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Genetic Divergence for Morphological and Biochemical Traits in 1% EMS Treated Tomato (*Lycopersicon esculentum* M.) *cv.* Arka vikas.

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ABSTRACT

Genetic diversity of 1.00 % EMS treated seeds of variety Arka vikas in tomato, was assessed using Mahalanobis D^2 statistic for 17 yield and quality characters in M_3 generation (109 M_3 families along with control with 45-50 plants per family) which indicated considerable diversity in the material. The maximum contribution per cent towards genetic divergence was by plant height, fruit weight, pericarp thickness, days to 50 % flowering, fruit shape index, no. of primary branches per plant, no. of fruits per plant, no. of locules per fruit and no. of flowers per cluster. The 109 M_3 families (unreplicated), along with control were grouped into 11 clusters using the Tocher's method and their distribution was at random. Although all the mutant lines were developed from the same mutagenic treatment (1.00 % EMS) and same parental genotype (Arka vikas) their grouping into different genetic clusters indicated that mutagenic treatment was effective in inducing diverse types of genetic changes due to the anomaly of the chromosomes in the seventeen traits studied. The inter-cluster distance was maximum between clusters III and X showing higher mean values for fruits per cluster and no. of fruits per plant, respectively. So, mutant lines from these clusters may be used in future hybridization programme.

Key words: *D^2 statistic, Genetic divergence, Tomato.*

The tomato is one of the most important versatile vegetables that belongs to the large and diverse *Solanaceae* family also called Nightshades. It is widely grown around the world and is used as both fresh market fruit and processed product. World wide it is the second most consumed vegetable after potato and is the most popular garden crop. It has wide usuage in Indian culinary tradition because of its special nutritive value and is an acknowledged model species for both basic and applied research.

Looking at commercial importance of tomato, there is utmost need to develop newer varieties/accessions/hybrids with higher yield, disease resistance and processing traits. For this purpose the breeders have to choose genetically distant parents, because the greater is parental diversity, the greater is the chance of developing high yielding breeding lines and providing better scope to isolate superior recombinants. Estimation of genetic divergence therefore allows breeders to eliminate some parents there by downsizing the scale of hybridization activities and concentrate their efforts in a smaller number of combinations. Although tomato is a self pollinated crop, there is genetic diversity not only in the morphological features but also in the quality attributes. Genetic diversity analysis reveals the redundancy of accessions with respect to a particular trait or combination of traits, which avoids wastage of resources. So, in the present study to attain genetic upgrading and sustainability, access is made for diversity present in 109 M_3 families along with control both for morphological and biochemical traits by employing Mahalanobis D^2 statistics. It has been extensively used as a quantitative measure for divergence studies.

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 methanesulfonate (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 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% 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). The genetic divergence was worked out by using Mahalanobis D^2 statistics given by Rao (1952) and 109 M_3 families along with control were grouped into different clusters by employing Tocher's method as outlined by Rao (1952).

RESULTS AND DISCUSSION

Coefficient of variation indicated sufficient variability in the material under study indicating considerable genetic diversity among 109 M_3 families. The per cent contribution towards genetic divergence by all the 17 characters is presented in Table -1. The knowledge on characters influencing divergence is an important aspect to a breeder. Character-wise rank has shown that no single character lonely had a greater contribution to total genetic divergence. Further analysis was done to estimate the D^2 values and on the basis of relative magnitude of D^2 values all the 109 M_3 families along with control were grouped into 11 clusters (Table 2 and Fig. 1) using the Tocher's method with the criterion that the intra-cluster average D^2 values should be less than the inter-cluster D^2 values.

The distribution of 109 M_3 families along with control into 11 clusters was at random with maximum number of families in cluster IV (34 families). Cluster II with control Arka vikas was the second largest with 27 families indicating that they did not possess enough divergence from the control (Arka vikas) in the seventeen characters

Table 1. Contribution of different characters towards genetic divergence in 109 M_3 families along with control in tomato (*Lycopersicon esculentum* M.) treated with 1% EMS.

S.No	Source	Times Ranked first	Contribution %
1	Plant height (cm)	2597	43.32
2	Fruit weight (g)	1301	21.70
3	â-carotene (mg/100g)	790	13.18
4	Pericarp thickness (cm)	339	5.65
5	Days to 50 % flowering	284	4.74
6	Fruit shape index	133	2.22
7	No. of primary branches per plant	124	2.07
8	No of fruits per plant	120	2.00
9	No of locules per fruit	91	1.52
10	No of flowers per cluster	85	1.42
11	Total soluble solids (°Brix)	41	0.68
12	No. of fruits clusters per plant	35	0.58
13	Lycopene (mg/100g)	21	0.35
14	Early fruit yield per plant (kg)	20	0.33
15	Titrable acidity **(g of citric acid/100 ml of juice)**	13	0.22
16	No. of fruits per cluster	1	0.02
17	pH	0	0.00

Table 2. Clustering of 109M_3 families along with control in tomato (*Lycopersicon esculentum* M.) treated with 1% EMS by Tocher's method.

Cluster No.	No. of families	Family no
I	8	e94-1,e303-9,e310-9,e368-11,e369-12,e372-9,e402-11,e415-5
II	27	**Arka vikas**,e1-10,e22-8,e34-16,e52-13,e54-13,e96-8,e99-2,e106-13,e149-8,e253-8,e255-10,e261-9,e281-1,e288-9,e292-5,e300-9,e301-8,e313-3,e349-9,e352-1,e357-6,e375-12,e380-6,e388-10,e392-4,e422-3,
III	1	e152-14
IV	34	e13-4,e30-3,e39-6,e69-8,e70-2,e81-6,e87-8,e122-7,e128-4,e137-8,e146-10,e172-8,e212-9,e213-8,e215-7,e227-10,e230-4,e233-9,e235-12,e274-9,e320-4,e323-9,e327-5,e331-1,e328-1,e330-6,e333-12,e337-7,e341-8,e347-9,e412-9,e440-11,e433-2,e436-9
V	20	e78-4, e79-3,e110-10,e116-4,e142-6,e159-6,e164-e10,e199-4,e202-6,e210-8,e224-8, e225-10,e260-1,e269-8,e270-3,e290-8,e295-8, e298-7,e319-8,e359-9,
VI	1	e193-8
VII	1	e6-16
VIII	1	e117-8
IX	15	e1-8,e40-2,e88-2,e97-3,e98-6,e133-6,e166-6,e188-6,e190-9,e220-15,e239-9, e246-9,e276-1,e280-6,e299-8
X	1	e237-8
X1	1	e3-15

Table 3. Average intra-and inter-cluster D^2 values among 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	**16.94 (4.12)**	46.11	22.71	71.49	47.18	142.56	60.79	103.33	96.68	205.27	135.02
II		**25.76 (5.08)**	40.59	46.09	49.57	97.23	71.09	85.37	95.02	128.86	96.83
III			**0.00 (0.00)**	64.55	45.56	116.03	62.34	98.50	94.69	215.23	152.69
IV				**42.88 (6.55)**	82.65	58.98	50.85	57.06	71.97	116.97	116.05
V					**44.96 (6.71)**	156.50	96.40	122.68	126.36	180.44	120.39
VI						**0.00 (0.00)**	63.93	56.06	73.66	149.57	180.79
VII							**0.00 (0.00)**	21.57	38.95	148.08	144.78
VIII								**0.00 (0.00)**	43.18	113.13	145.71
IX									**42.40 (6.51)**	153.20	153.88
X										**0.00 (0.00)**	60.31
XI											**0.00 (0.00)**

Note: Bold and diagonal values indicate intra-cluster D^2 distance; figures in parentheses are D values

studied to be classified as micro mutant lines. It is followed by cluster V (20 families), cluster IX (15 families) and cluster I (8 families). These lines not only exhibited genetic diversity from the control (Arka vikas) but also among themselves. Clusters III, VI, VII, VIII, X and XI were solitary clusters with nil intra-cluster D^2 values. The mutant lines developed from the same mutagenic treatment (1.00 % EMS) grouped into different clusters and grouping into different genetic clusters indicated that mutagenic treatment was effective in inducing diverse types of genetic changes due to the anomaly of the chromosomes in the seventeen traits studied. The mutual relationships between the clusters were represented diagrammatically by taking average intra and inter cluster D^2 values. The average intra and inter cluster D^2 values were estimated as per the procedure given by Singh and Chaudhary (1977) and were presented in the Table-3.

The maximum intra cluster distance was 44.96 in cluster V followed by 42.89 in cluster IV followed by 42.40 in cluster IX and 25.76 in cluster II and 16.94 in cluster I while, it was zero for clusters III, VI, VII, VIII, X and XI. Inter-cluster distances were worked out considering 17 characters and these distances ranged from 21.57 (between cluster VII and VIII) to 215.23 between cluster III and X. The pattern of cluster formation showed that there is a wide genetic diversity with regard to yield and its components in the mutant lines that were isolated on the basis of macro mutations (phenotypic variations) from the same family in M_2. Families grouped into the same cluster presumably differ little from one another as the aggregate of characters measured.

The cluster mean values for 17 characters are presented in Table- 4**.** Higher mean values for no. of fruit clusters per plant were seen in clusters

Table 4. Mean values of eleven clusters estimated by Tocher'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 % flowering	No. of flowers/ cluster	No. of fruit clusters/ plant	No. of fruits/ cluster	No. of fruits/ Plant	fruit weight (g)	Early fruit yield / plant (kg)
I	79.99	10.19	41.40	3.98	13.83	3.31	**45.11**	**31.33**	1.38
II	74.45	9.88	39.04	4.62	14.12	3.05	41.95	48.08	1.95
III	78.80	10.80	37.50	**5.00**	13.80	3.30	43.00	35.54	1.45
IV	59.94	10.27	41.53	4.49	14.20	3.19	42.98	47.41	2.02
V	**82.51**	10.42	38.40	4.48	12.72	3.15	39.80	41.07	1.57
VI	**41.18**	10.30	45.50	**3.10**	**14.80**	2.80	40.30	55.24	**2.19**
VII	52.88	10.80	40.90	4.00	13.00	**3.40**	41.60	32.26	1.39
VIII	44.28	**13.00**	**34.20**	**5.00**	13.50	2.60	31.90	41.22	**1.25**
IX	48.76	9.39	38.93	3.96	12.67	3.02	36.89	35.94	1.37
X	50.22	11.20	35.80	3.40	**12.40**	3.30	39.90	**56.26**	2.14
XI	69.66	**8.10**	**49.10**	**3.10**	14.10	**2.10**	**31.00**	46.24	1.38

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	0.29	**4.29**	0.67	5.35	0.40	4.65	3.84	0.13
II	0.37	4.78	0.73	5.26	0.41	4.52	4.92	0.19
III	**0.29**	4.60	1.22	5.81	**0.53**	4.56	4.35	**0.10**
IV	0.37	4.48	0.82	5.02	0.39	4.45	4.45	0.18
V	0.34	4.64	0.72	5.42	0.44	4.59	3.85	0.19
VI	0.31	4.50	**1.26**	5.86	0.39	4.64	**5.10**	0.10
VII	0.38	5.38	0.82	**4.11**	0.35	**4.20**	**1.73**	0.12
VIII	0.41	4.62	0.68	4.62	0.35	4.32	3.59	0.16
IX	0.36	4.41	0.85	5.05	0.39	4.44	4.29	0.16
X	**0.61**	4.32	0.64	4.13	**0.29**	4.21	3.04	**0.54**
XI	0.49	**5.62**	**0.61**	**5.91**	0.39	**4.82**	4.47	0.53

Note: Bold figures are minimum and maximum values

VI, IV and II while higher means for number of fruits per cluster were observed in clusters VII, I and III and higher mean value for no. of fruits per plant observed in clusters I, II and IV and higher mean values for fruit weight were observed in clusters X and VI which are major contributors in improving early fruit yield plant^{-1} in tomato. Based on mean values, series of crosses in diallel fashion may prove highly successful. The inter-cluster distance was maximum between clusters III and X showing higher mean values for fruits per cluster and no. of fruits per plant, respectively. So, mutant families from these clusters may be used in future hybridization programme. This study has clearly brought out in quantitative terms, the wide divergence induced in the mutant families isolated from the parental genotype through mutagen treatment. Thus, it could be concluded that while selecting mutant families from a particular cluster, the inter cluster distance, cluster mean and *per se* performance should be taken into consideration.

The success and usefulness of Mahalanobis' D^2 analysis in quantifying genetic divergence has been studied by Sanjeev *et al.* (2010), Meena *et al.* (2013), Rajasekhar Reddy *et al.* (2013), Manoj Kumar *et al.* (2014), Mukul *et al*. (2014), Srivastava *et al.* (2014) and Saleem *et al.* (2015) in tomato.

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