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Assessment of genetic diversity through D^2 analysis in tomato *(Solanum lycopersicon .L)*

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ABSTRACT: The present experiment was conducted during spring-summer 2013 to study genetic variability, correlation, path coefficient analysis, and genetic diversity for quantitative and qualitative traits in tomato in vegetable research farm Hisar. Using Mahalanobis D^2 statistics method, the 27 genotypes were grouped into nine clusters, indicating the presence of diversity for different traits. The cluster I had the highest number containing 16 genotypes followed by cluster III and VII containing three and two genotypes respectively. However, the cluster II, IV, V, VI, VIII and IX were solitary. The maximum intra-cluster distance was recorded within cluster III (10.88) and the maximum inter-cluster distance between cluster VI and VII (20.80), indicating the existence of wide genetic variability. Based on mean performances, the cluster VIII with single genotype ranked first and appeared to contain the potential genotype.The cluster VIII and II registered high plant height. The genotypes included in clusters V and VIII took less number of days to 50% flowering (29.67). The cluster III registered high fruit yield per plant (1004.60), average fruit weight (38.07), and ascorbic acid (28.7) can be utilised in breeding programme for enhancing their respective characters. The cluster IX had high number of fruits per plant (40.53). Based on cluster mean analysis these genotypes can be used in crop improvement programme in tomato for above-mentioned characters.

KEYWORDS: Genetic diversity, Tomato, *(Solanum lycopersicon .L)*.

1 INTRODUCTION

Tomato [*(Solanum lycopersicon .L)*], a member of Solanaceae family, is one of the most important vegetable crops grown widely all over the world. It is often called poor man's orange, because of its high nutritive value. The cultivated tomato originated in the Peru-Ecuador-Bolivia area of the South American (Vavilov, 1951). Its ripe fruits are consumed fresh as well as after cooking as a protective supplementary food and also utilized in the various value added durable products such as puree, paste, powder, ketchup, sauce and canned whole fruits, while the green unripe fruits are used for making pickles and chutney.

Tomato crop has wider adaptability, high yielding potential and multipurpose uses in fresh as well as processed food industries. It stands unique among vegetables because of its high nutritive values and innumerable uses (Vitamin A, C and Minerals). Its firmly ripened fruits are a source of lycopene (an antioxidant), ascorbic acid, and beta-carotene. Lycopene is treasured for its anticancer attribute It is reported to have properties as antiseptic and blood purifier. It acts as an antioxidant which is often colligated with carcinogenesis. Systematic study and evaluation of germplasm is of great importance for current and future agronomic and genetic improvement of the crop. Furthermore, if an improvement program is to be carried out, evaluation of germplasm is imperative, in order to understand the genetic background and breeding value of the

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available germplasm (Singh *et al.,* 2002). Reshuffling the genes through recombination is the principal way of developing improved genotypes in breeding programs.

Evaluation of germplasm is of immense important in genetic improvement of the crop. Genetic diversity analysis assists in interpreting the genetic background and breeding value of the germplasm. It was also said that plant breeders use a much less diverse genetic pool than the overall available genetic diversity within the crop (Joshi *et al.,* 2012). Heterogeneous local population of the genus forms an important source of genetic variation (Zeven, 1998). For the selection of parents in hybridization, diversity among parents for the character of interest, estimation of genetic distance is most important as diverse plants are supposed to give high hybrid vigour (Harrington, 1940). Estimation of genetic divergence also allows breeders to eliminate some parents in downsizing the gene pool available and concentrate their efforts in a smaller number of hybrid combinations (Fuzzato *et al*., 2002) Diversity relative to its use and production environments is high. However, the genetic base of cultivated tomato is narrow (Bai & Lindhout 2007). The multivariate analysis provides valuable information on the extent of variation present in the crop under improvement and usually helps a plant breeder in choosing desirable parents for breeding programme. Also inclusion of genetically diverse parents in any breeding programme is essential to generate new variability and desirable recombinants.

Among the various methods identified/developed to study the genetic divergence in the genotypes, the Mahalanobis D^2 (Mahalanobis, 1936) is reliable and most frequently used. D^2 analysis is a useful tool in quantifying the degree of divergence between biological population at genotypic level and to assess relative contribution of different components to the total divergence, both at the inter- and intra-cluster levels. Keeping these points in mind, the present study, the genetic divergence was estimated by using D^2 statistics suggested by Mahanlanobis (1936), which is based on multivariate analysis of quantitative traits. It is one of the very potential tools for measuring genetic divergence within a set of population using the concept of statistical distance employing multivariate measurements. The grouping of genotypes into different clusters is done by following Tocher's method as described by Rao (1952). Improvement in self-pollinated crops like tomato is normally achieved by selecting the genotypes with desirable character combinations existing in nature or by hybridization (Meena *et al.,* 2013). Such studies are also useful in the selection of parents for hybridization to recover superior transgressive segregates. Considering the above facts, the research has been planned with the following objective to assess the extent of genetic diversity in the available germplasm based on fifteen traits comprising of qualitative and quantitative traits.

2 MATERIALS AND METHODS

The present investigation was carried out at Research Farm of the Department of Vegetable Science, CCS Haryana Agricultural University, Hisar during spring-summer 2012-2013.The experimental materials comprised of twenty-seven genotypes (Table-1) of tomato collected from different sources. The experiment was laid out in a randomized block design with three replications accommodating 14 plants in each genotype. Seeds were transplanted at a spacing of 75x45 cm. The genotypes studied are Palam Pride, Palam, Pink, EC 620445, BBWR-11-1, BBWR-18-17, EC 620533, EC 620534, EC 620378, EC 620383, EC 620380,EC 620391, BBWR-10-3-17, BBWR-10-3-18, Punjab Varkha Bahar-2, Hisar Arun, Punjab Chhuhara, EC 620516, EC 620536, Arka Vikas, Saksham, Abhilash, Arka Meghali, US1196, US 3140, DVRT 2, S-12 and Hisar Lalit. All the recommended cultural practices were adopted for raising the crop successfully. The experimental details and observations to be recorded are as follows: The observation were recorded on five randomly selected plants per replication for each genotype on fifteen characters: i) plant height (cm), ii)days to 50% flowering, iii) average fruit weight (g), iv) number of branches per plant, v)polar diameter (cm), vi) equatorial diameter (cm), vii) number of locules per fruit, viii) number of flowers per cluster, ix) fruit yield per plant (g), x) number of clusters per plant, xi)number of fruits per plant, xii) number of fruit per truss, xiii) total soluble solids (%), xiv) acidity (%) and xv) ascorbic acid (mg/100 g). Mean across three replications were calculated for each traits and the analysis of variation was carried out. Multivariate analysis was done utilizing Mahalanobis D^2 statistic which are cited below (Mahalanobis, 1936) and genotypes were grouped into different clusters following Tocher's method. The inter and intra cluster distances were worked out as per method suggested by Murty and Arunachalam (1967) to find actual divergence within and between the clusters.

a) Mahalanobis D^2 analysis

Mahalanobis (1936) D^2 analysis was used for assessing the genetic divergence among the test entries involving quantitative characters. The generalized distance between any two populations is given by the formula.

$$D^2 = \sum\sum \lambda_{ij}\, \sigma_{ai}\, \sigma_{aj}$$

Where,

D^2 = Square of generalized distance

λ_{ij} = Reciprocal of the common dispersal matrix

$\sigma_{ai} = (\mu_{i1}-\mu_{i2})$

$\sigma_{aj} = (\mu_{j1}-\mu_{j2})$

μ=General mean

Since, the formula for computation requires inversion of higher order determinant, transformation of the original correlated un-standardized character mean (Xs) to standardized uncorrelated variable (Ys) was done to simplify the computational procedure. The D^2 values were obtained as the sum of squares of the differences between pairs of corresponding uncorrelated (s) values of any two uncorrelated genotypes (Rao, 1952).

b) Cluster of D^2 values

All n (n-1)/2 D^2 values were clustered using Tocher's method described by Rao (1952).

c) Intra cluster distance

$$\text{Square of the intra cluster distance} = \frac{\sum D^2 i}{n}$$

Where, $\sum D^2 i$ is the sum of distance between all possible combinations of the entries included in a cluster.

n = Number of all possible combinations

d) Inter cluster distance

$$\text{Square of the inter cluster distance} = \frac{\sum D^2 i}{n_i n_j}$$

Where,

$\sum D^2 i$ is the sum of distances between all possible combinations ($n_i n_j$) of the entries included in the clusters study.

ni = Number of entries in cluster i

nj =Number of entries in cluster j

3 RESULTS AND DISCUSSION

3.1 CLUSTERING

Clustering of genotypes under study is presented in Figure 1. Based on the D^2 values all the genotypes were grouped into nine clusters, signaling the presence of diversity for different traits. The cluster I had the highest number of genotypes (16) followed by cluster III (3) and cluster VII (2). The cluster II, IV, V, VI, VIII and IX were monogenotypic. The analysis of the Table 1 clearly indicated that clustering pattern there was no parallelism between geographical distribution of genotypes and genetic divergence. Therefore, geographical diversity could not be related to genetic diversity in the material investigated. This is an agreement with results of Singh et al. (2006), Reddy et al. (2013) and Basavaraj *et al.* (2010). So selection of genotypes for hybridization to generate diverse new gene combinations should be based on genetic diversity rather than geographic diversity (Pawar *et al.,* 2013). It is very difficult to establish the actual location of origin of a genotype. The diverse use of genetic material among the crop improvement programmes in the country makes it unmanageable to conserve the real identity of the genotypes. Mostly, breeding progenies incorporate genes from motleyed sources, resulting in casting off the basic geographical identity of the genotype (Meena *et al.*, 2013).

In perusal of the Table No.2 the intra-cluster distances indicates the divergence among the genotypes within the clusters and inter-cluster indicates diversity between clusters. The maximum intra-cluster distance was recorded within cluster III (10.88) followed by cluster I (9.94) and cluster VII (9.67). The maximum inter-cluster distance is observed between cluster VI &VII (20.80) followed by cluster III & VII (20.52), VII & VIII (19.98). These results suggest maximum divergence between genotypes of cluster VI with genotypes of cluster VIII, indicating the fact that the genotypes when used in hybridisation programme produce superior seggregants. The information obtained from inter-cluster distances may be used to select genetically diverse and superior genotypes. The genotypes possessing maximum genetic divergence is expected that more heterotic F_1 and most promising segregant in segregating generations. Intercrossing of divergent groups would lead to greater opportunity for crossing over, which may release hidden variability (Kumar et al., 2010). The minimum inter-cluster distance was observed between cluster III and II (9.56) followed by cluster IV & VIII (11.34). In general, less intra-cluster distance than inter cluster distance suggested homogenous and heterogeneous nature of the genotypes within and between the clusters, respectively Pawar *et al.,* (2013). These results are conformity with the findings by Veershetty (2004), Mehta and Asati (2008) and Kumar *et al.* (2010).

3.2 CONTRIBUTION OF CHARACTERS TOWARDS DIVERGENCE

The diversity among 27 genotypes was measured by employing D^2 statistic. The contribution of each character towards total genetic diversity is presented in Table 3. The characters, fruit yield per plant (20.51), total soluble solids (17.38), and equatorial diameter (15.38) contributed high for divergence. Thus, these characters may be given high emphasis while selecting the lines for hybridization programme to generate large variability and will provide immense scope for the improvement of yield through selection. The same has been suggested by Kumar *et al.* (2010). Other characters like number of flower clusters per plant (0.57%) and days to 50% flowering (1.14%) contributed very little for divergence.

3.3 CLUSTER MEAN ANALYSIS

The Table No.4 demonstrates the mean values for fifteen characters in nine clusters, which vary in their value differently from each other. The plant height was high for cluster VIII (130.33 cm). The genotypes included in cluster V and VIII are recorded minimum days to 50% flowering (29.67). Numbers of branches per plant were highest for cluster IV (9.33). Choice of parents is the most important aspect of crop improvement programme and highly diversified parents were selected based on the yielding ability of the respective parents. The economically important character high fruit yield per plant was supreme for the cluster III (1004.60) which indicates that the genotypes included in these clusters could effectively be used for the crop improvement programme for increasing yield-contributing characteristics. The number of fruits per plants and average fruit weight, which directly correlates with yield per plant, was high for the cluster IX (40.53) and cluster II (28.80) respectively. In case of ascorbic acid content of fruit, the cluster II (28.85 mg/ 100g), for TSS ºBrix the cluster VII (7.45 ºBrix) and for acidity the cluster VII (0.75 %) possess the highest values. Equatorial and Polar diameter is high in cluster VII (5.01) and cluster VI (5.17) respectively. It is suggested that hybridization among the genotypes of above said clusters would produce seggregants for more than one economic character. The potential lines are picked out from different clusters and used as parents in a hybridization programme. The choice should based on genetic distance and depending upon the objective of the breeding programme.

Many workers have observed that more diverse the parents within its overall limits of fitness, the greater are the chances of heterotic expression in F_1's and a broad spectrum of variability in segregating generations (Arunachalam,1981). In choosing parents for hybridisation programme the clustering pattern could be employed that would likely to render the maximum possible variability for various economic characters (Hazra *et al.* (2010) and Kumar *et al.* (2010). Moreover, it will be effective to intercross genotypes belonging to more diverse clusters like cluster VI and VII, cluster III and VII and cluster VIII and VII to create wide spectrum of variability and to produce transgressive segregates for tomato.

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Table 1: List of germplasm lines and standard released varieties included in the study

Sr. No.	Genotype/Origins	Sr. No.	Genotype/Origins
1.	Palam Pride/Palampur	15.	Hisar Arun/Hisar
2.	Palam Pink/Palampur	16.	Punjab Chhuhara/Hisar
3.	EC 620445/NBPGR	17.	EC 620516/ NBPGR
4.	BBWR 11-1/Bangalore	18.	EC 620536/ NBPGR
5.	BBWR 18-17/Bangalore	19.	Arka Vikas/IIHR
6.	EC 620533/NBPGR	20.	Saksham/Monsanto
7.	EC 620534/NBPGR	21.	Abhilash/Monsanto
8.	EC 620378/NBPGR	22.	Arka Meghali/IIHR
9.	EC 620383/NBPGR	23.	US 1196/Dharwad
10.	EC 620380/NBPGR	24.	US 3140/Dharwad
11.	EC 620391/NBPGR	25.	DVRT 2/IIVR
12.	BBWR 10-3-17/Bangalore	26.	S 12/Panjab
13.	BBWR 10-3-18/Bangalore	27.	Hisar Lalit/Hisar
14.	Punjab Varsha Bahar 2/Punjab		

Note: -National Bureau of Plant Genetic Resources (NBPGR), Indian Institute of Vegetable Research (IIVR), Indian Institute of Horticultural Research (IIHR)

Table 2. Intra-cluster distance of different groups.

Groups	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9
Group 1	9.94	12.48	13.24	11.86	12.38	12.57	15.77	13.69	14.40
Group 2		0.00	9.56	16.46	16.24	16.05	19.02	14.85	14.79
Group 3			10.88	16.02	15.92	15.27	20.52	13.99	14.19
Group 4				0.00	15.10	15.39	15.01	11.34	16.59
Group 5					0.00	12.73	13.05	18.64	13.72
Group 6						0.00	20.80	19.43	17.04
Group 7							9.67	19.98	18.01
Group 8								0.00	17.92
Group 9									0.00

Table 3. Contribution of 15 characters towards total genetic diversity of Solanum lycopersicon(Mill.) Wettsd.

Sr.No.	Source	Contribution (%)
1.	Plant height (cm)	5.13
2.	Number of branches per plant	2.56
3.	Days to 50% flowering	1.14
4.	Number of flowers per cluster	4.27
5.	Number of fruits per plant	3.13
6.	Average fruit weight (g)	1.71
7.	Number of fruits per truss	2.28
8.	Number of flower clusters per plant	0.57
9.	Polar diameter of fruit	14.53
10.	Equatorial diameter of Fruit	15.38
11.	Number of locules per fruit	6.55
12.	Fruit yield per plant (g)	20.51
13.	Total soluble solids (TSS)	17.38
14.	Acidity (%)	0.00
15.	Ascorbic acid (mg)	6.55

Table: 4 Cluster means for different characters in different cluster group's

Characters	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9
Plant height (cm)	72.96	117.93	107.91	84.53	68.20	61.13L	61.50	130.33H	61.93
Number of branches per plant	6.10	6.87	7.40	9.33H	4.84L	6.40	5.70	5.73	9.07
Days to 50% flowering	31.15	32.00	33.00	31.67H	29.67L	33.33	31.00	29.67L	30.00
Number of flowers per cluster	7.56	8.20	9.49	9.13	9.00	6.73	7.90	10.07H	8.07L
Number of fruits per plant	25.10	28.80	27.40	23.87	26.03	19.53L	25.13	20.93	40.53H
Average fruit weight (g)	31.23	31.47	38.07	30.15	22.07L	32.21	24.66	46.27H	24.90
Number of fruits per truss	3.34	2.53L	2.95	3.56	4.43	2.62	4.86H	2.78	4.63
Number of trusses per plant	7.76	11.40H	9.76	6.67	5.87	7.47	5.33L	7.53	8.73
Polar Diameter of fruit	4.15	4.98	5.16	3.90	3.49	5.17H	2.42L	4.43	3.39
Equatorial diameter of fruit	4.12	4.21	4.14	4.74	2.94	4.13	2.93	5.01H	2.68L
Number of locules per fruit	4.43	3.33	5.07H	2.70L	4.20	2.95	3.20	4.99	4.60
Fruit yield per plant (g)	776.25	905.67	1004.60H	715.27	572.00	624.00	577.37	969.00	943.13
TSS	5.41	3.91	5.04	5.13	6.39H	7.45	3.14L	4.99	6.22
Acidity (%)	0.69	0.59L	0.68	0.78H	0.71	0.67	0.68	0.75	0.66
Ascorbic acid (mg)	23.02	28.85H	28.27	23.06	24.03	26.99	22.02	19.63	15.05L

Note: H-maximum cluster and L-lowest cluster means

Figure 1. Clustering of Different genotypes of Solanum lycopersicon(Mill.) Wettsd. based on D^2 values.