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Research Article

Multivariate analysis in paprika (*Capsicum annuum* L.)

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Abstract
Forty four genotypes of paprika (*Capsicum annuum* L.) which were evaluated to understand the extent of genetic diversity through 13 yield attributing and 7 quality traits at Horticultural Research Station, Lam, Guntur, Andhra Pradesh, revealed that the analysis of variance showed significant differences among the genotypes for all the characters studied. Based on hierarchical cluster analysis, the 44 genotypes were grouped into 7 clusters. Among all the clusters, cluster I was the largest containing 13 genotypes followed by cluster II with 10 genotypes, cluster III and cluster V with 6 genotypes, cluster VII with 4 genotypes, cluster IV with 3 genotypes and cluster VI with 2 genotypes. The maximum inter-cluster distance was observed between cluster IV and cluster VII (1217.10) and the minimum inter-cluster distance was observed between cluster I and cluster II (424.49). The cluster VII exhibited highest intra-cluster distance (475.42) and cluster VI had minimum intra-cluster Euclidean^2 distance value of 231.65. The maximum per cent of contribution towards genetic divergence for yield contributing traits was shown by dry fruit yield per plant, weight of seeds per fruit followed by 1000 seed weight, fruit diameter, fruit length and number of fruits per plant. Whereas, higher contribution to the total divergence for quality traits were reported for total extractable colour followed by red carotenoids, yellow carotenoids, ascorbic acid, oleoresin content and capsaicin content. The principal component analysis revealed that first eight principal components with eigen value more than one were observed to contribute 83.62 per cent towards the total variability. Among the eight principal components, PC_1contributed maximum towards variability (25.18%).Considering the diversity studies, hierarchical cluster the genotypes *viz.*, Warangal chapata double patti, Warangal chapata single patti, LCA-511 and LCA-512 belonging to cluster I and genotypes of cluster IV (LCA 466, LCA 480 and Jangareddygudem local) were identified as promising parents and could be utilized for efficient hybridization programme for high yield coupled with good quality traits in paprika.

Key words
Paprika, genetic divergence, cluster analysis, principal component analysis.

Introduction

Paprika (*Capsicum annuum* L.), 2n = 24 is one of the most important commercial vegetable as well as spice crops grown all over the world. The genus Capsicum belongs to family Solanaceae comprising about 20 to 30 species of the new world tropics and subtropics. It is native to tropical areas of the Western Hemisphere, including Mexico, Central America, South America, and West Indies. Paprika, a form of chilli is mainly valued for its high colour, low or no pungency and oleoresins. In chilli, three major products *viz.*, paprika, oleoresins and dried chilli (both in whole and powder form) are traded in the world market. The pungent principle of paprika is capsaicin, an acid amide of Vanillylamine. They are widely used in curry powder, paste, pickles, sauces and ketchups for its characteristic pungency, color and aroma. As Paprika gains high value/market for it's characteristic quality traits, development of a variety with higher yield coupled with good quality traits is very important, which is possible only through the hybridization programme between genetically diversified parent for these traits. Thus, genetic divergence existing in the population helps in the selection of suitable parents for utilization in any crop breeding programme leading to reduction in the number of crosses (Guerra *et al.*, 1999). The information on the nature and degree of genetic divergence is essential for the breeder to choose the right type of parents for hybridization in heterosis breeding (Farhad *et al.*, 2010; Khodadabi *et al.*, 2011). In order to benefit transgressive segregation, the knowledge of genetic distance between parents is necessary (Khodadabi *et al.*,2011).

Hybrids produced from distantly related parents are expected to exhibit higher heterosis and minimize the inherent genetic vulnerability than those from closely related parents (Lahbib *et al.*, 2012). Ward's minimum variance dendrogram (Hierarchial cluster analysis) creates subgroup within a cluster, so relative position of the genotypes within the clusters can be examined by the dendrogram distance. In case of Mahalanobi'sD^2(1936)analysis, one can only know the intra-cluster distance but not relative position of the genotypes in the respective cluster. Principal component analysis facilitates in-depth analysis of genetic divergence between genotypes in terms of spatial distance. Thus, main objective of this study was to analyze the potential genetic diversity by Mahalanobi's D^2 statistics among genotypes of paprika, grouping of the genotypes into different clusters by cluster analysis and to classify the genotypes into different groups based on cluster analysis and principal component analysis for selection of suitable genotypes for further paprika hybridization programme.

Material and Methods

The present investigation was carried out during *kharif,*2016 at Horticultural Research Station, Lam, Guntur with 44 genotypes of paprika in a randomized block design with two replications. The nursery was raised during the first week of August and the seedlings were transplanted at a spacing of 75 cm × 30 cm of 4 m length during first fortnight of September, 2016. Each row consisted of 12 plants, of which five competitive plants were selected at random for recording the observations. The crop was raised as per the recommended package of practices. A total of 20 characters, consisting of 13 yield attributing characters *viz.,* plant height (cm), plant spread (cm), number of primary branches per plant, daysto50%flowering, days to maturity, numberoffruitsper plant, fruit length (cm), fruit diameter (cm), fruit pedicel length (cm), number of seeds per fruit, weight of seeds per fruit (g), 1000 seed weight (g), dry fruit yield per plant (g), and 7 quality traits such as ascorbic acid (mg/100g), oleoresin content (%), capsaicin content (%), total extractable colour (ASTA units), red carotenoids (%) , yellow carotenoids (%) and total carotenoids (%) were used in the study using various statistical procedures such as analysis of variance was carried out as per the procedure given by Panse and Sukhatme (1985), agglomerative hierarchical clustering technique (Ward's minimum variance) was followed for cluster analysis as given by Anderberg (1993) and principal component analysis (PCA) as per Jackson(1991).

Results and Discussion

The analysis of variance (ANOVA) revealed significant differences among 44 genotypes for quantitative and qualitative traits indicating the existence of variability among the genotypes for the characters studied (Table 1). The per cent contribution towards genetic divergence by all the 20 contributing characters is presented in Table 2.The maximum contribution towards genetic divergence by the yield contributing traits was shown by dry fruit yield per plant, weight of seeds per fruits followed by 1000 seed weight, fruit diameter, fruit length and number of fruits per plant. Whereas, higher contribution to the total divergence for quality characters were reported for total extractable colour followed by red carotenoids, yellow carotenoids, ascorbic acid, oleoresin content and capsaicin content. Hence, selection for divergent parents based on these characters will be useful for heterosis breeding in paprika.

Agglomerative cluster analysis revealed that 44 genotypes of paprika were grouped into seven clusters of which high number of 13 genotypes were grouped in cluster I (LCA 445, LCA 472, LCA 442, LCA 425, LCA 446, LCA 490, LCA 436, LCA 430, LCA 499, LCA 436, LCA 470, LCA 503 and LCA 424) followed by cluster II with 10 genotypes (LCA 439, LCA 504, LCA 457, LCA 506, LCA 488, LCA 501, LCA 447, LCA 437, LCA 465 and LCA 441), cluster III (LCA 440, LCA 498, LCA 510, LCA 476 and LCA 475) and cluster V (LCA 482, Byadagidubbi, LCA 450, Byadagikaddi, Kt-1, LCA 513) with 6 genotypes each respectively, followed by cluster VII with 4 genotypes (LCA 511, Warangal chapata single patti, LCA 512, Warngalchapata double patti), cluster IV with 3 genotypes (LCA 466, LCA 480, Jangareddygudem local) and cluster VI with 2 genotypes (LCA 443 and LCA 453)(Table 3 and Figure 1). The random distribution of genotypes indicated absence of parallelism between geographical and genetic diversity. Farhad *et al.* (2010) reported six clusters with 45 chilli genotypes and Shrilekha *et al.* (2011) reported seven clusters with 38 genotypes and Lahbib *et al.* (2012) grouped 11 landraces into three clusters and Janaki *et al.* (2015) grouped 63 genotypes into eight clusters and these findings support the results of this investigation. The intra and inter-cluster distance represents the index of genetic diversity among clusters (Table 4 and Figure 2).

The maximum inter-cluster distance was observed between cluster IV and cluster VII (1217.10) followed by cluster V and cluster VII (1112.55) and

cluster II and cluster VII (1105.94), cluster I and cluster VII (1105.19), cluster IV and cluster VI (1004.54) and cluster V& cluster VI (1000.77). While the minimum inter-cluster distance was observed between cluster I and cluster II (424.49). Thus, the cross between the genotypes from cluster IV and VII can be used in paprika breeding to achieve maximum heterosis and to obtain heterotic hybrids and desirable segregants. The minimum inter-cluster distance was observed between cluster I and cluster II (424.49) which can be used for backcross breeding programmes. The genotypes of cluster II and cluster V (482.89), cluster II and cluster III (553.68), cluster III and cluster V (559.59) and cluster II and cluster IV (581.84) also have recorded minimum inter-cluster distance. The lowest inter-cluster distance between these cluster pairs suggested close proximity of genotypes of one cluster with those of the other cluster in respect of their genetic constitution. The findings on inter and intra cluster distances are in agreement with earlier works of Roy and Sorma (1996), Mishra *et al*., (2004), Farhad *et al*. (2010), Kumar *et al*. (2010), Lahbib *et al.* (2012) and Janaki *et al*. (2015).

Of the 7 clusters formed, the mean intra-cluster D^2 values ranged from 231.65 to 475.42 where cluster VI had minimum intra-cluster Euclidean^2 distance value of 231.65. The high intra-cluster distance was observed in cluster VII (475.42) followed by cluster V (395.51) and cluster III (347.26) indicating the presence of wide genetic diversity among the genotypes within these clusters. The genotypes grouped into the same cluster presumably diverge very little from one another and crossing of genotypes belonging to the same cluster is not expected to yield desirable segregants. Consequently, a crossing programme should be conducted with putative parents. Thus, crosses between the members of clusters separated by inter-cluster distances are likely to be beneficial for further improvement.

Cluster I showed high mean values for plant spread , number of primary branches per plant, dry fruit yield per plant ; cluster II for days to 50% flowering, number of fruits per plant, fruit length and yellow caroteniods ; cluster III for red and total carotenoids ; cluster IV for number of primary branches per plant, ascorbic acid and oleoresin ; cluster VI for days to maturity, fruit pedicel length and capsaicin ; cluster VII for plant height, fruit diameter, number of seeds per fruit, weight if seeds per fruit and 1000 seed weight. The clusters V, I, III, VI, VII, IV and II were found superior for one or more characters. Therefore, a multiple crossing programme can be proposed involving genotypes from these clusters to isolate superior segregants in advanced generations with high genetic yield potential and other desirable characters in chilli.

In the present investigation, the principal components with eigen values more than one were retained and less than one were considered as non-significant. The first eight principal components with eigen values more than one contributed 83.62 per cent towards the total variability. The first PC explained 25.18 per cent of the total variability in the set of all variables and remaining accounted for progressively lesser amount of variation (Table 6). The characters viz., weight of seeds per fruit, fruit diameter, number of seeds per fruit, 1000 seed weight, number of fruits per plant and fruit length significantly loaded in PC_1 and contributed more towards variability.

The hierarchical cluster analysis and principal component analysis confirmed the findings of each other. Results of cluster analysis based on PCA scores were compared with the results of the principal component analysis on a visual aid in desecrating clusters in the 2D and 3D scattered diagrams (Figures 3 & 4). The genotypes falling in same cluster were present closer to each other in scattered diagram thereby confirming the results of cluster analysis. 2D and 3D graphs showed wide divergence between Warangal chapatta double patti and Byadagikaddi which are also distantly placed with LCA 512 and Warangal chapata single patti, signifying their usefulness in paprika breeding to develop high heterotic hybrids. Utilization of principal component analysis combined with clustering of Ward's method in genetic divergence studies of chilli is supported by Thul *et al.* (2009), Kadri *et al.* (2009), Farhad *et al*. (2010), Sudre *et al.* (2010), Shrilekha *et al.* (2011), Lahbib *et al.*(2012) and Janaki *et al*.(2015).

Both the methods of grouping revealed a single concept of non-correspondence of genetic divergence and geographic diversity. In a broad sense both the methods of classifying the genotypes into different groups are equally useful but hierarchical cluster analysis gave an additional advantage of identifying sub-clusters of the major groups at different levels so that each small group can be critically analyzed and exploited in the breeding programmes. Considering the diversity studies and hierarchical cluster the genotypes *viz.*, Warangal chapata double patti, Warangal chapata single patti, LCA-511 and LCA-512 belonging to cluster I and genotypes of cluster IV

(LCA 466, LCA 480, Jangareddygudem local) were identified as promising parents and could be utilized for efficient hybridization programme for high yield coupled with good quality traits in paprika, as maximum inter-cluster distance was observed between these two clusters.

However, paprika is mainly valued for its high colour (total carotenoids), low or no pungency (low pungency) and oleoresins. Thus, hybridization programme between the genotypes belonging to cluster I (LCA 470, LCA 472, LCA 490, LCA 445, LCA 436) and cluster II (LCA 506, LCA 501, LCA 439 and LCA 476) would result in high dry fruit yield per plant For low pungency crossing may be carried out among the genotypes belonging to cluster V (LCA 482 and LCA 450), cluster I (LCA 425 and LCA 472). For low oleoresin content genotype LCA 482 of cluster V can be used as a parent and for high colour genotypes containing high total carotenoids content genotypes of cluster V (LCA 450 and Byadagidubbi), LCA 512 of cluster VII and LCA 499 of cluster I can be used for hybridization programme.

So, they can be exploited for the development of heterotic hybrids in future breeding programmes. From this study, it may be concluded that a wide range of variation for almost all the economically important traits is observed in this crop. This implies a great potential for breeding through hybridization programme or direct use as variety for successful paprika production. Further, one or two promising genotypes from different clusters may be chosen for further genetic studies either by way of diallel or line x tester analysis. As, the greater genetic diversity we possess, the larger heterotic pool we can built for either positive or negatively related characters and most importantly economically viable characters. Hence, the genetically divergent genotypes may be used as mapping populations to detect diversity at molecular level and also to identify molecular markers linked to desirable traits for marker assisted selection(MAS).

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Table 1.Analysis of variance for various characters in paprika (*Capsicum annuum*L.)

S.No.	Character	Mean sum of squares		
		Replications	Genotypes	Error
1	Plant height (cm)	0.16	257.11**	122.38
2	Plant spread (cm)	12.45	698.05**	187.34
3	Number of primary branches per plant	0.02	0.17**	0.04
4	Days to 50 per cent flowering	0.40	52.46**	12.59
5	Days to maturity	405.92	840.97**	121.33
6	Number of fruits per plant	78.09	2217.27**	97.27
7	Fruit length (cm)	2.10	10.32**	0.78
8	Fruit diameter (cm)	0.13	2.92**	0.11
9	Fruit pedicel length (cm)	0.20	0.68**	0.13
10	Number of seeds per fruit	192.63	1204.12**	85.28
11	Weight of seeds per fruit (g)	0.001	0.09**	0.004
12	1000 seed weight (g)	1.73	16.86**	0.73
13.	Ascorbic acid (mg/100g)	145.89	4674.56**	151.61
14	Oleoresin content (%)	0.53	16.66**	0.66
15	Capsaicin content (SHU)	1784670.75	36198080.00**	157919.25
16	Total extractable colour (ASTAunits)	22.99	2376.67**	115.58
17	Red carotenoids (%)	0.0005	0.02**	0.0007
18	Yellow carotenoids (%)	0.00004	0.015**	0.0009
19	Total carotenoids (%)	0.0003	0.03**	0.0019
20	Dry fruit yield per plant (g)	0.10	2316.47**	178.91

**Significant at 1 per cent level

Table 2 . Relative contribution of different characters towards genetic divergence in paprika(*Capsicum annum* L.)

S.No	Source	Times Ranked 1st	Contribution %
1	Plant height (cm)	0	0.01
2	Plant spread (cm)	0	0.01
3	Number of primary branches per plant	0	0.01
4	Days to 50 per cent flowering	1	0.11
5	Days to maturity	0	0.01
6	Number of fruits per plant	10	1.06
7	Fruit length (cm)	10	1.06
8	Fruit diameter (cm)	28	2.96
9	Fruit pedicel length (cm)	2	0.21
10	Number of seeds per fruit	4	0.42
11	Weight of seeds per fruit (g)	56	5.92
12	1000 seed weight (g)	41	4.33
13	Ascorbic acid (mg /100g)	69	7.29
14	Oleoresin content (%)	66	6.98
15	Capsaicin content (SHU)	65	6.87
16	Total extractable colour (ASTA units)	204	21.56
17	Red carotenoids (%)	188	19.87
18	Yellow carotenoids (%)	132	13.95
19	Total carotenoids (%)	1	0.11
20	Dry fruit yield per plant (g)	69	7.29

Table 3.Clustering of 44 paprika genotypes (*Capsicum annuum* L.) by Ward's minimum variance method

Cluster No.	No. of genotypes	Name of genotypes
I	13	LCA 445, LCA 472, LCA 442, LCA 425, LCA 446, LCA 490, LCA 436, LCA 430, LCA 499, LCA 436, LCA 470, LCA 503 and LCA 424
II	10	LCA 439, LCA 504, LCA 457, LCA 506, LCA 488, LCA 501, LCA 447, LCA 437, LCA 465 and LCA 441
III	6	LCA 440, LCA 498, LCA 510, LCA 476 and LCA 475
IV	3	LCA 466, LCA 480, Jangareddygudem local
V	6	LCA 482, Byadagidubbi, LCA 450, Byadagikaddi, Kt-1, LCA 513
VI	2	LCA 443 and LCA 453
VII	4	LCA 511, Warangal chapata single patti, LCA 512, Warngalchapata double patti

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Table 4 .Average intra (bold) and inter cluster Euclidean^{2}values of seven clusters in paprika (*Capsicum annuum*L.)

Cluster	1 Cluster	2 Cluster	3 Cluster	4 Cluster	5 Cluster	6 Cluster	7 Cluster
I	**296.69**	424.39	678.65	838.02	571.97	679.94	1105.19
II		**242.37**	553.68	581.84	482.89	939.10	1105.94
III			**347.26**	695.20	559.59	815.43	850.93
IV				**282.82**	567.75	1004.54	1217.10
V					**395.51**	1000.77	1112.55
VI						**231.65**	839.90
VII							**475.42**

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Table 5. Mean performance of yield per plant and its component characters in various clusters of paprika (Ward's minimum variance method)

Cluster No.	PH	PS	NPBP	DFF	DM	NFP	FL	FD	FPL	NSP	WSP	1000 SW	AA	OC	CC	TEC	RC	YC	TC	DFYP
I	89.86	**138.00**	**3.70**	**56.92**	129.96	167.19	12.79	4.75	3.92	73.48	0.49	**3.78**	62.08	8.64	7764.11	132.71	0.20	**0.14**	0.34	**154.30**
II	92.29	129.50	3.56	**62.10**	144.95	**187.50**	**12.90**	4.29	3.78	57.74	0.40	5.37	66.68	8.82	9132.95	98.36	0.09	**0.26**	0.35	141.15
III	82.81	**120.15**	3.56	62.00	143.66	164.29	10.77	4.20	3.10	**50.55**	0.41	6.82	137.51	**6.88**	14640.91	**64.54**	**0.30**	0.16	**0.46**	124.33
IV	88.1	130.03	**3.70**	61.00	127.66	143.70	8.60	4.61	**3.00**	64.36	0.39	5.34	**171.15**	**11.26**	6983.50	91.37	**0.08**	0.22	0.30	114.33
V	**79.98**	121.26	**3.40**	59.33	**111.41**	119.70	11.36	**3.51**	3.28	59.66	**0.38**	7.10	139.69	8.78	**5772.00**	116.58	0.27	0.24	0.51	**92.75**
VI	93.55	123.15	3.50	60.50	**153.25**	112.75	**8.42**	6.00	**3.97**	92.70	0.53	5.76	**29.85**	8.22	**19381.00**	**151.76**	0.11	0.16	**0.28**	96.75
VII	**98.70**	124.35	3.45	61.50	121.87	**111.05**	10.67	**6.77**	3.12	**123.20**	**1.03**	**11.53**	97.02	10.93	8302.00	110.49	0.20	0.25	0.45	100.00

*****Bold values indicate maximum and minimum mean performance

Where

PH – Plant Height (cm), PS-Plant Spread(cm), NPBP – Number of Primary Branches per Plant, DFF – Days to 50 per cent Flowering , DM- Days to Maturity, NFP – Number of Fruits per Plant, FL – Fruit Length (cm), FD – Fruit Diameter (cm), FPL- Fruit Pedicel Length(cm), NSF – Number of Seeds per Fruit, WSF- Weight of Seeds per Fruit (g), 1000 SW- 1000 Seed Weight (g), AA -Ascorbic Acid(mg/100mg), OC- Oleoresin Content(%), CC- Capsaicin Content (SHU), TEC- Total Extractable Colour (ASTA units), RC- Red Carotenoids(%), YC- Yellow Carotenoids (%), TC- Total Carotenoids(%), DFYP – Dry Fruit Yield per Plant (g).

Table6.Eigen values, proportion of the total variance, cumulative per cent variance and component loading of different characters in paprika (*Capsicum annuum* L.)

	PC_1	PC_2	PC_3	PC_4	PC_5	PC_6	PC_7	PC_8
Eigene Value (Root)	**5.03**	**3.00**	**2.46**	**1.75**	**1.42**	**1.16**	**1.00**	**0.86**
% Var. Exp.	**25.18**	**15.02**	**12.33**	**8.79**	**7.12**	**5.84**	**5.00**	**4.31**
Cum. Var. Exp.	**25.18**	**40.20**	**52.54**	**61.33**	**68.45**	**74.30**	**79.30**	**83.62**
Plant Height (cm)	0.09	0.24	0.15	0.15	0.18	0.24	0.17	0.52
Plant Spread (cm)	-0.18	0.24	-0.02	0.06	0.19	0.28	-0.46	-0.45
Number of Primary Branches Per Plant	-0.14	0.12	0.003	0.33	0.10	0.57	0.19	0.12
Days to 50% Flowering	0.09	-0.30	0.24	0.34	-0.03	-0.12	-0.25	0.25
Days to Maturity	-0.03	-0.07	0.35	0.47	-0.02	-0.26	-0.09	0.00002
Number of Fruits Per Plant	-0.28	0.06	0.37	-0.05	0.15	0.02	0.21	-0.16
Fruit Length (cm)	-0.28	0.10	0.36	-0.07	-0.01	0.03	-0.17	-0.03
Fruit Diameter (cm)	0.37	0.05	0.12	0.03	0.19	-0.13	0.09	0.03
Fruit Pedicel Length (cm)	0.15	0.15	-0.31	0.40	-0.17	-0.11	-0.33	-0.05
Number of Seeds Per Fruit	0.35	0.17	0.06	-0.01	0.20	0.05	-0.34	-0.03
Weight of seeds Per Fruit (g)	0.38	0.08	0.09	-0.13	0.20	0.11	-0.18	0.02
1000 Seed Weight (g)	0.31	-0.16	0.01	-0.35	0.07	0.01	0.06	-0.05
Ascorbic Acid (mg /100g)	-0.10	-0.33	-0.34	0.18	-0.06	0.25	-0.01	-0.05
Oleoresin content (%)	-0.03	0.16	-0.29	0.08	0.54	-0.12	0.17	-0.12
Capsaicin content (SHU)	0.18	0.17	-0.009	0.10	-0.44	-0.02	0.29	-0.32
Total extractable colour (ASTA Units)	-0.25	0.37	-0.01	-0.15	-0.01	-0.24	-0.20	0.21
Red Carotenoids (%)	0.09	0.11	0.13	-0.25	-0.43	0.39	-0.22	0.14
Yellow Carotenoids (%)	-0.22	-0.31	0.14	-0.16	0.14	-0.08	-0.1	-0.05
Total Carotenoids (%)	-0.17	0.40	-0.18	-0.06	-0.15	-0.28	0.01	0.24
Dry Fruit Yield Per Plant (g)	0.16	0.26	0.33	0.14	-0.08	-0.03	0.22	-0.38

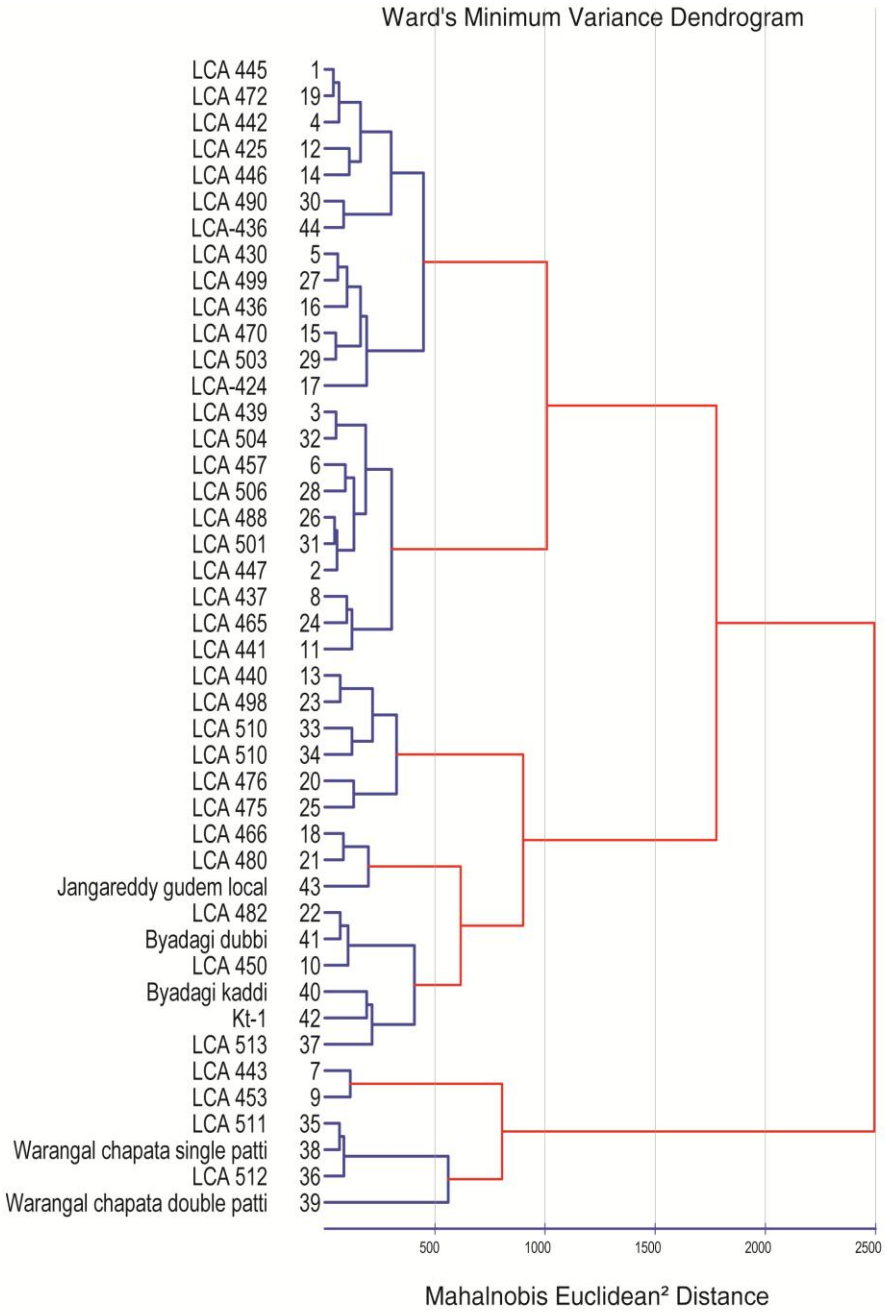

Fig. 1.Dendrogram showing clustering pattern of 44 paprika (*Capsicum annuum* L.) genotypes (Ward's minimum variance method).

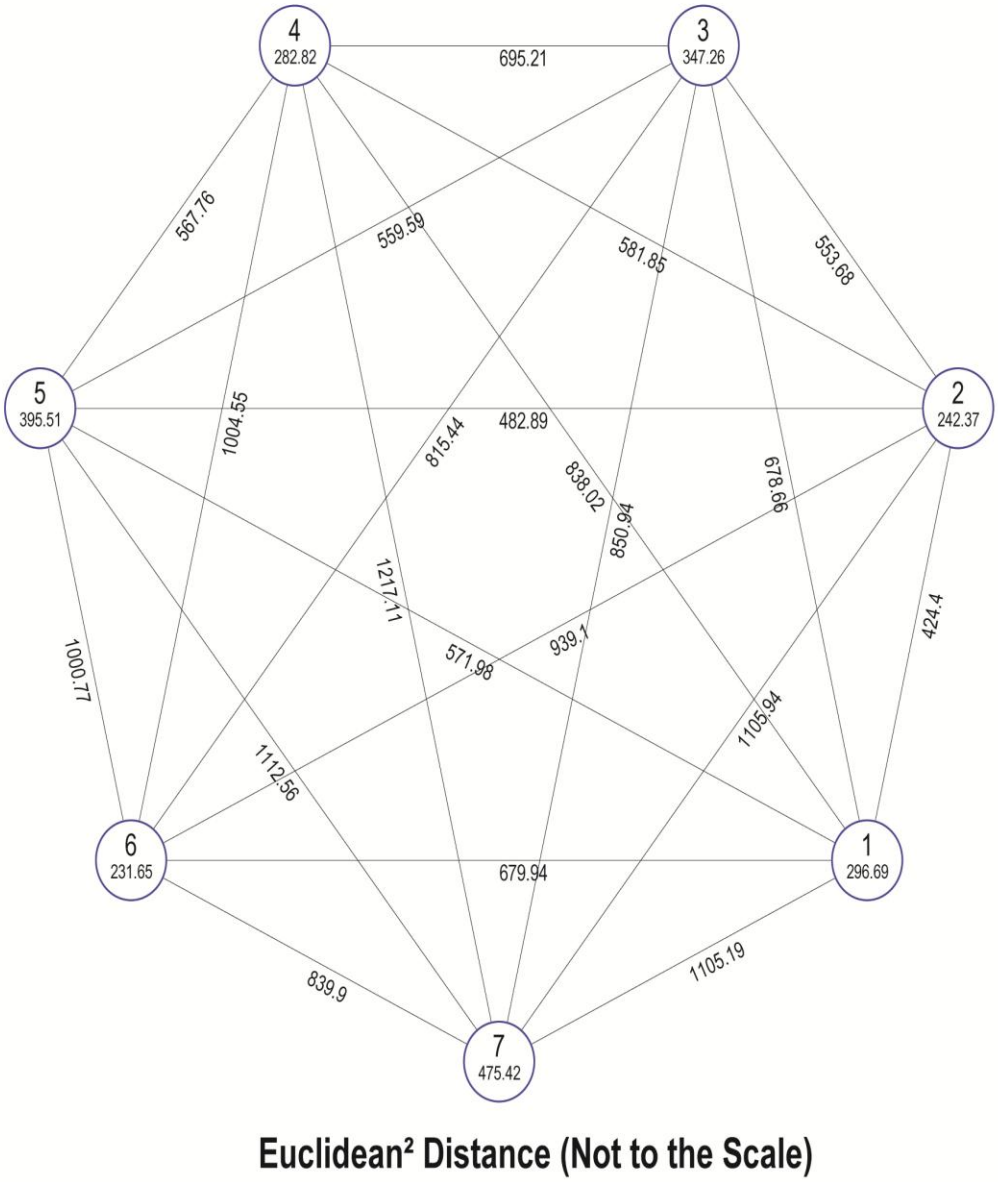

Fig. 2. Average intra and inter cluster Euclidean values of seven clusters in paprika(*Capsicum annuum L.)*

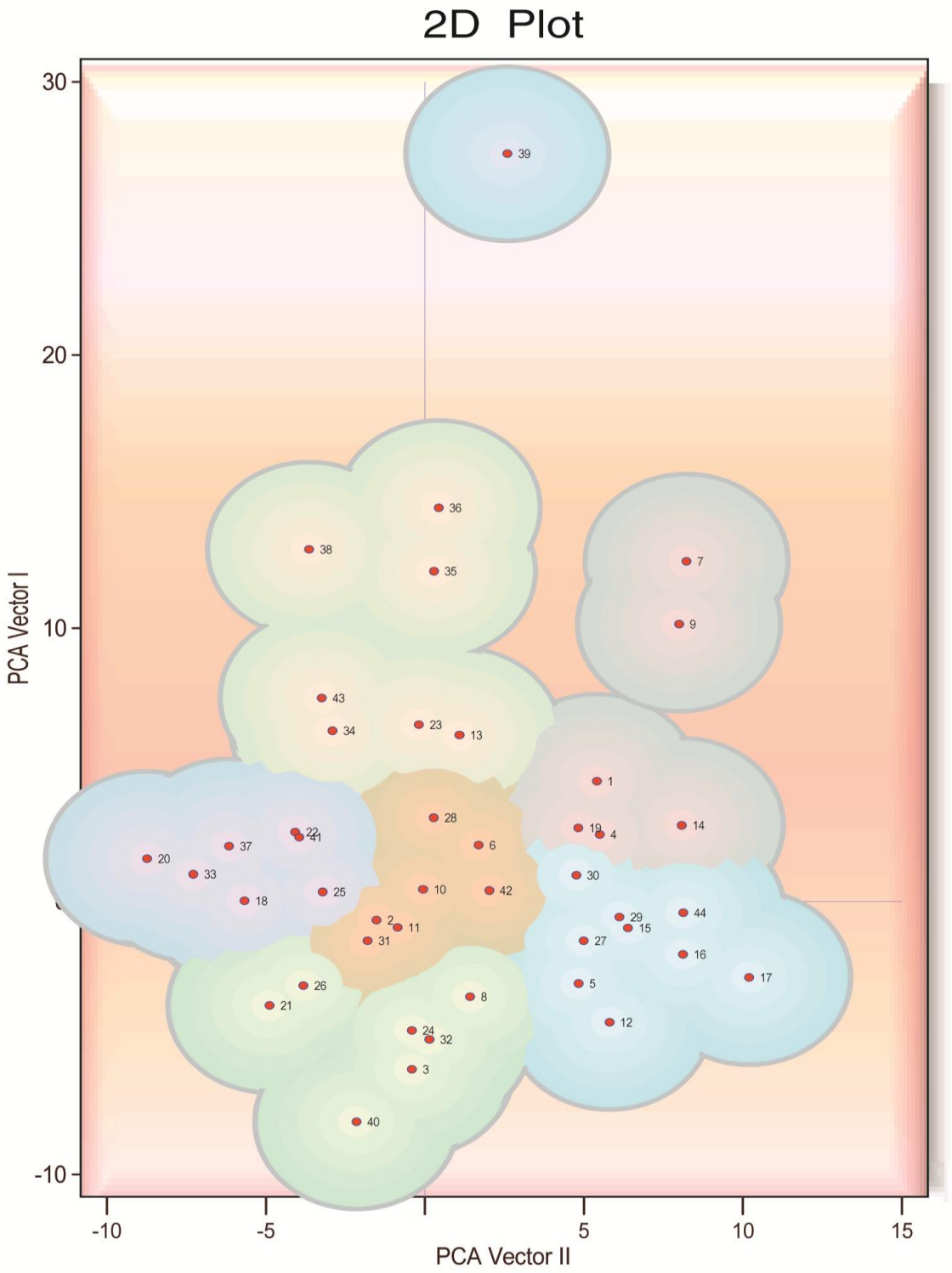

Fig. 3.Two dimensional graph showing relative position of 44 paprika (*Capsicum annuum* L.) genotypes based on PCA scores.

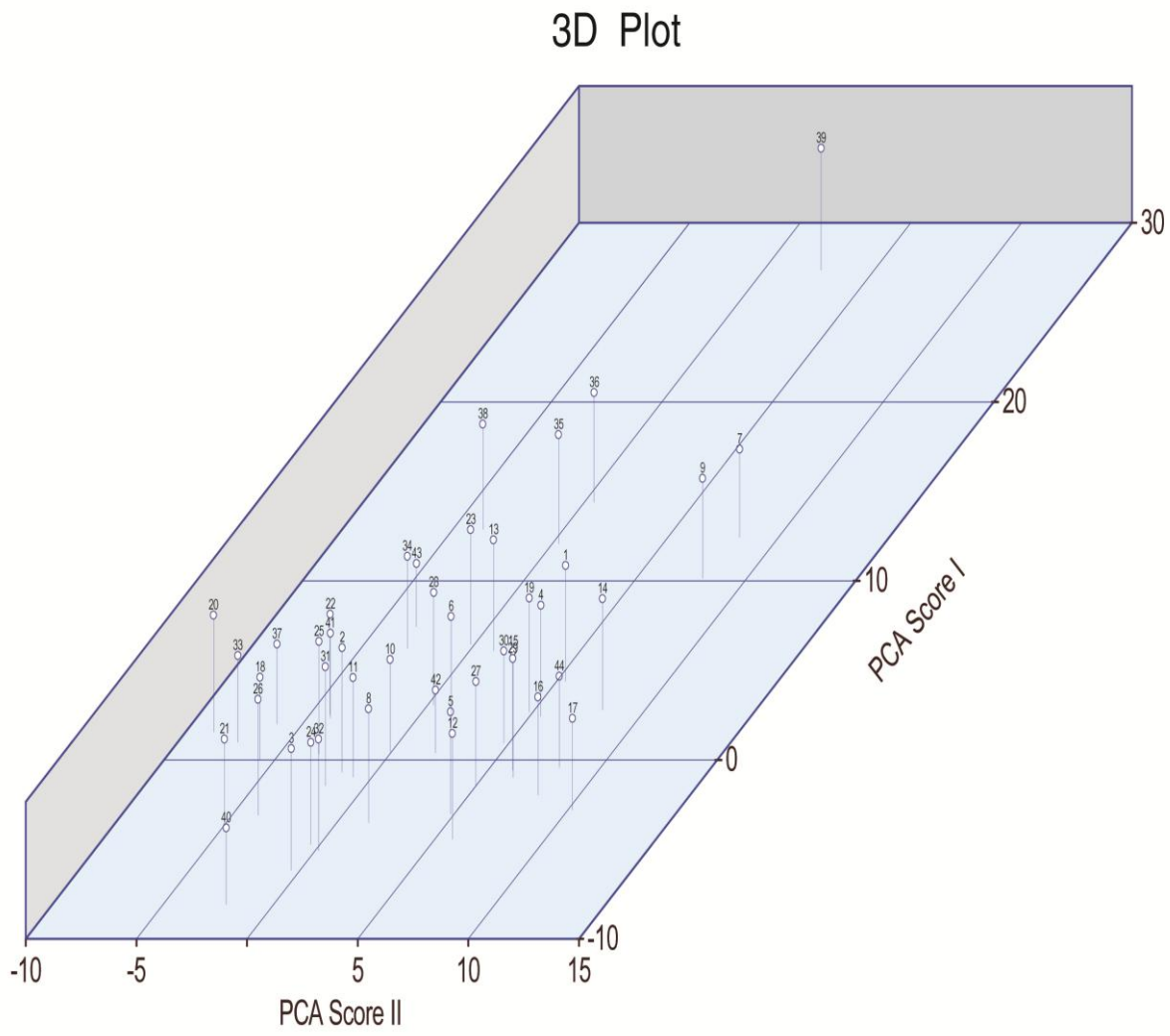

Fig.4.*Three dimensional graph showing relative position of 44 paprika (Capsicum annuum* L.*) genotypes based on PCA scores.*