execution of field/ lab experiments and data collection (MDM, GP); Analysis of data and interpretation (AR, RKS, MDM); Preparation of manuscript (MDM, RKS, RBS, PBK, RG).

Declaration

The authors declare no conflict of interest.

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