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Research Note

Genetic diversity study and estimation of iron and zinc content in red sorghum genotypes (*Sorghum bicolor* [L.] Moench)

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Abstract
A study involving 72 red sorghum genotypes was carried out during *Rabi*, 2021-22 with the objective of identifying high yielding red sorghum accessions with superior Fe and Zn content. These 72 genotypes were grouped into 10 clusters. All the four checks used in the study were grouped in cluster VIII (CO 4, Paiyur 2, Usilampatti local and AURS 013). Based on single plant yield, CO 4 was identified as the best check. Genotypes IS 29630, IS 30536 and 38 CS were grouped under cluster IX, and were better than the best check with respect to yield. These genotypes could be used in further breeding programmes. PCA identified five principal components that had Eigen value greater than one and accounted for about 72.5% of the total variation. Based on the mean performance, the genotypes *viz.,* IS 14775, IS 29630, 38 CS, IS 29322, IS 30536, 34 CS, IS 23390, SD 8348, IS 4966 and IS 21544, which had recorded high single plant yield were used for the estimation of iron (Fe) and zinc (Zn) content. High Fe and Zn were recorded in IS 21544 and IS 4966.

Keywords: Red sorghum, clustering, PCA, mean performance, Fe and Zn.

Sorghum (*Sorghum bicolor* [L.] Moench) is also called great millet, Indian millet, milo and durra. It is a versatile and important grain crop cultivated in many parts of Africa and Asia for food, feed and fodder. It is a staple diet for millions of people in tropical and semi-arid climates. It is the world's fifth most significant grain cereal and is an annual diploid crop species (2n=2x=20) (Hariprasanna and Patil, 2015). It is an excellent carbon fixer in high-temperature, low-moisture settings since it is a C4 plant. It is a resilient crop that may grow in marginal terrain areas (Kavipriya *et al.,* 2019).

Globally sorghum was cultivated in an area of 44.6 million hectares with a production of 68.3 million tonnes during 2020-21 (FAOSTAT, 2022), while in India, sorghum occupied an area of 4.24 million hectares with a production of 4.81 million tonnes (FAOSTAT, 2022). In Tamil Nadu, sorghum is grown as a rainfed crop and is cultivated in an area of about 3.97 lakh hectares with a production of 3.62 lakh tonnes (Season and crop report, Govt. of Tamil Nadu, 2022).

Sorghum grains have high vitamins, zinc (Zn) and iron (Fe). It is a preferred grain for optimal health, due to the high vitamin content. Compared to other cereal grains like barley and rice, it has significantly high fibre. Sorghum aids in the maintenance of calcium levels in the body because of its high magnesium (Mg) content . In order to develop high yielding red sorghum varieties, as a preliminary attempt, a study was conducted to evaluate

the available red sorghum genotypes from Ramaiah gene bank of Department of Plant Genetic Resources, Tamil Nadu Agricultural University, Coimbatore along with known red sorghum genotypes namely Co 4, Paiyur 2 and local collections namely, Usilampatti local and AURS 013 to identify the high yielding accessions with rich nutrient (Fe and Zn) content.
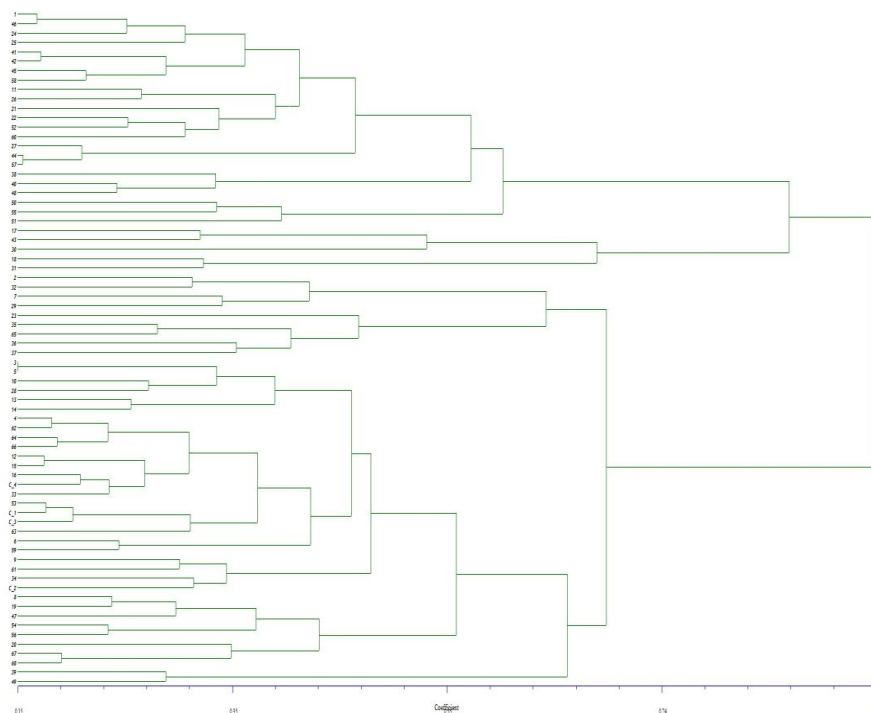
A field experiment was carried out to analyse genetic diversity and estimate iron (Fe) and zinc (Zn) content in red sorghum genotypes at Department of Millets, Tamil Nadu Agricultural University, Coimbatore during *Rabi*, 2021. The experiment was laid out in an augmented block design I (ABD I) with 68 red sorghum germplasm accessions and four checks. The checks were replicated five times. The red sorghum germplasm accessions and checks were raised in two rows each. The crop production practices were followed as per the recommendation. The data on 13 quantitative traits *viz.,* plant height, days to 50% flowering, flag leaf length, flag leaf width, number of leaves, stem diameter, panicle length, panicle width, panicle weight, days to maturity, hundred seed weight, dry fodder yield per plant and single plant yield were recorded on five randomly selected plants of all the genotypes studied. Hierarchical clustering by NTSYS and principal component analysis was employed to examine the percentage contribution of each trait towards total

genetic variation by GRAPES software based on the 13 yield contributing trait types. Among the 68 germplasms evaluated, the top yielders were utilized for estimating iron and zinc content. In this study grain Fe and Zn content was determined using the ground grain sample, by wet ashing technique *viz.,* triacid digestion method (Sahrawat *et al.,* 2002) in Atomic Absorption Spectrometer (AAS).

The clustering pattern of the 68 red sorghum germplasm accessions along with four checks based on quantitative traits (**Fig. 1; Table 1**) showed that the genotypes were grouped into 10 clusters at a similarity coefficient of 0.55. Cluster VIII was the largest with 25 accessions followed by Cluster I with 17 accessions. Similar findings were reported by Jain and Patel (2016), Rajarajan *et al*. (2016), Subramanian *et al.* (2019) and Navya *et al.* (2021). All the 10 clusters formed were divided into two sub-clusters *viz.,* sub-cluster A and sub-cluster B. The Cluster I, sub-cluster A had 14 accessions and sub-cluster B had three accessions. In Clusters II, sub-cluster A had only one genotype and sub-cluster B had two genotypes. Likewise, in both the clusters III and IV, sub-cluster A had two accessions and sub-cluster B had only one accession. Moreover, in clusters V and X, both the sub-clusters A and B had only one accession each. Furthermore, in Cluster VI, sub-clusters A and B had two accessions each. In Cluster VII, sub-cluster A had only one accession and

Table 1. Clustering pattern of red sorghum genotypes based on quantitative traits

Cluster	Sub-cluster	No. of genotypes	Germplasm accessions
I	A	14	TRs 18008, IS 13980, ISC 304-4, IS 20740, IS 1255, IS 29556, IS 20507, IS 29640, IS 13506, IS 19118, SD 8375, AS 5546, IS 4092, IS 8012
	B	3	IS 29315, ISC 304-1, ISC 304-3
II	A	1	IS 2346
	B	2	TRs 18003, SD 12885
III	A	2	IS 1052, IS 3076
	B	1	SD 776
IV	A	2	IS 29705, IS 19536
	B	1	SD 2033
V	A	1	03 CS
	B	1	IS 10930
VI	A	2	IS 1233, IS 30330
	B	2	Chen Cholam 1, IS 4105
VII	A	1	IS 14775
	B	4	IS 4956, IS 2583, IS 4966, Chen Cholam 2
VIII	A	21	IS 29218, IS 20603, SD 824, TRs 18010, SD 8374, IS 30450, IS 21544, IS 29690, IS 22764, IS 23390, TRs 18007, IS 22794, IS 30651, CO 4, IS 10718, Chen Cholam 3, AURS 013, Usilampatti local, MS 7863, IS 18195, IS 21713
	B	4	IS 30414, Red Irungu Cholam, Irungu Cholam, Paiyur 2
IX	A	5	IS 29630, IS 29322, IS 30536, 34 CS, SD 8348
	B	3	38 CS, TKSV 1008, IS 12804
X	A	1	SD 272
	B	1	55 CS

Fig. 1. Clustering pattern of red sorghum genotypes based on thirteen quantitative traits

sub-cluster B had four accessions. Likewise, in Cluster VIII, sub-cluster A was found to have 21 accessions, whereas sub-cluster B had four accessions. Finally, in Cluster IX, sub-cluster A had five genotypes but B had three genotypes. The four checks used in the study *viz.,* CO 4, Paiyur 2, Usilampatti local and AURS 013 were grouped in Cluster VIII. Among the four checks, Paiyur 2 and CO 4 were the best checks with respect to the quantitative characters. Among the accessions evaluated, IS 29630, IS 30536 and 38 CS, grouped under Cluster IX, were found to be superior over the checks for single plant yield. Hence, these identified accessions can be utilized for red sorghum improvement programmes.

The mean data of 72 red sorghum genotypes evaluated for 13 yield contributing traits were subjected to principal component analysis. The relative contributions of each principal component towards divergence with their Eigenvalues were presented in **Table 2**. Five principal components were extracted from PCA, as it had Eigen value greater than one. Similar findings were reported by Desmae *et al.* (2016) and Dossou-Aminon *et al.* (2015) in sorghum. Scree plot explained the percentage of variation associated with each principal component obtained by drawing a graph between Eigen values and principal component numbers. PC1 showed 26.24% variability with the Eigen value of 3.412. The Eigen values are gradually declined from PC1 to PC10. The percentage variation was shown in the scree plot (**Fig. 2**). Moreover, those five principal components accounted for about 72.49% of the total variation. The relative contribution of different characters towards divergence in extracted principal components is presented in **Table 3**.

The first principal component (PC 1) contributed about 26.24% of the total variation and had an Eigen value of 3.412. The traits *viz.,* single plant yield, dry fodder yield per plant, panicle weight, plant height and panicle width contributed maximum towards divergence in PC 1. The second principal component (PC 2) had an Eigen value

Table 2. Eigen values and percentage of total variances for different principal components

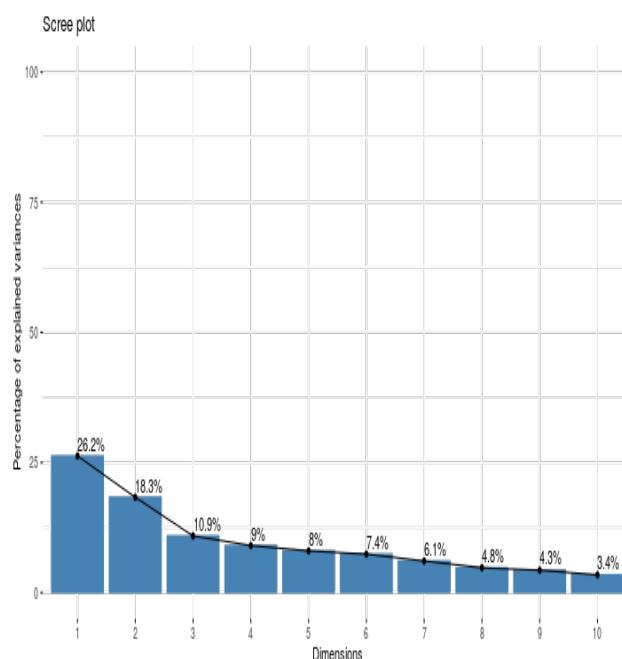| Principal components	Eigen value	Percentage of variance	Cumulative percentage of variance
PC1	3.412	26.244	26.244
PC2	2.38	18.311	44.554
PC3	1.414	10.879	55.433
PC4	1.175	9.035	64.468
PC5	1.042	8.016	72.485

Scree plot

Fig. 2. Scree plot showing Eigen value for each principal component

of 2.38 and accounted for about 18.311 per cent of the total variation. The major contributors in PC 2 were days to maturity, days to fifty per cent flowering and flag leaf length. The third principal component (PC 3) had an Eigen value of 1.414 and it explained about 10.879 per cent of the total variation. The major contributors in PC 3 were number of leaves, panicle length, hundred seed weight, flag leaf length and panicle width. The fourth principal component (PC 4) contributed about 9.035 per cent of the total variation and had an Eigen value of 1.175. The traits *viz.,* stem diameter, hundred seed weight and number of leaves specified their role towards divergence in PC 4. The fifth principal component (PC 5) had an Eigen value of 1.042 and accounted for about 8.016 per cent of the total variation. The major contributors in PC 5 were panicle length, number of leaves, hundred seed weight and plant height. The results obtained here were in concordant with Ahamed *et al.* (2015) findings.

Table 3. Relative contribution of thirteen characters towards divergence in extracted principal components in red sorghum

Characters	Principal components				
	PC1	PC2	PC3	PC4	PC5
Plant height (cm)	0.412	0.034	-0.179	-0.207	0.232
Days to 50% flowering	0.119	0.586	0.053	0.193	-0.201
Flag leaf length (cm)	0.185	0.291	-0.268	-0.172	0.43
Flag leaf width (cm)	0.124	0.182	-0.513	-0.217	-0.3
Number of leaves	-0.033	-0.127	-0.488	0.42	0.436
Stem diameter (cm)	0.113	0.069	0.155	0.598	0.114
Panicle length (cm)	0.171	0.161	0.395	0.051	0.513
Panicle width (cm)	0.366	-0.077	0.243	-0.095	-0.09
Panicle weight (g)	0.413	-0.236	0.087	0.126	-0.097
Days to maturity	0.087	0.598	0.046	0.164	-0.186
Hundred seed weight (g)	0.125	-0.165	-0.294	0.483	-0.271
Dry fodder yield per plant (g)	0.436	-0.023	-0.185	-0.137	0.029
Single plant yield (g)	0.452	-0.21	0.15	0.059	-0.198

Many traits were found to be contributing towards divergence from each principal axis. Hence, the traits with loaded factors more than 0.4 could be considered as significantly important. As a result, among the 13 yield contributing traits, 12 traits were specified their role towards divergence in the population screened except panicle width. Hence, those 12 traits can be used as an indicator while selecting plants for hybridization programme.

Ashok Kumar *et al.* (2013) reported that sorghum is an important source of dietary energy and micronutrients for the majority of individuals in Central India and Asia. It is one of the cheapest source of micronutrients (Fe and Zn). In the present study, among the 72 genotypes, 10 genotypes (IS 14775, IS 29630, 38 CS, IS 29322, IS 30536, 34 CS, IS 23390, SD 8348, IS 4966 and IS 21544) were selected based on the high single plant yield for iron and zinc estimation. The genotypes IS 21544 and IS 4966 showed high Fe and Zn, while IS 29630, IS 30536 and 38 CS had low Fe and Zn.

Among the 10 genotypes, IS 29322 (Fe – 42.8 ppm, Zn – 21.1 ppm) and IS 21544 (Fe – 40.3 ppm, Zn – 25.2 ppm) showed high Fe content and medium Zn content; IS 23390 (Fe – 31.8 ppm, Zn – 22.0 ppm) and IS 4966 (Fe – 38.5 ppm, Zn – 28.8 ppm) had medium Fe and Zn content; 34 CS showed high Zn (30.5 ppm) content but had low Fe (29.2 ppm) content, whereas IS 14775 (Fe – 30.2 ppm, Zn – 17.9 ppm) and SD 8348 (Fe – 34.3 ppm, Zn – 16.1 ppm) showed medium Fe content and low Zn content and IS 29630, 38 CS and IS 30536 genotypes showed low Fe and Zn content (**Table 4** and **Table 5**). Waters and Pedersen (2009) evaluated 95 accessions in sorghum reported 24.00 – 73.00 ppm Fe content and 15.00 – 59.00 ppm Zn content. Moreover, Hariprasanna *et al.* (2018) reported 6.30 - 168.10 mg kg^{1}and 2.30 – 5.50 mg kg^{-1} Fe and Zn contents, respectively in 113 germplasm accessions in sorghum. In general, the intensity of blue and red colours served as a reliable qualitative and semi-qualitative selection criterion for grain Fe and Zn content in red sorghum, respectively. The method was efficient in separating the genotype with high grain Fe and Zn content from those with low grain Fe and Zn content. When a large number of genotypes have to be screened for Fe and Zn content, this method will be highly efficient in discarding the genotypes with low Fe and Zn content or *vice versa*. Similar finding was reported by Velu *et al.* (2006) for the estimation of iron content and Velu *et al.* (2008) for the estimation of zinc content in pearl millet.

Table 4. The intensity of blue colour was determined for Iron (Fe) content using Atomic Absorption Spectroscopy (AAS) in red sorghum genotypes

S. No.	Genotypes	Class	Colour class	Fe content (ppm)
1.	IS 29322	High	More intense blue colour	42.8
2.	IS 21544	High	More intense blue colour	40.3
3.	IS 4966	Medium	Medium blue colour	38.5
4.	SD 8348	Medium	Medium blue colour	34.3
5.	IS 23390	Medium	Medium blue colour	31.8
6.	IS 14775	Medium	Less intense blue colour	30.2
7.	34 CS	Low	No colour	29.2
8.	38 CS	Low	No colour	29.0
9.	IS 29630	Low	No colour	28.6
10.	IS 30536	Low	No colour	25.5

Table 5. The intensity of red colour was determined for Zinc (Zn) content using Atomic Absorption Spectroscopy (AAS) in red sorghum genotypes

S. No.	Genotypes	Class	Colour class	Zn content (ppm)
1.	34 CS	High	More intense red colour	30.5
2.	IS 4966	Medium	Medium red colour	28.8
3.	IS 21544	Medium	Less intense red colour	25.2
4.	IS 23390	Medium	Less intense red colour	22.0
5.	IS 29322	Medium	Less intense red colour	21.1
6.	38 CS	Low	No colour	18.2
7.	IS 14775	Low	No colour	17.9
8.	IS 29630	Low	No colour	17.5
9.	IS 30536	Low	No colour	17.0
10.	SD 8348	Low	No colour	16.1

The study explored the genetic diversity among 72 red sorghum genotypes. Among the sorghum germplasm accessions studied, ten genotypes *viz.,* IS 14775, IS 29630, 38 CS, IS 29322, IS 30536, 34 CS, IS 23390, SD 8348, IS 4966 and IS 21544 were found to be high yielding. Moreover, high Fe and Zn contents were observed in IS 21544 and IS 4966. These genotypes could be further utilized in the red sorghum improvement programme.

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