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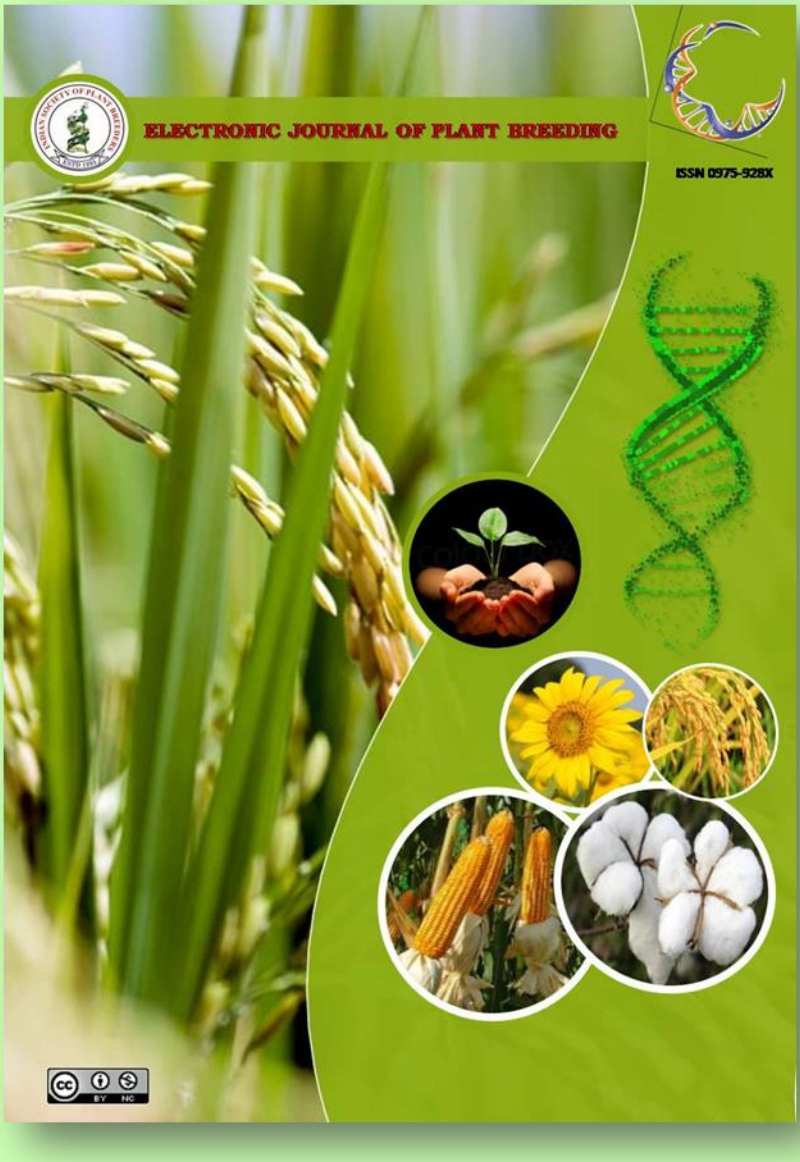

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Research Article

Genetic divergence studies in foxtail millet [*Setaria italica* (L.) Beauv.]

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Abstract

Fifty genotypes of foxtail millet were subjected to multivariate analysis through Mahalanobis' D^2 statistics (Tocher's method) and Wards' minimum variance method. Both D^2 analysis and Ward's method grouped the 50 foxtail millet genotypes into eight clusters each. However, the clusters are different in two different methods with respect to total number as well as genotypes included in each cluster. In D^2 analysis, cluster III is the largest with 13 genotypes followed by cluster II, IV, I, VI, V, VII and VIII. Whereas in Ward's method, the cluster VI is the largest with 14 genotypes followed by, cluster VII, I, II, IV, V, III and VIII. There was no solitary cluster in Ward's minimum variance method, where as in Tocher's method, three clusters *viz.*, cluster V, VII and VIII are solitary clusters. In both the methods, there is wide genetic diversity between the clusters. Considering the distribution of genotypes in both the methods it can be concluded that crosses, Ise-1605 x Ise-745 and Ise-1605 x Ise-1419 are having good chance of producing heterotic hybrids or transgressive segregants, depending on the gene action controlling the traits.

Keywords

Foxtail millet, Genetic divergence, D^2, Tocher's method, Wards' minimum variance

Introduction

Millet is one of the oldest cereals and resources of farmers in the drought prone area of the world. Millets are able to grow in poor soils in the drier regions and in the hottest climates, where no other cereal can grow and yield high (Howarth *et al*., 2002). Foxtail millet [*Setaria italica* (L.) Beauv.] is important underutilized small millet, grown in various parts of India. It grows well even under adverse agro climatic conditions. It is good source of dietary fiber and ß carotene. Foxtail millet ranks second in the world's total production of millets and is an important staple food for millions of people in Southern Europe and Asia (Marathee, 1993). In India small millets are cultivated in an area of 590,000 ha with a production of 390,000 t and productivity of 654 kg ha^{-1} while in Andhra Pradesh foxtail millet is grown in an area of 51,000 ha with a production of 48,000 t and productivity of around 945 kg ha^{-1} (Ministry of Agriculture, 2016).

The success of any plant breeding programme largely depends on the existence of diversity among the genotypes (Allard, 1960). The choice of parents is of greater importance in breeding programme. Assessment of a large number of germplasm for genetic diversity is of immense importance in selection of diverse genotypes for hybridization programme. The generalized distance concept of Mahalanobis' is based on multivariate analysis of quantitative traits. It is used to measure the genetic divergence and to classify the genetic stock into distinct groups. Intercrossing between more divergent parents is expected to generate a broad spectrum of variability and selection to be adopted in the segregating generations. Considering this, the present study was taken up in foxtail millet to understand the diversity available in the genetic stocks.

Material and Methods

Fifty germplasm collections of foxtail millet obtained from ICRISAT, Hyderabad were evaluated in Augmented Randomised Complete Block Design with three checks *viz*., Korra local, Prasad and Suryanandi in each block during *Kharif*, 2017-18 at RARS, Lam, Guntur. Each genotype was raised in a single row of four meter length with a spacing of 22.5x10 cm between and within the rows, respectively. Standard agronomic practices and recommended fertilizer doses were applied for normal crop growth. Data recorded from the total thirteen traits including seven yield component characters and six grain quality parameters. Single plant observations were recorded on five plants selected at random per genotype per replication and their means were used for the analysis. However, observations on test weight, days to 50% flowering and days to maturity and all the quality parameters *viz.*, protein, calcium, iron, fat, carbohydrate and phosphorus were recorded on plot basis. Genetic divergence analysis was done following the D^2 statistics proposed by Mahalanobis' (Mahalanobis, 1936). The genotypes were grouped on the basis of minimum generalized distance using Tochers' method as described by Rao (Rao, 1952). Hierarchial cluster analysis was also done for the same 50 foxtail millet genotypes and these genotypes were grouped into eight clusters using the Ward's minimum variance (Anderberg, 1993)

Results and Discussion

Mahalanobis' D^2 cluster analysis based on Tocher's method grouped all the 50 foxtail millet accessions of the present investigation into eight distinct non-overlapping clusters (Table 1 and Fig. 2). The distribution of 50 genotypes into eight clusters was at random with maximum number of 13 genotypes in cluster III. Cluster II and IV are the second largest clusters with 10 genotypes each. Cluster I was the third largest cluster having nine genotypes followed by cluster VI with five genotypes each. As could be seen from the results (Table 1.) that clusters V, VII and VIII were solitary clusters. The formation of distinct solitary clusters may be due to the fact that geographic barriers preventing gene flow or intensive natural and human selection for diverse and adaptable gene complexes must be responsible for this genetic diversity (Arunachalam and Ram, 1967). The estimate of intra and inter cluster distance (Table 2.) indicate that the highest intra cluster distance is of cluster VI (779.17) followed by cluster IV (480.21), cluster III (347.08), cluster II (220.24) and cluster I (129.34). Cluster V, Cluster VII and cluster VIII are having one genotype each hence the intra cluster distance for these three clusters were found to be (0.00). The maximum inter-cluster distance was observed between clusters VI and VII (5631.27), followed by clusters V and VII (4712.38), clusters V and VIII (3768.97), clusters II and VII (3433.42), clusters III and VII (3321.13), clusters VI and VIII (3299.94), clusters VII and Cluster VIII (3000.14) and cluster III and VIII (2661.90). This suggested that there is wide genetic diversity between these clusters (Table 2 and fig 1). The genotypes, Ise-1605 and Ise-769, belonging to same geographical area *i.e.* India were grouped in two different faraway clusters, V and VIII, respectively. Similarly in a single cluster *viz.*, cluster IV genotypes from different geographical areas *viz.*, India, Afganistan and Republic of Korea are present. This is clearly indicating that geographical divergence and genetic diversity are not correlated. Similar conclusions were drawn by different scientists in various crops [Gupta *et al*. (1991) in mustard; Amurrio *et al.*(1995) in pea; Iqbal *et al.* (2015) in maize; Sheriff (1992) and Kumar *et al.* (2010) in finger millet and Sandhyakishore *et al.* 2007 in paddy].

The cluster means of the eight clusters (obtained by Tocher's method) for all the thirteen characters under study are presented in Table 3. The data indicated a wide range of mean values between the clusters. Days to 50% flowering had a range of 45.26 days for cluster VII to 75.37 days for cluster VIII, plant height had a range of 89.08 cm for cluster VII to 154.57 cm for cluster VI, panicle length had a range of 5.79 cm for cluster VII to 18.16 cm for cluster V, number of productive tillers per plant had a range of 1.63 for cluster VII to 8.30 for cluster V, days to maturity varied from 77.33 days for cluster VII to 110.22 days for cluster VIII, test weight varied from 1.13 g for cluster VII to 3.63 g for cluster V, protein ranged from 6.36 g for cluster VII to 14.06 g for cluster V, fat varied from 1.97 g for cluster VII to 3.72 g for cluster II, carbohydrate varied from 52.32 g for cluster VII to 73.00 g for cluster V, iron varied from 3.42 mg for cluster VIII to 19.91 mg for cluster VII, phosphorus varied from 0.11 g for cluster VII to 0.33 g for cluster I, calcium varied from 6.91 mg for cluster VII to 19.30 mg for cluster VI and grain yield per plant varied from 4.66 g for cluster VII to 39.83 g for cluster V. The per cent contribution of all the thirteen characters in the 50 genotypes towards genetic divergence is presented in Table 4. Among the characters studied plant height (47.76%) recorded maximum contribution followed by iron (16.00%), grain yield per plant (13.80%), calcium (11.92%), days to maturity (7.18%), carbohydrate (1.22%), days to 50% flowering (1.06%), protein (0.98%) and fat (0.08%) while panicle length, number of productive tillers per plant, test weight and phosphorus (0.01) contributed least towards the genetic divergence.

Hierarchial cluster analysis (using Ward's method) for the same 50 foxtail millet genotypes also resulted in eight clusters and the clusters are formed using $Euclidean^2$ distances (Table 5 and Fig 3). Among all the clusters, cluster VI was the largest containing fourteen genotypes followed by cluster VII containing eleven genotypes, cluster I, II, IV and V each containing five genotypes, cluster III containing three genotypes and cluster VIII having two genotypes. The estimate of intra and inter cluster distance (Table 6 and Fig 4) revealed that the highest intra cluster distance is of cluster IV (18.20) followed by cluster III (17.50), cluster VIII (15.76), cluster VII (14.30), cluster V (12.34), cluster II (10.34), cluster VI (9.21) and cluster I (7.56). The maximum inter-cluster $Euclidean^2$

distance was observed between clusters I and VIII (76.41) followed by clusters I and IV (71.56) and clusters V and VIII (66.83) suggesting wider genetic divergence between these clusters. The two farthest clusters *viz.,* cluster I and cluster VIII are accommodating genotypes from same country (same geographical areas) *i.e.* India and cluster VII is accommodating genotypes from different geographical areas *viz.,* India, Kenya, Iran and U.S.A. Similarly, cluster IV is also accommodating the genotypes from different geographical areas *viz.,* India, Afghanistan and Lebanon. This once again support the conclusion drawn under Tocher's method of clustering *i.e.,* geographical and the genetic distances are least correlated or even not correlated.

Cluster means were computed for the thirteen characters studied by Ward's minimum variance method are presented in Table 7. Cluster I registered highest mean value for panicle length (18.12 cm), number of productive tillers per plant (6.83), test weight (4.10g), carbohydrate (70.01 g/100g) and grain yield per plant (29.86 g). Cluster II recorded lowest mean values for protein (8.13 g/100g) and fat (2.63 g/100g). Cluster III recorded highest mean values for plant height (157.96 cm) and protein (13.46 g/100g) and lowest mean values for iron (3.49 mg/100g) and phosphorus (0.17 g/100g). Cluster IV recorded lowest mean values for days to 50 per cent flowering (45.13), plant height (109.16 cm), panicle length (7.33 cm), number of productive tillers per plant (2.09) and days to maturity (77.51), test weight (1.52 g) and grain yield per plant (5.42 g). Cluster V registered highest mean value for calcium (29.21 mg/100g). Cluster VI recorded highest mean value for iron (17.93 mg/100g). Cluster VII recorded highest mean values for fat (4.27 g/100g) and phosphorus (0.34 g/100g). Similarly cluster VIII recorded highest mean values for days to 50 per cent flowering (72.82), days to maturity (108.28) and lowest mean values for carbohydrate (55.15 g/100g) and calcium (12.06 mg/100g). Thus, cluster I showed highest mean values for important yield contributing traits *viz.,* panicle length, number of productive tillers per plant, test weight and grain yield per plant while this cluster also registered highest mean value for one of the quality traits *i.e.* carbohydrate. So the genotypes from this cluster can be used for foxtail millet yield improvement programme.

Divergence analysis using Tocher's method revealed maximum divergence between clusters VI and VII followed by clusters V and VII. It was evident that the cluster VII recorded least mean values for all the characters, hence it is not wise to select genotypes from this cluster for hybridization. Among clusters VI and V, cluster V registered higher mean values for majority of yield contributing traits indicating the superiority of the genotypes present in cluster V. The cluster V had maximum genetic distance with cluster VII followed by cluster VIII and cluster IV. But clusters VII and VIII had least mean values for yield and yield contributing traits. Hence genotypes from the next farthest cluster i.e. cluster IV are to be used for hybridizing with the sole genotype Ise-1605 of cluster V. Among the genotypes present in cluster IV, Ise-745 exhibited good *per se* (data not presented for brevity) for majority of yield contributing traits. Therefore cross between Ise-1605 and Ise-745 may result in superior hybrids or transgressive segregants depending on the gene action.

Ward's minimum variance method indicated that the genotypes grouped under cluster I are farthest from the genotypes that are grouped under cluster VIII. Therefore hybridization is to be planned between the genotypes of these two clusters. Among the genotypes of cluster I Ise-1605 had maximum *per se* values (data not presented for brevity) for majority of yield contributing traits. Similarly in cluster VIII, the genotype Ise-1419 recorded better *per se* values (data not presented for brevity) for important yield contributing traits. Hence cross between Ise-1605 and Ise-1419 may be productive.

As per the clustering of Tocher's method, Ise-1605 x Ise-745 is considered as the best cross possible. Similarly, as per Ward's method cross Ise-1605 x Ise-1419 is considered as the best possible cross. However, by taking both methods of clusterings into consideration it can be concluded that crosses, Ise-1605 x Ise-745 and Ise-1605 x Ise-1419 are having good chance of producing heterotic hybrids or transgressive segregants, because genotype, Ise-1605 which is present in cluster V and I of Tocher's method and wards method respectively is having considerable genetic distance from Ise-745 (present in cluster IV of Tocher's method) and from genotype Ise-1419 (present in cluster VIII of Ward's method). Therefore the above mentioned crosses may be productive. However, one should test these genotypes for their combining ability and gene action using different mating designs so that one can decide whether to go for hybrid varieties (in presence of dominance gene action) or to target superior transgressive segregants (in presence of additive gene action)

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Table 1. Clustering pattern of 50 foxtail millet [*Setaria italica* (L.) Beauv.] genotypes by Tocher's method

Cluster No.	No. of genotypes	Name of genotype (S)
I	9	Ise-931, Ise-1846, Ise-1629, Ise-1406, Ise-144, Ise-1780, Ise-1402, Ise-795, Ise-362
II	10	Ise-796, Ise-985, Ise-525, Ise-869, Ise-375, Ise-1687, Ise-364, Ise-507, Ise-254, Ise-909
III	13	Ise-1806, Ise-1820, Ise-1000, Ise-1805, Ise-1851, Ise-31, Ise-1892, Ise-1881, Ise-1900, Ise-179, Ise-1269, Ise-907, Ise-840
IV	10	Ise-1354, Ise-995, Ise-838, Ise-1408, Ise-936, Ise-813, Ise-785, Ise-458, Ise-1593, Ise-745
V	1	Ise-1605
VI	5	Ise-1059, Ise-195, Ise-200, Ise-160, Ise-1419
VII	1	Ise-1026
VIII	1	Ise-769

Table 2. Average intra and inter-cluster distances (D^2 values) among eight clusters (obtained by Tocher's method) of 50 foxtail millet [*Setaria italica* (L.) Beauv.] genotypes

Cluster No.	I	II	III	IV	V	VI	VII	VIII
I	**129.34**	323.85	351.98	568.07	887.51	1199.40	2408.99	2175.14
II		**220.24**	549.36	1037.82	1038.14	869.99	3433.42	2498.59
III			**347.08**	1058.48	729.34	1122.25	3321.13	2661.90
IV				**480.21**	1920.78	2424.32	1277.35	2324.33
V					**0.00**	1392.70	4712.38	3768.97
VI						**779.17**	5631.27	3299.94
VII							**0.00**	3000.14
VIII								**0.00**

Note: Diagonal values are intra-cluster distances. Off-diagonal values are inter-cluster distances

Table 3. Mean values of eight clusters estimated by Tocher's method in 50 foxtail millet [*Setaria italica* (L.) Beauv.] genotypes

Cluster no.	Days to 50% flowering	Plant height (cm)	Panicle length (cm)	No. of productive tillers/plant	Days to maturity	Test wt (g)	Protein (g/100g)	Fat (g/100g)	Carbo-hydrate (g/100g)	Iron (mg/100g)	Phosphorus (g/100g)	Calcium (mg/100g)	Grain yield/plant (g)
I	48.64	131.66	13.94	4.73	81.44	2.60	10.84	3.36	66.34	13.38	**0.33**	15.93	14.11
II	51.14	142.87	12.69	3.52	84.38	2.41	12.34	**3.72**	64.42	16.48	0.32	14.39	11.76
III	47.50	136.18	14.95	5.42	80.92	3.24	11.94	3.63	67.56	6.61	0.26	17.81	20.04
IV	46.06	117.56	10.18	3.13	78.55	2.35	12.06	3.56	61.73	17.70	0.27	15.51	9.38
V	49.26	140.41	**18.16**	**8.30**	82.33	**3.63**	**14.06**	2.39	**73.00**	15.25	0.27	16.24	**39.83**
VI	54.90	**154.57**	15.46	5.78	88.53	2.95	10.95	3.24	60.60	8.09	0.22	**19.30**	18.59
VII	**45.26**	**89.08**	**5.79**	**1.63**	**77.33**	**1.13**	**6.36**	**1.97**	**52.32**	**19.91**	**0.11**	**6.91**	**4.66**
VIII	**75.37**	112.71	9.98	2.86	**110.22**	1.96	9.61	2.76	60.53	**3.42**	0.31	15.88	6.26

Note: Bold figures indicate minimum and maximum values in each character

Table 4. Contribution of different characters towards genetic divergence in 50 foxtail millet [*Setaria italica* (L.) Beauv.] genotypes

S. No.	Source	No. of times ranked first	Per cent contribution
1	Days to 50% flowering	13	1.06
2	Plant height (cm)	585	47.76
3	Panicle length (cm)	0	0.01
4	No. of productive tillers/plant	0	0.01
5	Days to maturity	88	7.18
6	Test wt (g)	0	0.01
7	Protein (g/100g)	12	0.98
8	Fat (g/100g)	1	0.08
9	Carbohydrate (g/100g)	15	1.22
10	Iron (mg/100g)	196	16.00
11	Phosphorus (g/100g)	0	0.01
12	Calcium (mg/100g)	146	11.92
13	Grain yield/plant (g)	169	13.80

Table 5. Clustering pattern of 50 foxtail millet [*Setaria italica* (L.) Beauv.] genotypes by Ward's minimum variance method

Cluster No.	No. of genotypes	Name of genotype (S)
I	5	Ise-31, Ise-1881, Ise-1605, Ise-1892, Ise-200
II	5	Ise-1806, Ise-362, Ise-1851, Ise-1820, Ise-1000
III	3	Ise-195, Ise-840, Ise-160
IV	5	Ise-785, Ise-813, Ise-936, Ise-458, Ise-1026
V	5	Ise-907, Ise-1269, Ise-179, Ise-1059, Ise-1593
VI	14	Ise-931, Ise-1406, Ise-254, Ise-375, Ise-1408, Ise-1846, Ise-909, Ise-1354, Ise-364, Ise-745, Ise-144, Ise-1629, Ise-1402, Ise-995
VII	11	Ise-796, Ise-869, Ise-507, Ise-1805, Ise-838, Ise-525, Ise-985, Ise-1687, Ise-795, Ise-1780, Ise-1900
VIII	2	Ise-769, Ise-1419

Table 6. Average intra and inter cluster distances (Euclidean^2 values) among eight clusters (obtained by Ward's method) in 50 foxtail millet [*Setaria italica* (L.) Beauv.] genotypes

Cluster No.	I	II	III	IV	V	VI	VII	VIII
I	**7.56**	21.47	22.67	71.56	32.51	24.46	32.05	76.41
II		**10.34**	22.72	40.15	22.48	16.31	23.41	58.71
III			**17.50**	52.99	25.50	26.53	26.77	63.54
IV				**18.20**	34.49	29.54	34.50	66.29
V					**12.34**	18.47	19.20	66.83
VI						**9.21**	14.77	56.68
VII							**14.30**	50.36
VIII								**15.76**

Note: Diagonal values are intra-cluster distances. Off-diagonal values are inter-cluster distances

Table 7. Mean values of eight clusters estimated by Ward's minimum variance method in 50 foxtail millet [*Setaria italica* (L.)Beauv.] genotypes

Cluster no.	Days to 50% flowering	Plant height (cm)	Panicle length (cm)	No. of productive tillers/plant	Days to maturity	Test wt (g)	Protein (g/100g)	Fat (g/100g)	Carbo-hydrate (g/100g)	Iron (mg/100g)	Phosphorus (g/100g)	Calcium (mg/100g)	Grain yield/plant (g)
I	49.93	142.36	**18.12**	**6.83**	83.60	**4.10**	13.33	2.98	**70.01**	12.90	0.22	15.76	**29.86**
II	47.33	134.69	14.96	5.83	80.51	2.72	**8.13**	**2.63**	67.38	4.67	0.27	13.62	18.25
III	49.30	**157.96**	16.10	6.15	82.30	2.74	**13.46**	4.03	61.89	**3.49**	**0.17**	19.67	19.52
IV	**45.13**	**109.16**	**7.33**	**2.09**	**77.51**	**1.52**	9.69	3.57	56.43	13.07	0.23	14.69	**5.42**
V	46.97	133.11	12.22	4.12	78.40	2.92	11.81	3.83	65.43	9.58	0.30	**29.21**	12.12
VI	47.99	129.91	13.02	4.45	80.84	2.76	12.04	3.09	65.70	**17.93**	0.29	13.10	13.32
VII	50.87	139.52	13.00	3.45	84.68	2.48	12.61	**4.27**	64.88	14.33	**0.34**	16.04	13.60
VIII	**72.82**	125.39	11.57	4.08	**108.28**	2.05	9.61	3.13	**55.15**	3.50	0.30	**12.06**	9.93

Note: Bold figures indicate minimum and maximum values in each character

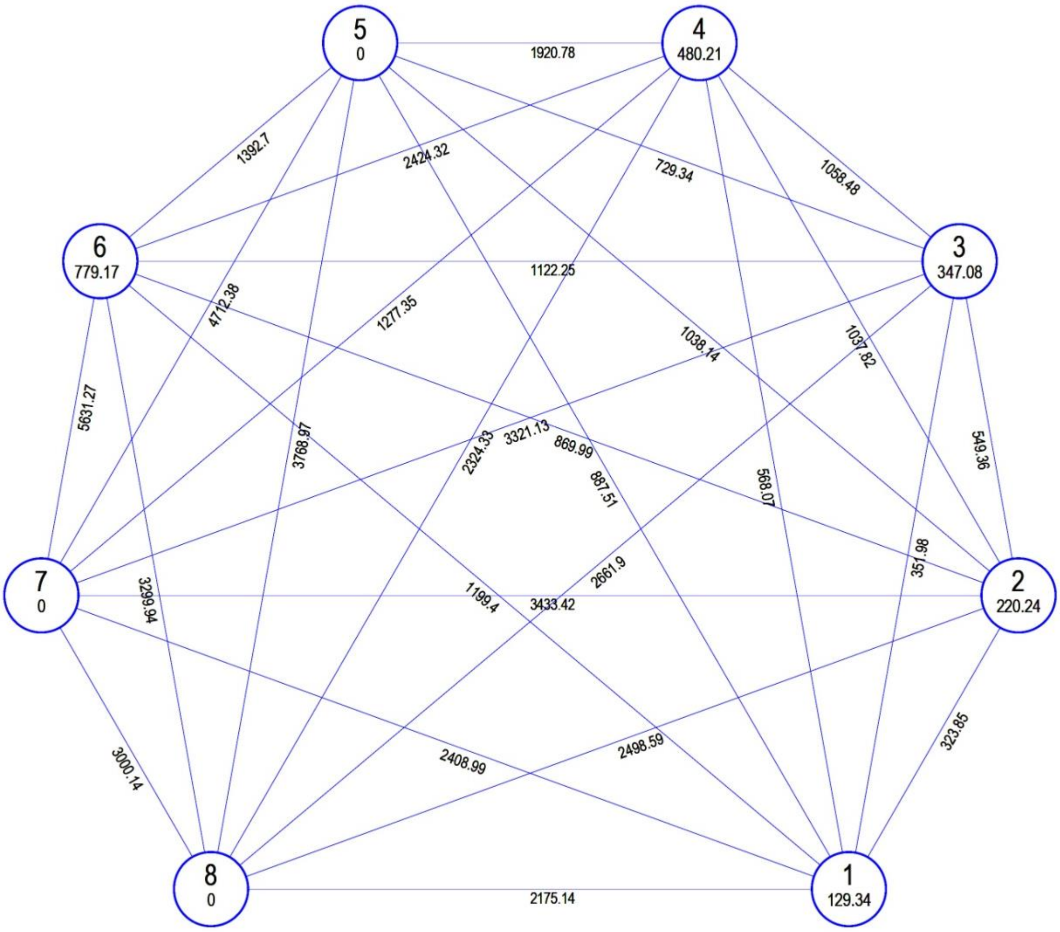


Fig. 1. Intra and inter-cluster distance of 50 foxtail millet [*Setaria italica* (L.) Beauv.] genotypes in eight clusters based on Tocher's method

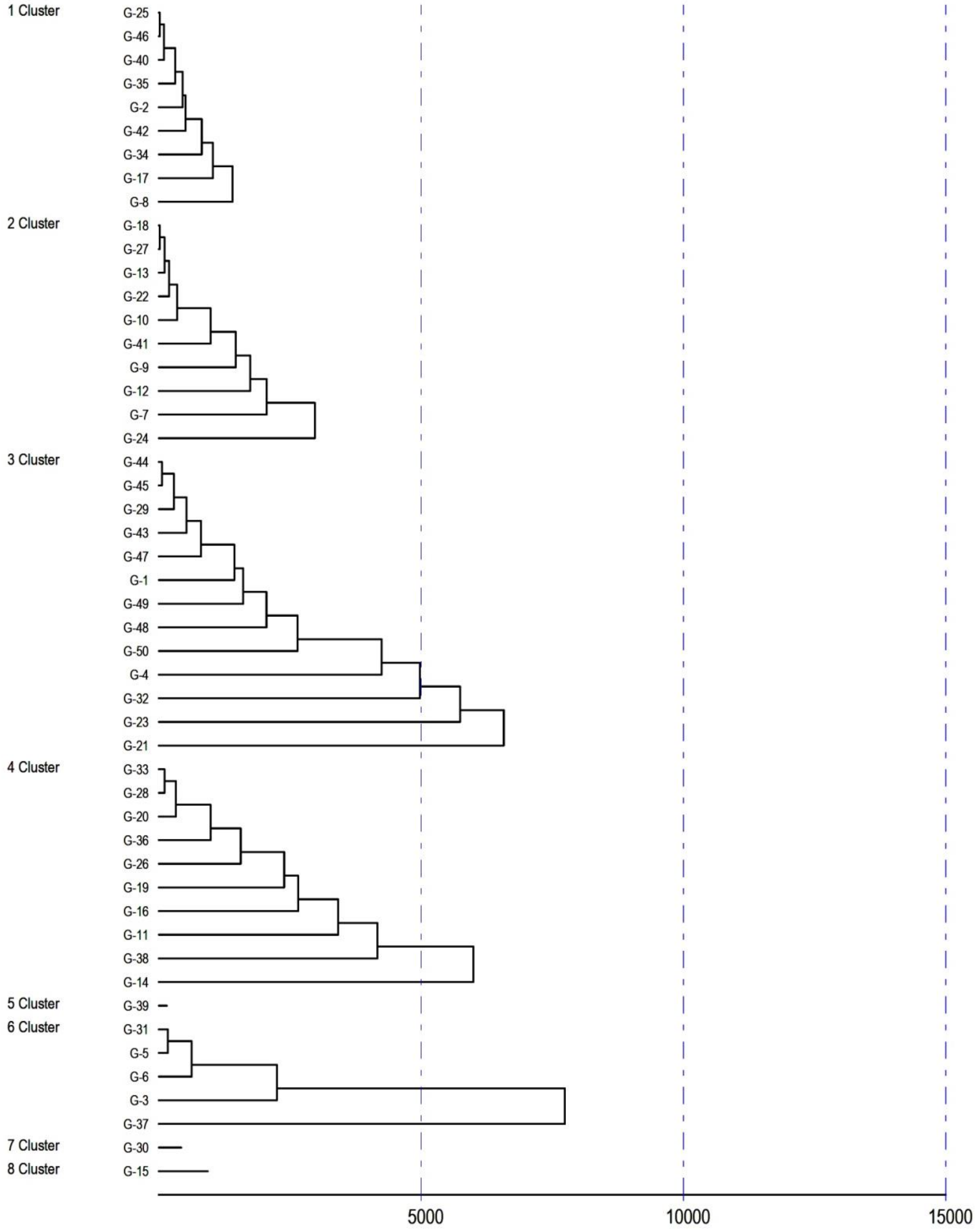


Fig. 2. Dendrogram showing relationship among 50 foxtail millet [*Setaria italica* (L.) Beauv.] genotypes in eight clusters based on Mahalanobis' D^2 values using Tocher's method

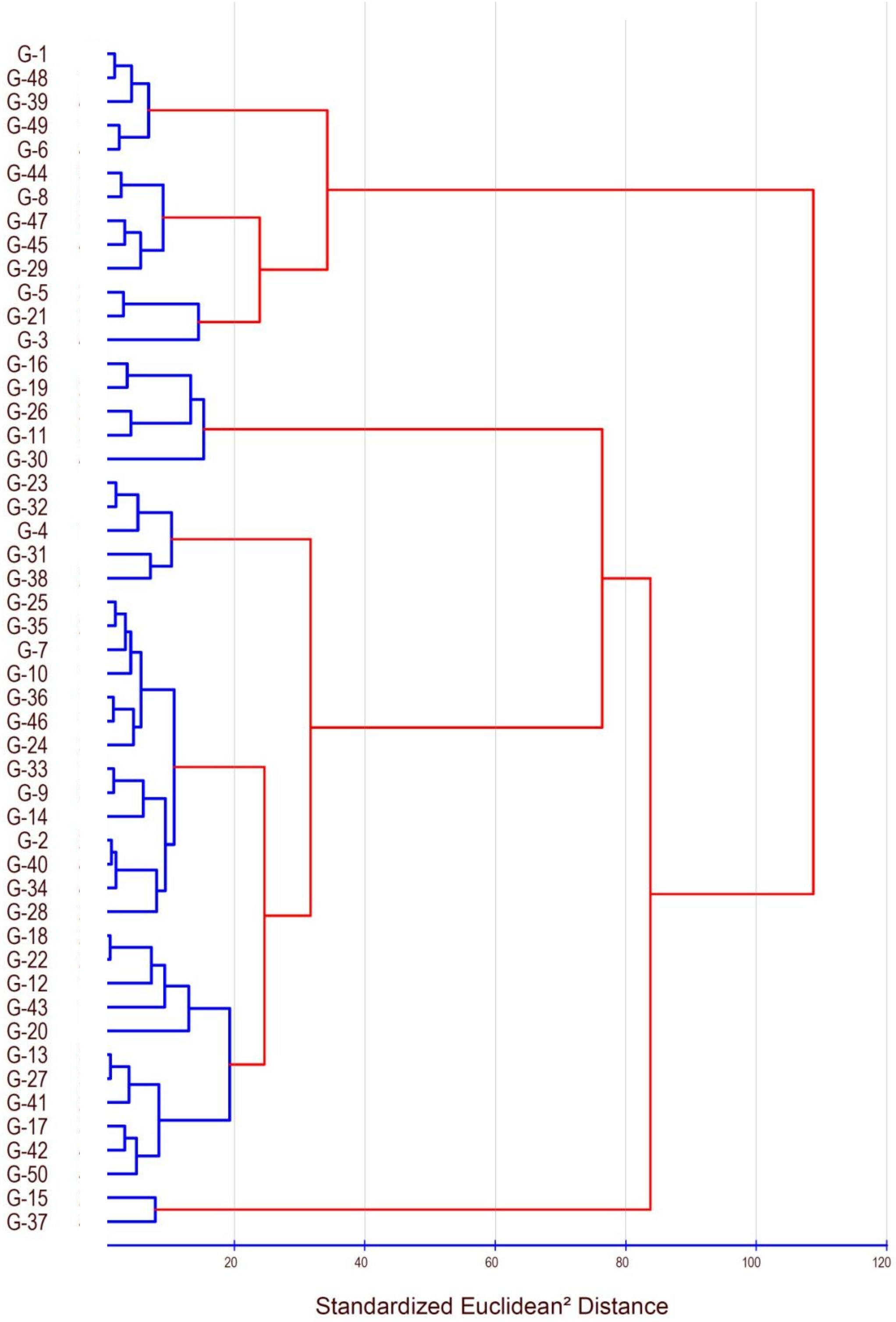


Fig. 3. Dendrogram showing relationship of 50 foxtail millet [*Setaria italica* (L.) Beauv.] genotypes in eight clusters based on Euclidean^2 distance of ward's method

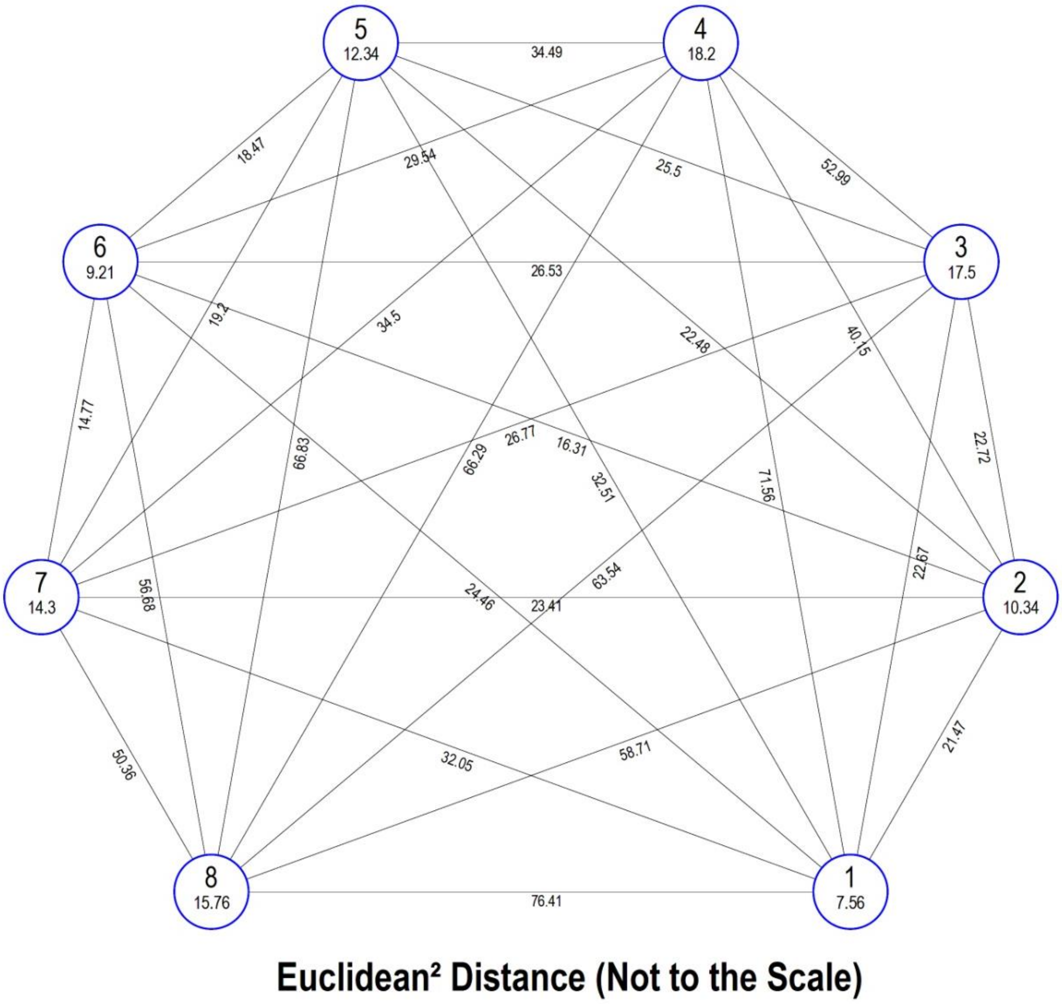
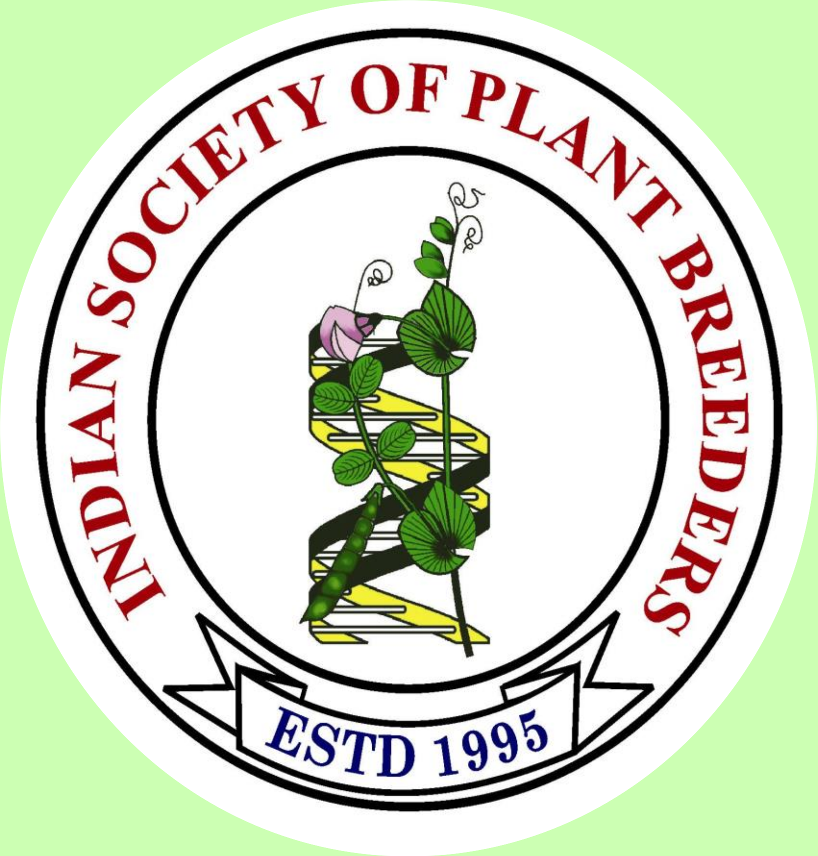


Fig. 4. Intra and inter-cluster distances of 50 foxtail millet [*Setaria italica* (L.) Beauv.] genotypes in eight clusters based on Euclidean^2 distances of Ward's method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