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# Genotype × environment interactions for grain micronutrient contents in sorghum [*Sorghum bicolor* (L.) Moench]

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**Abstract**

**Assessment of the stability of grain iron (Fe) and zinc (Zn) content across growing regions is a pre-requisite in breeding for micronutrient enrichment in grains. Grain Fe and Zn contents were estimated in a set of 10 sorghum genotypes grown at six locations. The Fe and Zn in grain ranged from 28.9-34.9 mg/kg and 20.4-25.7 mg/kg, respectively, across the locations. Significant genotype × environment (G × E) interactions were observed for both grain Fe and Zn, indicating differential nutrient accumulation by the genotypes. Additive main effects and multiplicative interaction (AMMI) analysis indicated that the first two principal components were significant and contributed more than 75% of G × E sum of squares. Though environment (linear) variance was significant indicating linear sensitivity, significant pooled deviation for both the traits suggested that expression in some of the genotypes fluctuated significantly from their respective linear path of response to environments. The study shows the necessity of multi-location as well as multi-season evaluation of genotypes for identifying stable donors that can be used in breeding programmes for micronutrient enrichment.**

**Key words**: Grain iron, grain zinc, micronutrients, sorghum

## Introduction

Micronutrient deficiency, also known as hidden hunger, affects nearly two billion people world-wide [1], and is particularly concentrated in the semi-arid tropics [2]. Among the micronutrient deficiencies, iron (Fe) deficiency is the most common in the world and the main cause of anemia, and approximately 50% of all anemia can be attributed to Fe deficiency. Zinc (Zn) deficiency is the second common micronutrient deficiency, which leads to anorexia, depression and psychosis, impaired growth and development, altered reproductive biology, gastro-intestinal problems and impaired immunity [3]. In India, micronutrient malnutrition has been a persistent problem. The intake of micronutrients in daily diet is less than 50% RDA in over 70% of the Indian population [4]. The magnitude of micronutrient deficit is particularly alarming among children, women of reproductive age, and pregnant and lactating women [5]. Anemia due to iron deficiency is very common among Indians and its prevalence varies from 75% among children and women to 45% among adult males [6]. The prevalence of Zn deficiency has not been adequately investigated, partly due to the lack of suitable biomarkers. Using disability adjusted life years (DALYs) technique; it has been found that Zn deficiency in India is a highly relevant health problem and responsible for the loss of 2.8 million DALYs annually [7].

Sorghum is the main source of dietary energy in central parts of the country contributing nearly 50% of the total cereal intake (75 kg grain per head per year), especially by rural consumers in the inlands of Maharashtra and Karnataka. In terms of nutrient intake, sorghum accounts for about 35% of the total intake of calories, protein, Fe and Zn in the dominant production/ consumption regions [8]. Considering the high prevalence of micronutrient deficiency in the rural population, particularly in the dominant sorghum consumption regions of the country, biofortification – development of cultivars with increased nutritional value – with grains enriched for Fe and Zn offers the most vital intervention. Breeding for grain micronutrient enrichment requires sufficient genetic variability for grain

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Fe and Zn in the available germplasm as well as the information on genetic control of grain micronutrient content in seed. Apart from this, the knowledge on stability of the trait across growing conditions or seasons is another important pre-requisite, though not given adequate attention in most of the breeding programmes beforehand. Assessment of the stability of expression of grain micronutrients is important for effective utilization of identified genotypes in breeding programmes. Hence, an attempt was made to study the stability of grain Fe and Zn contents in selected sorghum genotypes across six growing conditions. To our knowledge, the present research effort is the first of its kind in India in case of sorghum.

**Materials and methods**

A set of 10 sorghum genotypes comprising six released cultivars, three germplasm accessions and a parental line was grown at six locations during the rainy season of 2010. The genotypes were raised in a randomized block design with two replications and single row plots per replication. Standard agronomic practices were followed for raising and maintenance of plants. The crop was harvested at maturity and replicated grain samples (50 g each) were collected from each location after proper drying. The concentrations of grain Fe and Zn in the samples were analyzed at Agharkar Research Institute, Pune following standard protocols. It consisted of sample digestion and readings taken on Atomic Absorption Spectrophotometer (AAS-Perkin Elmer 5000AA, USA). Dried grain sample was accurately weighed and digested on a heating mantle using the three acid mixture (nitric acid, perchloric acid and sulphuric acid in 3:2:1 proportion). Standard solutions of Fe and Zn were prepared as stock of 1000 ppm and were suitably diluted to get a series of working solutions in the range of 0.1-10 ppm using metal free water (Millipore Q). Certified reference rice flour samples from Center for Environmental Measurement and Analysis, National Institute for Environmental Studies (NIES), Tsukuba-City, Ibaraki, Japan were used as standards to check the precision and accuracy of the method. The measured concentrations of Fe and Zn were considered appropriate when they agreed well with the certified values.

Individual datasets from the six locations were subjected to analysis of variance (ANOVA) and comparison of means, and pooled analysis was taken up to detect the genotype × environment interaction. Additive Main effects and Multiplicative Interaction (AMMI) and stability analyses based on the Eberhart and Russell [9] model were undertaken using WINDOSTAT Ver. 7.5 statistical package.

**Results and discussion**

Among the locations, the overall mean grain Fe content was the highest in samples from Coimbatore (38 mg/kg) and the least was from Solapur (26.3 mg/kg). The coefficient of variation (C.V.) for grain Fe was high in samples from Hyderabad (17.5%) followed by Solapur and Deesa, while it was around 6% in Akola and Palem (Table 1a). The environmental index (deviation of site mean from general mean) was positive for Akola, Coimbatore and Deesa, while for rest of the locations it was negative indicating the unfavourable environment for accumulation of grain Fe. In case of grain Zn, the highest mean value was 27.1 mg/kg (Coimbatore) and least was 20.6 mg/kg (Solapur). The variation within the locations was higher for grain Zn compared to Fe content and the C.V. was high (10-19%). Environmental index was positive for Coimbatore and Deesa, indicating better overall grain Zn status or favourable environment in these two locations (Table 1b).

Analyses of variance revealed significant variation for grain Fe at all the locations, while Zn concentration was significant at three locations (Hyderabad, Palem and Solapur) at 5% level of significance (Table 2). Presence of significant variation for grain Fe and Zn in sorghum has been reported [2, 10-12], and different ranges of values have been presented for both grain Fe and Zn in these studies. The status of soil Fe and Zn in the test locations is given in Table 2. Among the six locations, Akola had high soil Fe while Deesa had high soil Zn content.

Significant correlation between grain Fe and Zn content was observed in individual datasets from Coimbatore, Hyderabad, Palem and Solapur ($r = 0.784$, 0.715, 0.705, 0.866, respectively) as well as in pooled analysis ($r = 0.688$). However, the correlation between grain Fe and Zn content was non-significant at Akola and Deesa. High and positive correlation between grain Fe and Zn content has also been reported earlier [2, 10, 12-13], which suggests ample scope for simultaneous improvement of both the micronutrients.

The mean grain Fe content in genotypes pooled over locations ranged from 28.9 to 34.9 mg/kg while the grain Zn had a range of 20.4 to 25.7 mg/kg (Fig. 1, Tables 1a & 1b). Among the 10 genotypes, GGUB 39 and EP 95 recorded high grain Fe contents (34.9 and 34.8 mg/kg, respectively) over the locations (Fig. 1). However, when individual locations were considered,

**Table 1a:** Mean grain Fe concentrations (mg/kg) of the entries in different locations and mean estimates of stability parameters

| Genotype | Akola | Coimbatore | Deesa | Hyderabad | Palem | Solapur | Mean±SEm | bi | $S^2di$ |
|---|---|---|---|---|---|---|---|---|---|
| EP 95 | 34.97 | 33.33 | 43.40 | 39.05 | 34.55 | 23.32 | 34.77±1.96 | 0.581 | 41.9955 *** |
| EP 5 | 33.40 | 31.70 | 38.10 | 41.75 | 26.90 | 22.40 | 32.38±2.09 | 0.425 | 52.7495 *** |
| CSV 20 | 27.92 | 31.10 | 41.10 | 22.17 | 25.47 | 25.60 | 28.90±2.13 | 0.827 | 31.5724 *** |
| CSV 23 | 30.70 | 45.96 | 27.90 | 23.58 | 29.00 | 46.47 | 33.94±2.73 | 0.180 | 114.0368 *** |
| CSV 216R | 47.60 | 33.90 | 27.10 | 23.50 | 30.10 | 23.50 | 30.95±2.58 | 1.358 | 47.1002 *** |
| CSV 17 | 35.57 | 40.35 | 29.10 | 29.58 | 30.77 | 20.23 | 30.93±2.13 | 1.251 | 9.2401* |
| CSV 18 | 39.95 | 37.75 | 41.17 | 28.00 | 34.10 | 24.08 | 34.18±1.98 | 1.314 | 6.0811 |
| CSV 15 | 34.35 | 37.35 | 37.00 | 21.77 | 25.47 | 25.75 | 30.28±1.87 | 1.304 | 4.8687 |
| C 43 | 37.80 | 46.20 | 25.30 | 26.00 | 26.07 | 24.90 | 31.05±2.54 | 1.523 | 29.9221 *** |
| GGUB 39 | 39.42 | 42.30 | 35.45 | 26.75 | 38.75 | 26.50 | 34.86±1.90 | 1.237 | 9.4528* |
| Mean | 36.17 | 37.99 | 34.56 | 28.22 | 30.12 | 26.27 | 32.22 | 1.000 | |
| SEm | 1.53 | 2.42 | 2.57 | 3.49 | 1.33 | 2.29 | 2.82 | 0.602 | |
| CD (0.05) | 4.88 | 7.73 | 8.23 | 11.16 | 4.25 | 7.33 | | | |
| C.V. (%) | 5.96 | 9.00 | 10.52 | 17.49 | 6.24 | 12.34 | | | |
| Env. index | 3.95 | 5.77 | 2.34 | -4.01 | -2.10 | -5.95 | | | |

bi regression coefficient; $S^2di$ deviation from regression; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

**Table 1b:** Mean grain Zn concentrations (mg/kg) of the entries in different locations and mean estimates of stability parameters

| Genotype | Akola | Coimbatore | Deesa | Hyderabad | Palem | Solapur | Mean±SEm | bi | $S^2di$ |
|---|---|---|---|---|---|---|---|---|---|
| EP 95 | 24.38 | 25.55 | 25.92 | 26.97 | 23.95 | 23.35 | 25.02±0.79 | 0.312 | -3.8637 |
| EP 5 | 22.32 | 23.05 | 25.47 | 29.77 | 22.53 | 22.83 | 24.33±1.08 | 0.200 | 4.8509 |
| CSV 20 | 22.02 | 23.18 | 30.95 | 15.57 | 20.80 | 18.33 | 21.81±1.51 | 1.124 | 18.0567** |
| CSV 23 | 15.92 | 31.44 | 23.82 | 21.95 | 22.25 | 38.85 | 25.71±2.42 | 0.348 | 76.3307*** |
| CSV 216R | 24.25 | 29.25 | 24.97 | 25.55 | 22.15 | 18.02 | 24.03±1.20 | 1.140 | 0.8938 |
| CSV 17 | 18.45 | 25.65 | 20.95 | 22.62 | 18.95 | 16.33 | 20.49±1.06 | 1.094 | -1.9202 |
| CSV 18 | 21.20 | 23.30 | 24.92 | 23.10 | 26.55 | 15.85 | 22.49±1.20 | 0.678 | 7.9685 |
| CSV 15 | 16.92 | 27.20 | 25.20 | 21.23 | 20.23 | 20.80 | 21.93±1.30 | 1.303 | -3.0449 |
| C 43 | 21.52 | 32.05 | 22.32 | 16.67 | 15.88 | 13.85 | 20.38±1.91 | 2.056 | 12.4007* |
| GGUB 39 | 21.55 | 29.85 | 31.05 | 25.00 | 25.05 | 17.52 | 25.00±1.64 | 1.744 | 0.2116 |
| Mean | 20.85 | 27.05 | 25.56 | 22.85 | 21.83 | 20.57 | 23.12 | 1.000 | |
| SEm | 1.66 | 2.22 | 1.82 | 1.97 | 1.63 | 2.82 | 1.82 | 0.689 | |
| CD (0.05) | 5.32 | 7.09 | 5.82 | 6.31 | 5.23 | 9.03 | | | |
| C.V. (%) | 11.28 | 11.58 | 10.07 | 12.21 | 10.58 | 19.41 | | | |
| Env. index | -2.26 | 3.93 | 2.44 | -0.27 | -1.29 | -2.55 | | | |

bi regression coefficient; $S^2di$ deviation from regression; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

the highest values were recorded in different genotypes. Similar is the case with respect to grain Zn, where GGUB 39 and EP 95 along with CSV 23 exhibited about 25 mg/kg grain Zn on pooled basis (Fig. 1). The differential response of the genotypes over the locations necessitated the genotype × environment (G × E) interaction study.

The pooled ANOVA revealed significant variation due to locations and genotype × environment (G × E) interaction for both grain Fe and Zn concentrations (Table 3). Ashok Kumar *et al*. [11, 13] also reported significant cultivar × year interactions for grain Fe and Zn contents in sorghum. The sum of squares for G × E

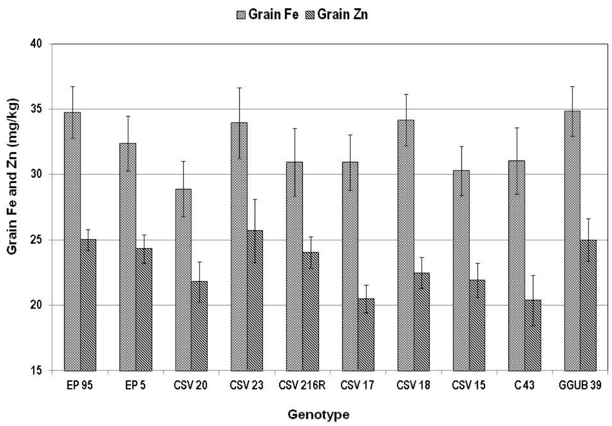


**Fig. 1. Mean grain Fe and Zn content in sorghum genotypes over locations**

for grain Fe was as high as 52% of the total sum of squares, while for the grain Zn, it was slightly low at 47%. The high proportion of G × E variance is perhaps indicative of the sensitivity of the grain micronutrient contents to soil or environmental conditions. However, reports suggest that soil type or micronutrient application with Fe and Zn fertilizers have only a limited influence on sorghum grain Fe and Zn concentrations when the soils are not deficient in these minerals [14-15]. We did not observe significant correlation between the soil micronutrient status (Table 2) and mean grain micronutrient content at the locations.

As the G × E interaction was significant for both the grain Fe and Zn concentrations, AMMI analysis [16] was undertaken for estimation of variance components. In case of grain Fe, the first two principal component axes (PCA I and PCA II) were significant at P = 0.01 and 0.05 level, respectively (Table 4), and cumulatively contributed to more than 75% (50% and 26.6%) of

**Table 2.** ANOVA for grain Fe and Zn content and soil micronutrient status in the test locations

| Location | Mean sum of squares | | Soil Fe (mg/kg) | Soil Zn (mg/kg) |
|---|---|---|---|---|
| | Grain Fe | Grain Zn | | |
| Akola | 59.75** | 16.45 | 7.40 | 0.48 |
| Coimbatore | 62.47* | 23.61 | 5.90 | 0.94 |
| Deesa | 88.78** | 21.10* | 0.41 | 3.52 |
| Hyderabad | 95.84* | 38.11* | 1.80 | 0.10 |
| Palem | 40.34** | 18.95* | NA | NA |
| Solapur | 107.46** | 100.73** | 3.72 | 0.44 |

*p < 0.05 and **p < 0.01

**Table 3.** Pooled ANOVA and interaction components for grain Fe and Zn content in sorghum

| Source of variation | d.f. | Mean Sum of Squares | |
|---|---|---|---|
| | | Grain Fe | Grain Zn |
| Replication | 1 | 0.39 | 27.91 |
| Location | 5 | 440.89** | 139.05** |
| Genotype | 9 | 52.97 | 45.45 |
| Genotype x location | 45 | 80.33** | 34.70** |
| Error | 59 | 10.55 | 10.37 |

*p < 0.05 and **p < 0.01

interaction sum of squares. Similarly, the first two component axes captured nearly 78% of interaction sum of squares for grain Zn, with PCA I accounting for 54.7% and PCA II for 23.2% of sum of squares. The mean squares for the PCA 1 and PCA 2 were significant at P = 0.01 and 0.05 level, respectively (Table 4). Thus, the interaction of 10 genotypes with six locations was best predicted by the first two principal components of genotypes and environments. The AMMI biplot demonstrated that Deesa and Hyderabad had less G × E interaction, while the interaction was high in Coimbatore and Solapur for grain Fe. Among the genotypes, EP 95 and EP 5 exhibited nearly the same location-wise grain Fe, while CSV 23 performed differently depending on the location. In case of grain Zn, Hyderabad and Palem exhibited less G × E interaction, while the interaction was high in Solapur. Genotype-wise, again CSV 23 exhibited very high differential response for grain Zn.

The stability analysis following Eberhart and Russell model [9] undertaken to study the behaviour of these traits indicated that the variation due to environment (linear) and pooled deviation were highly significant (Table 4) for both grain Fe and Zn, while G × E (linear) was non-significant. The sum of squares for pooled deviation for grain Fe and Zn was almost 50% of total sum of squares. The significant environment (linear) variance implies that the variation among environments was linear, which signify unit changes in environmental index for each unit change in the environmental conditions. Insignificant G × E (linear) variance also indicates linear sensitivity. However, significant pooled deviation for both the traits suggests importance of non-linear component in the manifestation of G × E interaction, or in other words, expression of some of the genotypes fluctuated significantly from their respective linear path of response to environments. This

**Table 4.** ANOVA and variance components for grain Fe and Zn content in sorghum (based on AMMI & Eberhart and Russell model)

| Source of variation | d.f. | Mean Sum of Squares | |
|---|---|---|---|
| | | Grain Fe | Grain Zn |
| Genotypes | 9 | 26.48 | 22.72 |
| Environments | 5 | 220.44** | 69.52** |
| Gen. × Env. | 45 | 40.17 | 17.35 |
| PCA I | 13 | 69.61** | 32.83** |
| PCA II | 11 | 43.15* | 16.48* |
| PCA III | 9 | 27.93 | 12.82 |
| Residual | 12 | 14.71 | 4.77 |
| Env.+ (Gen.× Env.) | 50 | 58.19 | 22.57 |
| Env. (Lin.) | 1 | 1102.19*** | 347.62*** |
| Gen.× Env.(Lin.) | 9 | 23.52 | 13.31 |
| Pooled Deviation | 40 | 39.89*** | 16.52*** |
| Pooled Error | 54 | 5.66 | 4.25 |

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

may also be due to the difference in the soil micronutrient status in the test locations, though no significant association between soil Fe and Zn status and overall location mean was observed. More elaborate experiments may be necessary to understand the differential response of the genotypes for grain Fe and Zn content. This genotype × location interaction can be better understood by studying the performance of genotypes in multiple locations as well as at different growing seasons. Though G × E interactions for grain Fe and Zn contents appear to play an important role, it is possible to identify the genotypes with stable micronutrient concentrations across environments, and thus it could be feasible to combine high micronutrient traits with high grain yield [17].

Among the 10 genotypes, the regression coefficient for grain Fe was near to unity in six, but deviation from regression was significant or highly significant in all except two genotypes (Table 1a). Considering the regression coefficient and deviation from regression for grain Fe, CSV 18 and CSV 15 were the stable genotypes, but only CSV 18 has high mean value (Fig. 2). The genotypes having higher mean value with high regression coefficients reflect the higher sensitivity of specific genotypes for environmental changes, and such genotypes could be performing better in more favourable environments. Among the genotypes tested, GGUB 39 had high mean grain Fe content, above unity regression and minimum deviation

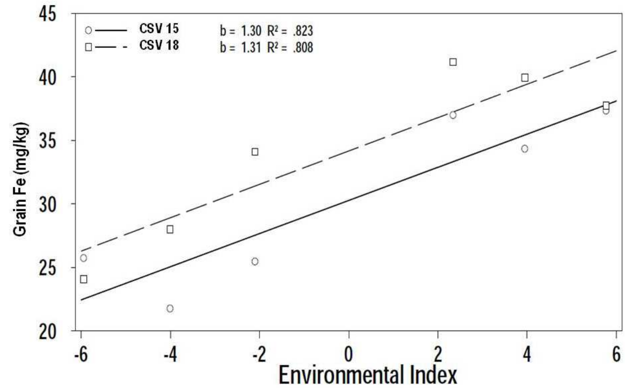


**Fig. 2. Performance of stable genotypes for grain Fe content**

from regression, and thus is expected to perform better under favourable environments. EP 95 also had high mean grain Fe; however, the regression coefficient was very low and the deviation from linearity was highly significant. Hence, this genotype may accumulate more Fe in the grain under poor soil conditions, but this has to be confirmed through further experimentations.

For grain Zn, four genotypes had near to unity regression coefficient, but only three (CSV 216R, CSV 17 and CSV 15) had non-significant deviation from regression (Table 1b) indicating linear response. High mean value was observed in CSV 23 followed by EP 95 and GGUB 39, but both CSV 23 and EP 95 had very low regression coefficient while GGUB 39 had high regression indicating non-stable performance. Relatively high mean grain Zn with near unit regression and no deviation from linearity was observed only for CSV 216R (Fig. 3), indicating stable performance.

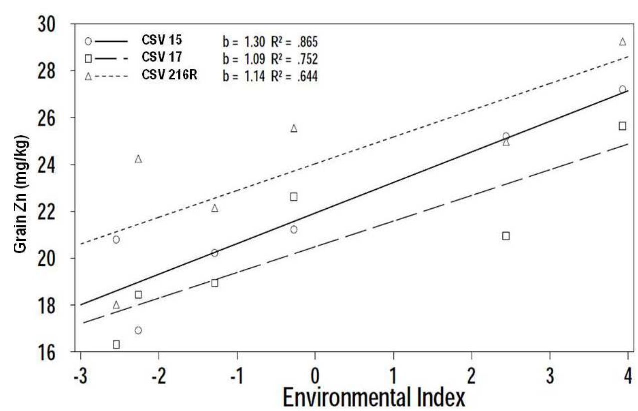


**Fig. 3. Performance of stable genotypes for grain Zn content**

To conclude, the G × E interactions play a significant role in the expression of grain Fe and Zn content in sorghum. As grain sorghum is grown in different soil types with varying levels of soil fertility and management in India, it is necessary to examine the stability of grain micronutrient content across different soil types and soil fertility. The environmental conditions, especially soil micronutrient status may affect the grain Fe and Zn content, but no significant association was observed, thus reiterating the differential response of genotypes with regards to Fe and Zn accumulation in the grains. Though linear response to environmental conditions was observed in some of the genotypes, non-linear response was also equally evident in other genotypes, necessitating multi-location as well as multi-season evaluation of genotypes before identifying stable genotypes that can be used as donor parents in breeding for micronutrient enrichment in sorghum. Proper understanding of micronutrient accumulation in the grains, genetic control and identification of genotypes that accumulate high Fe and Zn contents irrespective of growing conditions will pave the way for development of micronutrient rich sorghum varieties to address the problem of micronutrient malnourishment among the target populations.

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