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Multivariate Analysis in 1% EMS Treated Tomato (*Lycopersicon esculentum* M.) *cv.* Arka vikas.

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ABSTRACT

An experiment was conducted to analyze the genetic diversity among 1.00 % EMS mutagen treated seeds of variety Arka vikas for 17 morphological and biochemical characters in tomato at Agricultural College Farm, Bapatla, Andhra Pradesh in 109 M_3 families along with control (untreated). The 109 M_3 families along with control with an optimum stand of 45-50 plants per family (unreplicated), were grouped into 11 clusters based on hierarchial cluster analysis. Among all the clusters, cluster II was the largest with 21 families followed by cluster I (with control Arka vikas) and X each with 14 families, cluster VIII with 11 families, cluster III with 10 families, cluster IX with 9 families, cluster IV and XI each with 8 families, cluster V and VII each with 6 families and cluster VI with 4 families. This random distribution of mutant families indicated that genetic diversity is existed not only from parent but also among themselves due to chromosomal anomalies for the seventeen characters studied. In the principal component analysis the first seven principal components with eigen values more than one contributed 74.53 per cent towards the total variability. It was therefore inferred that the essential features of data set had been represented in the first seven principal components. The first principal component contributed maximum towards variability (15.57 %).

Key words: *Ethyl methane sulfonate, Genetic divergence, Hierarchial cluster analysis and Principal component*

Tomato (*Lycopersicum esculentum* Mill.) belongs to the nightshade family *Solanaceae* and is considered one of the most important and world's major traded vegetable. It has wide usuage in Indian culinary tradition because of its special nutritive value and occupies an area of 1,204 hectares with a production and productivity of 19,402 million tons and 16.10 million tons per hectare respectively.

The success of any breeding method depends on the availability of genetic diversity in the base population. Utilization of diverse parents in hybridization programmes has been observed to yield better hybrids. Hierarchial cluster analysis highlights the nature of relationship between any type of samples described by any type of descriptors. It could serve as a basis for selection of parental types that could result into superior hybrids. Principal component analysis is frequently used to determine the relative significance of different variables of classification, prior to cluster analysis (Jackson, 1991). Additionally PCA also gives a reduced dimension model that would point out the measured differences among different groups and leads to understanding of variables by telling how much of the total variance is explained by each one. It facilitates in depth analysis of genetic divergence between varieties in terms of spatial distance. The objective of this study is to analyze the genetic diversity among 109 M_3 families along with control in tomato and to classify the families into different groups based on Euclidian distance and principal component analysis.

MATERIAL AND METHODS

The present investigation was taken up during *rabi* 2012-13, *kharif* 2013, *rabi* 2013-14 and *kharif* 2014 at Agricultural College Farm, Bapatla, Andhra Pradesh. The soils were red sandy loam. Recommended doses of fertilizers were applied in split doses.

Ethyl methane sulfonate (EMS) mutagenesis:

An Indian cultivar of *Solanum lycopersicum cv.* Arka vikas (Sel 22), was used to develop the ethyl methane sulfonate (EMS) induced mutagenized population. Breeder seed of Arka vikas was procured from IIHR, Bangalore. Batches of ~10,000 seeds (M_0 seeds) were soaked in distilled water for 24 h at room temperature. After removing

Table 1. Clustering of 109 M_3 families along with control in tomato (*Lycopersicon esculentum* M.) treated with 1 % EMS by Ward's minimum variance method.

Cluster No	No. of families	Family no.
I	14	**Arkavikas**,e1-10,e69-8,e78-4,e99-2,e116-4,e133-6,e149-8,e225-10,e255-10,e372-9,e375-12,e380-6,e392-4
II	21	e52-13,e54-13,e96-8,e106-13,e146-10,e159-6,e164-10,e253-8,e261-9,e281-1,e288-9,e292-5,e300-9,e301-8,e319-8,e347-9,e349-9,e352-1,e357-6,e359-9,e440-11
III	10	e34-16,e227-10,e215-7,e230-4,e233-9,e341-8,e388-10,e412-9,e422-3,e436-9,
IV	8	e110-10,e202-6,e260-1,e269-8,e270-3,e290-8,e295-8,e313-3
V	6	e22-8,e70-2,e320-4,e323-9,e327-5,e443-8
VI	4	e3-15,e128-4,e235-12,e237-8
VII	6	e79-3,e88-2,e97-3,e98-6,e142-6,e220-15
VIII	11	e94-1,e152-14,e199-4,e224-8,e298-7,e303-9,e310-9,e368-11,e369-12,e402-11,e415-5
IX	9	e1-8,e30-3,e81-6,e87-8,e172-8,e190-9,e193-8,e239-9,e280-6
X	14	e6-16,e13-4,e122-7,e137-8,e210-8,e212-9,e213-8,e274-9,e330-6,e328-1,e331-1,e333-12,e337-7,e433-2,
XI	8	e39-6,e40-2,e117-8,e166-6,e188-6,e246-9,e276-1,e299-8,

excess water, seeds were submerged in freshly prepared 500 ml solution of EMS at a concentration of 1 % for 12 h in dark with gentle shaking at 25±2°C. The mutagenized seeds (M_1) were placed in muslin cloth bag and extensively washed under running tap water for 8 h. The M_1 seeds were sown in nursery bed containing red loam sandy soil prepared in the open field conditions. A batch of 1000 seeds were used as a control and processed through the same procedures as mentioned above without EMS treatment. The M_1 plants were grown and were allowed to self-pollinate. Each fertile M_1 plant was treated as independent line and was numbered with the tags. Fruits were collected from individual M_1 plant and the M_2 seeds were extracted. The dried seeds were placed in the aluminium foil bags with their respective tags and then kept each of them inside a polythene zip lock bags. Finally the M_2 seed packets were serially arranged in plastic boxes and stored at -20 °C in freezers. About 20-25 seeds were taken out from each M_2 seed packet and placed in petri plates and surface sterilized with 20% (v/v) sodium hypochlorite solution for 15-20 min, then washed thoroughly under running tap water. The surface sterilized seeds were transferred in to portrays (germination trays) filled with soil rite mix (vermiculite and peat mixture. Each individual M_2 line was tagged with tear proof labels after transplantation. About 420 M_2 families (each family with an optimum plant stand of 16-20 plants) were screened for viable mutants by maintaining optimum population (16-20 plants) for each family. Every plant in the M_2 generation was visually phenotyped according IBPGR descriptors to study viable phenotypic (macro) mutants. About 109 M_2 plants showing phenotypic variation from control were identified after screening 420 M_2 families. Fruits were collected from these 109 individual M_2 plants and M_3 seeds were extracted. About 60 seeds were taken out from each M_3 seed packet and placed in petri plates and surface sterilized with 20% (v/v) sodium hypochlorite solution for 15-20 min, then washed thoroughly under running tap water. The surface sterilized seeds were transferred into portrays (germination trays) filled with soil rite mix (vermiculite and peat mixture. Optimum size of the population (about 45-50 plants for each individual M_3 family) was maintained. 3-4 weeks old seedlings were transplanted at a spacing of 50 x 50 cm along with control and are allowed to self-pollinate in open field as unreplicated trail. Each individual M_3 line was tagged with tear proof labels as described above. The data was recorded on 10 randomly selected competitive plants per family for all the progeny rows in M_3 generation for 17 quantitative and biochemical parameters *viz.,* plant height (cm), no. of primary branches per plant, days to 50%

Table 2. Average intra and inter- cluster Eucledian^2 values among the eleven clusters in 109 M_3 families along with control in tomato (*Lycopersicon esculentum* M.) treated with 1% EMS.

Cluster No	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
I	**291.95**	405.58	405.89	571.21	453.97	928.77	549.71	483.72	760.27	470.44	806.02
II		**301.98**	370.96	487.24	449.56	995.09	865.17	593.11	918.45	597.73	1191.69
III			**262.88**	739.21	401.05	961.05	627.14	622.44	558.36	413.12	899.45
IV				**359.70**	728.61	1150.65	1133.42	612.24	1622.15	1063.99	1684.75
V					**196.07**	505.56	840.36	867.60	622.51	513.44	870.16
VI						**429.57**	1367.10	1478.63	1105.56	957.29	1142.32
VII							**391.08**	536.68	804.21	575.48	657.90
VIII								**241.81**	1298.29	750.91	1104.37
IX									**280.44**	439.13	612.15
X										**311.64**	531.79
XI											**368.10**

Bold and diagonal values indicate intra-cluster distance

flowering, no. of flower clusters per plant, no. of fruit clusters per plant, no. of fruits per cluster, no. of fruits per plant, fruit weight (g), early fruit yield per plant, no. of locules per fruit, fruit shape index, pericarp thickness (cm), TSS (° Brix), titrable acidity, pH, lycopene (mg/100g) and â-carotene (mg/100g). Agglomerative heirarchial clustering technique (Ward's minimum variance) was followed for cluster analysis as given by Anderberg (1993). PCA was performed as per Jackson (1991).

RESULTS AND DISCUSSION

Coefficient of variation indicated sufficient variability in the material under study indicating considerable genetic diversity among 109 M_3 families. The 109 M_3 families along with control were grouped into 11 clusters. The distribution of genotypes into 11 clusters is presented in Table-1 . Among all the clusters, cluster II was the largest with 21 families followed by cluster I (with control Arka vikas) and X each with 14 families, cluster VIII with 11 families, cluster III with 10 families, cluster IX with 9 families, cluster IV and XI each with 8 families, cluster V and VII each with 6 families and cluster VI with 4 families. This random distribution of mutant families indicated that genetic diversity is existed not only from parent but also among themselves due to chromosomal anomalies for the seventeen characters studied. The mutual relationship between clusters is represented diagrammatically by taking average intra- and inter-cluster Euclidean^2 distances.

The intra- and inter- cluster distance represent the index of genetic diversity among clusters (Table 2). Of the 11 clusters formed, cluster V has minimum intra cluster Euclidean^2 distance value of 196.07 followed by cluster VIII (241.81), cluster III (262.88), cluster IX (280.44), cluster I (291.95), cluster II (301.98), cluster X (311.64), cluster IV (359.70), cluster XI (368.10), cluster VII (391.08) and cluster VI (429.57). The inter cluster Euclidean^2 distances varied from 370.96 (between cluster II and cluster III) to 1684.75 (cluster IV and XI). Cluster means were computed for the 17 characters studied on pooled basis and are presented in Table-3. Cluster V showed high mean values for yield and most of the yield contributing traits like no. of flowers per cluster (5.20), no. of fruit clusters per plant (15.82), no. of fruits per plant (45.22), early fruit yield per plant (2.24) and total soluble solids (5.54) and cluster VI recorded high mean values for no. of primary branches per plant (10.90)

Table 3. Mean values of eleven clusters estimated by Ward's method in 109 M_3 families along with control in tomato (*Lycopersicon esculentum* M.) treated with 1 % EMS.

Cluster No	Plant height (cm)	No. of primary Branches/ Plant	Days to 50 % flower-ing	No. of flow-ers/ Cluster	No. of fruit clusters/ plant	No. of fruits/ cluster	No. of fruits/ Plant	fruit weight (g)	Early fruit yield /plant (kg)
I	71.43	10.38	**35.81**	4.77	**12.16**	3.06	**36.49**	42.70	1.55
II	77.32	9.88	40.99	4.71	13.59	**3.28**	43.02	50.45	2.09
III	66.92	9.96	**44.72**	4.33	14.98	3.02	41.79	48.96	1.97
IV	**90.70**	9.69	38.44	4.09	12.70	3.20	39.28	45.12	1.80
V	61.96	9.32	37.83	**5.20**	**15.82**	3.07	**45.22**	49.73	**2.24**
VI	57.34	**10.90**	40.55	**3.60**	14.73	**2.90**	41.15	49.45	1.94
VII	62.45	**9.27**	40.05	3.83	13.57	2.87	38.15	**28.97**	1.17
VIII	81.78	10.43	40.48	4.23	14.04	3.25	44.93	30.31	**1.28**
IX	**44.51**	9.60	40.02	4.31	13.06	3.08	39.38	**50.61**	1.98
X	57.66	10.89	40.79	3.94	14.51	3.04	43.28	43.57	1.89
XI	45.80	9.99	37.61	4.54	12.73	3.21	39.31	33.19	1.33

Cluster No	Pericarp thickness (cm)	No. of locules/fruit	Fruit shape index	Total soluble solids (^{0}Brix)	Titrableacidity (g of citric acid/100 ml of juice)	pH	Lycopene (mg/ 100g)	β-Carotene (mg/ 100g)
I	0.35	**5.02**	0.78	5.18	0.40	4.42	4.37	0.18
II	0.36	4.63	0.65	5.20	0.42	4.51	4.70	0.18
III	0.29	4.24	0.91	4.98	0.41	4.47	4.63	0.17
IV	0.41	5.00	0.69	5.53	0.42	4.57	3.87	0.27
V	0.38	4.73	0.81	**5.54**	0.39	4.61	4.52	0.31
VI	**0.54**	4.78	**0.59**	4.94	**0.33**	**4.41**	4.66	**0.46**
VII	**0.25**	4.50	0.97	**4.76**	0.39	4.48	**5.12**	0.15
VIII	0.30	**4.14**	0.74	5.46	0.41	**4.66**	3.90	0.14
IX	0.29	4.33	**0.94**	5.00	0.39	4.45	4.37	0.14
X	0.40	4.71	0.79	5.03	0.40	4.45	**3.86**	**0.12**
XI	0.48	4.22	0.74	5.35	**0.44**	4.55	3.95	0.15

Note: Bold figures are minimum and maximum values

and pericarp thickness (90.54). So mutant families from cluster V (e1-8, e30-3, e81-6, e87-8, e172-8, e190-9, e193-8, e239-9 and e280-6) and cluster VI (e3-15, e128-4, e235-12 and e237-8) can be used for tomato yield improvement programme. Among the quality attributes, high mean values for total soluble solids, â-carotene, lycopene, pH and titrable acidity were recorded each by cluster V, VI, VII, VIII and XI, respectively. So, the mutant families from these clusters depending on the objective can be used for quality improvement.

In the present investigation, the principal components with eigen values >1 were retained and <1 were considered as non-significant (Legendre and Legendre, 1984). The first seven principal components with eigen values more than one contributed 74.53 per cent towards the total variability. The first PC explained 15.57 per cent of the total variability in the set of all variables and remaining ones accounted for progressively lesser amount of variation (Table 4).

The hierarchial cluster analysis and PCA 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 scattered diagram. The mutant families falling in same cluster were present closer

Table 4. Eigen values, proportion of the total variance represented by first eight principal components, cumulative per cent variance in tomato (*Lycopersicon esculentum* M.)

	PC_1	PC_2	PC_3	PC_4	PC_5	PC_6	PC_7
Eigene Value (Root)	2.65	2.25	2.00	1.68	1.62	1.35	1.13
% Var. Exp.	15.57	13.22	11.75	9.87	9.53	7.94	6.65
Cum. Var. Exp.	15.57	28.79	40.54	50.41	59.94	67.87	74.53

to each other in scattered diagram there by confirming the results of cluster analysis. Bernousi *et al.* (2011) , Evgenidis *et al.* (2012), Glogovac *et al.* (2012), Chernet *et al. (* 2014), Iqbal *et al.* (2014) and Osei *et al.* (2014) studied the utilization of principal component analysis combined with clustering of Ward's method in genetic divergence studies in tomato.

Both the methods of grouping revealed that no definite relationship of mutagenic origin and clustering of mutant families was observed. The mutant families developed from the same mutagenic treatment (1.00% EMS) often grouped into different clusters indicating that this particular treatment was effective in inducing diverse types of changes/chromosomal anomalies in the seventeen traits studied. The hybridization program between the divergent mutant families *i.e.* with more inter-cluster distance is expected to give promising and desirable segregants in subsequent generations. Usually, higher genetic diversity between any two mutant families is indication of good combiners for cross ability and might ultimately yield appreciable recombinants. But these crosses should be tested to get final conclusion.

LITERATURE CITED

Anderberg M R 1993 *Cluster Analysis for Application*. Academic Press, New York.

Bernousi I, Aliyeh E, Tajbakhsh M, Darvishzadeh R and Henareh M 2011 Studies on genetic variability and correlation among the different traits in *Solanum lycopersicum* L. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca,* 39 (1): 152-158.

Chernet S, Belew D and Abay F 2014 Genetic diversity studies for quantitative traits of tomato (*Solanum lycopersicon* L.) genotypes in Western Tigray, Northern Ethiopia. *Journal of Plant Breeding and Crop Science,* 6 (9):105-113.

Evgenidis G, Traka-Mavrona E and Koutsika-Sotiriou M 2011 Principal component and cluster analysis as a tool in the assessment of tomato hybrids and cultivars. *International Journal of Agronomy*, 4 (5): 1-10.

Glogovac S, Adam Takaè, Šumiæ A L Z, Varga J G, Janko Èervenski, Mirjana Vasiæ and Vukašin Popoviæ 2012 Principal component analysis of tomato genotypes based on some morphological and biochemical quality indicators. *Journal of Field and Vegetable Crops Research,* 49 (3): 296-301.

Iqbal Q, Saleem M Y, Amjad Hameed and Muhammad Asghar 2014 Assessment of genetic divergence in tomato through agglomerative hierarchical clustering and principal component analysis. *Pakistan Journal of Bot*any, 46 (5): 1865-1870.

Jackson J E 1991 A User's Guide to Principal Components. *John Wiley and Sons Inc., New York.*

Legendre L and Legendre P 1984 Ecologia Numerique. *Presses de I'Universite du Quebec, Canada,* Vol 1-2.

Osei M K, Bonsu KO, Agyeman A and Choi H S 2014 Genetic diversity of tomato germplasm in Ghana using morphological characters. *International Journal of Plant and Soil Science,* 3 (3): 220-231.

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