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Genetic diversity of wheat (*Triticum aestivum* L.) genotypes with grain zinc and iron content for yield and its attributing traits

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ABSTRACT

Wheat (*Triticum aestivum* L.) is crucial for global food security, providing essential calories for about one-third of the world's population and a great source of micronutrients like zinc (Zn) and iron (Fe). This study focuses on the screening of thirty wheat germplasms for Zn and Fe content, and their association with yield and related traits. Analysis of variance showed significant variation among the genotypes for all studied traits including Zn and Fe content. The phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were very close for micronutrient indicating fruitful for selection of these traits, whereas great differences for yield and yield attributing traits. High heritability coupled with high genetic advance was also observed for Zn and Fe content, but low for yield per plant. Genotype BAW897 and DSN117 for Zn, BAW1006 and SADH-22 for Fe and BAW1006 and Sonalika for yield were selected based on the mean performance, and BAW667 was best considering all three traits, suggesting their suitability and adaptability for cultivation to fulfil the agricultural demand. Zn and Fe content showed negative associations with canopy temperature, chlorophyll content yield and its different contributing characters. Principal Component Analysis revealed that zinc had a positive value in PCA1, iron showed a positive value in PCA2, and total yield was positively associated with PCA2, with the first five components explaining 77.1% of the cumulative variance. The thirty genotypes were clustered in four major groups, having maximum number in cluster 1 and minimum in cluster 4. Cluster 1 consists of the most promising genotypes having higher micronutrient content and high yielding ability. The identified genotypes can be utilized in forthcoming breeding to ensure both food and nutritional security in Bangladesh.

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INTRODUCTION

Wheat is a vital cereal crop that plays a significant role in providing essential food calories for approximately one-third of the world's population (IDRC, 2010). The nutritional value of the wheat grain is notably high, containing significant proportions of protein, fats, starch, and minerals (Peterson, 1965). Key micronutrients crucial for human health include iron (Fe), zinc (Zn), iodine (I), selenium (Se), copper (Cu), calcium (Ca), and fluoride (F), alongside various vitamins (Cakmak & Kutman, 2018). Micronutrient malnutrition affects over 3 billion people worldwide, with iron (Fe) and zinc (Zn) deficiencies standing out as predominant factors (Krishnaswamy *et al.*, 2016). Roughly 20.5% of the world's population is estimated to be at risk of zinc deficiency (Wuehler *et al.*, 2005). In Bangladesh, wheat holds the position of the second most important cereal crop following rice (FAO, 2002). At present, zinc deficiency is the primary concern among micronutrient disorders, with over 70% of cultivated soils in Bangladesh showing inadequate levels of zinc (Jahiruddin *et al.*, 1981; FPMU, 2011). Inadequate consumption of zinc results in a range of negative outcomes such as significant weight loss, depression, psychosis, diarrhea, stunted growth and development, changes in reproductive biology, gastrointestinal disturbances, and compromised immunity (Solomons, 2003). Anemia caused by iron deficiency affects approximately one-fourth of the global population (de Benoist *et al.*, 2008). Inadequate iron intake impacts physical growth and cognitive function (Bouis, 2003) as well as reproductive health and productivity in work (Bouis, 2002). Wheat generally has lower-than-ideal levels of micronutrients, with iron (Fe) and zinc (Zn) being particularly deficient (Borrill *et al.*, 2014). The iron content in wheat typically adheres to a standardized range of 29 to 73 mg kg^{-1}, while the zinc content falls within a standardized range of 7 to 85 mg kg^{-1} (Peleg *et al.*, 2009; Shewry & Hey, 2015). To satisfy the nutritional requirements of humans, it is preferred that the average iron (Fe) and zinc (Zn) levels in grain are approximately 40 and 60 mg kg^{-1}, respectively (Ansari & Thapa, 2019). To fulfill the demand for additional micronutrients in food, it is an urgent need to develop micronutrient biofortified varieties. Micronutrient biofortification is largely depends on the availability of germplasm with higher content and the presence of wider genetic diversity among them (Rajshree & Singh, 2018). Genetic variability and character association studies are very important for any breeding programs (Samsuddin, 1985), as they are the key indicators of successful biofortification breeding. Keeping the above facts in mind, the study aimed to assess the local wheat genotypes for Zn and Fe content, and also different morphological and yield attributing traits, and the association among them to select suitable breeding strategies for the development of micronutrient biofortified high yielding wheat variety in Bangladesh.

MATERIALS AND METHODS

Plant Materials and Experimentation

Thirty wheat genotypes namely Sonalika, Ananda, Sourav, Sonora, Kheri, Wuhan, Kalyan Sona, Sebia, Pavan, Chyria, Opata, Sawghat, Ning-3517, O-4, BL-1020, NK-5, KAV-2, DSN-117, SA-2, NE-3, BAW-667, BAW-897, BAW-898, BAW-966, BAW-1008, BAW-1006, DSN-76, SADH-12, SADH-22, and Gourav, were obtained from the Plant Molecular Genetics laboratory of the Genetics and Plant Breeding Department, Bangladesh Agricultural University (BAU), for the experiment. The experiment took place at the Farm Laboratory of the Genetics and Plant Breeding Department, BAU, Mymensingh, from November 2022 to April 2023. The field's coordinates were 24° 75' N latitude and 90° 50' E longitude, with an elevation of 18 meters above sea level. The area features non-calcareous dark grey floodplain soil, sandy loam texture, and a pH of 6.5. The climatic conditions during the experiment featured sparse rainfall and low temperatures typical of the Rabi season. The experiment was conducted using a randomized complete block design (RCBD) with three replications; all agronomic practices like fertilization, irrigation, weeding, etc. were done properly following standard procedures.

Data Collection

Data were collected across different growth stages from ten plants of each plot. Parameters comprised days to germination, 50% heading, 50% flowering, grain filling, and maturity. Additionally, canopy temperature was measured using an infrared thermometer in degrees Celsius, while chlorophyll content was assessed via a SPAD meter. Other metrics included plant height, tillers per plant, flag leaf dimensions, spike length measured in centimeters, hundred grain weight, and total yield determined with an electric balance. Iron and Zinc content underwent standardized analysis (grinding, digestion using HNO_3 and $HCLO_4$, filtering and collecting clear solution) using an atomic absorption spectrophotometer at the agricultural chemistry laboratory of BAU.

Statistical Analysis

The statistical analysis was performed using RStudio statistical software, version 4.0.3, for computing various genetic parameters such as mean square value, a range of genetic parameters such as genotypic variance (GV), phenotypic variance (PV), broad-sense heritability (h^2b), genotypic coefficient of variance (GCV), phenotypic coefficient of variance (PCV), genetic advance (GA), and genetic advance as a percentage of mean (GA%). Additionally, the software facilitated calculations for the mean performance, correlation coefficient, principal component analysis (PCA), and heatmap analysis. MS Office Excel was employed for organizing data, managing records, and creating graphics.

RESULTS

Analysis of Variance (ANOVA), Genetic Variability, Heritability (h^2b) and Genetic Advance

The analysis of variance (Table 1) revealed that the studied genotypes showed significant variation among them concerning all the studied characters, clearly indicates the genotypes were genetically variable and considerable amount of variability existed among them, offered opportunities for further improvement *via* selection. Genetic parameters such as genotypic variance (GV), phenotypic variance (PV), phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability (%), genetic advance (GA) and genetic advance as a percentage of mean (GA%) of the recorded traits were estimated and presented in Table 2. The GCV values were lower than PCV values for all the traits indicating the environmental influence on the expression of the characters. The analysis of various traits in wheat genotypes revealed significant genetic variability and a heritability across different parameters. Notably, traits such as days to grain filling (95.0%) and maturity (97.7%) exhibited high heritability estimates, indicating a strong genetic basis. Additionally, traits related to yield components, such as grain per plant and hundred grain weight, showed substantial genetic variability and significant genetic advances (35.02%, 31.94%), suggesting potential for improvement through breeding programs. The PCV and GCV for Zn and Fe content are almost the same (28.76 & 28.83; 31.31 & 31.31 respectively) with a high heritability (99.5%) and genetic advance, whereas, yield and yield contributing traits have low/moderate heritability with high PCV than GCV.

Mean Performance of the Genotypes

The performance of 30 wheat genotypes exhibited diverse responses across various traits, with certain genotypes demonstrating notably superior performance compared to

Table 1: Mean square values for different studied traits of 30 wheat genotypes

Source of variation	Replication (df=2)	Genotype (df=29)	Error (df=58)
DG	0.178	1.988***	0.327
DFH	8.811	63.551***	3.639
DFF	1.244	115.510***	4.244
DGF	2.344	44.320***	0.758
DM	1.011	41.184***	0.321
CT	1.190	1.582***	0.439
CC	0.875	53.074***	0.281
PH	0.79	370.76***	0.61
NT	0.128	1.311***	0.064
FLL	0.825	29.809***	0.501
FLW	0.029	0.026*	0.013
SL	0.222	3.023***	0.072
NSPS	0.754	5.578***	0.279
GPS	29.831	83.622***	24.968
GPP	1224.3	4591.0***	445.4
HGW	0.094	0.816***	0.039
TY	4.3443	8.3697***	3.1625
FeC	0.18	2365.3***	0.28
ZnC	1.119	223.1***	0.383

'***'=Significant at 0.1% level of probability, '**'=Significant at 1% level of probability, '*'=Significant at 5% level of probability, df=Degree of freedom, DG=Days to germination, DFH=Days to 50% heading, DFF=Days to 50% flowering, DGF=Days to grain filling, DM=Days to maturity, CT=Canopy temperature, CC=Chlorophyll content, PH=Plant height (cm), NT=Number of tillers, FLL=Flag leaf length (cm), FLW=Flag leaf width (cm), SL=Spike length (cm), NSPS=Number of spikelets spike^{-1}, GPS=Grain spike^{-1}, GPP=Grain plant^{-1}, HGW=Hundred grain weight (g), TY=Total yield plant^{-1} (g), FeC=Iron (Fe) content (μg/g), ZnC=Zinc (Zn) content (μg/g).

Table 2: Estimation of genetic parameters for morphological traits in thirty wheat genotypes

Traits	GV (σ^2_p)	PV(σ^2_g)	GCV (%)	PCV (%)	h^2b (%)	GA	GA (%)
DG	0.55	0.88	19.63	24.77	62.9	1.22	32.07
DFH	19.97	23.61	6.99	7.61	84.6	0.84	13.25
DFF	37.08	41.33	9.07	9.58	89.7	11.88	17.71
DGF	14.52	15.27	4.14	4.24	95.0	7.65	8.31
DM	13.62	13.94	3.29	3.33	97.7	7.51	6.70
CT	0.38	0.82	2.85	4.18	46.5	0.86	4.01
CC	17.59	17.87	12.42	12.52	98.4	8.57	25.39
PH	123.39	123.99	11.94	11.97	99.5	22.82	24.54
NT	0.41	0.47	11.22	12.05	86.7	1.23	21.52
FLL	9.76	10.27	10.40	10.66	95.1	6.27	20.91
FLW	0.004	0.02	7.52	15.21	24.4	0.06	7.66
SL	0.98	1.05	11.41	11.81	93.2	1.97	22.69
NSPS	1.76	2.04	10.53	11.34	86.3	2.54	20.17
GPS	19.55	44.51	13.08	19.74	43.9	6.03	17.86
GPP	1381.88	1827.26	19.54	22.47	75.6	66.59	35.02
HGW	0.25	0.29	16.62	17.82	86.9	0.97	31.94
FeC	788.34	788.62	31.31	31.31	99.9	57.82	64.48
ZnC	74.23	74.62	28.76	28.83	99.5	17.70	59.09
YPP	1.73	4.89	22.28	37.43	35.44	1.61	27.32

DG=Days to germination, DFH=Days to 50% heading, DFF=Days to 50% flowering, DGF=Days to grain filling, DM=Days to maturity, CT=Canopy temperature, CC=Chlorophyll content, PH=Plant height (cm), NT=Number of tillers, FLL=Flag leaf length (cm), FLW=Flag leaf width (cm), SL=Spike length (cm), NSPS=Number of spikelets spike^{-1}, GPS=Grain spike^{-1}, GPP=Grain plant^{-1}, HGW=Hundred grain weight (g), FeC=Iron (Fe) content (μg/g), ZnC=Zinc (Zn) content (μg/g), YPP=Total yield plant^{-1} (g).

others, as depicted in Supplementary Table 1. For instance, in terms of days to germination, which ranged from 3 DAS to 7 DAS, DSN-117, BAW-966, and Wuhan exhibited outstanding performance. Similarly, for days to 50% heading, spanning from 55.667 DAS to 73.667 DAS, DSN-117 and Gourav emerged as top performers. Likewise, BAW-898 and DSN-117 excelled in days to 50% flowering, ranging from 58.33 to 82.33 DAS, while DSN-117 and BAW-898 displayed superior performance in days to grain filling, ranging from 86.67 to 100.00 DAS. Additionally, canopy temperature ranging from 20.45 °C to 22.95 °C showcased genotype Ananda with the highest canopy temperature, while chlorophyll content, varying from 23.79 to 43.087, demonstrated Gourav with the highest levels. In terms of plant height, ranging from 81.0 cm to 118.667 cm, Sebia showcased the tallest genotype, while the number of tillers per plant, ranging from 4 to 7, was highest in SADH-22, BL-1020, and Sonalika. Furthermore, regarding flag leaf length, spanning from 24.87 cm to 38.87 cm, BAW-667, NE-3, and SA-2 exhibited the longest, while for flag leaf width, ranging from 0.598 cm to 1.03 cm, Sonora and BAW-1006 displayed the widest. Moreover, spike length, ranging from 6.33 cm to 11.4 cm, demonstrated that genotypes such as SA-2, Ananda, and Sonalika exhibited the longest spikes (Figure 1a), while the number of spikelets per spike, ranging from 9.3 to 15.27 was highest in Chyria. Grains per spike, ranging from 23.57 to 45.73 (Figure 1b), showcased BAW-1006 and Sonora with the highest counts, while hundred grain weight, ranging from 1.56 g to 4.137 g, demonstrated Chyria as the highest performer. Regarding micronutrient content, iron content, ranging from 59.85 μg/g to 178.7 μg/g, displayed SADH-22 and BAW-1006 (Figure 1d) with the highest levels, while zinc content, ranging from 17.88 μg/g to 50.173 μg/g, exhibited BAW-897 and DSN-117 (Figure 1c) as top performers. Finally, BAW-1006, Sonalika, and BL-1020 demonstrated the highest total yield per plant, while Chyria and Sebia exhibited the lowest yield, ranging from 2.08 g (Chyria) to 8.75 g (BAW 1006) (Figure 1e). Overall, these findings highlight the diverse genetic potential of different wheat genotypes for various agronomic and nutritional traits.

Correlation Coefficient and Principal Component Analysis (PCA)

The zinc content displayed a significant negative association with both chlorophyll content and canopy temperature, while iron content showed a significant negative correlation only with canopy temperature (Figure 2). Although, zinc and iron exhibited a positive but statistically insignificant correlation with each other, suggesting the potential for simultaneous enhancement of both nutrients. Total yield per plant demonstrated a highly significant positive correlation with grain per spike, grain per plant, number of tillers, flag leaf width, and hundred grains weight and non-significant correlation with iron content, but a significant negative correlation with days to 50% heading, days to 50% flowering, days to grain filling, and days to maturity and non-significant correlation with zinc content. Hundred grain weight exhibited a significant positive correlation with yield per plant and number of tillers, but

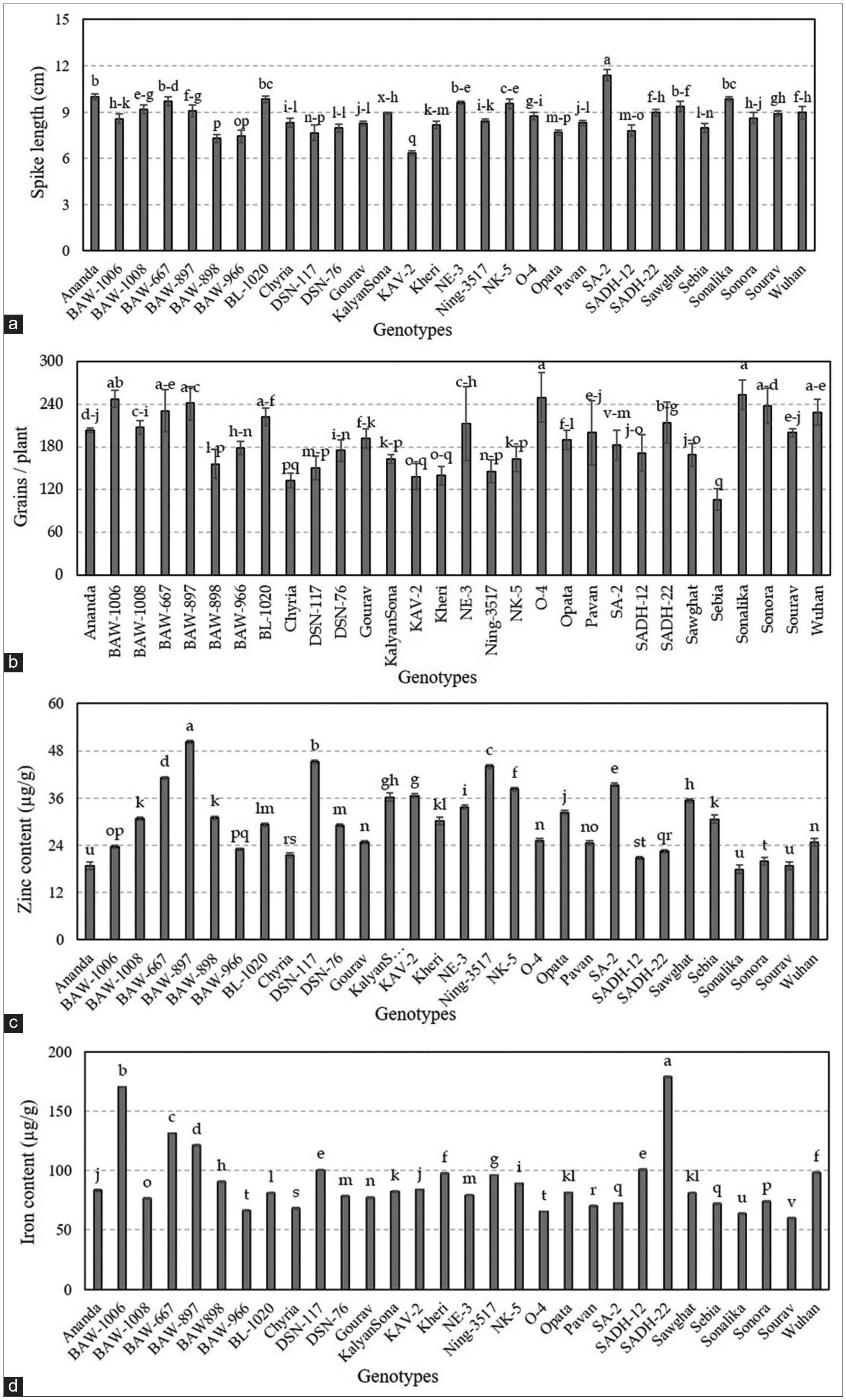

Figure 1: Bar graph showing the mean performance of thirty wheat genotypes for a) spike length, b) grains/spike, c) Zinc content, d) Iron content and e) yield per plant. Plotted data represent the average of three replicates of each genotype (n=5). Vertical bar indicates standard error and different letter states the significant differences at 5% level of probability following Tukey's test

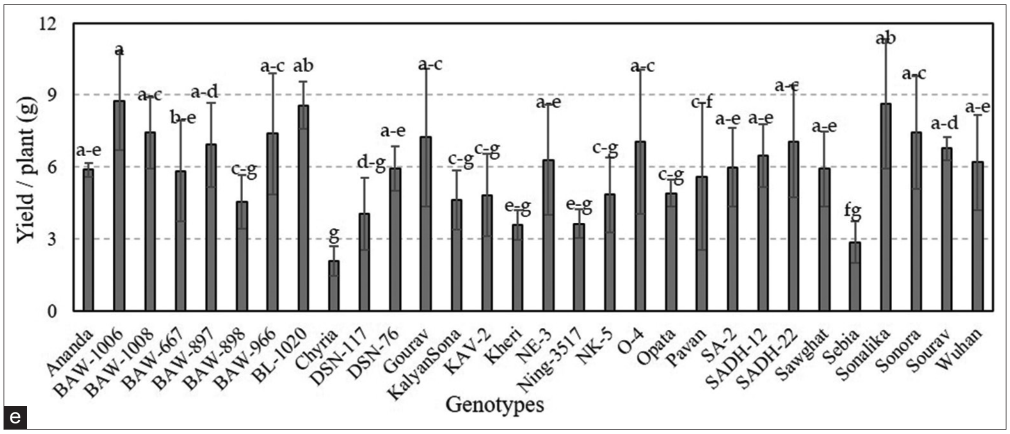

Figure 1: *(Continued)*

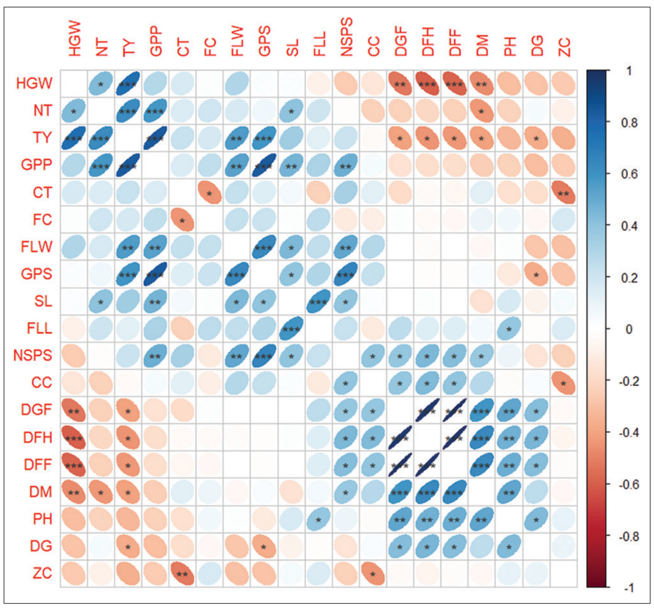

Figure 2: Pearson correlation coefficient matrix among the studied traits of thirty wheat genotypes. Red and blue color indicate negative and positive correlations, respectively, with increasing color intensity reflects a higher coefficient. *, ** and *** indicates significant at 5%, 1% and 0.1% level of significance

Table 3: Eigen values and percentage of variation for corresponding 19 characters in 30 wheat genotypes

PCA	Eigen value	Variance (%)	Cumulative variance (%)
PCA1	5.51	29.04	29.04
PCA2	4.17	21.95	50.99
PCA3	2.33	12.30	63.30
PCA4	1.40	7.38	70.69

negative associations with days to 50% heading, days to 50% flowering, days to grain filling, and days to maturity. Grain per spike showed a highly significant positive correlation with total yield per plant, grain per plant, flag leaf width, and number of spikelets per spike.

Principal Component Analysis (PCA) was employed to investigate the correlations among different traits. The first five principal components displayed eigenvalues exceeding 1, with the first component (PCA1) elucidating 30.5% of the variance, and the second component (PCA2) describing 19.0%. Collectively, up to the fifth component (PC5), 77.1% of the cumulative variance was explained (Table 3). The PC1 exhibited about 29% of total variability positively explained by NSPS, CC, DM, DG, PH, DFFH and Zn content, whereas, GPP, GPS, FLW, SL, TY, NT, HGW and Fe content. In case of PC2 covering about 22% variability and most of the traits are positively contributed except DG, HGW and Zn content. Zn content exhibited a positive value in PCA1, while iron displayed a positive value in PCA2. Additionally, total yield was positively associated with PCA2. Thirty genotypes were grouped into four major clusters C1, C2, C3 and C4. C1 consists of maximum 14 (46.33%), C4 is 8 (26.66%), C2 is 4 (13.33%) and C3 is also 4 (13.33%) genotypes (Figure 3).

Heatmap Cluster Analysis

In the hierarchical clustering analysis, all the genotypes were segregated into two main clusters, each containing different subgroups as indicated in the heatmap dendrogram (Figure 4). While most genotypes exhibited average Z-scores (white color) for various elements, some displayed above-average Z-scores (dark orange color), while others showed below-average Z-scores (dark black colors). The second sub-cluster primarily comprised zinc and iron, while the fourth sub-cluster included total yield, grain per plant and hundred grain weight, among others. BAW-1006 and SADH-22 demonstrated higher iron content, whereas Ning-3517, DSN-117, and SA-2 exhibited elevated zinc content (Figure 4). Notably, BAW-1006, Sonalika, and BL-1020 showed superior performance in terms of total yield.

DISCUSSION

The analysis of variance (ANOVA) for the investigated wheat genotypes revealed highly significant variation (p<0.001) across all the traits especially zinc (Zn) and iron (Fe), as depicted in Table 1. Thus, breeding efforts can be made to improve different quantitative traits including micronutrient Zn and Fe in wheat grain using the studied genotypes. Wild relatives and cultivated genotypes having high genetic diversity and

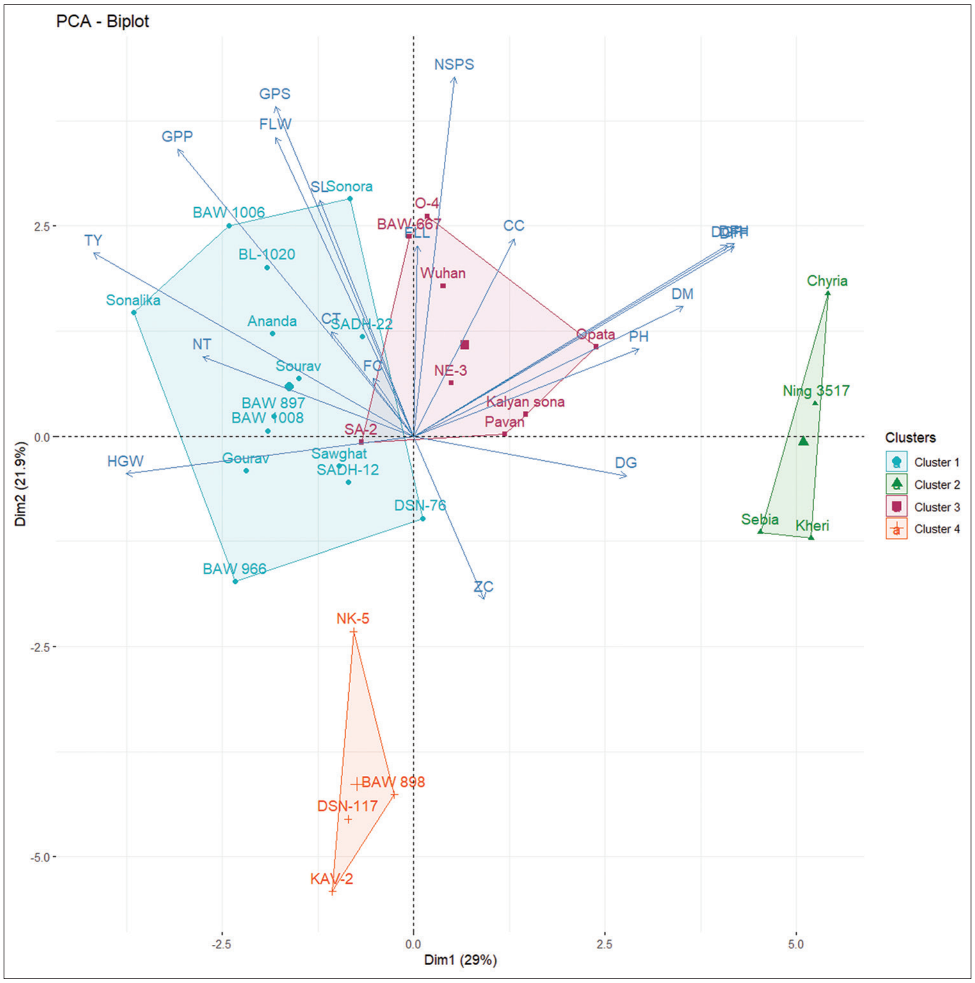

Figure 3: PCA-Biplot of 19 morpho-physiological traits in 30 wheat genotypes. Genotypes positioned in different ordinates based on dissimilarity. Vector length indicate the contribution of the traits in genotype diversity. Different color showed clustering of the genotypes

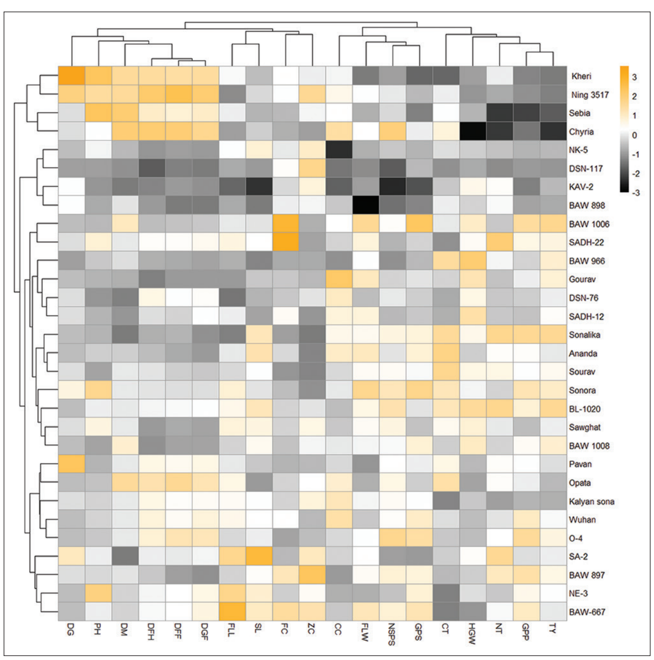

Figure 4: Heatmap of studied morpho-physiological traits along with genotype performance indicates trait and genotype association together

variation for micronutrient like Zn, Fe, Se content provide great source of genetic bio-fortification breeding for those traits (Tripathi *et al.*, 2011; Gupta *et al.*, 2022). In 2006, a fundamental initiative was taken by Harvestplus for the estimation of genetic variability of micronutrients like Zn, Fe and Se content in six crops and to facilitate varietal development in the breeding programs. Generally a wide range of variation in grain Zn and Fe concentration was also observed in land races and unadopted wheat germplasms (Heidari *et al.*, 2017; Goel *et al.*, 2018). Recently also a novel source of variation was observed for grain Zn and Fe content in advanced breeding lines created from the crosses of different landraces in United Kingdom (Khokhar *et al.*, 2020). Heritability is an inherent traits and very much useful for selection in breeding from one generation to next (Pujar *et al.*, 2020). In this study high heritability coupled with high GA were observed for Zn and Fe content, indicating a suitable trait of selection for breeding. High variability is also reported for both iron and zinc concentration in wild emmer wheat (Peleg *et al.*, 2008; Gomez-Becerra *et al.*, 2010), followed by durum wheat (Velu *et al.*, 2011; Badakhshan *et al.*, 2013). In pearl millet, high heritability for Zn and Fe content was also reported by Vagadiya *et al.* (2014). Micronutrient content is generally negatively correlated with the yield in wheat

(Badakhshan *et al.*, 2013). Several studies showed the increase of grain yield was accompanied with significant decrease in grain micronutrient concentration (Amiri *et al.*, 2015). A positive correlation between Fe and Zn concentrations and strong association of grain iron and protein content of wheat grains in our experiment was concurrent with some previous studies performed on bread wheat (Morgounov *et al.*, 2007; Zhao *et al.*, 2009; Ali *et al.*, 2015; Pandey *et al.*, 2016), Similarly, heat-map based grouping results are also consistent, showing a robust positive correlation between iron (Fe) and zinc (Zn) content (Gu *et al.*, 2016; Gupta *et al.*, 2020). Positive and significant correlation for Zn and Fe content was also reported in millet (Rai *et al.*, 2014). Principal Component Analysis (PCA) displays the importance and contribution of different component of variance information (Sanni *et al.*, 2012), in our study first five components contribute most of the variability for studied traits among the genotypes. Clustering of genotypes will be helpful to plan breeding scheme for micronutrient content and also yield, as it facilitates the selection of diverse genotypes for getting maximum heterosis (Pujar *et al.*, 2020). Hierarchical grouping techniques are a useful tool to categorize the Indian and Turkish wheat genotypes into two primary clusters (Morgounov *et al.*, 2007). Our studied 30 genotypes were clustered into four groups having different number of germplasms, C1 and C4 are more diverse in terms of micronutrient content, showing their potential use in breeding program to enhance micronutrient content.

CONCLUSION

The study underscores the intricate relationships among zinc, iron, and yield content in wheat genotypes, highlighting superior performers like BAW-1006, Ning-3517, and BL-1020. The study outcomes underscore a broad spectrum of variations across the examined traits. Basic selection techniques could effectively enhance these characteristics. Furthermore, certain genotypes might be deemed promising candidates for parental roles in breeding programs, especially with respect to their nutritional attributes and yield contributions.

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AUTHORS' CONTRIBUTION

GHMS: Design, formulation, supervision of experiment and writing of manuscript. SKS, TA, KMM: Conducting experiments, Data collection, Lab analysis, and writing of manuscript. SA: Data analysis and manuscript preparation.

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SUPPLEMENTARY TABLE

Supplementary Table 1: Mean performance with lettering of thirty wheat genotypes based on 19 yield attributing traits

DG			DFH			DFF			DGF			DM		
VAR	Mean	Group	DFH	Mean	Group	VAR	Mean	Group	VAR	Mean	Group	VAR	Mean	Group
Kheri	6.667	a	Ning3517	73.667	a	Ning3517	82.333	a	Ning3517	100.000	a	Sebia	119.667	a
Pavan	5.667	b	Chyria	73.333	a	Chyria	79.333	ab	Chyria	98.333	b	Chyria	119.333	a
Ning3517	5.333	bc	Kheri	70.667	ab	Opata	77.000	bc	Kheri	97.667	bc	Kheri	117.667	b
SA-2	4.667	cd	Opata	69.667	bc	Kheri	75.667	c	Opata	96.667	cd	Ning-3517	117.667	b
Sonora	4.333	de	Sebia	68.333	bcd	0-4	74.667	cd	0-4	96.333	cde	Opata	117.667	b
BAW898	4.000	def	0-4	67.667	bcde	Sebia	72.000	de	Sebia	95.667	def	BAW-1006	116.000	c
KAV-2	4.000	def	Wuhan	67.333	cdef	Pavan	70.000	ef	Wuhan	95.000	efg	BAW-1008	115.333	c
BAW897	3.667	efg	DSN-76	66.667	cdefg	Wuhan	69.667	ef	BAW-667	94.667	fgh	BL-1020	112.000	d
Chyria	3.667	efg	KalyanSona	66.667	cdefg	KalyanSona	69.333	efg	NE-3	94.333	fghi	KalyanSona	112.000	d
DSN-76	3.667	efg	Pavan	66.667	cdefg	SADH-22	69.333	efg	Pavan	94.333	fghi	0-4	112.000	d
KalyanSona	3.667	efg	SADH-22	65.667	defgh	BAW-667	69.000	efg	SADH-22	94.000	ghi	Pavan	112.000	d
SADH-12	3.667	efg	BAW-667	64.667	efghi	NE-3	69.000	efg	KalyanSona	93.667	ghij	SADH-22	112.000	d
SADH-22	3.667	efg	BL-1020	64.333	fghij	DSN-76	68.667	efgh	DSN-76	93.333	hij	Sawghat	112.000	d
Sawghat	3.667	efg	NE-3	64.333	fghij	BL-1020	68.000	fghi	BL-1020	93.000	ij	Sonora	112.000	d
Sebia	3.667	efg	SA-2	64.000	ghij	SA-2	67.000	fghij	SADH-12	93.000	ij	Sourav	112.000	d
Sourav	3.667	efg	Sonora	63.333	hijk	Sonora	66.000	ghijk	SA-2	92.333	jk	Wuhan	112.000	d
Ananda	3.333	fg	Sourav	63.333	hijk	SADH-12	65.333	hijkl	Sonora	91.333	kl	BAW-897	111.667	de
BAW-667	3.333	fg	SADH-12	63.000	hijk	Sourav	65.000	ijklm	BAW1006	90.333	l	BAW-898	111.667	de
BAW1006	3.333	fg	BAW897	62.000	ijkl	BAW1006	64.000	jklmn	Sourav	90.333	l	BAW-966	111.333	def
BAW1008	3.333	fg	Ananda	61.333	jklm	Sonalika	62.667	klmn	BAW1008	88.667	m	BAW-667	111.000	ef
BL-1020	3.333	fg	BAW1006	61.333	jklm	Ananda	62.000	lmno	BAW966	88.667	m	NE-3	111.000	ef
Gourav	3.333	fg	BAW966	60.333	klmn	BAW966	62.000	lmno	Sawghat	88.667	m	Ananda	110.667	f
NE-3	3.333	fg	Sonalika	60.333	klmn	Sawghat	62.000	lmno	Ananda	88.000	mn	NK-5	109.667	g
NK-5	3.333	fg	BAW898	59.333	lmn	BAW1008	61.667	mnop	Gourav	88.000	mn	Gourav	109.333	g
0-4	3.333	fg	Sawghat	59.333	lmn	NK-5	61.333	nop	NK-5	88.000	mn	SADH-12	108.000	h
Opata	3.333	fg	NK-5	59.000	lmn	BAW897	61.000	nop	BAW897	87.667	mn	DSN-117	107.667	hi
Sonalika	3.333	fg	BAW1008	58.667	mno	Gourav	60.667	nop	KAV-2	87.667	mn	DSN-76	107.000	ij
Wuhan	3.333	fg	KAV-2	58.000	no	KAV-2	60.667	nop	Sonalika	87.667	mn	KAV-2	106.667	j
BAW966	3.000	g	Gourav	57.667	no	DSN-117	58.667	op	BAW898	86.667	n	SA-2	106.667	j
DSN-117	3.000	g	DSN-117	55.667	o	BAW898	58.333	p	DSN-117	86.667	n	Sonalika	106.667	j

CT			CC			PH			NT			FLL		
VAR	Mean	Group	VAR	Mean	Group	VAR	Mean	Group	VAR	Mean	Group	VAR	Mean	Group
Ananda	22.950	a	Gourav	43.087	a	Sebia	118.667	a	SADH-22	7.067	a	BAW-667	38.867	a
Sourav	22.860	ab	Chyria	39.767	b	Kheri	118.533	a	BL-1020	6.867	ab	NE-3	35.493	b
Sonalika	22.767	ab	Wuhan	39.377	bc	NE-3	113.733	b	Sonalika	6.833	ab	SA-2	35.400	b
BAW-966	22.700	abc	KalyanSona	38.577	cd	Sonora	111.633	c	SA-2	6.800	ab	Sonora	32.647	c
Opata	22.617	abc	DSN-76	37.960	de	Ning-3517	109.613	d	BAW-897	6.467	bc	Sawghat	32.307	cd
Sonora	22.483	abcd	Ananda	37.687	ef	SADH-22	102.033	e	Sourav	6.167	cd	SADH-22	32.267	cd
BL-1020	22.467	abcd	Opata	37.500	ef	Sawghat	100.560	f	Ananda	6.000	de	Opata	32.060	cd
Chyria	22.200	abcde	SADH-12	37.030	fg	Chyria	95.547	g	KAV-2	6.000	de	Wuhan	31.847	cde
Pavan	22.067	abcdef	Sonalika	36.213	gh	NK-5	94.133	h	Opata	6.000	de	BL-1020	31.753	cde
Sawghat	21.983	abcdef	Ning-3517	36.163	h	BAW-667	93.467	h	Pavan	6.000	de	KalyanSona	31.367	def
Sebia	21.800	bcdefg	Sebia	35.000	i	BL-1020	93.400	h	0-4	5.933	def	NK-5	30.800	efg
Ning-3517	21.683	cdefgh	BAW-1006	34.863	ij	SA-2	93.400	h	BAW-667	5.867	defg	Kheri	30.533	fg
BAW-898	21.683	cdefgh	Kheri	34.123	jk	BAW-897	90.133	i	BAW-898	5.867	defg	SADH-12	30.227	fgh
BAW-1006	21.633	cdefgh	BAW-1008	33.733	kl	KalyanSona	89.733	i	BAW-966	5.800	defgh	Sebia	30.173	gh
Wuhan	21.517	defghi	Sonora	33.687	kl	Wuhan	89.733	i	Kheri	5.767	defgh	BAW-1006	30.000	gh
NK-5	21.500	defghi	0-4	32.910	lm	Ananda	88.867	ij	Sawghat	5.733	efgh	Ananda	29.947	gh
Gourav	21.417	defghi	Sourav	32.800	mn	BAW-966	88.167	j	Wuhan	5.667	efgh	Pavan	29.833	gh
BAW-1008	21.383	efghi	Pavan	32.767	mn	0-4	88.067	j	BAW-1008	5.533	fghi	0-4	29.333	h
SA-2	21.383	efghi	SA-2	32.643	mn	Opata	87.667	j	Sonora	5.533	fghi	Sourav	29.333	h
0-4	21.350	efghi	SADH-22	32.633	mn	BAW-1006	86.160	k	BAW-1006	5.467	ghi	BAW-897	29.200	h
KAV-2	21.333	efghi	BAW-667	32.070	mno	SADH-12	85.567	k	DSN-76	5.467	ghi	BAW-1008	29.120	h
BAW-897	21.200	efghi	BAW-898	32.067	mno	Sonalika	85.473	k	NE-3	5.467	ghi	BAW-966	27.620	i
SADH-12	21.100	fghi	Sawghat	31.967	no	Gourav	85.167	k	SADH-12	5.467	ghi	BAW-898	27.467	i
DSN-76	21.053	fghi	BL-1020	31.423	o	Pavan	85.133	k	Gourav	5.400	hi	DSN-117	27.467	i
DSN-117	20.833	ghi	NE-3	30.477	p	BAW-898	83.600	l	NK-5	5.400	hi	Chyria	26.960	ij
SADH-22	20.817	ghi	BAW-897	29.577	q	BAW-1008	83.400	l	Ning-3517	5.200	ij	Gourav	26.860	ij
KalyanSona	20.700	hi	BAW-966	29.053	q	Sourav	83.400	l	KalyanSona	5.133	ij	Ning-3517	26.180	jk
NE-3	20.683	hi	DSN-117	27.510	r	DSN-117	82.467	l	DSN-117	4.933	j	Sonalika	25.860	jkl
BAW-667	20.667	hi	KAV-2	26.387	s	KAV-2	81.133	m	Chyria	4.267	k	DSN-76	25.400	kl
Kheri	20.453	i	NK-5	23.790	t	DSN-76	81.000	m	Sebia	4.267	k	KAV-2	24.867	l

(Contd...)

Supplementary Table 1: *(Continued)*

FLW			Spike Lenth			NSPS			GPS			GPP		
VAR	Mean	Group	VAR	Mean	Group	VAR	Mean	Group	VAR	Mean	Group	VAR	Mean	Group
Sonora	1.033	a	SA-2	11.400	a	Chyria	15.273	a	BAW-1006	45.737	a	Ananda	203.467	defghij
BAW-1006	1.023	a	Ananda	10.000	b	O-4	14.863	ab	Sonora	43.070	ab	BAW-1006	247.467	ab
BAW-667	0.980	ab	Sonalika	9.840	bc	BL-1020	14.357	bc	O-4	41.333	abc	BAW-1008	207.467	cdefghi
Ananda	0.973	ab	BL-1020	9.833	bc	Sonora	14.153	bcd	BAW-667	40.177	abcd	BAW-667	230.733	abcde
Gourav	0.967	abc	BAW-667	9.687	bcd	BAW-667	13.763	cde	Wuhan	38.693	abcde	BAW-897	241.400	abc
SADH-22	0.940	abcd	NE-3	9.600	bcde	BAW-897	13.760	cde	Ananda	38.153	abcdef	BAW-898	156.067	lmnop
SADH-12	0.940	abcd	NK-5	9.533	cde	Sonalika	13.553	cdef	NE-3	38.017	abcdef	BAW-966	178.933	hijklmn
BL-1020	0.933	abcd	Sawghat	9.367	def	Sawghat	13.507	cdef	BAW-1008	37.947	abcdef	BL-1020	222.067	abcdef
Sonalika	0.920	abcde	BAW-1008	9.167	efg	Opata	13.430	def	BAW-897	37.613	abcdef	Chyria	132.733	pq
BAW-897	0.913	abcde	BAW-897	9.067	fg	KalyanSona	13.233	efg	Sonalika	37.613	abcdef	DSN-117	150.933	mnop
Sawghat	0.907	abcde	SADH-22	8.967	fgh	Sourav	13.230	efg	KalyanSona	35.067	bcdefg	DSN-76	175.000	ijklmn
Sourav	0.907	abcde	Wuhan	8.967	fgh	BAW-1006	13.117	efgh	Sourav	34.777	cdefgh	Gourav	192.200	fghijk
Chyria	0.900	abcde	KalyanSona	8.947	fgh	Wuhan	13.090	efgh	Pavan	33.940	cdefgh	KalyanSona	163.333	klmnop
SA-2	0.897	abcde	Sourav	8.900	gh	Pavan	13.020	efghi	Chyria	33.787	cdefgh	KAV-2	138.600	opq
BAW-966	0.893	abcde	O-4	8.733	ghi	BAW-1008	12.797	fghij	Gourav	33.333	cdefgh	Kheri	139.733	opq
O-4	0.893	abcde	Sonora	8.600	hij	Ning-3517	12.563	ghijk	BL-1020	33.313	cdefgh	NE-3	212.933	cdefgh
Ning-3517	0.887	abcde	BAW-1006	8.567	hijk	DSN-76	12.537	ghijk	SADH-22	32.380	defghi	Ning-3517	145.900	nop
BAW-1008	0.880	abcde	Ning-3517	8.427	ijk	Ananda	12.467	ghijk	DSN-76	31.983	efghi	NK-5	163.333	klmnop
KalyanSona	0.880	abcde	Chyria	8.320	ijkl	Sebia	12.330	hijk	Opata	31.840	efghi	O-4	249.400	a
Wuhan	0.827	bcde	Pavan	8.287	jkl	Gourav	12.193	ijkl	SADH-12	31.490	efghij	Opata	190.165	fghijkl
DSN-76	0.820	bcde	Gourav	8.267	jkl	NK-5	12.000	jklm	Sawghat	31.063	efghij	Pavan	199.933	efghij
NE-3	0.800	bcde	Kheri	8.160	klm	NE-3	11.937	jklm	DSN-117	30.457	fghij	SA-2	182.800	ghijklm
NK-5	0.800	bcde	DSN-76	7.967	lmn	SADH-12	11.750	klmn	BAW-966	30.447	fghij	SADH-12	171.400	jklmno
Opata	0.800	bcde	Sebia	7.967	lmn	SADH-22	11.710	klmn	Ning-3517	30.353	fghij	SADH-22	214.333	bcdefg
Sebia	0.800	bcde	SADH-12	7.800	mno	Kheri	11.333	lmno	NK-5	30.187	fghij	Sawghat	169.000	jklmno
KAV-2	0.780	cdef	Opata	7.667	nop	SA-2	11.320	mno	SA-2	28.467	ghij	Sebia	106.400	q
Pavan	0.767	def	DSN-117	7.660	nop	BAW-966	11.020	nop	BAW-898	27.070	ghij	Sonalika	253.133	a
DSN-117	0.760	def	BAW-966	7.440	op	BAW-898	10.487	op	Sebia	26.783	hij	Sonora	237.467	abcd
Kheri	0.740	ef	BAW-898	7.280	p	DSN-117	10.183	p	Kheri	25.103	ij	Sourav	200.267	efghij
BAW-898	0.593	f	KAV-2	6.333	q	KAV-2	9.300	q	KAV-2	23.573	j	Wuhan	228.733	abcde

HGW			Iron Content			Zinc Content			Yield/Plant		
VAR	Mean	Group	Gen	Mean	Group	VAR	Mean	Group	VAR	Mean	Group
Ananda	2.890	ijk	Ananda	83.270	j	Ananda	18.730	u	Ananda	5.880	abcde
BAW-1006	3.550	bcdef	BAW-1006	170.260	b	BAW-1006	23.623	op	BAW-1006	8.753	a
BAW-1008	3.580	bcde	BAW-1008	76.370	o	BAW-1008	30.723	k	BAW-1008	7.430	abc
BAW-667	2.513	lm	BAW-667	131.280	c	BAW-667	41.093	d	BAW-667	5.833	bcde
BAW-897	2.883	ijk	BAW-897	121.180	d	BAW-897	50.173	a	BAW-897	6.930	abcd
BAW-898	2.900	ijk	BAW898	90.520	h	BAW-898	30.993	k	BAW-898	4.553	cdefg
BAW-966	4.137	a	BAW-966	66.270	t	BAW-966	22.963	pq	BAW-966	7.383	abc
BL-1020	3.853	ab	BL-1020	81.130	l	BL-1020	29.180	lm	BL-1020	8.573	ab
Chyria	1.567	n	Chyria	68.050	s	Chyria	21.470	rs	Chyria	2.080	g
DSN-117	2.640	klm	DSN-117	100.380	e	DSN-117	45.160	b	DSN-117	4.060	defg
DSN-76	3.373	efg	DSN-76	78.510	m	DSN-76	28.993	m	DSN-76	5.927	abcde
Gourav	3.703	bcd	Gourav	77.320	n	Gourav	24.733	n	Gourav	7.237	abc
KalyanSona	2.830	ijkl	KalyanSona	82.080	k	KalyanSona	36.240	gh	KalyanSona	4.640	cdefg
KAV-2	3.347	efg	KAV-2	84.100	j	KAV-2	36.610	g	KAV-2	4.837	cdefg
Kheri	2.553	lm	Kheri	97.650	f	Kheri	30.110	kl	Kheri	3.583	efg
NE-3	2.984	hij	NE-3	79.220	m	NE-3	33.720	i	NE-3	6.300	abcde
Ning-3517	2.499	m	Ning-3517	96.100	g	Ning-3517	43.990	c	Ning-3517	3.637	efg
NK-5	2.877	ijk	NK-5	89.090	i	NK-5	38.200	f	NK-5	4.850	cdefg
O-4	2.770	jklm	O-4	65.440	t	O-4	25.170	n	O-4	7.050	abc
Opata	2.600	klm	Opata	81.600	kl	Opata	32.370	j	Opata	4.903	cdefg
Pavan	2.747	jklm	Pavan	70.190	r	Pavan	24.560	no	Pavan	5.593	cdef
SA-2	3.243	fgh	SA-2	72.450	q	SA-2	39.290	e	SA-2	5.993	abcde
SADH-12	3.753	bc	SADH-12	100.740	e	SADH-12	20.743	st	SADH-12	6.470	abcde
SADH-22	3.293	efgh	SADH-22	178.700	a	SADH-22	22.433	qr	SADH-22	7.060	abc
Sawghat	3.483	cdef	Sawghat	81.240	kl	Sawghat	35.350	h	Sawghat	5.923	abcde
Sebia	2.703	jklm	Sebia	71.860	q	Sebia	30.630	k	Sebia	2.870	fg
Sonalika	3.340	efg	Sonalika	63.770	u	Sonalika	17.880	u	Sonalika	8.633	ab
Sonora	3.107	ghi	Sonora	73.880	p	Sonora	19.960	t	Sonora	7.447	abc
Sourav	3.393	defg	Sourav	59.850	v	Sourav	18.830	u	Sourav	6.767	abcd
Wuhan	2.770	jklm	Wuhan	98.000	f	Wuhan	24.780	n	Wuhan	6.187	abcde