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Exploiting the plant characters association of grain iron and zinc under diverse environments

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Abstract
Malnutrition is a rising issue in the present era. Biofortification is a process used to improve the nutritional content of the crop, it enhances the levels of essential vitamins and minerals such as iron, zinc, and provitamin A (beta-carotene) in the grain and make it more nutritious without extra supplements. In regards to this, to elevate the grain iron and zinc nutrient status in sorghum, the present study was conducted at two locations i.e. Sorghum Research Station, Akola and Agriculture Research Station, Washim in the rabi season 2023. The present study comprised of ten parents and 45 crosses (obtained by half diallel method) laid out in randomized block design with three replications at both the locations having diverse environments. Amongst the parents, Parbhani Shakti exhibited superior performance in desirable direction along with high iron and zinc content along with satisfactory grain yield per plant along with the parents AKSV-442, M-35-1 and PKV Kranti. Further, from amongst the crosses, the cross CSV-26R X CSV-22R was recorded promising for grain iron and zinc content along with grain yield per plant in desirable direction. Correlation studies inferred that, the micro-nutrients such as grain iron and zinc content recorded significantly positive correlation with each other indicating simultaneously improvement of the character. Whereas, on the other hand, both grain iron and zinc content recorded significantly negative association with grain yield which indicated that genetic enhancement for grain iron and zinc content does not have yield enhancement. This low yield in the rabi sorghum was due to the diverse environmental conditions faced by the rabi sorghum as compared to the kharif sorghum. Also, phytic acid had positive association with grain iron and zinc content and negative correlation with grain yield which attributed to less germination due to phytic acid content in the seed. The study led to a final conclusion that, breeding for higher levels of micronutrients can be achieved simultaneously for grain iron and zinc content without compromising on the grain yield in the rabi sorghum. Therefore, it will be possible to develop cultivars with high grain iron and zinc content along with high yield and appropriate phytic acid levels having low anti nutritional factors by the use of recombination breeding.

Keywords: Rabi sorghum, correlation studies, kharif sorghum, iron, zinc, grain yield

Introduction
Sorghum, 'The King of Millets,' is one of the major staple crop cultivated in India and one of the most important major millet grown in Africa, Asia, and America. Sorghum being a C4 plant, is photosynthetically efficient, drought tolerant and resistant to water-logging Doggett (1988) [1], and grows in various soil conditions Dillon *et al.*, (2007) [2]. Sorghum is a significant industrial crop, animal feed, and staple food Bhosale *et al.*, (2011) [3] and a crop that grows best in arid and semi-arid regions of the world Robert *et al.*, (2009) [4] and is mostly resistant to heat and drought Ahamed *et al.*, (2015) [5]. It is extensively cultivated in marginal rainfall areas of the tropics and subtropics, and selected varieties are widely grown in temperate climates.

Increased temperatures and unpredictable rainfall patterns brought on by climate change have an impact on crop productivity, but sorghum's ability to withstand drought has made it a valuable crop in the fight against these impacts. Its low input cultivation, versatility of uses, and excellent tolerance to a wide range of ecological circumstances make it the most important grain after wheat, maize, rice, and barley. Over 500 million people across 30 nations in Africa and Asia rely on it as a staple food. In India, the postrainy season sorghum grain is mainly used for human consumption, whereas the rainy season grain is mainly used

as animal feed. It contributes more than 50% of the Fe and Zn and is among the least expensive sources of protein, energy, Fe, and Zn. With a yield of about 65 million tons, sorghum is farmed on more than 40 million hectares worldwide Goldschein *et al*., (2011) [6]. It is the second most economical energy-rich and micro-nutritive source of Iron (Fe) and Zinc (Zn) after pearl millet. Among the poorer and vulnerable sections of society which cannot afford or have access to nutrient-rich foods such as vegetables, fruits, and animal products especially in India, sorghum is still one of the major food staples acting as a source of both calories and micronutrients.

Over two-thirds of the world's population, particularly women of reproductive age, pregnant and lactating women as well as preschool children are affected by micro-nutrient malnutrition leading to nutritional blindness and anemia, so the programs are being implemented for fortification of foods directed towards the most vulnerable groups of populations. The World Health Organization (WHO) of the United Nations has identified that these two micronutrients (zinc and iron) along with pro-vitamin A (as β-carotene) are critical for human nutrition and health. Also, nutritionists have targeted three principal interventions in order to alleviate micronutrient malnutrition viz., supplementation, fortification, and dietary diversification Underwood (2000) [7], Sharma (2003) [8] and Welch and Graham (2004) [9].

Biofortification is the most sustainable way of providing micronutrients to combat deficiencies, as one does not have to pay for the micronutrient which has been already consumed continually without any extra cost to the consumer in developing countries. Micronutrient-dense staple crop cultivars achieved by efficient, low-cost traditional breeding enhanced by modern biotechnology constitute a potent, sustainable and long-term approach for increasing the micronutrient benefits achievable for target populations and thereby improving health status. Study of the interrelationship of these micronutrients with various biochemical and anti-nutritional factors give researchers an idea regarding the association of iron and zinc with the different characters which help in selection of characters for appropriate breeding strategy.

Material and Methods

The present study was conducted during Rabi season 2023-24, at two locations i.e. at Sorghum Research Unit, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola and Agricultural Research Station, Washim. The experimental material comprised of 45 crosses (F_1) obtained by 10 X 10 half diallel (excluding reciprocals) (Method-2, Model-1) Griffing (1956) [11] along with their parents. These 45 crosses and the 10 parents were evaluated in three replications at two locations in randomized block design. The two locations chosen had diverse environmental conditions with Akola situated at altitude of 285 m above mean sea level and Washim at an altitude of 546 m above sea level belonging to a hilly region. The soil at Akola and Washim also shows diversity with former having vertisol, deep black to medium deep black soil derived mostly from basalt rocks and later consisting of deep black to dark grey brown clay soil. The different parents used for the present experiment were viz., AKSV-438, AKSV-440, AKSV-442, AKSV-439 Parbhani Shakti (varieties found to be high in iron and zinc content), CSV-26R, CSV-22R, CSV-29R (dual purpose varieties), M-35-1 and PKV Kranti (high grain yielding varieties).

Observations recorded

The average value per plant was worked out by recording observations on randomly selected five plants in all crosses and parents. The observations per plant was taken by tagging the main shoot in the experimental plot.

Morphological characters

- **Days to 50% flowering:** The number of days required for 50 percent flowering of the plants in row on the plot from date of sowing was recorded.
- **Plant height (cm):** The height of fully matured five plants was measured from ground level to the tip of the ear head in centimeters and their average was worked out from the five observed plants.
- **Grain yield per plant (g):** The weight of grains per plant was recorded in grams and was averaged over five observed plants.
- **100 seed weight (g):** The weight of 100 randomly selected seeds from the bulk of the panicle was measured using an electric weighing balance in grams and was average over five observed samples.

Nutritional characters

Iron and Zinc content (ppm)

The iron and zinc content in the grain samples was determined by using the di-acid digestion method whereas, the iron and zinc content in the aliquot was determined by Atomic Absorption Spectrometer (ASS) by Pequerul *et al*., (1993) [12].

Biochemical characters

Proline content (g/100 g) Bates *et al*., (1973) [13]

Proline content was estimated by Bates *et al*., (1973) [13] where seed sample was homogenized in sulphosalicylic acid. Acid ninhydrin and glacial acetic acid were added in equal quantities to the filtrate. The contents after transferring to separating funnel toluene was added and mixed vigorously. The coloured toluene fraction was separated and measured at 520 nm in spectrophotometer. A blank was maintained with all the reactants except sample extract. Proline content was calculated by the equation:

$$\text{Proline} = \frac{34.11 \text{ x OD520 x V}}{2 \text{ x f}}$$

Where,
V = total volume of extract
f = grams of fresh leaf

Protein content (g/100 g)

The powdered seeds samples were taken for estimation of protein and digested in the Kjeldhal apparatus Anantakrishnan *et al*., (1952) [14] in the block digester till a transparent liquid was obtained. The transparent liquid was then cooled by adding sufficient quantity of distilled water. Further, for sample distillation, boric acid was taken in the conical flask and placed in the distillation unit of Kjeldhal apparatus. The colour of boric acid in conical flask changed from green to pink. This boric acid was then titrated with $H2SO_4$ till boric acid changed colour to pink. The reading on the burette were recorded and the total nitrogen and crude protein was calculated by the following formulas:

$$\text{Total Nitrogen} = (\text{A-B}) \text{ x } 0.014 \text{ x Normality of acid x } \frac{100}{\text{Weight of sample}}$$

Here,
A = Reading of seed sample solution
B = Reading of blank sample solution

The protein content was estimated by using the nitrogen to protein conversion factor i.e. 6.25
Crude Protein = 6.25 x Total Nitrogen

Carbohydrate content (g/100 g)
Carbohydrates was estimated by utilizing Anthrone method Dreywood (1946) [15]. Carbohydrates were first hydrolysed into simple sugars using dilute hydrochloric acid. In hot acidic medium glucose was dehydrated to hydroxymethyl furfural. This compound forms with anthrone a green colored product with an absorption maximum at 630nm.

Antinutritional factors
Tannins (%) (Chlorox Bleach Test)
For tannin% the Chlorox bleach test Waniska *et al*., (1992) [16] was used where the whole sorghum kernels were used. Enough bleaching reagent was used to fully immerse sorghum kernels and covered the top of the beaker with aluminum foil. The kernels were placed on a paper towel and gently patted to remove excess water. The sorghum kernels were allowed to dry at room temperature. Evaluate the sorghum kernels. Kernels that are completely black have tannins and kernels that are white or lightly colored have no tannins. Count the number of kernels that are completely black. Expressed the results as percentage of tannin sorghums. Duplicates should not differ by more than 5.0%

Phytic acid (ppm)
Phytic acid estimation has been performed by modified colorimetric method Vaintraub and Lapteva (1988) [17]. Sample was added to 3% trichloroacetic acid and shaked using rotary shaker. After centrifuging the solution the supernatant was transferred to flask and ferric chloride was added. The precipitate obtained after keeping in the hot water bath was dissolved using trichloroacetic acid and repeated twice. After that sodium hydroxide was added and solution was heated in the boiling bath. Then the precipitate was dissolve in nitric acid and volume was made up using distilled water. 1 ml aliquot was taken diluted and 4 ml potassium thiocyanate was added and absorbance was taken within 1 min at 480 nm using UV-vis Spectrophotometer.

Statistical analysis
The data analysis was performed in the specially made Excel sheet where raw data was used to perform the pooled analysis. The correlation coefficient at genotypic level and phenotypic level was worked out by using the r studio software.

Result
Pooled mean performance of parents and their F_1 crosses for all the characters under study
Mean performance of parents and their F_1 crosses evaluated at two locations (Akola and Washim) obtained by using 10x10 half diallel design (excluding reciprocals) was studied during the *rabi* season 2023, and pooled analysis of the data was performed and is presented in the Table 1 and Table *2* .
From among the parents under evaluation (Table 1), the parent AKSV-438 and AKSV-440 (70.5 days and 118.5 cm height, 124 cm height respectively) were observed to be the earliest flowering parents with short height. The parent CSV-29R (80.5 gm) recorded the highest grain yield per plant followed by Parbhani Shakti (79 gm), whereas, 100 seed weight was recorded highest in the parent AKSV-440 (4.4 gm). Highest protein content and carbohydrate was recorded in the parent CSV-29R (12.33%) and Parbhani Shakti (87.57 g/100 g), respectively. The highest grain iron and zinc content was recorded in the parent Parbhani Shakti (43.58 ppm and 28.75 ppm, for iron and zinc content, respectively). Proline content was observed to be lowest in the parent M-35-1 (0.46 g/100 g) and lowest phytic acid was observed in the parent CSV-29R (2.06 ppm) whereas, lowest tannin percent was observed in the parent CSV-29R, PKV Kranti and Parbhani Shakti (2%).
Among the 45 crosses (Table 2) evaluated, the cross AKSV-440 X M-35-1 (63.5 days) was observed to be the earliest flowering cross and AKSV-438 x Parbhani Shakti (148.5 cm) recorded the shortest plant height. However, the cross CSV-26R x M-35-1 (90 gm) recorded highest grain yield per plant followed by the cross CSV-29R x Parbhani Shakti (88 gm). Highest protein content was recorded in the cross PKV-Kranti X Parbhani Shakti (9.32%) and the highest carbohydrate content was observed in the cross CSV-22R X CSV-29R (90.28 g/100 g). The highest grain iron and zinc content was observed in the cross AKSV-442 X Parbhani Shakti (43.95 ppm) and AKSV-440 x AKSV-442 (28.10 ppm), respectively. Proline content and phytic acid was observed to be lowest in the cross CSV-26R x CSV-22R (0.34 g/100 g) and M-35-1 X AKSV-439 (3.02 ppm), respectively. Whereas, tannin content was observed to be lowest in the cross CSV-22R x PKV Kranti (1%).

Table 1: Pooled mean of parents for various characters evaluated over the environments (Akola and Washim) during the 2023 rabi season.

S. N	Genotypes	Pooled Mean Value										
		Days to 50% flowering	Plant height (cm)	Grain yield per plant (g)	100 Seed weight (g)	Iron content (ppm)	Zinc content (ppm)	Protein content (%)	Proline (g/100 g)	Phytic acid (ppm)	Carbohydrates (g/100 g)	Tannins %
1	AKSV-438	70.50	124.00	29.50	2.95	38.40	25.48	4.01	1.59	5.44	73.65	5.00
2	AKSV-440	70.50	118.50	41.50	4.40	33.30	21.85	6.42	1.05	8.19	81.63	84.00
3	AKSV-442	72.50	191.67	36.50	3.30	38.75	25.83	9.60	1.70	17.33	77.92	88.00
4	CSV-26R	78.00	196.33	33.33	3.60	31.75	19.17	7.97	1.42	7.04	71.32	4.00
5	CSV-22R	81.00	232.67	74.50	3.75	28.80	17.74	7.35	0.87	7.78	73.46	4.00
6	M-35-1	73.00	177.33	45.50	3.40	38.25	21.08	10.32	0.46	8.22	72.24	4.00
7	CSV-29R	81.50	208.67	80.50	4.00	24.45	20.22	12.33	0.78	2.06	44.22	2.00
8	PKV Kranti	78.50	184.67	63.50	3.45	26.90	21.86	7.29	0.74	6.80	58.77	2.00
9	Parbhani Shakti	80.50	134.67	79.00	3.25	43.58	28.75	6.74	1.43	10.18	87.57	2.00
10	AKSV-439	70.50	144.83	61.50	3.60	33.19	19.56	5.40	1.48	3.09	72.16	95.50
	Parental mean	75.65	171.33	54.53	3.57	33.73	22.15	7.74	1.15	7.61	71.29	29.05

Table 2: Pooled mean of crosses for various characters evaluated over the environments (Akola and Washim) during the 2023 rabi season.

S. N	Genotypes	Pooled mean values										
		Days to 50% flowering	Plant height (cm)	Grain yield per plant (g)	100 Seed weight (g)	Iron content (ppm)	Zinc content (ppm)	Protein content (%)	Proline (g/100 g)	Phytic acid (ppm)	Carbohydrates (g/100 g)	Tannins %
1	AKSV-438 X AKSV-440	69.00	151.67	18.50	3.10	37.43	27.82	6.50	1.27	15.75	56.31	88.17
2	AKSV-438 X AKSV-442	71.50	162.50	28.83	3.40	34.88	26.27	7.00	1.19	19.16	30.92	33.00
3	AKSV-438 X CSV-26R	72.50	161.67	39.00	3.40	33.65	23.99	6.35	0.86	11.37	49.07	9.00
4	AKSV-438 X CSV-22R	76.50	172.17	37.50	3.37	27.85	20.06	6.04	0.83	8.42	37.52	2.00
5	AKSV-438 X M-35-1	73.00	186.17	59.50	3.40	30.10	21.50	5.94	0.87	4.39	55.18	4.00
6	AKSV-438 X CSV-29R	76.00	203.67	63.00	3.45	35.15	23.08	6.15	0.55	13.54	35.93	2.00
7	AKSV-438 X PKV-Kranti	70.50	173.83	37.00	3.65	39.35	23.08	6.90	0.42	13.77	43.30	2.00
8	AKSV-438 X Parbhani Shakti	70.50	148.50	40.50	2.90	33.45	21.52	6.86	0.79	7.84	61.46	2.00
9	AKSV-438 X AKSV-439	71.50	152.00	24.00	3.60	30.70	21.39	6.83	0.82	12.11	51.30	2.00
10	AKSV-440 X AKSV-442	71.50	184.67	39.00	3.85	36.73	28.10	5.88	1.31	15.74	36.15	89.00
11	AKSV-440 X CSV-26R	70.00	187.50	47.50	4.00	31.37	24.55	5.67	0.86	9.31	48.54	81.00
12	AKSV-440 X CSV-22R	80.00	189.17	44.50	3.54	25.50	18.68	4.20	1.16	16.86	54.61	80.00
13	AKSV-440 X M-35-1	63.50	186.50	35.00	4.10	33.00	20.49	6.85	0.72	18.18	51.65	95.00
14	AKSV-440 X CSV-29R	71.50	192.33	50.50	4.20	27.80	18.93	6.69	0.76	21.17	77.70	90.00
15	AKSV-440 X PKV-Kranti	69.00	196.00	40.50	4.00	34.25	23.50	6.25	1.08	29.78	77.55	80.00
16	AKSV-440 X Parbhani Shakti	73.00	161.83	68.00	3.93	30.75	24.17	6.71	1.02	25.51	81.97	82.00
17	AKSV-440 X AKSV-439	68.50	152.33	19.50	3.80	35.05	20.79	7.13	1.77	37.60	69.22	45.67
18	AKSV-442 X CSV-26R	65.50	190.00	37.00	4.05	32.10	23.66	7.00	0.71	42.90	69.25	27.00
19	AKSV-442 X CSV-22R	67.00	201.33	47.50	4.37	36.60	25.51	6.29	0.92	10.73	68.04	41.00
20	AKSV-442 X M-35-1	71.00	203.17	43.00	4.45	27.90	24.37	6.52	0.96	19.30	72.35	16.00
21	AKSV-442 X CSV-29R	73.00	210.00	43.50	4.40	37.30	26.54	6.98	0.98	30.94	80.52	14.00
22	AKSV-442 X PKV-Kranti	78.50	216.00	39.00	4.08	34.50	24.06	6.96	1.23	37.24	78.13	22.00
23	AKSV-442 X Parbhani Shakti	72.00	177.17	36.00	3.75	43.95	25.31	6.49	1.50	34.73	81.34	38.00
24	AKSV-442 X AKSV-439	73.00	156.33	43.50	3.80	27.64	21.86	6.13	1.14	23.46	69.21	90.00
25	CSV-26R X CSV-22R	79.50	219.50	75.50	4.10	22.40	23.22	8.58	0.34	18.98	85.10	2.50
26	CSV-26R X M-35-1	73.00	155.00	90.00	4.35	20.50	17.79	7.89	1.03	21.94	72.14	3.33
27	CSV-26R X CSV-29R	81.50	205.33	46.50	3.78	18.80	17.95	7.55	0.50	15.02	70.65	2.50
28	CSV-26R X PKV-Kranti	78.50	220.50	71.00	4.00	24.55	18.89	6.73	0.92	13.26	80.55	2.50
29	CSV-26R X Parbhani Shakti	70.50	190.50	60.00	3.60	29.70	19.69	7.99	1.29	13.59	82.28	1.67
30	CSV-26R X AKSV-439	68.00	183.83	42.50	4.15	23.05	15.59	7.92	1.38	12.84	83.76	6.67
31	CSV-22R X M-35-1	75.00	202.17	77.67	3.95	24.95	11.94	7.46	0.40	14.66	89.19	1.33
32	CSV-22R X CSV-29R	76.00	168.67	63.00	3.45	20.00	15.11	6.75	0.90	12.99	90.28	1.67
33	CSV-22R X PKV-Kranti	70.00	196.33	58.00	4.13	14.80	16.99	7.70	1.10	15.78	86.19	1.00
34	CSV-22R X Parbhani Shakti	76.00	216.17	55.00	3.80	25.26	24.97	8.64	1.51	15.42	45.82	1.50
35	CSV-22R X AKSV-439	70.50	159.67	67.50	4.00	35.75	20.96	7.43	1.43	14.44	50.83	1.67
36	M-35-1 X CSV-29R	74.00	204.00	78.50	4.35	25.25	18.42	7.45	0.90	9.39	75.87	3.17
37	M-35-1 X PKV-Kranti	71.50	180.33	61.50	4.10	23.15	16.22	8.59	0.59	18.98	64.15	1.67
38	M-35-1 X Parbhani Shakti	69.00	174.83	50.00	3.60	23.70	15.52	4.58	0.64	12.29	80.12	1.17
39	M-35-1 X AKSV-439	81.50	172.00	27.00	3.40	23.95	10.83	6.00	0.60	3.02	85.77	28.00
40	CSV-29R X PKV-Kranti	80.00	205.67	48.50	3.75	16.85	15.39	5.69	0.96	5.31	80.66	4.67
41	CSV-29R X Parbhani Shakti	78.50	217.00	88.00	3.55	17.45	10.37	9.16	0.57	8.50	77.76	4.50
42	CSV-29R X AKSV-439	67.50	159.33	30.50	3.95	25.40	15.98	7.19	0.94	9.15	79.89	20.00
43	PKV Kranti X Parbhani Shakti	82.00	199.67	60.00	3.55	21.95	12.76	9.32	1.12	10.31	76.26	1.33
44	PKV Kranti X AKSV-439	80.50	156.50	26.00	3.70	32.20	17.16	4.61	1.25	7.62	52.71	6.00
45	Parbhani Shakti X AKSV-439	72.00	172.50	27.00	3.90	39.20	13.96	4.32	1.33	6.11	62.22	4.00
	Cross mean	73.18	183.91	48.57	3.82	29.02	20.20	6.79	0.96	16.43	66.87	25.24

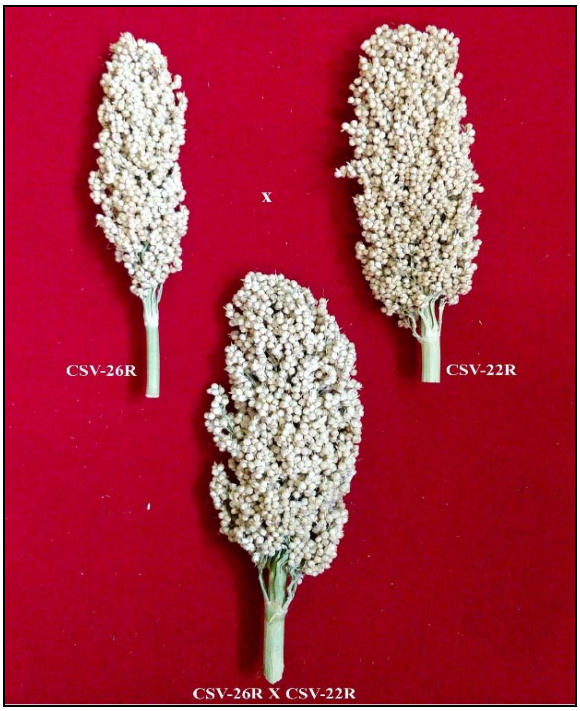

Fig 1: Cross CSV-26R X CSV-22R at Washim location

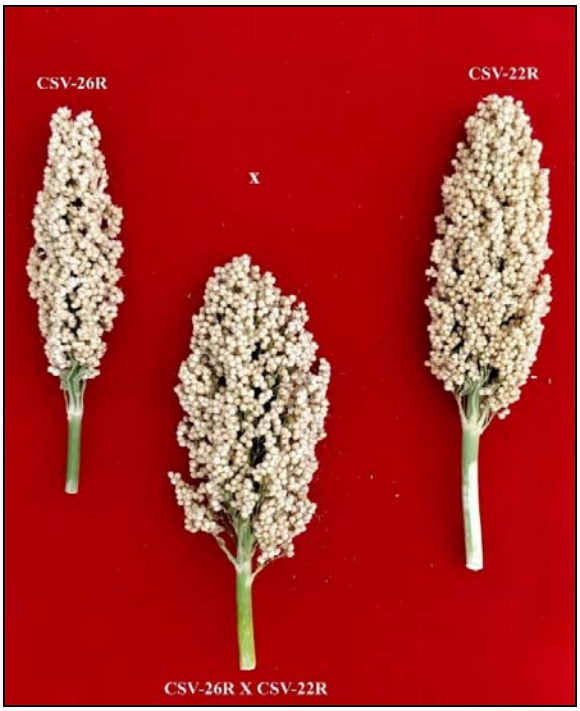

Fig 2: Cross CSV-26R X CSV-22R at Akola location

Correlation Studies: (Table 3) (Figure 3)

The degree and direction of association between the characters has been studied. The positive value of r (correlation coefficient) indicated change of two variables in same direction, whereas, negative value of variables the r value changed in opposite direction. Singh *et al.*, (2017) [18]

Days to 50% flowering recorded significantly positive correlation with plant height (0.4373 and 0.2545 at genotypic and phenotypic level, respectively) and grain yield per plant (0.4206 and 0.3222 at genotypic and phenotypic level, respectively). On the other hand, days to 50% flowering recorded negatively significant correlation with tannin percent (-0.3863 and-0.3566 at genotypic and phenotypic level, respectively), grain iron content (-0.3595 and-0.3245 at genotypic and phenotypic level, respectively) and grain zinc content (-0.3054 and-0.2717 at genotypic and phenotypic level, respectively).

Plant height recorded positively significant correlation with 100 seed weight (0.3909 and 0.308 at genotypic and phenotypic level, respectively), protein (0.4473 and 0.2916, at genotypic and phenotypic level, respectively) and grain yield per plant (0.3426 and 0.3953, at genotypic and phenotypic level, respectively). However, plant height recorded negatively significant correlation with proline (-0.5048 and-0.1558 at genotypic and phenotypic level, respectively), grain iron content (-0.4298 and-0.3326 at genotypic and phenotypic level, respectively) and grain zinc content (-0.1621 at phenotypic level)

100 seed weight recorded positively significant correlation with phytic acid (0.5384 and 0.1624, at genotypic and phenotypic level respectively), carbohydrates (0.1819 at phenotypic level) and grain yield per plant (0.2717 at phenotypic level) and recorded negatively significant correlation with grain iron content (-0.2846 and-0.1637, at genotypic and phenotypic level, respectively)

Protein recorded positively significant correlation with grain yield per plant (0.4346 and 0.3549, at genotypic and phenotypic level, respectively) whereas, it recorded negatively significant genotypic correlation with proline (-0.1764 at phenotypic level), tannin percent (-0.2751 and-0.2113, at genotypic and phenotypic level, respectively) and grain iron content (r =-0.2702 and-0.197, at genotypic and phenotypic level, respectively).

Proline recorded positively significant correlation with tannin percent (0.3258 and 0.2178, at genotypic and phenotypic level, respectively), grain iron content (0.4801 and 0.2883, at genotypic and phenotypic level, respectively) and grain zinc content (0.4027 and 0.2227, at genotypic and phenotypic level, respectively). Proline also recorded negatively significant correlation with grain yield per plant (-0.3949 and-0.2492, at genotypic and phenotypic level, respectively).

Table 3: Correlation analysis for different characters at two environments studied during rabi season 2023

s		Days to 50% flowering	Plant height	100 seed weight	Grain Density	Protein	Proline	Phytic acid	Carbohydrates	Tannin percent	Grain yield per plant	Iron content	Zinc content
Days to 50% flowering	G	1	0.4373**	-0.2529	0.2349	0.2172	-0.1517	-0.3529**	0.0964	-0.3863**	0.4206**	-0.3595 **	-0.3054 *
	P	1	0.2545 **	-0.1616 *	0.1687 *	0.1686 *	-0.1563 *	-0.2667 **	0.0584	-0.3566 **	0.3222 **	-0.3245 **	-0.2717 **
Plant height	G		1	0.3909**	-0.0438	0.4473**	-0.5048**	0.204	0.0962	-0.2573	0.3426*	-0.4298 **	-0.194
	P		1	0.308 **	-0.0065	0.2916 **	-0.1558 *	0.0202	0.0635	-0.1931 *	0.3953 **	-0.3326 **	-0.1621 *
100 seed weight	G			1	-0.3773*	0.1377	-0.1304	0.5384**	0.2575	0.1259	0.192	-0.2846 *	-0.077
	P			1	-0.2918 **	0.1316	-0.1091	0.1624 *	0.1819 *	0.0932	0.2717 **	-0.1637 *	-0.0633
Grain Density	G				1	0.092	-0.0855	-0.2415	0.0615	-0.1843	0.1057	-0.1194	-0.0972
	P				1	0.0538	-0.0896	-0.1773 *	0.0895	-0.1708 *	0.0861	-0.111	-0.0599
Protein	G					1	-0.1672	-0.005	0.1194	-0.2751*	0.4346**	-0.2702 *	-0.0992
	P					1	-0.1764 *	0.0298	0.0391	-0.2113 **	0.3849 **	-0.197 *	-0.0663
Proline	G						1	0.1882	0.0299	0.3258*	-0.3494**	0.4801 **	0.4027 **
	P						1	0.1252	0.0199	0.2178 **	-0.2492 **	0.2883 **	0.2227 **
Phytic acid	G							1	0.1905	0.3074*	-0.1604	0.236	0.406 **
	P							1	0.0828	0.2244 **	-0.2007 **	0.1604 *	0.2776 **
Carbohydrates	G								1	-0.0646	0.2872*	-0.2847 *	-0.3651 **
	P								1	-0.0607	0.2008 **	-0.2587 **	-0.316 **
Tannin percent	G									1	-0.3357*	0.3031 *	0.3504 **
	P									1	-0.2651 **	0.296 **	0.3398 **
Grain yield per plant	G										1	-0.4932 **	-0.2636
	P										1	-0.3587 **	-0.2164 **
Iron content	G											1	0.6944 **
	P											1	0.6706 **
Zinc content	G												1
	P												1

Here, G – Genotypic corelation; P – Phenotypic correlation

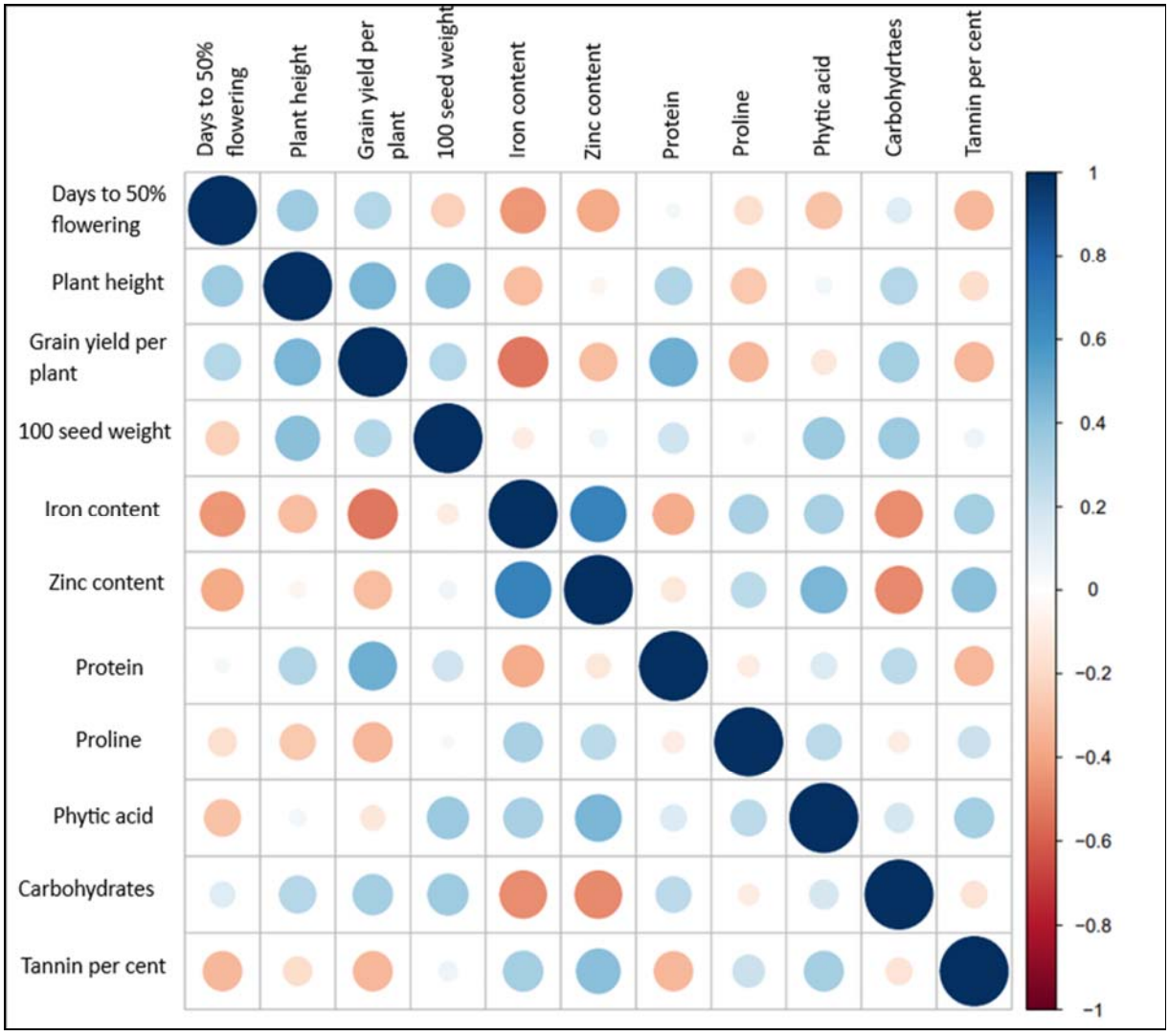

Fig 1: Correlation matrix pooled over two environments during rabi season 2023

Phytic acid recorded positively significant correlation with 100 seed weight (0.5384 and 0.1624, at genotypic and phenotypic level, respectively), tannin percent (0.3074 and 0.2244, at genotypic and phenotypic level, respectively), grain zinc content (0.406 and 0.2776, at genotypic and phenotypic level, respectively) and with grain iron content (0.1604 at phenotypic level only). However, phytic acid was negatively correlated with days to 50% flowering (-0.3529 and-0.2667, at genotypic and phenotypic level, respectively) and with grain yield per plant (-0.2007 at phenotypic level).

Carbohydrates recorded positively significant correlation with grain yield per plant (0.2872 and 0.2008, at genotypic and phenotypic level, respectively) but, recorded negatively significant correlation with grain iron content (-0.2847 and-0.2587, at genotypic and phenotypic level, respectively) and grain zinc content (-0.3651 and-0.316, at genotypic and phenotypic level, respectively).

Tannin percent had positively significant correlation with grain iron content (0.3031 and 0.296, at genotypic and phenotypic level, respectively) and grain zinc content (0.3504 and 0.3396, at genotypic and phenotypic level, respectively) however, on the other hand, tannin percent recorded negatively significant correlation with grain yield per plant (-0.3357 and-0.2651, at genotypic and phenotypic level, respectively).

Grain yield per plant recorded negatively significant correlation grain iron content (-0.4932 and-0.3587, at genotypic and phenotypic level, respectively) but had negative correlation with grain zinc content (-0.2164 at phenotypic level) which can also be visible with the orange colour circle in the Figure 3. Grain iron content recorded positively significant correlation with grain zinc content (0.6944 and 0.6706, at genotypic and phenotypic level, respectively) which can also be observed from the Figure 3, where dark blue colour circles highlight the intensity of correlation between these micronutrients.

Discussion

Amongst the parents, Parbhani Shakti, AKSV-442, M-35-1 and PKV Kranti showed promising performance for grain iron and zinc content. However, from among them Parbhani Shakti exhibited superior performance in desirable direction for iron and zinc content along with grain yield per plant and for various different characters under study at both the locations such as Akola and Washim and as well as in pooled environments. The superiority of Parbhani Shakti due to the higher values of yield attributing characters along with good mean performance gave the impression to be promising for seed yield and other yield attributing characters.

Further, from amongst the crosses, the crosses AKSV-438 X M-35-1, AKSV-438 X CSV-29R, AKSV-438 X PKV Kranti, AKSV-440 X AKSV-442, AKSV-440 X Parbhani Shakti, AKSV-442 x CSV-22R, AKSV-442 x CSV-29R, CSV-26R X CSV-22R, AKSV-442 x Parbhani Shakti, CSV-22R X ASKV-439 recorded significant iron and zinc content. However, from among these crosses, the cross CSV-26R X CSV-22R (Figure, Figure 2) recorded desirable iron and zinc content along with grain yield per plant along with superior performance in desirable direction for various characters under study at both the locations Akola and Washim as well as in the pooled environments.

From the overall correlation study, it was observed that, the micro-nutrients such as grain iron content and zinc content were significantly and positively correlated. This association between grain iron and zinc content indicated that these two traits can be simultaneously improved as their physiological mechanisms may be interconnected. Thus, development of high grain iron and zinc cultivars with high yield having different maturity backgrounds is feasible Kumar *et al*., (2012) [19], Gupta *et al*., (2021) [20], Kumar *et al*., (2009) [21], Kumar *et al*., (2010) [22], Reddy *et al*., (2010) [23], Kumar *et al*., (2013) [24], Venkateswarlu *et al*., (2018) [25], Chavan *et al*., (2023) [26], Kumar *et al*., (2012) [19] and Phuke *et al*., (2017) [27].

It was also observed that, both grain iron and zinc contents recorded significant negative association with grain yield which indicated that genetic enhancement for grain iron and zinc content does not have yield penalty Kumar *et al*., (2012) [19]. It was also inferred that, breeding for higher levels of micronutrients can be achieved simultaneously for grain iron and zinc content without compromising on the grain yield Gupta *et al*., (2021) [20] and Thribhuvan *et al*., (2023) [29]. However, on the other hand, contradictory statement regarding positive correlation between grain yield and iron and zinc content was recorded by some researchers Chavan *et al*., (2023) [26]. This contradiction can be due to difference in the season of cultivation. As the studies conducted to improve the rabi sorghum yield did not result in significant gain as compared to the rainy season i.e. kharif sorghum. The major reasons highlighted for low yield in rabi season might be continuously receding soil moisture, poor nutrient availability and low light temperatures Seetharama *et al*., (1990) [30].

It was also observed from the present study that phytic acid had negatively significant correlation with grain yield which may be due to the effect of phytic acid levels on germination, emergence, and seed filling which eventually leads to less grain yield Badigannavar *et al*., (2015) [31]. On the other hand, it was observed that phytic acid had positive correlation with grain zinc and iron content which has contradicted the findings that phytic acid acts as a chelating agent and binds to the micronutrients by making them unavailable Badigannavar *et al*., (2015) [31].

Conclusion

It was inferred from the present study that the parent Parbhani Shakti and the cross CSV-26R X CSV-22R recorded promising performance among all the parents and crosses, respectively which can be used in the further breeding program to enhance the micronutrient contents with optimum yield level. It was also observed that, grain iron and zinc content were significantly and positively correlated with each other and can be simultaneously improved. The development of varieties with high grain iron and zinc content is desirable without compromising on the grain yield, as negative association between grain yield and micro nutrients was recorded. It was also inferred that the low grain yield in the rabi sorghum as compared to the kharif sorghum was attributed to the diverse environmental factors in the rabi season. However, phytic acid content was found to be positively correlated with the micronutrients and negatively associated with grain yield due to effect of phytic acid on the germination of seeds. Therefore, accordingly, recombination breeding strategy methods need to be formulated which will enhance the micronutrient status of the crop along with satisfactory yield level and low level of anti nutritional factors. The present study will thus be useful

for further exploitation of the segregating material and development of new high yielding cultivars along with micronutrient densification and lower antinutritional contents.

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