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

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**ABSTRACT**

*A study was conducted at Vegetable Research Farm, Dr. RPCAU, Pusa, Samastipur, Bihar during rabi 2015-16 to evaluate the genotypes of tomato (Solanum lycopersicum L.) for yield and quality. Investigation was carried out on variability, character association, path analysis and genetic divergence for morpho-physiological characters. 24 genotypes were grown in Randomized Block Design with three replications. The 24 genotypes of tomato were grouped into five clusters using Tocher method. The genotypes in cluster IV and cluster II followed by cluster III and II and cluster V and II, due to maximum inter cluster distance between them, exhibited high degree of genetic diversity and thus may be utilized under inter varietal hybridization programme (transgressive breeding) for getting high yielding recombinants. Cluster IV was suitable for number of primary branches per plant, diameter of fruit, length of fruit, average fruit weight, yield per plant and yield per hectare. Therefore, selection of parents from this cluster for these traits would be effective. Maximum contribution towards divergence was obtained by lycopene content, average fruit weight, & ascorbic acid. All together they have contributed 67%.*

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## INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is a member of solanaceous family. Tomato 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. It originated in wild form in the Peru-Ecuador-Bolvia region of Andes (South America) and is grown in almost every corner of the world (Robertson and Labate, 2007). It is typical day neutral plant and is mainly self pollinated, but a certain percentage of cross-pollination also occurs (Depra *et al*., 2014). Tomato is universally known as "Protective Food" (Thamburaj and Singh, 2013). 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.

Systematic study and evaluation of germplasm is of great importance for current and future agronomic and genetic improvement of 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 available germplasm (Singh *et al*., 2002). Reshuffling the genes through recombination is the principle way of developing improved genotypes in breeding programs.

Evaluation of germplasm is of immense important in genetic improvement of the crop. Genetic diversity analysis assist 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).

The $D^2$ statistics developed by Mahalanobis (1936) is a potential tool for obtaining quantitative estimates of divergence among biological populations and has extensively been utilized to assess diversity.

Moreover, the relative contribution of different yield components to total divergence using Mahalanois $D^2$ analysis helps in the identification of selection parameter to be used as criteria for the improvement in the yield. Hybridization between divergent parents is likely to produce wide variability and transgressive segregation with high heterotic effects. $D^2$ 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. The progenies derived from diverse parents are expected to show a broad spectrum of genetic variability and provide better scope to isolate superior recombinants. Therefore, genetically diverse genotypes per genotypes should be used in a hybridization programme to get superior recombinants.

## MATERIALS AND METHODS

The present investigation was carried out at Vegetable Research Farm, Dr. RPCAU, Pusa, Samastipur, Bihar during *rabi* 2015-16. The experimental materials comprised of twenty-four genotypes (Table-1) of tomato collected from two different sources. The experiment was laid out in a randomized block design with three replications accommodating 10 plant in each. Seeds were transplanted at a spacing of 60×45 cm. The genotypes studied are given in table-1. All the recommended cultural practices were adopted for raising the crop successfully. The experimental details and observations to be recorded as follows: The observations were recorded on five randomly selected plants per replication for each genotype on eighteen characters: i) plant height at maturity (cm), ii) number of primary branches per plant, iii) number of days to flower initiation, iv) number of days to fruit initiation, v) number of days to fruit maturity at physiological stage, vi) diameter of fruit (cm), vii) length of fruit(cm), viii) number of locules per fruit, ix) number of fruits per plant, x) average fruit weight (g), xi) yield per plant (kg), xii) yield per hectare (quintal) xiii) total soluble solids (%), xiv) titrable acidity (%), xv) zinc content (mg/100g), xvi) iron content (mg/100g), xvii) lycopene content (mg/100g) and xviii) ascorbic acid content (mg/100g). Mean across the replications were calculated for each traits and the analysis of variation was carried out. Multivariate analysis was done utilizing Mahalanobis $D^2$ statistics which are cited below (Mahalanobis, 1936) and genotypes were grouped into different clusters following Tocher's method. The inter and intra cluster distance were worked out as per method suggested by Singh and Chaudhary (1985) to find actual divergence within and between the clusters. The contribution of individual characters towards genetic divergence was computed by using the method given by Singh and Chaudhary (1985).

**Clustering of genotypes using $D^2$ values**

All the genotypes used were clustered in to different groups by following Tocher's method (**Rao, 1952**). The intra and inter cluster distance were also computed. The criterion used in clustering by this method was that any two varieties belonging to the same cluster at least on an average show a smaller $D^2$ values then those belonging to two different clusters

The device suggested by Tocher (**Rao, 1952**) was strated with two closely associated populations and find a third population which had the smallest average of $D^2$ from the first two. Similarly, the fourth was chosen to have a smallest average of $D^2$ value from the first three and so on. If at any stage increase in average $D^2$ value exceeded the average of already included, because of addition of new genotypes, then the genotype was deleted. The genotypes those are included already in that group were considered as the first cluster. This procedure was repeated till $D^2$ values of the other genotypes were exhausted omitting those, that were already included in former cluster and grouping them in to different clusters.

**The generalized distance between any two populations is defined as:**

$$D^2p=b_1d_1 + b_2d_2+\ldots\ldots\ldots\ldots\ldots\ldots + bpdp$$

Where, $X_1$, $X_2$, $X_3$……. Xp as a multiple measurements available on each individual $d_1$, $d_2$ ……$d_2$ as $X_1^{-1}$, $X_2^{-1}$ - $X_2^{-2}$ …… $Xp^{-1}$ - $Xp^{-2}$, respectively, is being the difference in the means of two populations.

In term of variance and covariance, the $D^2$ value is obtained as follows:

$$D^2P = Wij\ (Xi^{-1} - Xj^{-1})\ (Xj^{-1} - Xj^{-2})$$

Where,

Wij is the inverse estimated variance covariance matrix.

### 2.1 Intra and Inter cluster distance

Based on $D^2$ values, average intra and inter cluster distances were calculated as per Euclidean method

#### 2.1.1 Intra cluster distance:

The average intra cluster distances were calculated by the formula given by **Singh and Chaudhary (1985)**:

$$\text{Inter cluster distance} = \frac{\Sigma Di2}{n}$$

Where,

$\Sigma Di^2$ = Sum of distance between all possible combinations

n = number of all possible combinations

**2.1.2 Inter cluster distance:**

The average inter distances were calculated by the formula given by **Singh and Chaudhary (1985)**.

**Inter cluster distance =** $\frac{\sum D_1^2}{n_i n_j}$

ni = Number of entries in cluster i

nj = Number of entries in cluster j

**2.2 Contribution of Individual characters**

The character contribution towards genetic divergence was computed by using the method given by **Singh and Chaudhary (1985)**. In all the combination, each character is ranked on the basis of

$$di = yi^j - yi^k$$

Where,

di = mean deviation

$yi^j$ = mean value of $j^{th}$ genotype for the $i^{th}$ character

$yi^k$ = mean value of $k^{th}$ genotype for the $i^{th}$ character

Rank 'I' is given to the highest mean difference and rank 'p' is given to the lowest

**Mean difference**

Where,

P is the total number of characters.

Finally, number of times that each character appeared in the first rank is computed and per cent contribution of characters towards divergence was estimated.

**RESULT AND DISCUSSION**

**3.1 Clustering pattern**

The twenty four genotypes taken for genetic divergence analysis differed significantly with regard to the characters studied and displayed marked divergence and grouped into five clusters following Tocher's method (Table 2). Cluster I had twelve genotypes viz., Sweet 72, Nandhi, PT-2009-08, EC-519758, Masina, EC-519778, CN-2237 A, EC-519770, Arka Meghali, Big Oval 2009, CO-3, Azad T-5. Cluster II had two genotypes viz., Arka Alok, Arka Abha. Cluster III had five genotypes viz., cherry tomato, CLN-2870 A, S-108, Sherozi, EC-519823. . Cluster IV had two genotypes viz., PT-41, Avinash-221. Cluster V had three genotypes viz., Utkal Pallavi, CLN-1154 R, CLN-2123 E. Similar studied based on $D^2$ statistic was also performed by **Dharmatti *et al.* (2001), Mahesh *et al*. (2006), Mehta and Asati (2008), Jogi *et al*. (2008), Rana and Singh (2010), Nalla *et al*. (2014) and Lekshmi and Celine (2016).**

**3.2 Cluster Means for Different Characters**

Cluster mean in respect of eighteen quantitative characters of twenty four genotypes were presented in Table 3. From the perusal of Table, it was observed that cluster mean value for days to flower initiation, total soluble solid, iron content and ascorbic acid content was maximum for cluster I (63.81), (4.98), (0.52) and (22.21) respectively and minimum cluster mean value for plant height at maturity (79.19) and lycopene content (1.29). Cluster II had maximum cluster mean value for days to fruit maturity at physiological stage (132.25) and titrable acidity (0.57) and minimum cluster mean value for days to flower initiation (55.52), number of fruits per plant (12.05), zinc content (0.28). Cluster III had maximum mean value for plant height (111.41) ) and minimum cluster mean value for number of primary branches per plant (4.58), diameter of fruit (3.12), length of fruit (3.18), number of locules per fruit (2.22), average fruit weight (21.92), yield per plant (0.69) and yield per hectare (212.70). Cluster IV had maximum cluster mean value for number of primary branches per plant (8.45), days to fruit initiation (84.92), diameter of fruit (4.55), length of fruit (4.55), number of fruits per plant (68.39), average fruit weight (25.55), yield per plant (1.70) and yield per hectare (525.67) and minimum cluster mean value for total soluble solid (4.58) and iron content (0.49). Cluster V had maximum cluster mean value for number of locules per fruit (3.86), zinc content (0.312) and lycopene content (5.03) and minimum cluster mean value for days to fruit initiation (75.53), days to fruit maturity at physiological stage (100.92), titrable acidity (0.38) and ascorbic acid content (16.99). Therefore, this cluster may be chosen for transferring the traits having high mean values through hybridization programme. Selection of genotypes based on cluster mean for the better exploitation of genetic potential also reported by **Rai *et al*. (1998), Joshi and Kohli (2003) and Sharma *et al*. (2006) .**

**3.3 Intra and Inter Cluster Distances**

The mean intra and inter cluster $D^2$ values among the twelve clusters are given in (Table 4). The intra cluster $D^2$ value ranged from 203.353 (Cluster V) to 709.013 (Cluster IV). The cluster IV had the

maximum $D^2$ value (709.013) followed by cluster I (479.068), cluster II (354.461) and cluster III (301.176) while it was least in cluster V (203.353).

The inter cluster $D^2$ values of the five clusters revealed that highest inter cluster generalized distance (3072.639) was between cluster IV and cluster II followed by cluster III and cluster II (2493.978), cluster V and cluster II (2031.663), cluster II and cluster I (1412.612), cluster V and cluster IV (1308.436), cluster V and cluster I (1269.787), cluster IV and cluster I (1192.489), cluster V and cluster III (938.533) and cluster IV cluster III (894.675) while the lowest (830.841) was between cluster III and cluster I. These results of genetic diversity study were in agreement with that of **Mahesh *et al*. (2006), Prashanth *et al*. (2007), Reddy *et al.* (2013), Nalla *et al*. (2014), Lekshmi and Celine (2016)**. They also suggested that genotypes of most diverse cluster may be used as parents in hybridization programmes to develop high yielding varieties.

### 3.4 Contribution Percentage of Each Character towards Total Divergence

The contribution percentages of traits under studied towards total divergence are tabulated in Table 5. Contribution of different plant character for genetic divergence is important for the purpose of further selection and choice of parents for hybridization. The highest contribution in the manifestation of genetic divergence was exhibited by lycopene content (27.90) followed by average fruit weight (25.00), ascorbic acid content (15.94), titrable acidity (15.58), number of fruits per plant (8.70), number of locules per fruit (2.54), total soluble solid (1.45), zinc content (1.45), plant height at maturity, number of primary branches per plant, days to fruit maturity at physiological stage and fruit yield per plant had minimum contribution (0.36) towards total divergence. The contribution of remaining trait in manifestation of genetic divergence was zero. These result are in consonance with the findings of **Lekshmi and Celine (2016)** in tomato.

**Table 1 : List of genotypes/treatments**

| | GENOTYPE | SOURCES |
|---|---|---|
| **1.** | Sweet 72 | GBPUA & T, Pantnagar |
| **2.** | PT2009-08 | GBPUA & T, Pantnagar |
| **3.** | EC-519823 | GBPUA & T, Pantnagar |
| **4.** | EC-519778 | GBPUA & T, Pantnagar |
| **5.** | CN-2237A | GBPUA & T, Pantnagar |
| **6.** | ArkaAlok | GBPUA & T, Pantnagar |
| **7.** | Cherry Tomato | GBPUA & T, Pantnagar |
| **8.** | PT-41 | GBPUA & T, Pantnagar |
| **9.** | CLN-2123E | GBPUA & T, Pantnagar |
| **10.** | UtkalPallavi | GBPUA & T, Pantnagar |
| **11.** | ArkaAbha | GBPUA & T, Pantnagar |
| **12.** | EC-519770 | GBPUA & T, Pantnagar |
| **13.** | EC-519758 | GBPUA & T, Pantnagar |
| **14.** | CLN-1154R | GBPUA & T, Pantnagar |
| **15.** | CLN-2870A | GBPUA & T, Pantnagar |
| **16.** | Big Oval | GBPUA & T, Pantnagar |
| **17.** | S-108 | GBPUA & T, Pantnagar |
| **18.** | Sherozi | GBPUA & T, Pantnagar |
| **19.** | Nandhi | IIVR, Varanasi |
| **20.** | CO-3 | IIVR, Varanasi |
| **21.** | Azad T-5 | IIVR, Varanasi |
| ***22.*** | Avinash-2-2-1 | IIVR, Varanasi |
| **23.** | ArkaMeghali | IIVR, Varanasi |
| **24.** | Masina | Local |

**Table 2 : Clustering pattern of 24 genotypes of tomato on the basis of $D^2$ statistic**

| Cluster No. | No. of Genotypes within cluster | Genotypes in cluster |
|---|---|---|
| I | 12 | Sweet 72, Nandhi, PT-2009-08, EC-519758, Masina, EC-519778, CN-2237 A, EC-519770, Arka Meghali, Big Oval 2009, Co-3, Azad T-5 |
| II | 2 | Arka Alok, Arka Abha |
| III | 5 | Cherry Tomato, CLN-2870 A, S-108, Sherozi, EC-519823 |
| IV | 2 | PT-41, Avinash-221 |
| V | 3 | Utkal Pallavi, CLN-1154 R, CLN-2123 E, |

**Table 3 : Cluster mean for eighteen characters in tomato**

| | PH | PB/P | DFI | DFr.I | DFr.M | Fr.D | Fr.L | Lo/Fr. | Fr./P | Av. Fr. Wt. (g) | Y/P (kg) | Y/H (q) | TSS (%) | TA (%) | Zinc | Iron | Lycopene | A.A |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Cluster I** | 79.19 | 7.43 | 63.81 | 79.77 | 109.00 | 4.12 | 4.19 | 2.75 | 27.55 | 53.48 | 1.44 | 444.83 | 53.48 | 4.98 | 0.47 | 0.31 | 0.52 | 1.29 |
| **Cluster II** | 79.63 | 5.38 | 55.52 | 76.52 | 132.25 | 3.35 | 3.35 | 3.87 | 12.05 | 88.83 | 0.99 | 304.22 | 88.83 | 4.23 | 0.57 | 0.28 | 0.51 | 2.08 |
| **Cluster III** | 111.41 | 4.58 | 62.01 | 80.57 | 103.94 | 3.12 | 3.18 | 2.21 | 33.55 | 21.92 | 0.69 | 212.70 | 21.92 | 4.13 | 0.47 | 0.30 | 0.51 | 1.75 |
| **Cluster IV** | 90.15 | 8.45 | 63.36 | 84.92 | 109.12 | 4.55 | 4.55 | 3.42 | 68.38 | 25.55 | 1.70 | 525.67 | 25.55 | 4.00 | 0.48 | 0.29 | 0.49 | 2.59 |
| **Cluster V** | 88.97 | 5.46 | 59.58 | 75.53 | 100.92 | 3.37 | 3.58 | 3.86 | 26.28 | 32.38 | 0.86 | 265.26 | 32.38 | 4.44 | 0.38 | 0.31 | 0.51 | 5.03 |

**Table 4 : Mean intra and inter cluster distance ($D^2$) among five clusters in tomato**

| Cluster | 1 Cluster | 2 Cluster | 3 Cluster | 4 Cluster | 5 Cluster |
|---|---|---|---|---|---|
| **1 Cluster** | 479.068 | 1412.612 | 830.841 | 1192.489 | 1269.787 |
| **2 Cluster** | | 354.461 | 2493.978 | 3072.639 | 2031.663 |
| **3 Cluster** | | | 301.176 | 894.675 | 938.533 |
| **4 Cluster** | | | | 709.013 | 1308.436 |
| **5 Cluster** | | | | | 203.353 |

**Table 5 : Contribution percentage of eighteen characters towards genetic divergence in tomato**

| Sl. No. | Source | Times ranked 1st | Contribution % |
|---|---|---|---|
| 1 | Plant height at maturity (cm) | 0.36 | 0.36 % |
| 2 | No. of primary branches/plant | 0.36 | 0.36 % |
| 3 | No. of Days to flower initiation | 0.01 | 0.00 % |
| 4 | No. of Days to fruit initiation | 0.01 | 0.00 % |
| 5 | No. of Days to fruit maturity at physiological stage | 0.36 | 0.36 % |
| 6 | Diameter of Fruit (cm) | 0.01 | 0.00 % |
| 7 | Length of Fruit (cm) | 0.01 | 0.00 % |
| 8 | No. of locules/fruit | 2.54 | 2.54 % |
| 9 | No. of fruits/plant | 8.70 | 8.70 % |
| 10 | Average fruit weight (g) | 25.00 | 25.00 % |
| 11 | Fruit yield/plant (kg) | 0.36 | 0.36 % |
| 12 | Fruit Yield/hectare (quintal) | 0.01 | 0.00 % |
| 13 | Total Soluble Solid (%) | 1.45 | 1.45 % |
| 14 | Titrable Acidity (%) | 1.58 | 15.58 % |
| 15 | Zinc content (mg/100g) | 1.45 | 1.45 % |
| 16 | Iron content (mg/100g) | 0.01 | 0.00 % |
| 17 | Lycopene content (mg/100g) | 27.90 | 27.90 % |
| 18 | Ascorbic Acid content (mg/100g) | 15.94 | 15.94 % |

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