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Original Research Article

Genetic divergence in micronutrient rich wheat– A tool to identify diverse parents

ABSTRACT

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Aim: 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. Forty five of the forty nine high Zn and Fe lines were clubbed in one cluster as two of the ten traits studied i.e., zinc and iron were high for all these lines and not diverse. The traits that contributed mast 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 greatest inter-cluster distance, followed by Cluster III and Cluster IV, Cluster II and Cluster V, and Cluster II and Cluster IV.

Conclusion: Wheat varieties with enhanced iron and zinc content is one of the most sought objective 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. The Harvest Plus programme of the CIMMYT aims at developing and distributing zinc and iron rich wheat lines to the breeding fraternity. An experiment was designed to develop micronutrient rich wheat lines in the genetic background of rust resistance.

Keywords: wheat, cluster, Genetics contribution, PCA

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 (Kumar et al.,2011).However they are deficient in micronutrient like Zn and Fe (Alan et al., 2000; Shewry and Hey, 2015).

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As per World Health Organization (WHO) report, iron (Fe), zinc (Zn) and vitamin-A are the three nutrients that are limiting to human body (Ortiz-Monasterio et al., 2007) and approximately a quarter of the population across the globe suffers from anemia caused by iron deficiency (WHO, 2008) and Zn deficiency related diseases (Wessel's and Brown, 2012).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. Development of varieties in any crop necessitates the identification of diverse lines/ genotype that can be utilized in hybridization. With this background, preliminary studies on genetic diversity of micronutrient rich lines obtained from Harvest Plus breeding programmealong with commercial varieties was done using Mahalanobis's D^2 which helps breeder in identification of parents for specific breeding objectives. Multivariate analysis is a useful tool in quantifying the degree of divergence between biological population at genotypic level and to assess relative contribution of different components to the total divergence both at intra- and inter-cluster levels.

2. MATERIAL METHODS

The experimental material for the present study comprised of 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 and74°48E; having Sub-tropical climate with cold winterand dry summers) under normal fertilization regime (150kg N+80 kg P_2O_5 +60 kg K_2O kg/ha) with spacing of 20 x 10 cm. Data of morho-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(Datta *et al.* 2017)

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2.1 Micronutrients Profiling for Zinc and Iron

Grain samples were washed with tap water followed by dilute HCl (0.01 *N*) and finally rinsed with distilled water. Samples were dried in hot air oven at 60±5 ^oC. After attaining constant weight, grain were ground and used for subsequent chemical analysis. Grain samples were digested with HNO_3 using microwave digestion with 0.5 g of sample taken into PTFE-TFM vessel, to which 7 ml suprapure HNO_3 was added and pre-digested overnight. The vessel was then closed and put in microwave digestion the operational conditions and the heating program were set at a ramp time of 25 min to reach 180 to 190 ^oC and a hold time of 25 min at 180 to 190^oC ().The samples were cooled at room temperature followed by addition of Milli-Q water and shaken thoroughly to ensure the complete transfer of content. The resultant mixture was filtered through Whatman No. 42 and final volume was made up to 100 ml in volumetric flask with Milli-Q water containing 1% suprapure HNO_3. Total Zn and Fe in digest were determined with atomic absorption

spectrophotometer (AAS). Similar procedure without sample was carried out for preparing a reagent blank.

2.2 Statistical analysis

Statistical analyses was done using the Windostat Statistical Software 4.1 version. Genetic distance among the test entries was measured by using Mahalanobis's (1936) D^2-statistic.The clustering was done by using Tocher's method as described by Rao (1952) while the intra and inter cluster distance was calculated using formula given by Singh and Choudhary (1985).

3. Result and discussion

Based on degree of divergence among genotypes, the accessions could be grouped into five clusters (Table 1 & Fig 1) with moderate diversity among accessions as indicated by D^2-value range of 70.16 to 2434.13. However, the distribution pattern of genotypes showed that cluster I (70.16) had maximum number of genotypes (48) while cluster II, cluster III and IV and V with one genotype each had no intra cluster distance (Table 2&Fig. 2). 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 and ascending inter cluster distance of Cluster III, IV and V with Cluster I indicate greater diversity in the order of clusters which indicates that the genotypes involved in these clusters have wide genetic diversity and thus can be used in hybridization programme for improving high zinc and iron. The relative association among the different genotypes is presented in the form of Wards Minimum Variance Dendrogram which was prepared using the rescaled distance. The resemblance coefficient between two genotypes is the value at which their branches join. The dendrogram elaborate the relative magnitude of resemblance among the genotypes as well as the 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 (fig 1). 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 (Mishra et al., 2015; Poudel et al., 2017; Khan et al., 2020; Shaygan et al., 2021). 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 Figure 3. 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., (2009) also reported that grain yield per plant and number of tillers per plant contributed maximum to genetic diversity.

Principal factors were carried out using principal component (PC) method for factor extraction. Differentiation among populations occurs in stages, or in other words in different axes of differentiation which accounts for total divergence. Theoretically as many as axes of differentiation can be envisaged as there are characters contributing to total variation, but it is not absolutely. It is possible that most of the variation is accounted for by the first two or more axes of differentiation. In the present investigation only the first three principal components showed eigen values more than one and cumulatively they explained 55.46 % variability (Table 5). The first principal component explained 22.41% of the total variation while second and third principal components explained 21.04 and 12.04 % variation, respectively. The first principal component (λ1) absorbed and accounted for maximum (22.41%) proportion of variability and remaining accounted for progressively lesser and lesser amount of variation for λ2, λ3 and λ4, respectively. The study through canonical analysis revealed that there are three effective axes (vectors) λ1 + λ2 + λ3 = 55.47 %. In first axis (vector 1), plant height (cm) with element value 0.47; in second axis, iron with element value 0.41 and in third axis, effective grain yield per plant with element value 0.43. Similar works on principal component analysis have been reported by Saif et al (2013) and Dargicho et al. (2015) by grouping wheat genotypes.PCA scores and Euclidian distance matrix reflected highest diversity between HP-12 and HP-48.

Genetic divergence between genotypes is measured in terms of spatial distance and resulted in formation of two dimensional (2D) representation based on three PCA scores (λ1, λ2 and λ3 graphs) as depicted in Figure 4. Three principal factors scores were used to plot all the fifty two wheat genotypes using PCA1, PCA2 and PCA3. i.e., 2D plot which accounted for most important component traits namely zinc, iron, yield and yield contributing traits.

4. Conclusion

Multivariate analysis was performed to decipher the genetic diversity among ~~fifty two~~fifty-two genotypes for yield and yield contributing traitsalong with zinc and iron content. Genotypes HP 44, HP 49, HP 14 and HP 13 show significant genetic variability among tested genotypes indicating the presence of excellent

opportunities for improvement through hybridization. The information obtained from this study can be used to plan crosses and maximize the use of genetic diversity and expression of heterosis.

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

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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**

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 50percent 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**

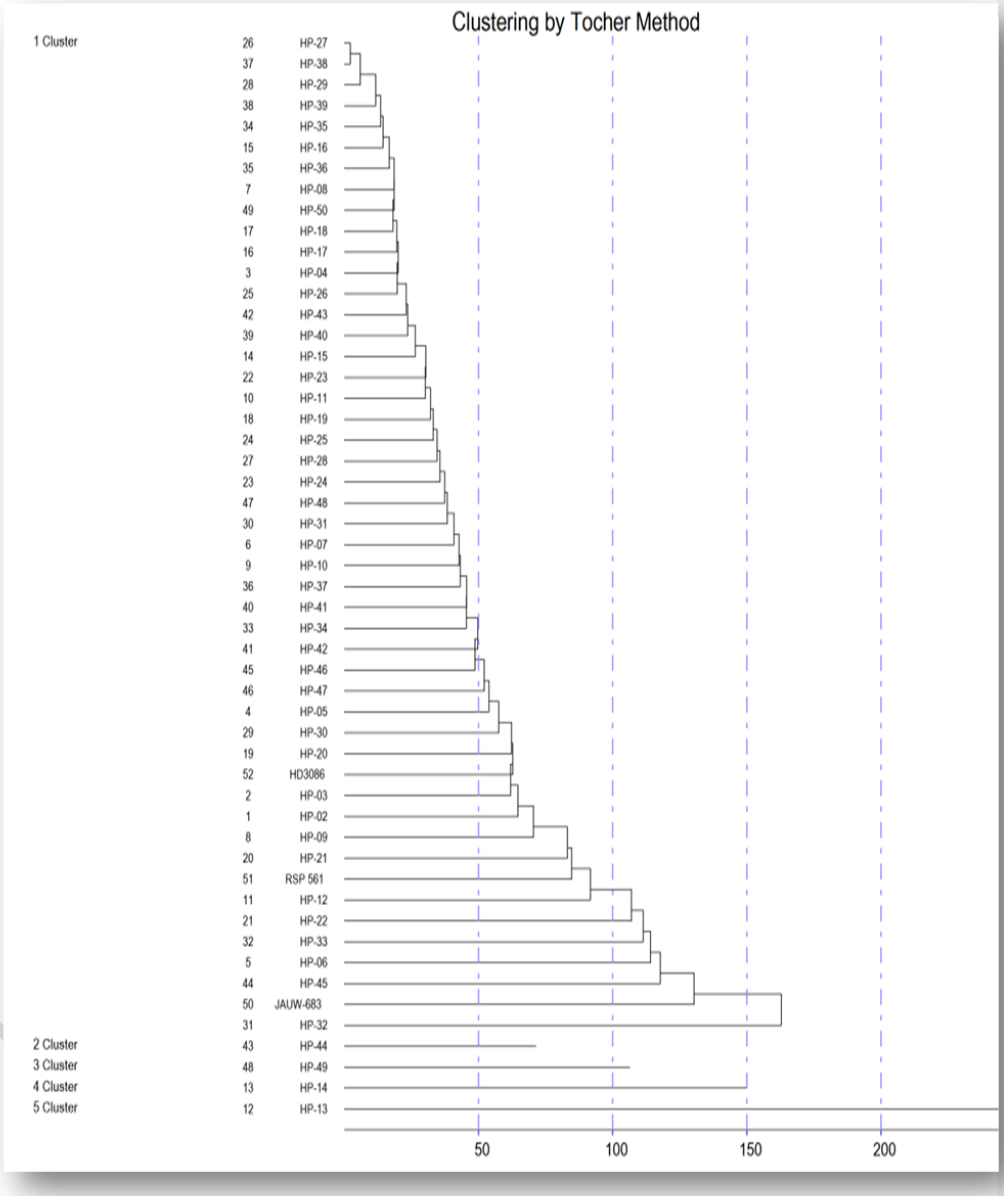

Figure 1: Dendrogram showing genetic relationship among wheat genotypes based on Euclidean distance.

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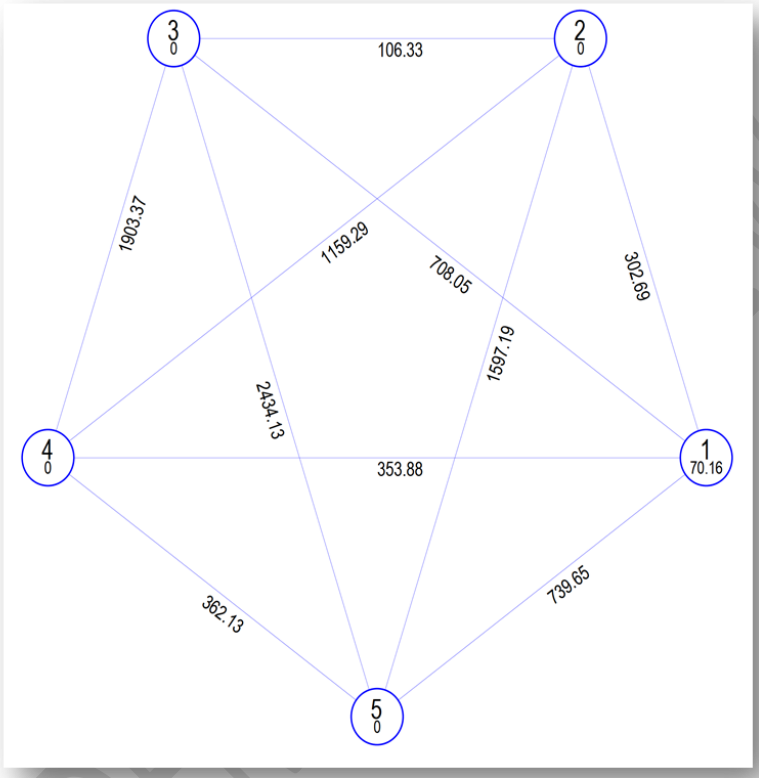

Figure 2: Intra- and inter cluster distances related to genetic divergence (D^2) among five clusters of wheat genotype.

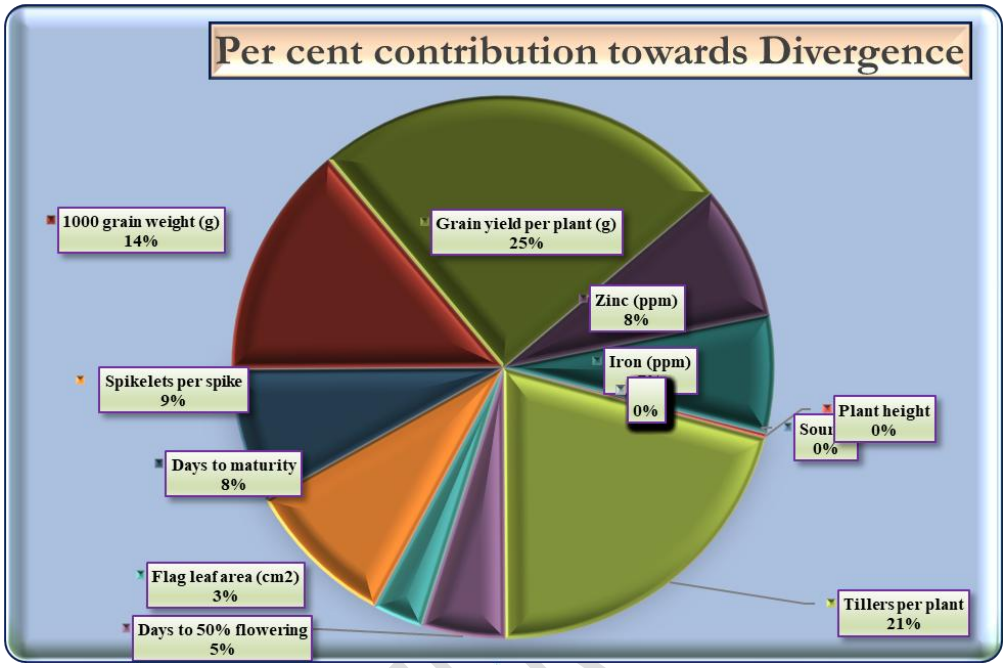

Figure 3: Contribution of morpho-physiological and micronutrient traits towards total diversity in wheat genotypes.

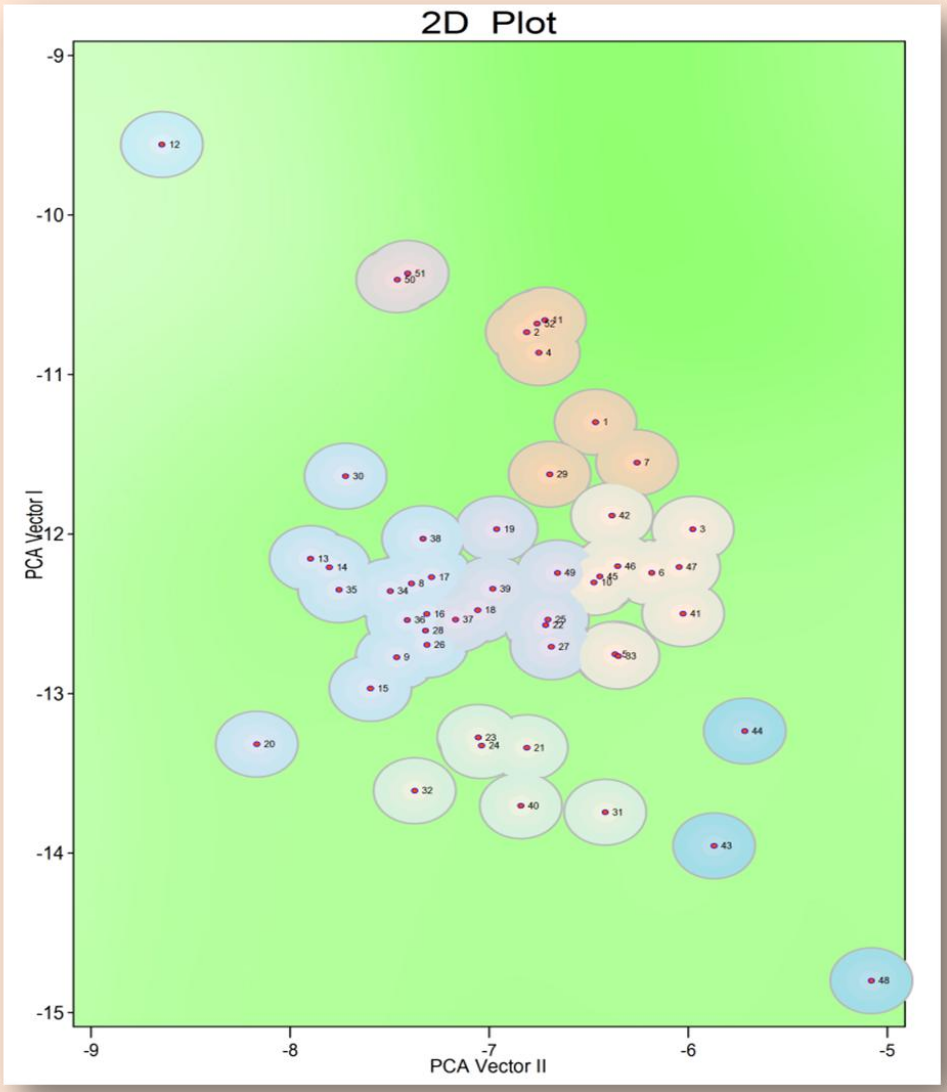

Figure 4. Two dimensional representations of genotypes using 3 principal component based on wheat genotypes.

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