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CHARACTERIZATION OF RANCIDITY INDICATORS IN SELECTED PEARL MILLET GENOTYPES BY MULTIVARIATE ANALYSIS

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Abstract

In the present study, a comparative assessment of genetic variation for various rancidity indicators in pearl millet was determined by using multivariate techniques. Free fatty acid (42.4%) and fat acidity (41.6%) were identified for high variation while fat content (9.8%) for lowest variation. A dendrogram constructed with data on the rancidity of the representative samples divided the populations of pearl millet into three clusters. Cluster II included maximum (26) genotypes while cluster I and III have lowest number of genotypes i.e. 3 and 5 respectively. The maximum distance (205.9) between cluster II and cluster III showed that these clusters are more heterogeneous in respect to the genotypes. The genotypes ISK 48 and HBL0825-01 have highest rancidity characters while genotype HBL0828-01 has lowest rancidity. A measurable distinction between the classes of pearl millet genotypes was observed in the present study which determined the impacts of various rancidity variables on quality of stored flour and other possible correlations that overall influencing the intra-specific variation among the genotypes with rancidity development and would help in picking the lines with low rancidity characteristics.

***Key words*:** Rancidity indicators, pearl millet genotypes, PCA, AHC and Genetic divergence

Introduction

Pearl millet forms a major part in diet in some parts of India and in African region but traditionally it has been considered as a "poor man's bread" but now situation has been changed, which is made possible due to its climate supportive nature (tolerance to drought, heat and soil salinity and high water use efficiency) and additional nutritional rich properties like high level of calcium, iron, zinc, lipids and high quality proteins (Nambiar *et al.*, 2011). However, apart from being a nutritionally balanced crop, its utilization on public and industrial sector is quite restricted, which is due to its limited storability and development of rancidity. Only upon grinding of the grains, the meal quality rapidly stagnates as the meal develops a mousy acidic odour and flavour, which makes it unappealing for use but its intact grains, can be stored for long periods without significant changes in quality (Cepkova *et al.*, 2014). Fat content is considered as prime suspect for the stagnation of pearl millet flour because during the grinding process germ and kernel are not completely separated and major fraction of lipids is found in the germ, which is mixed during milling. Lipase activity and fat acidity function together in development of rancid flavour in ground pearl millet flour as increase in fat acidity after milling is mainly due to action of lipase, which in turn cause bitterness and make flour unacceptable (Arora *et al.,* 2002; Nantanga *et al.,* 2008; Yadav *et al.,* 2012; and Tiwari *et al*., 2014). The milling of grains leads to tissue damage and contact between enzymes and fat substrate increase (Dvoracek *et al.,* 2010). It is observed that activity of peroxidase is positively correlated with volatile components (tentative 3-methylbutanal, tentative 2-methyl butanal) indicate that enzymatic reactions are important in flavour generation (Suzuki *et al.*, 2010). Lipoxygenase catalyses oxidation of unsaturated fatty acid results in deterioration in flavour and quality (Wang *et al.*, 2014). The repulsive grey colour of pearl millet is mainly contributed by the polyphenols which also limit the bioavailability of protein and starch and limit utilization (Thompson and Yoon, 1984; Pawar and Parlikar, 1990). Generation of free fatty acids during storage is considered as the main contributor of acidic flavor produced and its amount depend on the temperature and storage period (Kaced *et al.*, 1984).

The association of diversity patterns is quite necessary for the establishment of evolutionary relationships because it could contribute various desired traits like increased productivity, insect resistance and development of other

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desirable quality related characters, which are required in crop improvement programmes (Liu *et al.*, 2003). Multivariate statistical techniques have become a multipurpose tool in determining the genetic diversity as it can analyze multiple measurements together (Dong *et al.*, 2007 and Grahic *et al.*, 2013). Multivariate analysis had been used for genetic diversity analysis in pearl millet (Sathya *et al*, 2013; Chaudhary *et al.*, 2015 and Kiprotich *et al*., 2015). However, multivariate analysis for the rancidity indicators in pearl millet has not been reported yet. Therefore, the present study was done to evaluate and identify genetic variation in the rancidity indicators like fat, fat acidity, free fatty acid, peroxidise, lipoxygeanse and phenols in pearl millet genotypes, which would further help in exploiting the suitability and popularity in food security and breeding programmes by selecting the lines as parents with reduce rancid character.

Materials and Methods

Plant material and experimental design

The experimental material for present study comprised of 34 genotypes of pearl millet developed at the *Bajra section*, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar.

Statistical analysis

All the analyses were performed in duplicate (n=2). Descriptive statistics such as Mean, Standard Deviation (SD) and Coefficient of Variation (CV) for each trait were calculated. Multivariate analysis techniques (cluster analysis (CA) and principal component analysis (PCA)) were performed with statistical package programs XLSTAT-18.02 (2016). Clustering of genotypes into similarity groups based on their rancidity indicators traits were performed using Agglomerative Hierarchical Clustering (AHC). In order to identify the patterns of morphological variation and to estimate the relative contribution of various traits for total variability, principal component analysis (PCA) was used. Principal components with eigen values greater than one were selected as proposed by Jeffers (1967).

Results and Discussion

The genetic divergence for 34 genotypes was determined for their biochemical constitution, which is required for the pre-selection of variety in future breeding programmes and to limit control the rancidity character that is a big hurdle in wide adaptability of pearl millet flour. Therefore, in the present study an attempt has been made to select those genotypes that had lowest rancid character by evaluating the rancidity indicators, *i.e.*, fat acidity (FA), free fatty acids (FFA), lipoxygenase (LOX), peroxidise (POX), phenols and oil content using multivariate techniques.

Descriptive statistics

Genetic diversity plays an important role for a crop to adapt in a new environment during evolution and further use of this diversity using molecular markers could help in the selection of germplasm for crop improvement and breeding programmes. Hence, study of genetic diversity along with their biochemical characters could be helpful in calculating the breeding potential of a particular variety for desired traits. The descriptive statistics for rancidity traits for the period of study are given in Table 1. Highest variation was observed in FFA (42.40%) and FA (41.60%) followed by POX (21.13%), LOX (20.73%), and phenol (19.15%) and lowest in oil content (9.75%).

In the present study, the oil content has a variation ranged from 4.80 to 7.10 %, respectively, with an average 5.95 %. Cepkova *et al*. (2014) reported genotypic variation (3.96-5.24%) in crude fat content in pearl millet genotypes while Chaudhary and Kapoor (1984) reported 6 to 8 per cent fat in pearl millet cultivars. Most of genotypes had high phenol content, which is considered for its unappealing grey colour. The total phenol content varied from 143 to 293 mg/100 g flour with mean value of 208 mg/100 g. Similar, high phenol content was reported by Nambiar *et al.*, 2011 with range of 268.5-420 mg/100 g in raw pearl millet cultivars. Fat acidity (FA) is considered as one of main contributor in rancidity development as more FA indicates the high rancid character (Nantanga *et al.*, 2008; Yadav *et al.*, 2012; Cepkova *et al*., 2014; Tiwari *et al*., 2014). The FA varied from 34.11to 204.05 mg KOH/ 100 g in tested 34 genotypes with an average of 102.45 mg KOH/ 100 g. Both high and low values compared with present results of FA has been reported, which implies a wide variation and correlation of various environmental and storage factors in generation of FA (Kaced *et al.*, 1984 and Palande *et al.*, 1996). Development of acidic flavour in stored pearl millet flour is generally due to generation of (FFA) free fatty acids (Kaced *et al*., 1984). The FFA ranged from 14.78 to 96.48 mg KOH/g with average of 58 mg KOH/g. Peroxidase activity is well related with rancid flavour generation in soybean (Anil and Tilak, 2004), butterbur (*Petasites japonicas*) (Ibaraki *et al*., 1989) and buckwheat flour (Suzuki *et al.*, 2010), lettuce (Altunkaya and Gokemen, 2011). All the genotypes showed very high activity of POX, it ranged from 288.37 to 728.16 units/g, and mean value was 518.47 units/g. During the storage of pearl millet flour various complex reactions occurs like lipid hydrolysis, oxidation and polymerization reaction, which results in quality failure and rancidity development. These reactions made favourable environment for the action of lipoxygenase (LOX), which is known for its deterioration in flavour and quality (Whitaker, 1996).

Principal Component Analysis (PCA)

Principal component analysis with correlation matrix is best to determine the principal factors, as it does not require the normal distribution assumption of populations (Kholghi *et al*., 2011; Tabrizi *et al*., 2011). The prime objective in the present study was to identify the genotypes with lowest rancidity parameters. The principal component analysis grouped the rancidity indicators (FA, FFA, LOX, POX and phenol) into 6 principal components (PCs) that account for entire variability (100%), however only two principle component were found significant for having eigen value more than one as eigen value greater than 1 are considered significant only. Eigen value, reflects the quality of projection from n-dimensional initial table (n=6 in present case). First two PCs correspond to high per cent (%) of variance, which ensures that maps based on these two factors would contribute to good quality projection regarding the initial multi-dimensional table (Figure 1). First principle component (PC1) with eigen value of 2.27 alone explained 37.99% of total variability, which means that if we represent the data only on one axis, we will still be able to see 38% of the total variability of the data. Variation in PC1 was mainly due to persuade of LOX and phenol with positive loadings whereas negatively correlated with POX and FFA, which represent that the genotypes with high PC1 would have high values of LOX and phenol. The sign here indicates the relationship between variable and principle components. The second principal component (PC2) accounted 21.13% of the total variability and was negatively associated with FFA, FA and oil content, while positively associated with POX, LOX and phenol (Figure 2). The characters FA, oil and phenols all three are positively correlated with each other. The characters contributed to the variation in the first principal component, forms a larger percentage in the variation in all genotypes. Both the components were negatively associated with FFA, which indicates that the genotypes with high values of these components would have less amount of FFA and that is desired. A decrease in FFA content will lead to reduced lipase activity, which is main branch point and provide substrate for the activity of lipoxygenase and peroxidase and positively correlated with fat acidity. Figure 3 also showed a projection of variables in factors space, which tells about the correlation between them like, if two variables, away from the centre but close to each other, is correlated as in present study phenol with oil content and fat acidity. Variables on the opposite side are significantly and negative correlated like free fatty acids with other variables. Out of 34 genotypes only nine genotypes had positive value for PC1 and only six genotypes had positive value for PC2, which showed that they would be demonstrating less rancid characters. Out of 34 genotypes, only ten genotypes, namely ISK 48 (1), HBL 0551 (2), HBL 0566 (3), HPT 10-129 (10), HMS 55B (11), HBL 0837 (15), HBL 0825-1 (19), HBL 0830-2 (21), HBL 0843-2 (24) and ICMB 05222 (26) have positive values for both main components, which means that these genotypes would contribute to more rancid character and must be avoided for use in future exploitations. However, eleven genotypes were identified for having negative value for the both major components namely HBL 0828-1 (20), HMS 18B (5), 78/711 (7), HMS 60B (12), HBL 72(16), HBL 0832 (23), ICMB 03888 (27), BRBC 1005(30), LPBL/10/120 (31), H12/1009 (32) and H12/1013 (34), may be potential parents for breeding programmes for reduced rancidity.

PCA results are generally displayed as a biplot, where the axes correspond to the new system of coordinates, and both samples (dots) and taxa (arrows) are represented. The direction of arrow denotes the maximum change in great quantity and the length can be related with the rate of change occur. Figure 3 represents the principal component loading plots, which classified the studied genotypes into four quadrants based on the rancidity indicators, *viz*, FA, FFA, LOX, POX and phenol and showed the genetic variation in their formation. The genotypes on top left quadrant were related in POX only while on top right related with LOX and phenol. The bottom left quadrant showed association for FFA, while bottom right for oil and FA. The degree of similarity or dissimilarity can be predicted by the distance between genotypes in score plot.

The scatter plots could be highly beneficial to select the best varieties and helps to look at the observations on a 2D map and to identify trends, which in turn depends upon the variables present in the major principle components by depicting the similarity and dissimilarities between the studied variable. It was observed that demographics of genotypes ISK 48, HBL0825-1 and HBL0828-1 were unique and most divergent as genotypes HBL0825-1 and ISK 48 shares the common characteristics and having maximum oil content, fat acidity and phenols while genotype HBL0828-1 had opposite characters from others in having lowest fat acidity, fat content, free fatty acids and phenol (Figure 3). The genotypes, which are closer, are similar while those near to origin are distinctive. The genotypes with overlapping in principle components are desired for breeding purposes due to large variation in their biochemical characters.

In present experiment, PC score was used for ranking the genotypes. Here the genotypes with least value are desired, as these will have minimum amount of rancidity indicator so less chances to produce rancidity in flour. The values of PC score along with the rank of genotypes are given in Table 2. Here genotypes with lowest value of PC score were considered for the future selection, as this would represent the lowest value of rancidity indicators. The genotype ISK 48(1) was found to

be having maximum value but also least desired genotype followed by HBL-0825-1(19), HBL 0902 (29), HBL 0566 (3) and H12/1007(14), whereas genotype HBL-0828-1(20) was on the last place but desired one followed by the BRBC 1005(30), LPBL/10/120 (31), ICMB 93333 (25) and HBL 72(16). The genotype HBL 0843-2(24), HBL0830-2(21), ICMB 052222(26), HBL 0837(15) and 78/711(7) were found to be moderate genotype. The results are similar as observed by biplot determination.

Cluster analysis

Cluster analysis represented a clear differentiation between genotypes, which is demonstrated by difference among the clusters by comparing cluster means for studied rancidity indicators and the class centroid/cluster for the each characteristics resemblance is also given (Table 3). The maximum cluster mean was observed in POX (455.5) and lowest in oil (6.2). Based on the most correlated rancidity indicators in pearl millet genotypes a dendrogram was generated by clustering that divided the present data into three major clusters. In the second cluster 26 individuals of same biochemical constitution, while in first and third classes 3 and 5 individuals, respectively were grouped together.

The variance decomposition, which denotes the optimal classification values was 72.24% for within class variation while 27.29 % for the between class differences (Table 4). The distance between clusters shows the Euclidean distance (dissimilarity) between clusters for studied variables (Table 5). The most of the genotypes were included in cluster III (26 genotypes) and 5 genotypes in third cluster while cluster II contains only 3 genotypes. The maximum inter-cluster distance was observed between clusters III and II (205.90), which represents the maximum genetic divergence among the genotypes present in these clusters. The minimum cluster distance (135.10) found between cluster I and III suggested a close relationship between the individuals placed in clusters. The association among different genotypes is presented in the form of dendrogram (Figure 4). Crossing between individuals from clusters with maximum inter-cluster distance may result in high heterosis. The genotype HBL 0837(15) found the extreme place from HMS 55B (11) meaning thereby that they had maximum genetic distance between them. Cluster I had higher mean values for oil, FA and phenol whereas lowest for FFA. Cluster II had higher mean values for POX and FFA. On the other hand cluster III had highest mean value for LOX, while lowest values for POX and phenol. As a result, the genotypes with contrast mean performance from these clusters as could be utilized as potential parents in the development of new varieties with less rancidity.

Conclusion

A wide variation was observed for all the studied characters of 34 genotypes of pearl millet, which implies that genotypes will maximize opportunities to obtain desired segregating progenies due to presence of unique advantageous alleles at various loci. In the present study, it is suggested that, selection of parents for hybridization need to be based on genetic diversity. From all the examined genotypes, only ten have negative values for both main components so having less value of the studied rancidity indicators. The genotypes HBL0828-1 and 30 were found with low rank with (there is only one ranking method) as having lowest values for rancidity variables therefore, these genotypes can be used in future such as parent genotypes for desirable traits, which have less rancid character. Hence, indirect selection based on rancidity indicators may lead to create better genetic recombinants for reduced rancidity with improved quality *per se.*

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Table 1: Descriptive statistics for different rancidity indicators traits

Variable	Observations	Minimum	Maximum	Mean	Std. deviation	CV (%)
OIL	34	4.80	7.10	5.95	0.58	9.75
FA	34	34.11	204.05	102.45	42.62	41.60
FFA	34	14.78	96.48	58.00	24.59	42.40
LOX	34	242.67	516.33	384.91	79.80	20.73
POX	34	288.37	728.16	518.47	109.55	21.13
Phenol	34	143.00	293.05	208.46	39.93	19.15

Table 2: Ranking of genotypes based on analyzed PC Scores of pearl millet genotypes

No. of Genotype	PC1	Rank	No. of Genotype	PC1	Rank	No. of Genotype	PC1	Rank
1	3.767	1	9	0.411	13	18	-1.131	25
19	3.538	2	11	0.280	14	6	-1.234	26
29	2.197	3	24	0.162	15	23	-1.499	27
3	1.930	4	21	0.123	16	12	-1.669	28
14	1.443	5	26	0.037	17	34	-1.702	29
13	1.171	6	15	0.026	18	16	-1.888	30
22	1.128	7	7	-0.017	19	25	-1.971	31
10	1.083	8	28	-0.141	20	31	-1.993	32
4	1.059	9	5	-0.351	21	30	-2.022	33
17	0.832	10	8	-0.498	22	20	-2.296	34
2	0.575	11	32	-0.860	23			
33	0.509	12	27	-0.999	24			

Table 3: Class Centroids

Class	OIL	FA	FFA	LOX	POX	Phenol
1	6.680	179.523	37.775	386.333	440.687	276.905
2	5.790	88.751	60.844	371.974	555.974	202.326
3	6.365	127.443	55.354	451.333	370.112	199.294
Mean	6.278	131.905	51.324	403.214	455.591	226.175

Table 4: Variance decomposition for the optimal classification

	Absolute	Percent
Within-class	16282.378	72.74%
Between-classes	6102.243	27.26%
Total	22384.621	100.00%

Table 5: Distance between the class centroids

Clusters	1	2	3
1	0	166.829	135.095
2	166.829	0	205.862
3	135.095	205.862	0

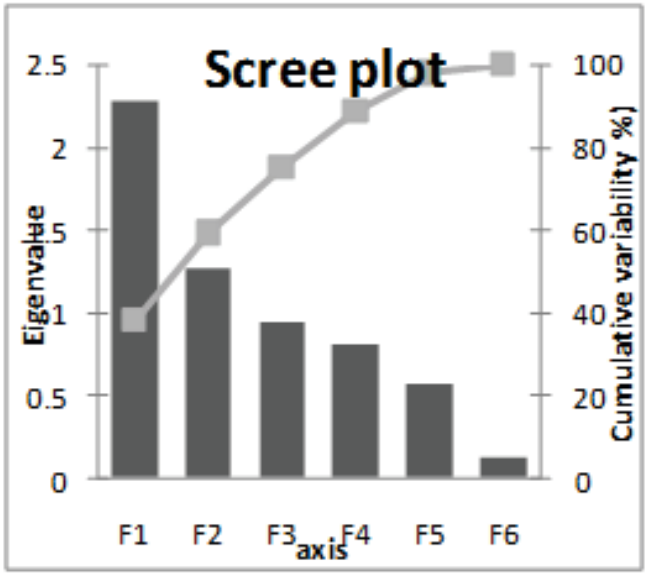

Figure 1: Scree Plot (Variability w.r.t. PCs)

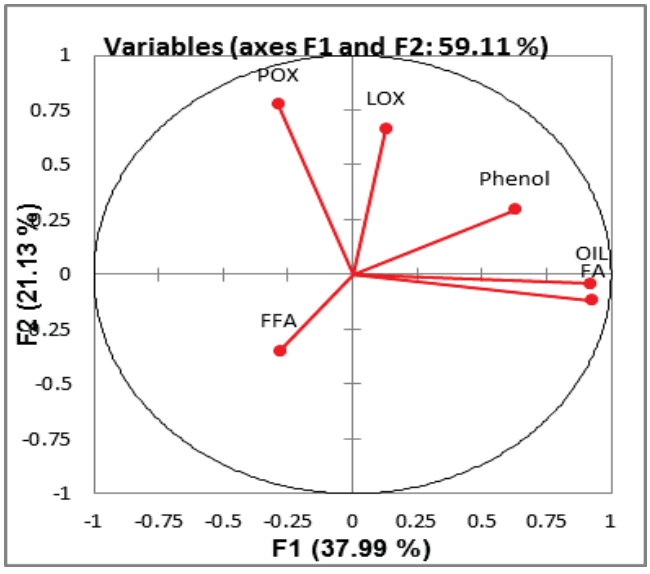

Figure 2: Correlation of PC1 and PC2 with variables

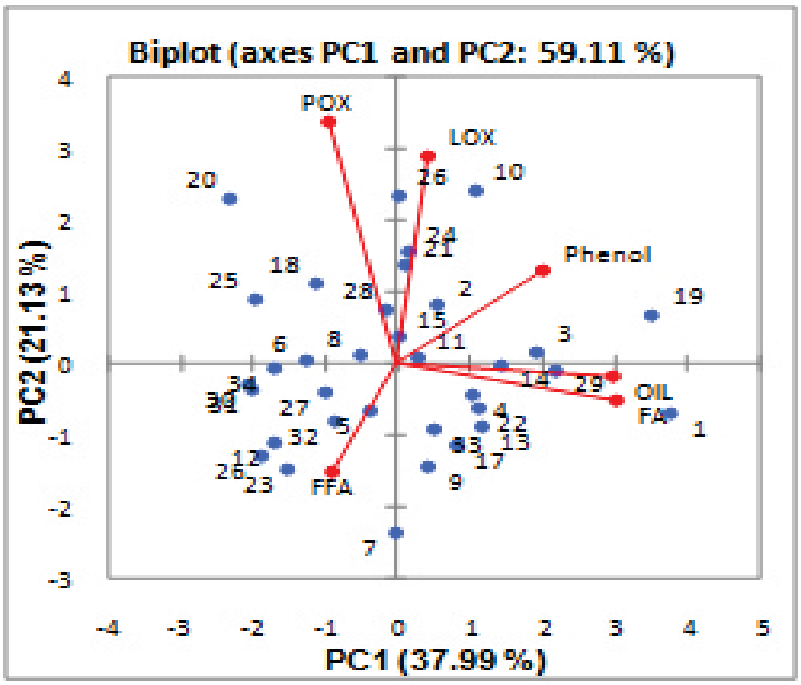

Figure 3: Principal Component loading plot of PC1 and PC2

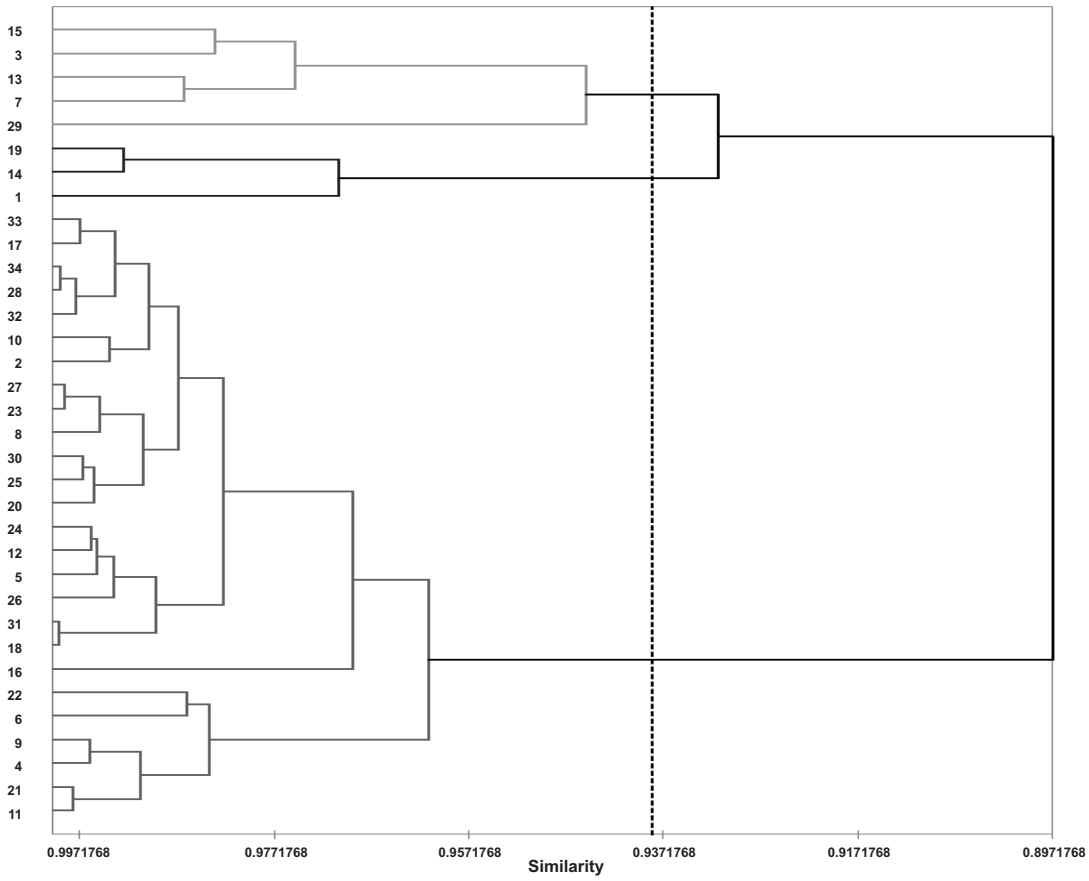

Figure 4: The association among different genotypes in the form of dendrogram