



Genetic Divergence in Micronutrient Rich wheat– A Tool to Identify Diverse Parents

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To Study genetic divergence in the micronutrient rich lines to identify diverse parents for hybridization.

Study Design: Randomized Complete Block Design

Place and Duration of Study: Division of Plant Breeding and Genetics, She-e-Kashmir University of Agricultural Sciences and Technology of Jammu during *rabi* 2019-20.

Methodology: Nature and magnitude of variability in forty-nine zinc/iron rich genotypes along with three commercial varieties with stripe rust resistance could be grouped into five clusters using D^2 -Statistics.

Results: Fifty-two lines were placed in five cluster with most of the micronutrient rich lines lying in cluster I except for HP-44, HP 49, HP 14 and HP 13 placed in cluster II, III, IV and V respectively indicating them to be divergent than the rest. Further HD 3086, RSP 561 and JAUW-683 were in the cluster I indicating them to be less diverse with respect to traits under study. The traits that contributed most towards divergence were grain yield per plant (25) percent followed by number of tillers per plant (20.7) percent and 1000 grain weight (14) percent zinc and iron add little contribution of 8 and 7 percent respectively to the total divergence hence could not classified the genotypes into different cluster based on inter cluster distance Cluster III and Cluster V had the

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greatest inter-cluster distance, followed by Cluster III and Cluster IV, Cluster II and Cluster V, and Cluster II and Cluster IV.

Conclusion: Developing wheat varieties with enhanced content of iron and zinc is one of the most sought objectives in a present world to alleviate micronutrient malnutrition. Modern wheat varieties exhibit little diversity in zinc and iron levels in the grain, but large-scale screening has found significant amounts of zinc and iron in wild relatives and progenitors of cultivated wheat.

Keywords: *Wheat; cluster; genetics contribution; PCA; randomized block design.*

1. INTRODUCTION

Wheat grains have a unique place in human diet as it fulfills 55 per cent of carbohydrate and 20 per cent of calorific demand of world population [1]. However they are deficient in micronutrient like Zn and Fe [2].

The World Health Organization (WHO) estimates that over 820 million people are hungry, two billion are deficient in micronutrients, and another two billion are fat or overweight. [3]. Combating micronutrient malnutrition through fortified food enriched with micronutrients is important but not in the reach of common people, so the most economically viable strategy is the availability of staple food crop varieties enriched in micronutrients. The identification of various lines/genotype that can be used in hybridization is required for the development of varieties in any crop. With this background, preliminary studies on genetic diversity of micronutrient rich lines obtained from Harvest Plus breeding programme along with commercial varieties was done using Mahalanobis's D^2 which aids for identification of parents required for certain objectives of breeding. Multivariate analysis is a useful method for assessing the proportionate contribution of various components to overall divergence at intra- and inter-cluster levels, as well as quantifying the degree of divergence between populations at the genotypic level. As a result, the current study has the following aims: 1) to investigate genetic diversity in wheat germplasm for several morph physiological and micronutrient properties, and 2) to select genotypes with high micronutrient content (Zn & Fe) based on genetic diversity.

2. MATERIALS AND METHODS

The experimental material for this investigation included fifty-two genotypes of wheat (*Triticum aestivum* L.) including forty nine zinc and / or iron enriched Harvest plus genotypes along with three adapted varieties viz., HD3086, JAUW 683, RSP 561. The experiment was laid down in Randomized Block Design (RBD) for during *Rabi*

season (2019–20) with three replication at the research farm of SKUAST-Jammu, India (32°40N and 74°48E; having Sub-tropical climate with cold winter and dry summers) under normal fertilization regime (150 Kg N+80 Kg P_2O_5 +60 Kg K_2O Kg/ha) with spacing of 20 x 10 cm. Data of morpho-physiological traits viz., plant height, number of tillers per plant, flag leaf area, spikelets per spike and grain yield per plant was recorded from mean of five randomly selected plants from each genotype. Days to 50 percent flowering, days to maturity and 1000 grain weight were recorded on plot basis. Further three sets of zinc and iron estimation from each genotype was utilized for micronutrient profiling [4].

2.1 Micronutrients Profiling for Zinc and Iron

The micronutrient content (Zn & Fe) was determined by using the atomic absorption spectrophotometer (AAS) instrument as described by Datta et al., 2017 [4].

2.2 Statistical Analysis

Statistical analyses were done using the Windostat version 9.3. D^2 -statistic given by Mahalanobis [5] was used to measure genetic distance among the test entries. Tocher's method given by Rao [6] was used for clustering while the formula given by Singh and Choudhary (1985) was used to calculate intra and inter cluster distance.

3. RESULT AND DISCUSSION

The genotypes may be divided into five clusters (Table 1) based on the degree of divergence among genotypes, with moderate diversity among genotypes as indicated by D^2 -value ranges of 70.16 to 2434.13. However, the genotype distribution pattern revealed that cluster I (70.16) had the most genotypes (48), while clusters II, III, IV, and V, each with one genotype, had no intra cluster distance (Table 2 fig. 1). The inter cluster distance ranged from 302.69 (between cluster I and cluster II) to

2434.13 (between cluster III and cluster V). The maximum inter cluster distance was recorded between cluster III and cluster V (2434.13) followed by cluster III and cluster IV (1903.37), cluster II and cluster V (1597.19), cluster II and cluster IV (1159.29). Low inter cluster distances were observed between cluster I and cluster II (302.69), cluster II and cluster IV (353.88) and cluster IV and cluster V (362.13). Low inter cluster distance between Cluster I and II indicate proximity of genotype HP-44 of cluster II with members of Cluster I. Similarly, the ascending inter cluster distance between Cluster III, IV, and V and Cluster I indicates greater diversity in the order of clusters, indicating that the genotypes involved in these clusters have a wide genetic diversity and can thus be used in a hybridization programme to improve high zinc and iron levels. The relative association between the genotypes is calculated using rescaled distance and displayed as a Wards Minimum Variance Dendrogram. The similarity coefficient is the point at which two genotypes' branches meet. The dendrogram elucidates the relative level of resemblance between genotypes and clusters. It is clear from the perusal of wards minimum variance dendrogram that "fence sitter" single genotype, grouped by Tocher method in cluster number I is entirely different from cluster number II, III, IV and V. The cluster means analyzed for ten traits have been presented in Table 3. Cluster II, IV and V exhibit highest mean value for days to maturity (137.67), Cluster I and III showed the highest mean value for days to maturity (136.6) and (136.33) respectively. Five diverse clusters were formed during this analysis and it showed that all the clusters are differently performing from each other. The members of each cluster were grouped in a way that they had a greater level of similarity within themselves while greater level of dissimilarity between all the clusters. Crossing of genotypes from these groups can increase the micronutrient concentration by combining the diverse genes controlling different morphological traits into single plant. Such recommendations in wheat breeding has been advocated by different researchers in past by [7,8,9,10]. Genotype HP-44, HP-49, HP-13 and HP-14 lying in different clusters can be ideal candidate lines for utilization in hybridization with adapted varieties namely, HD-3086, RSP-561 and JAUW -683 provided they have high zinc and iron content.

The analysis of the contribution of each trait towards the expression of genetic divergence is

presented in Table 4 and Fig. 2. Perusal of the data indicate that grain yield per plant (g) followed by number of tillers per plant contributed maximum of 25 and 20.7 per cent to the total genetic divergence among the twenty two bread wheat genotypes studied. These traits were followed by 1000 grain weight (14), spikelets per spike (9), number of days to maturity (8) and zinc content (8), the sum of which accounted for 84.7 per cent of total genetic divergence. Iron content (7), days to 50 percent flowering (5), flag leaf area (3) and plant height (0.3) Lal et al., [11] also reported that grain yield per plant and number of tillers per plant contributed maximum to genetic diversity.

For factor extraction, the principle components (PC) approach was used to calculate the major factors. Differentiation occurs in stages, or in other words, along multiple axes of differentiation, which account for overall divergence. In theory, as many axes of differentiation as there are characters contributing to total variation can be imagined, however this is not always the case. It's probable that the first two or more axes of differentiation account for the majority of the variance. Only the first three main components in this study had eigen values greater than one, explaining 55.46 percent of the variance (Table 5). The first principal component contributed for 22.41 percent of total variation, whereas the second and third principal components accounted for 21.04 and 12.04 percent, respectively. The first principal component (1) absorbed and accounted for the greatest variance (22.41 percent), while the other components contributed for progressively less and less variance for 2, 3, and 4, correspondingly. The investigation discovered three effective axes (vectors): $1 + 2 + 3 = 55.47$ percent by canonical analysis. Plant height (cm) with element value 0.47 in the first axis; iron with element value 0.41 in the second axis; and effective grain yield per plant with component value 0.43 in the third axis. Saif et al., [12] and Dargicho et al., [13] used principal component analysis to categorise wheat genotypes in similar ways. Between HP-12 and HP-48, the PCA scores and Euclidian distance matrix revealed the most diversity [14].

As shown in Fig. 3, genetic divergence between genotypes is assessed in terms of spatial distance, resulting in the construction of a two-dimensional (2D) representation based on three

PCA scores. PCA1, PCA2, and PCA3 were used to plot all fifty-two wheat genotypes using three primary factors scores, resulting in a 2D plot that accounted for the most relevant component traits, namely zinc, iron, yield, and yield contributing variables [15].

Table 1. Clustering pattern of fifty two wheat genotypes on the basis of non-hierarchical Euclidean cluster analysis

Cluster Group	No. of genotype	List of genotypes
Cluster I	48	HP-27, HP-38, HP-29, HP-39, HP-35, HP-16, HP-36, HP-08, HP-50, HP-18, HP-17, HP-04, HP-26, HP-43, HP-40, HP-15, HP-23, HP-11, HP-19, HP-25, HP-28, HP-24, HP-48, HP-31, HP-07, HP-10, HP-37, HP-41, HP-34, HP-42, HP-46, HP-47, HP-05, HP-30, HP-20, HD3086, HP-03, HP-02, HP-09, HP-21, RSP 561, HP-12, HP-22, HP-33, HP-06, HP-45, JAUW-683, HP-32
Cluster II	1	HP-44
Cluster III	1	HP-49
Cluster IV	1	HP-14
Cluster V	1	HP-13

Table 2. Average intra and inter cluster distances among grouped fifty-two wheat genotypes

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V
Cluster I	70.16	302.69	708.05	353.88	739.65
Cluster II		0	106.33	1159.29	1597.19
Cluster III			0	1903.37	2434.13
Cluster IV				0	362.13
Cluster V					0

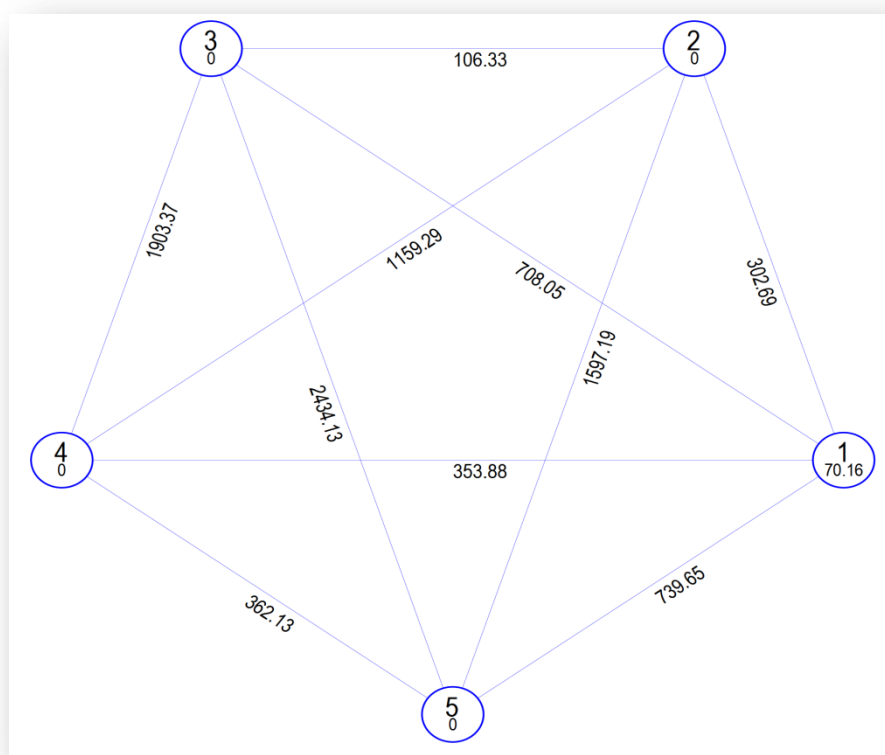


Fig. 1. Intra- and inter cluster distances related to genetic divergence (D^2) among five clusters

Table 3. Cluster means of different clusters for the morpho-physiological and micronutrient traits in wheat

Clusters	Plant height (cm)	No. tillers per plant	Days to 50 per cent flowering	Flag leaf area (cm ²)	Spikelets per spike	Days to maturity	1000 grain weight (g)	Zinc (ppm)	Iron (ppm)	Grain yield per plant (g)
Cluster I	84.92	5.65	99.17	29.76	18.90	136.6	38.45	13.19	34.99	42.58
Cluster II	82.67	6.33	98.33	28.13	17.33	137.67	41.57	17.40	34.67	71.00
Cluster III	84.33	4.67	98.33	29.43	19.33	136.33	43.37	13.93	38.33	88.33
Cluster IV	67.67	6.67	102.33	29.3	18.67	137.67	31.97	11.63	36.00	12.00
Cluster V	82.33	6.67	105.00	37.2	23.00	137.67	39.00	11.57	8.00	9.67

Table 4. Contribution of individual morpho-physiological and micronutrient traits towards genetic divergence

Sl.no	Source	Contribution %
1	Plant height (cm)	0.3
2	Number of tillers per plant	20.7
3	Days to 50 percent flowering	5
4	Flag leaf area (cm ²)	3
5	Spikelets per spike	9
6	Days to maturity	8
7	1000 grain weight (g)	14
8	Grain yield per plant (g)	25
9	Zinc (ppm)	8
10	Iron (ppm)	7

Table 5. Eigen values and Eigen vectors of the first four principal components (PCs) for 10 different morpho-physiological and micronutrient traits wheat

Traits	PC1	PC2	PC3	PC4
Plant height (cm)	0.47	0.28	0.13	0.11
Number of tillers per plant	0.38	0.02	0.07	-0.63
Days to 50 percent flowering	-0.10	-0.52	-0.01	-0.01
Flag leaf area (cm ²)	0.34	-0.18	-0.52	0.24
Spikelets per spike	0.02	-0.50	0.18	0.50
Days to maturity	-0.43	-0.16	-0.17	-0.25
1000 grain weight (g)	0.20	0.15	-0.66	0.12
Grain yield per plant (g)	0.27	0.13	0.43	0.32
Zinc (ppm)	-0.32	0.38	0.09	0.09
Iron (ppm)	-0.32	0.41	-0.16	0.31
Eigene Value	2.24	2.10	1.20	0.96
Proportion of total variance (per cent)	22.41	21.04	12.02	9.65
Cumulative variance (per cent)	22.41	43.45	55.46	65.11

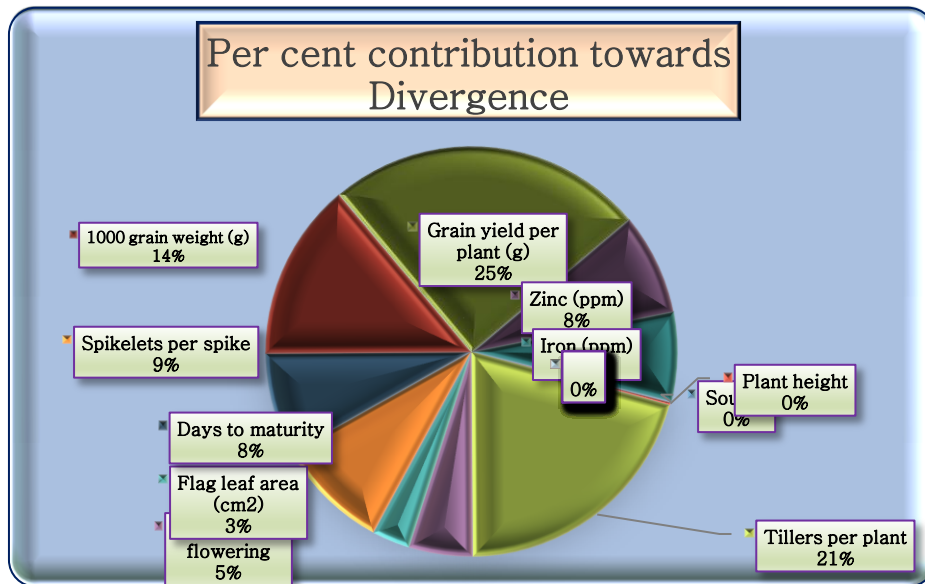


Fig. 2. Contribution of morpho-physiological and micronutrient traits towards total diversity in wheat genotypes

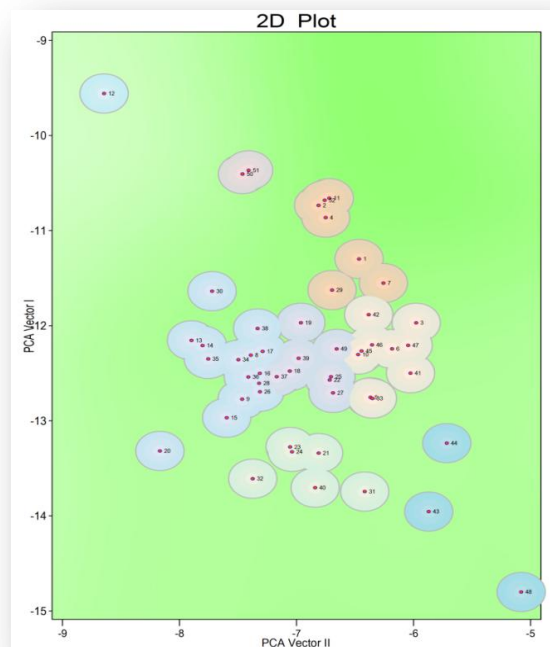


Fig. 3. Two dimensional representations of genotypes using 3 principal component based on wheat genotypes

4. CONCLUSION

Multivariate analysis was performed to decipher the genetic diversity among genotypes for yield and yield contributing traits along with zinc and iron content. Most of the genotypes grouped in

one cluster showed the minimum differences among themselves based upon their traits data and maximum differences with the members of the other clusters. This concluded that clusters are very much different in their performance to other clusters. Sometimes genotypes of

heterogeneous origin are clustered in the same group which indicates the presence of partial ancestral relationship among genotypes. The information obtained from current study can be used to choose appropriate parents for crosses by maximizing the usage of genetic diversity and expression of heterosis.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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