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Genetic divergence for horticultural traits and ToLCV resistance in tomato (*Solanum lycopersicum* L.)

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Abstract

Genetic divergence analysis of elite germplasm provides a correct picture of the extent of variation which is a prerequisite for choosing promising and genetically diverse lines for desirable traits. Multivariate analysis following Mahalanobis D^2 statistics revealed considerable genetic diversity among 30 genotypes of tomato (*Solanum lycopersicum* L.) for quantitative characters pertaining to growth, earliness, yield and ToLCV. Diversity within and between clusters were observed. Fruit weight, number of fruit per plant and plant height differentiated the germplasm. Six clusters were developed from the D^2 analysis using Tocher's method. Cluster II was the largest cluster contained 13 accessions accounting 43.33% of total genotype followed by cluster III and IV both having 5 accessions, and cluster I with 3 genotypes which were accounting 16.66, 16.66 and 10.00%, respectively. Beside these four clusters, remaining two clusters V & VI have two genotypes each. The selected lines exhibited diversity despite common parentage. The maximum inter-cluster distance was between cluster VI and I (15078.88) followed by cluster VI and V (13901.29). ToLCV incidence recorded minimum in the genotype H-24 (13.72%) followed by H-88-78-1 (15.12%); however, the severity percentage was observed minimum in genotype H-88-78-1 (7.50%) followed by VRT-3-2-1 (8.17%), Cheku Grand (9.17%), H-24 (13.33%) and Inden 2013-4 (13.53%). Pusa Sadabahar showed the maximum incidence and severity percentage of 52.58 and 38.67%, respectively. The assessment of variation guides the breeder to select superior parents to initiate an effective and fruitful crossing programme to improve the genotypes.

Keywords: *Solanum lycopersicum*, ToLCV, D^2 analysis, diversity, germplasm, Solanaceae

Introduction

Tomato stand unique and highly praised vegetable because of its high nutritive values and myriad uses and is referred as "Protective Food" and "Poor man's orange". It is grown worldwide for its edible fruit which can be consumed either fresh or cooked and also in the form of various processed products like juice, ketchup, sauce, puree, powder, essence, cocktail, etc. it belongs to the nightshade family Solanaceae with chromosome number 2n=24. Leading tomato producing states in India are Andhra Pradesh (36%) followed by Karnataka (10%), Orissa (8%), West Bengal (6%), Bihar (6%), Gujarat (6%), Maharashtra (4%), Chhattisgarh (4%), Tamil Nadu (3%), Jharkhand (2%) and 15% shared by other other states (NHB 2015). Besides soil and climate factors, the cultivar itself is very important in respect of his performance regarding earliness, disease resistance and yield. Tomato Yellow leaf curl virus (ToLCV), transmitted by whitefly, causes major disease losses to tomato crops in tropical and subtropical regions of the world reported by Gonzalez-Cabezuelo et al. (2012) . Screening is best promising techniques for obtain ToLCV resistance varieties which is an urgent need of the researcher. Although tomato is a self-pollinated crop, there is genetic diversity not only in the morphological features but also in the quality attributes. Success of crop improvement depends on the extent of genetic variability, choice of parents for hybridization, and selection procedure. Breeders are dependent on the nature,and magnitude of genetic variance of the character under consideration and interrelationships among characters.

Mahalanobilis (1936) D^2 statistic method is used to discern genetic divergence among groups based on expression of multiple characters and applied to estimate the nature and magnitude of genetic diversity among accessions. The divergence analysis has a definite role to play in an efficient choice of divergent parents for hybridization to exploit maximum heterosis.The

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adoptability and yielding capacity of the cultivars is not the same in all regions. Hence, it is important to find out the most suitable cultivar to a particular region and make the farmers aware of it. The present study was undertaken to evaluate the nature and magnitude of variability for yield and other characters present in germplasm pool owing to genetic and non-genetic causes, is an important basic pre-requisite for starting any systematic breeding programme in identifying superior lines or varieties.

Materials and Methods

Thirty tomato accessions originated at different geographical locations were obtained from ICAR-Indian Institute of Vegetable Research, Varanasi, UP, and ICAR-Indian Agricultural Research Institute, New Delhi. The experiment was carried out at the Vegetable Research Farm, Department of Horticulture, Sam Higginbottom University of Agriculture, Technology & Science, Allahabad, UP located at an elevation of 78 m above sea level at 25.87 °North latitude and 81.15 °E longitude. This region has a sub-tropical climate with both the low and high extremes of temperature. In cold winters, the temperature sometimes is as low as 32°F in December-January and very hot summer with temperature reaching up to 115°F in the months of May-June. The average rainfall is around 1013.4 mm, with maximum concentration during July to September months with occasional showers in winter. The soil of the experimental field was sandy loam in texture, poor in nitrogen, comparatively rich in phosphorus and medium in potash with slightly alkaline reaction. To grow seedlings, the raised nursery beds of 5.0 m length, 1.0 m width and 0.15 m height were prepared after bringing the soil to a fine tilth. Well decomposed FYM was properly mixed into top soil of the bed @ 3 kg/m^2. A fertilizer mixture of 0.5 kg of NPK/bed was also mixed in the soil. The seedbeds were also treated with 40% formalin @ ½ l/m^2. For raising good and healthy seedlings, the seeds were treated with carbendazim @ 2.0g/kg seed. After that, the seeds of each genotype were sown in lines 10 cm apart on September 25, 2015. The experimental field was ploughed once with a mould board plough in the beginning followed by cross harrowing with disc cultivator and finally by planking to prepare the field to fine tilth. FYM @ 250 q/ha incorporated before one month of transplanting. Half dose of nitrogen, full dose of phosphorus and potash of the recommended doses i.e. N:P:K fertilizers (120:60:60 kg/ha) were applied as basal dose with the last ploughing. The remaining half dose of nitrogen was applied as top dressing in two equal split doses i.e. at 30 and 45 DAT. Four weeks old healthy and uniform seedlings were transplanted in the experimental plots after allotting entries randomly in each replication. Before transplanting, seedlings were dipped in imidacloprid (2.0g/l) for five minutes to control insect pests.

The experiment was arranged in a completely randomized block design with 3 replications during the September 2015 to March 2016. About four week old 20 seedlings were transplanted at a distance of 60×45 cm between plants in 1.2×4.5 m plot. Besides, the good agricultural practices and plant protection measures were applied time to time as recommended for commercial tomato production. Five plants from each plot were randomly tagged and observations recorded on plant height, days to first flowering, days to 50% flowering, number of flowers per cluster, number of flower cluster per plant, fruit set per cluster, number of fruit per plant, average weight of fruit, and fruit yield per plant, and incidence and severity of ToLCV. In order to assess symptom severity, an observation scale with 5 classes (0, 1, 2, 3 and 4) was adopted (Lapidot et al. 1997 and Anonymous 2006) i.e. 0: no symptoms, 1: slight leaf curl, 2: substantial curl with or without yellowing, 3: substantial curl with substantial yellowing and 4: substantial curl with yellowing and stunting or death of the plant. These classes were determined for all the plants observed after 45 DAT. ToLCV was rated according to this severity scale: 1- light leaf yellowing, 2 - moderate plant is stunting and leaf curling and yellowing, 3 - severe plant stunting and leaf curling/ yellowing (Camra et al. 2013). Analysis of variance analysed was followed Panse and Sukhatme (1978) methods and genetic divergence determined using the Mahalanobis D^2 statistic. On the basis of magnitude of D^2 values accessions were grouped into clusters by the Tocher's method (Rao 1952). Significance of differences among associations was tested using Wilk's criterion (1932).

Results and Discussion

The analysis of dispersion for the test of significance of difference in the mean values based on Wilk's criterion revealed significant differences among accessions for the aggregate 10 characters. The thirty tomato genotypes were categorized into six distinct clusters using Tourch's method (Fig. 1) and their Euclidean^2 distance using D^2 (Fig. 2). It is clear evident from the (Table 1) cluster II is the largest cluster contained 13 accessions accounting 43.33% of total genotype followed by cluster III and IV both having 5 accessions and cluster I, with 3 genotypes which were accounting 16.66, 16.66 and 10.00%, respectively. Beside these four clusters, remaining two clusters V & VI both have two 2

Table 1: Composition of accessions in clusters of tomato

Cluster	Number of accessions	Accession
I	3	CH-155, Angoor Lata, Hisar Lalit
II	13	S-22, Tripura Local, Azad-T-6, CLN-1621-L, Co-3, Pusa Sadabahar, Arka Vikas, Ageta, H-88-78-5, CLN-2011, Pusa Rohit, VRT-97-384, DS-122
III	5	Azad-T-5, Cheku Grand, Rio Grande, EC-668883, H-88-78-1
IV	5	VRT-97-754, Hata-8, Pant-T-7, Pusa Ruby, S-21
V	2	Arka Abha, VRT-3-2-1
VI	2	H-24, Indan-2013-4

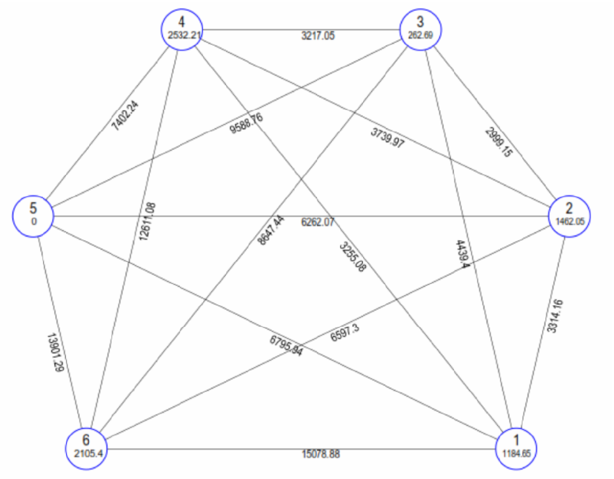

Figure 1: Euclidean distance (Not to the Scale)

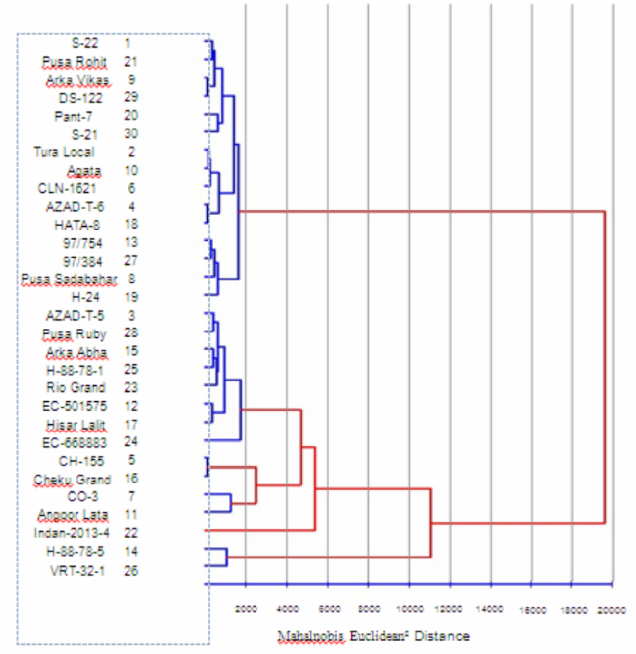

Figure 2: Ward's Minimum Variance Dendrogram

genotypes. The lines selected exhibited diversity despite common parentage. The genetic diversity among accessions may be due to history of selection, heterogeneity, selection under diverse environments and genetic drift. The range of intra-cluster distance was from minimum 0.0 in V cluster to maximum of 2532.21 in the IV cluster (Table 2). This apparently indicates that cluster IV have genotypes that are relatively distant from each other than the other clusters which have lower D^2 distances except cluster V, had only two genotypes. The maximum inter-cluster distance of 15078.88 was observed among the first and sixth cluster indicating large genetic differences among genotypes of these two clusters. Minimum inter-cluster distance of 2999.15 was observed between the II and III clusters indicating significantly lesser genetic differences among the genotypes of these clusters. Maximum inter-cluster distance is indicative that genotypes falling in these clusters had wide diversity and can be used for hybridization programme to get better recombinants in the segregating generations. Low level of intra-cluster distance was indicative of narrow genetic variation within the cluster. Genotypes of same cluster would not yield desirable recombinants. Different intra- and inter-cluster distances were recorded previously for various vegetable crops. Tomato has received a great deal of attention in genetic studies by the number of researchers (Mahesha et al. 2006, Sharma et al. 2006, Mehta and Asati 2008 and Prashath et al. 2008).

Table 2: Intra- and inter-cluster distances between accessions of tomato

Cluster	I	II	III	IV	V	VI
I	1184.65	3314.16	4439.40	3255.08	6795.94	15078.88
II		1462.05	2999.15	3739.97	6262.07	6597.30
III			262.69	3217.05	9588.76	8647.44
IV				2532.21	7402.24	12611.08
V					0.00	13901.29
VI						2105.40

The character means were also worked out for the genotypes falling in these eight clusters (Table 3). Cluster V was found to be the cluster consisting of average fruit weight (177.4), fruit yield per plant (5.64). Similarly, cluster IV with the highest flower cluster per plant (12.28), number of fruits per plant (110.25), while minimum days to 50% flower (31.68). Cluster III was characterised with maximum number of flower per cluster (12.28), fruit set per cluster (7.19) while, minimum days to first flower (19.24). Maximum height (135.11 cm) was found in cluster VI. Similar findings were also reported by Joshi and Kohli (2003) and Mohanty and Prusti (2001) for number of fruits/plant and average fruit weight, Sharma and Verma (2001) for reported that characters like fruit yield per plant, pericarp thickness and fruit diameter, played an important role in divergence between the populations. Cluster based mean estimations are very useful in targeting the genotypes for breeding programme, as they prevent the tedious efforts of screening the inferior germplasm lines. Hence, genotypes from desirable clusters could be directly used for final field evaluation in advanced breeding experiments.

In a breeding program aimed at crop improvement, the choice of parents is important and only component characters of yield should be taken into account for selecting genetically divergent parents. Genotype of specific clusters for respective traits can be utilized as donor parents to enhance yield directly and indirectly. To improve earliness, number of flower cluster per plant and less ToLCV severity, genotypes in cluster III should be used. For more fruit weight and yield genotypes should come from cluster V. To improve plant height and less ToLCV incidence genotypes from cluster VI should be used. For more fruit per plant genotypes should be selected from cluster IV. In field screening of thirty genotypes against ToLCV, It is evident from data graphically illustrated in Fig. 3 reveals that disease incidence are minimum in the genotype H-24 (13.72%) followed by H-88-78-1 (15.12%), VRT-3-2-1 (14.45%), Rio Grand (18.72%) and H-88-78-5 (18.86%), and are classified as resistance to ToLCV disease. However, genotype Pusa Sadabahar (61.71%) was found moderately susceptible to ToLCV disease. Similarly, the minimum disease severity was in genotype H-88-78-1 (7.50%) followed by VRT-3-2-1 (8.17%), Cheku Grand (9.17%), H-24 (13.33%) and Inden 2013-4 (13.53%). Nevertheless, maximum disease severity was observed that Pusa Sadabahar (38.67%). Similar finding were also reported by Kalloo and Banerjee (2000). Hence, these genotypes H-24 and H-88-78-1showed resistance to ToLCV and could be utilized as parents in further hybridization programme. To increase in production and productivity of tomato, varieties must be early, high yielder and resistance to ToLCV. For future experiment, traits contributing maximum to genetic diversity such as fruit weight and fruit size should be given top priority as selection parameter and diverse genotypes identified in the present study may be utilize for attempting hybrid breeding and developing ToLCV resistant hybrids/ varieties.

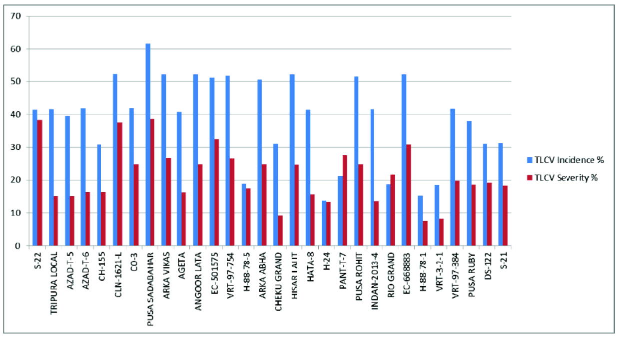

Figure 3: Screening of tomato genotypes for the incidence and severity of ToLCV

सारांश

टमाटर की उत्कृष्ट प्रभेदों का अनुवांशिक रूप से विविध वंशक्रमों का वांछित गुणों के चयन हेतु अनुवांशिक विचलन विश्लेषण आवश्यक है। महालैनोबिस डी² सांख्यिकी द्वारा बहुभिन्न रूपी विश्लेषण से टमाटर (*सोलेनम लाइकोपर्सिकम* एल.) के 30 प्रभेदों में मात्रात्मक गुणों जैसे–वृद्धि, अगेतीपन व उपज के लिए सार्थक विविधता स्पष्ट हुई। समूह के भीतर और बीच में विविधता पायी गयी। जननद्रव्यों को फलभार, फलों की संख्या, प्रतिपौध व पौध ऊँचाई के आधार पर विभेदित किये गये। टोचर विधि द्वारा डी² विश्लेषण कर कुल 6 समूह बनाये गये जिनमें समूह– II सबसे बड़ा समूह बना जिसके अन्तर्गत 13 प्रभेदों को समाहित किया गया जो कुल प्रभेदों का 43.33 प्रतिशत है। इसके उपरान्त समूह– III व IV जिनमें 5 प्रभेद एवं समूह–I में 3 प्रभेद समाहित हुए व IV में 2 प्रभेद थे। चयनित वंशक्रमों में सामान्य पितृ के बावजूद विविधता प्रदर्शित किए व समूह– IV व I में अन्तः समूह दूरी (15078.88) था तथा इसके बाद समूह– IV व V (13901. 29) का स्थान था। प्रभेद एच.–24 (13.72 प्रतिशत) में सबसे कम टी.

Table 3: Cluster means of qualitative and quantitative traits in tomato

Cluster	Plant Height (cm)	Days to First Flower	Days to 50 % Flowering	Flowers/ Cluster	Fruits/ plant	Fruit Weight (g)	Fruit Yield/ Plant (kg)	TLCV Incidance %	TLCV Severity %
I	53.623	22.934	29.475	6.534	41.036	51.544	2.123	41.039	23.602
II	85.027	23.462	29.505	7.887	58.341	41.375	2.378	39.669	22.000
III	82.112	19.243	24.343	12.282	73.675	17.667	1.295	31.055	12.750
IV	71.925	20.242	24.293	7.817	110.247	29.500	3.247	47.083	25.000
V	84.717	25.523	31.677	5.143	31.780	177.400	5.643	41.587	13.533
VI	135.110	22.355	30.057	11.962	60.455	27.083	1.715	18.652	12.817
Mean	71.586	22.697	28.907	7.679	53.426	47.669	2.301	38.937	21.490
Treatment MSS	3687.534	12.415	25.557	26.382	2585.168	5170.095	4.048	289.384	115.045
Error MSS	87.167	6.572	6.208	4.674	557.008	202.010	1.486	155.575	65.424
F Ratio	42.304	1.889	4.117	5.644	4.641	25.593	2.725	1.860	1.758
Probability	0.000	0.144	0.011	0.002	0.006	0.000	0.052	0.149	0.169

एल.सी.वी. का प्रकोप प्रतिशत पायी गया इसके उपरान्त एच.–88–78–1 (15.12 प्रतिशत) में पाया गया। नुकसान का प्रतिशत प्रभेद एच.–88.78–1 में 7.50 प्रतिशत रहा इसके बाद वी.आर.टी.–30–2–1 (8.17 प्रतिशत), चंकु ग्रेन्ड (9.17 प्रतिशत), एच.–24 (13.33 प्रतिशत) तथा इण्डेन–2013–14 (13.33 प्रतिशत) रहा। पूसा सदाबहार में सबसे ज्यादा प्रकोप तथा उग्रता 52.58 व 38.67 प्रतिशत क्रमशः पाया गया। विविधता मल्यांकन से स्पष्ट हुआ कि उच्च पित्रों का चयन कर प्रभावी एवं उद्देश्य परक प्रजाति सुधार हेतु प्रजनन कार्यक्रम में अपनाया जा सकता है।

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