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Genetic divergence in root system architecture of tomato genotypes at vegetative stage

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ABSTRACT

Root system architecture (RSA) is important for supplying water and nutrients for crop growth and production. In the present investigation an attempt has been made to study the genetic variability present in root system architecture of tomato genotypes at vegetative stages. Fifteen tomato genotypes were evaluated in CRD with three replications at AICRP on Vegetable Crops, OUAT, Bhubaneswar during the year 2020-21. Three seedlings per pot were grown and observations were recorded on thirteen RSA traits. Analysis of variance indicated presence of significant differences among the tested genotypes in respect of different RSA traits. Number of root crossings showed the highest GCV (59.35). Broad sense heritability was the highest (97.56 %) in case of volume of fine roots. The results of D^2 analysis revealed 4 numbers of clusters. Results on principal component analysis revealed that PC1 and PC2 had 65.32 % and 26.33 % of contribution towards total variability.

***Keywords*:**Tomato, root system architecture, heritability, genetic advance, principal component analysis

Plant root system plays crucial role in plant growth and production by transporting diverse resources from the soil. The shape and structure of root system in the soil is described through root system architecture (RSA). Its importance in plant productivity lies in the fact that major soil resources are heterogeneously distributed in the soil, so that the spatial deployment of roots will substantially determine the ability of a plant to secure edaphic resources (Lynch *et al.*, 1995). Root traits are usually described as root system architecture (RSA), referring to the shape and physical space of the roots (Ye *et al*., 2018). Root system architecture (RSA) is an important developmental and agronomic trait, which plays vital roles in plant adaptation and productivity under water limited environments (Ye *et al*., 2018). A deep and proliferative root system helps extract sufficient water and nutrients under these stress conditions.

In tomato breeding programme root system architecture is rarely considered as a selection criteria as it is difficult to take observations on root characters. But root system architecture is important for supplying water and nutrients for crop growth and production. The first step in utilizing the traits for practical breeding programme starts with the exploration of genetic diversity. Very little work on variability in RSA traits of tomato genotypes has been performed.Therefore in the present investigation an attempt has been made to study the genetic variability present in root system architecture of tomato genotypes at vegetative stages.

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MATERIALS AND METHODS

In the present investigation root morphology of fifteen tomato genotypes was studied in pot culture condition. The genotypes taken for the study were BT 1 (V1-Utkal Pallavi), BT 2 (V2-Utkal Deepti), BT 10 (V3-Utkal Kumari), Utkal Raja (V4), BT-101 (V5), BT-136 (V6), BT-317 (V7), BT 12-2 (V8), BT 112-1 (V9), BT 428-3 (V10), BT 442-2 (V11), BT 506-1 (V12), BT 22-4-1 (V13), BT 19-1-1-1 (V14-Kalinga tomato 121) and Arka Vikash (V15).

Experimental details

The seeds of tomato genotypes were collected from AICRP on Vegetable Crops, OUAT, Bhubaneswar. Seeds of different genotypes were treated with Carbendazim and sown in nursery bed on 05.11.20.Twenty five days old seedlings were transplanted into the round poly pots (dimensions: 45 cm height × 45 cm top diameter × 42 cm base diameter) filled with sandy loam soil (pH range 4.58-5.44), FYM, DAP and MOP as basal dose. 10.0 g DAP and 15.0 g MOP were applied to each pot as basal dose. The pot mixture was prepared 10 days before transplanting. Three seedlings were transplanted into each pot. During growth, the pots were watered regularly and fertilizer application was done at vegetative stage and fruit initiation stages. Water soluble fertilizer (19:19:19) was applied at a concentration of 0.3 % (3 g per litre of water) at vegetative and fruit initiation stage.

Root sample extraction

Roots were extracted at vegetative stage (20 days after transplanting). For sampling of roots, the poly pots were removed carefully and soaked in water tank over night to loosen the soil. From each replication two plants were selected for each genotype. Then the plants were uprooted and separated as roots and shoots at root collar region. After separation, the roots were washed in tap water properly to remove the soil and debris attached to the root. Then the roots were kept in a zipped poly packet to avoid desiccation.

Root morphological trait analysis using root analyser

The washed roots were taken out from the zipped poly packet and placed on a transparent cleaned plastic tray of size 30 x 40 cm. The tray was filled with deionised water for complete immersion of the roots taking care to separate the roots and to avoid any overlapping. Then the tray was placed on the EPSON professional scanner to capture the image. From the scanned images, the component traits of root system architecture were estimated using Win Rhizo Pro – 2016a (Regent Instrument Inc., Quebec, Canada) Root Analysis System, which was used to investigate root morphology based on images (400 Dots per Inch, DPI; Magalhaes *et al.*, 2011). Two plant root samples per replication were taken for the study. Data on different root traits were generated through Win Rhizo Pro – 2016a software. Observations were recorded on total root length (TRL, cm), projected root area (PRA, cm^2), root surface area (SA, cm^2), root diameter (RD, mm), root volume (RV, cm^3), root length density (RLD,cm cm^{-3}), root growth rate in volume (mm^3day^{-1}) root tips, root forks, root crossings, total root length, surface area, and volume of fine roots (roots having < 0.5 mm diameter are considered as fine roots) of tomato genotypes at harvesting stages.

Statistical analysis

Analysis of variance for pot culture was done with the Windostat software, version 9.3 for 13 root traits.

RESULTS AND DISCUSSION

The mean genotypic sum of squares for fifteen tomato genotypes with respect to 13 RSA traits revealed presence of significant ($P < 0.01$ & $P < 0.001$) differences among the tested genotypes in respect of all the traits studied.

Root system of tomato genotypes at vegetative stage

At vegetative stage the genotypes showed significant differences in respect of 13 root traits under study (Table 1). Total root length of the test genotypes ranged from 54.88 to 298.98 cm; projected root area varied from 4.39 to 22.20 cm^2; root surface area varied from 13.79 to 69.74 cm^2; average root diameter ranged from 0.50 to 1.17 mm; root volume varied from 0.18 to 1.38 cm^3; root length density ranged from 86.92 to 584.68 cm cm^{-3}; number of root tips floated between 138.33 to 684.67; root forks varied from 288.0 to 1339.67; number of root crossings varied from 11.67 to 174.33; root growth rate in volume varied from 3.31 to 25.97 $mm^{-3}day^{-1}$ total root length of fine roots varied from 25.38 to 172.90 cm; surface area of fine roots varied from 2.16 to 16.26 cm^2 and volume of fine roots varied from 0.017 to 0.140 cm^3 (Table 1). The mean value in respect of total root length, projected root area, root surface area, average root diameter, root volume, root length density, root tips, root forks, root crossings, root growth rate in volume, total root length < 0.5mm, root surface area < 0.5 mm and root volume < 0.5mm were 139.51 cm, 10.187 cm^2, 32.08 cm^2, 0.75 mm, 0.61 cm^3, 265.93 cm cm^{-3}, 323.09, 794.04,75.62, 11.592 mm^3 day^{-1}, 87.209 cm, 6.787 cm^2 and 0.051 cm^3, respectively (Table 2). The released variety, Utkal Pallavi (V1) recorded low value (value less than mean) for all root traits except root length density (RLD). Utkal Deepti (V2) recorded low value (value less than mean) for the entire trait except average root diameter (0.76 mm). The released variety, Utkal Kumari (V3) recorded high value (value more than mean) for all traits except root length density (210.87 cm cm^{-3}) and root diameter (0.72 mm). The highest total root length (289.98 cm) was observed in case of Utkal Kumari (V3).

Estimates of genetic parameters

The estimates of genetic parameters at vegetative stage are presented in Table 2. In case of total root length the GCV, PCV, heritability, genetic advance as percent of mean were 43.87, 48.16, 82.99 % and 82.34. High genetic advance along with high heritability revealed that heritability is due to additive gene actions and selection for this trait may be achievable. GCV and PCV in case of projected root area were 42.55 and 46.35 indicating environmental effect is non-negligible. High heritability (84.37%) along with high genetic advance as per cent of mean (80.20) indicated that heritability is due to additive gene effects and selection for projected root area may be effective. In case of root surface area the GCV, PCV, heritability, genetic advance as percent of mean were 42.72, 44.09, 93.88% and 85.37. High heritability along with high genetic advance indicated that heritability is due to additive gene effects and selection for this trait may be rewarding. In case of average root diameter, GCV and PCV were low (25.14, 29.81). High heritability (80.00%) with low genetic advance as percent of mean (49.13) indicated non-additive gene action. In case of root volume, the GCV, PCV, heritability, genetic advance as percent of mean

were 48.99, 54.37, 81.82% and 91.84. High heritability along with high genetic advance indicated that this trait is governed by additive gene action. The GCV, PCV, heritability, genetic advance as per cent of mean in case of root length density were 52.12, 59.99, 75.49% and 92.68. High genetic advance as per cent of mean is due to high phenotypic variance. In case of root tips, the GCV value (45.80) was closer to PCV value (47.20) which indicated that this character is least influenced by the environment. This character showed high heritability (94.17%) and high GA as per cent of mean (91.40).

The GCV, PCV, heritability, genetic advance as per cent of mean in case of root forks were 42.71, 48.89, 76.30 % and 76.55. In case of root crossings, both GCV and PCV were maximum as compared to other traits. The difference in PCV and GCV value was high which reflected the influence of environment in the expression of this trait. Heritability and genetic advance as per cent of mean were 82.95% and 111.43. The GCV, PCV, heritability, genetic advance as per cent of mean in case of root growth rate in volume (RGRV) were 48.99, 54.42, 81.05% and 90.80. High heritability along with high genetic advance indicated that this trait is governed by additive gene action. Total root length in case of fine roots (<0.5mm root diameter) showed moderately high heritability (74.19%) and genetic advance as per cent of mean (78.76). The difference in PCV and GCV value was high which indicated influence of environment in the expression of this trait. Surface area of fine roots (< 0.5 mm root diameter) exhibited high genetic advance as percent of mean (92.08) and moderately high heritability value (78.59%). Root volume of fine roots (<0.5mm root diameter) showed the highest heritability (97.56), highest GA as per cent of mean (141.60) and also the highest PCV value (70.14) as compared to other traits. The wide gap between PCV and GCV value indicated that this trait was also highly influenced by environment. At vegetative stage, most of the characters exhibited high heritability and high genetic advance because of the presence of high genetic variability.

Divergence analysis in RSA traits of tomato genotypes at vegetative stage

From the results of D^2 analysis it was observed that fifteen genotypes were grouped into 4 numbers of clusters (Fig.1) of which three clusters (cluster 2, 3 & 4) were monotypic and cluster 1 comprised of 12 genotypes at the squared Euclidean distance level of 700. But at the lower Euclidean distance level of 200 the genotypes were classified into 10 groups of which 9 clusters were monotypic. Lower distance level indicates closer relationship and higher distance level indicates further (or distant) relationship. From the dendrogram (Fig.1, clustering by Tocher's method) it was observed that number of clusters at lower squared Euclidean distance level (200) had almost matched to the number of clusters formed in 2D plot (Fig. 2) and the 3D plot (Fig. 3) graphs in which the principal component analysis (PCA) scores or canonical vectors are taken in different axes.

Number of clusters formed in 3D and 2D plot was 9 due to fusion of V2 and V12 in a single cluster where as number of clusters formed in Fig.3 at 200 distance level was 10 because of separation of V2 and V12.Cluster 1 comprised of 12 genotypes (V1, V2, V4, V6, V7, V8, V10, V11, V12, V13, V14 & V15); V9 is included in Cl -2; V5 in Cl- 3 and V3 in Cl-4. Maximum inter cluster distance (2040.47) was observed between Cl-1 and Cl-3. Minimum inter cluster distance (439.7) was observed between Cl-2 and Cl-3. At vegetative stage total root length had maximum contribution towards divergence (18.24 %) followed by total root length of fine roots (< 0.5mm root diameter; 9.0 %).

Principal component analysis

In principal component analysis, Eigen values are used to determine how many factors are to be retained. The theorem is that sum of Eigen values is equal to the number of variables used. The first component therefore is expected to gather maximum information. In the present investigation the total variances were divided into thirteen components. The principal components having Eigen values more than one (Eigen value>1) were considered as significant. In the present investigation out of 13 components or factors the first two components PC1& PC2 had Eigen value>1 (8.492, 3.423) and they contributed 91.66 % of total variation (Table 2). PC3 and PC4 had Eigen values of 0.723 & 0.205 and they were not significant. PC1 accounted for 65.32 % and PC2 accounted for 26.33 % of total variation.

In order to identify the characters which has greater influence on the PCA value we have to look for individual loadings. The loading values which are closer to unity in a given component represent the influential character for that component. The percentage contribution of each observation was determined by the ratio of the squared factor score (PC score) of this observation by the Eigen-value associated with that component. In the present study first component was highly positively influenced by total root length (0.991, 11.56 %), SA <0.5mm root diameter (0.964, 10.95 %), projected root area (0.947, 10.57 %), root surface area (0.947, 10.57 %) and negatively influenced by average root diameter (-0.274, 0.89%). The second component was highly positively influenced by root length density (0.963, 27.09 %). The distribution of the genotypes and the root traits is depicted in the biplot of PC1 and PC2 (Fig. 4**).** The scatter plot of PC1 and PC2 showed that the tomato genotypes were dispersed in all four quarters, indicating

Table 1: Root traits of tomato genotypes at vegetative stage

Genotype	TRL (cm)	PRA (cm^2)	SA (cm^2)	Av. Root Dia. (mm)	Root Vol. (cm^3)	RLD ($cm\ cm^{-3}$)	Root Tips	Root Forks	Root Crossings	RGRV ($mm^{-3}\ day^{-1}$)	TRL (cm) <0.5mm root diameter	SA (cm^2) <0.5mm root diameter	Root Vol. (cm^3) <0.5 mm root diameter
V1	87.24	4.39	13.79	0.50	0.18	498.41	167.67	413.67	58.33	3.307	66.953	5.140	0.037
V2	86.12	6.53	20.50	0.76	0.39	219.43	140.33	445.67	41.00	7.397	50.860	4.053	0.031
V3	289.98	22.20	69.74	0.72	1.38	210.87	368.67	1339.67	130.33	25.970	172.900	16.263	0.140
V4	156.27	10.38	32.62	0.66	0.55	284.24	253.67	677.33	69.33	10.390	105.257	8.533	0.065
V5	195.15	10.65	33.45	0.52	0.34	584.68	481.33	1222.33	174.33	6.360	143.307	9.493	0.064
V6	120.19	9.16	28.79	0.74	0.56	214.80	345.33	669.33	60.33	10.563	82.850	6.183	0.044
V7	178.59	11.89	37.35	0.66	0.63	284.48	435.00	839.33	82.00	11.840	117.253	8.483	0.061
V8	128.14	11.81	37.10	0.91	0.90	143.25	307.00	746.67	63.33	16.923	75.950	5.807	0.044
V9	207.50	14.57	46.82	0.73	0.85	245.14	684.67	1273.67	140.67	15.980	106.963	9.277	0.064
V10	87.05	6.65	20.88	0.73	0.40	221.76	236.00	475.33	29.67	7.553	50.357	3.923	0.030
V11	54.88	6.55	20.58	1.17	0.63	86.92	138.33	288.00	11.67	11.873	25.377	2.153	0.017
V12	107.64	5.87	18.45	0.54	0.25	428.40	256.67	776.00	95.00	4.770	75.430	5.413	0.039
V13	85.89	9.43	29.96	1.10	0.84	101.79	245.33	479.33	24.33	15.903	46.860	3.583	0.027
V14	142.28	11.01	34.59	0.77	0.67	211.46	313.33	838.67	85.00	12.703	84.513	6.240	0.046
V15	165.80	11.67	36.65	0.71	0.65	253.36	473.00	750.67	69.00	12.347	103.310	7.260	0.051
CD(0.01)	**18.47**	**1.43**	**4.06**	**0.10**	**0.09**	**34.59**	**42.39**	**94.80**	**8.43**	**1.696**	**10.99**	**0.89**	**0.006**
CV(%)	**5.90**	**6.25**	**5.63**	**6.05**	**6.30**	**5.79**	**5.84**	**5.64**	**4.96**	**6.486**	**5.61**	**5.82**	**5.65**

Table 2 : Estimates of genetic parameters in respect of RSA traits of tomato genotypes at vegetative stage

Root trait	Vegetative stage					
	Mean	GCV	PCV	Heritability (Broad Sense) (%)	GA of Mean (%)	Exp. Mean next Generation
Total root length (TRL)	139.51	43.87	48.16	82.99	82.34	140.91
Projected root area (PRA)	10.18	42.55	46.35	84.37	80.20	10.28
Root surface area (SA)	32.09	42.72	44.09	93.88	85.37	32.41
Average root diameter (RD)	0.75	25.14	29.81	80.00	49.13	0.76
Root volume (RV)	0.61	48.99	54.37	81.82	91.84	0.62
Root length density (RLD)	265.93	52.12	59.99	75.49	92.68	268.59
Root tips	323.09	45.80	47.20	94.17	91.40	326.32
Root forks	749.05	42.71	48.89	76.30	76.55	756.54
Root crossings	75.62	59.35	65.17	82.95	111.43	76.38
RGRV(root growth rate in volume)	11.59	48.99	54.42	81.05	90.80	11.71
TRL < 0.5mm root diameter	87.21	44.50	51.67	74.19	78.76	88.08
SA < 0.5mm root diameter	6.79	50.18	56.58	78.59	92.08	6.86
RV < 0.5mm root diameter	0.05	56.74	70.14	97.56	141.60	0.0505

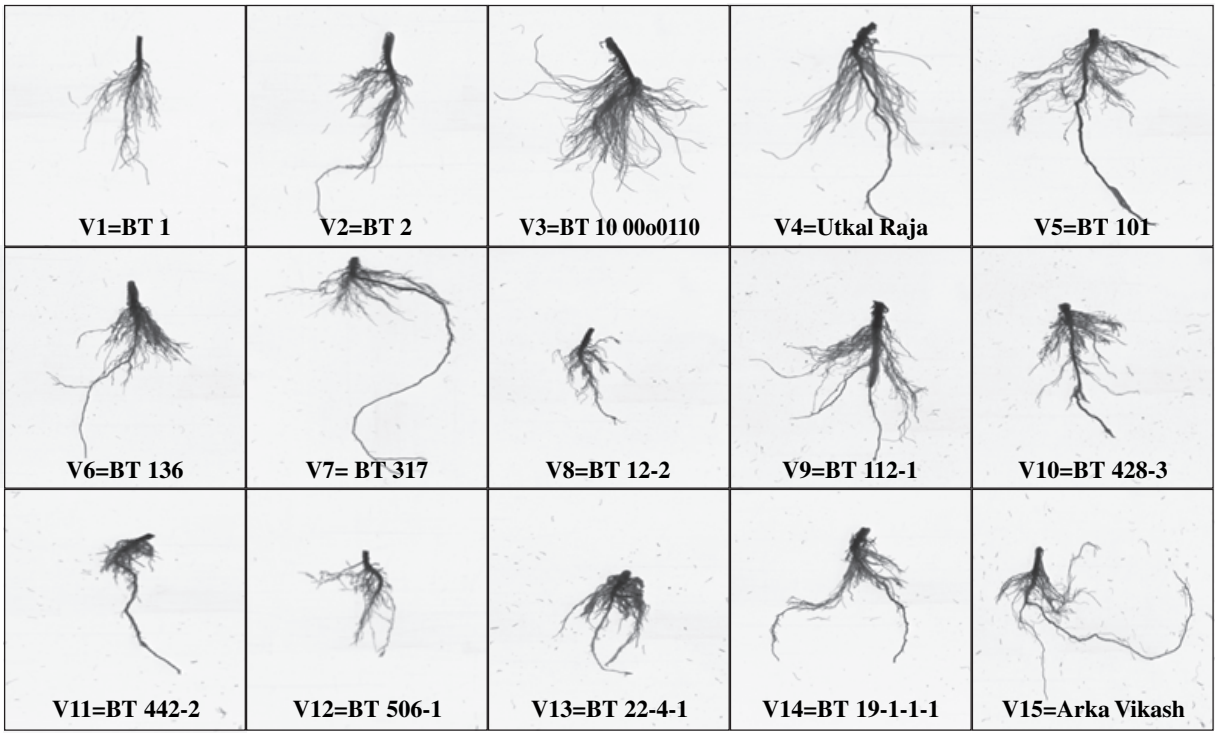
Table 3: Principal component analysis showing relative contribution of characters towards divergence at vegetative stage (Loading values and their variability percentage)

Root trait	PC1(F1)	PC2(F2)	PC3(F3)	PC4(F4)
Total root length (TRL)	0.991 (11.56%)	0.093 (0.25%)	-0.032	-0.056
Projected root area (PRA)	0.947 (10.57%)	-0.316 (2.91%)	-0.032	-0.023
Root surface area (SA)	0.947 (10.57%)	-0.316 (2.93%)	-0.018	-0.023
Root diameter (RD)	-0.274 (0.89%)	-0.897 (23.51%)	0.194	0.236
Root volume (RV)	0.687 (5.56%)	-0.719 (15.08%)	-0.025	0.037
Root length density (RLD)	0.106 (0.13%)	0.963 (27.09%)	-0.086	0.157
Root tips	0.716 (6.03%)	0.185 (1.00%)	0.638	-0.200
Root forks	0.933 (10.25%)	0.223 (1.46%)	0.214	0.131
Root crossings	0.790 (7.35%)	0.517 (7.81%)	0.208	0.242
RGRV	0.687 (5.56%)	-0.718 (15.08%)	-0.025	0.037
TRL <0.5mm root diameter	0.932 (10.24%)	0.291 (2.48%)	-0.119	-0.031
SA <0.5mm root diameter	0.964 (10.95%)	0.118 (0.41%)	-0.223	-0.034
RV <0.5mm root diameter	0.937 (10.34%)	0.011 (0.004%)	-0.338	-0.014
Eigen value	**8.492**	**3.423**	**0.723**	**0.205**
Variability (%)	**65.322**	**26.334**	**5.558**	**1.580**
Cumulative %	**65.322**	**91.657**	**97.215**	**98.795**

a high level of genotypic variation. From Fig. 4 and Table 2, it is evident that all the traits had positive influence on PC1 except average root diameter. In case of PC2, projected area, root surface area, root volume, root diameter and RGRV had negative influence and other traits had positive effect. The genotypes and traits which are located close to the axis line are considered to have more variation than others. Therefore the genotypes V2, V10, V6, V9 and V15 and the traits like total root length, surface area of fine roots (<0.5 mm root diameter), volume of fine roots ((< 0.5 mm root diameter) showed more variation.

The aim of carrying out root system architecture (RSA) studies in crop plants is to understand areas of interest within the root system and incorporate this information in crop improvement programs. Roots collect water and nutrients from the soil. Hence, the morphological and physiological characteristics of roots play an important role in determining shoot growth and overall production (Ghosh and Xu, 2014). The genetic

Scanned root images of tomato genotypes showing variability at vegetative stage



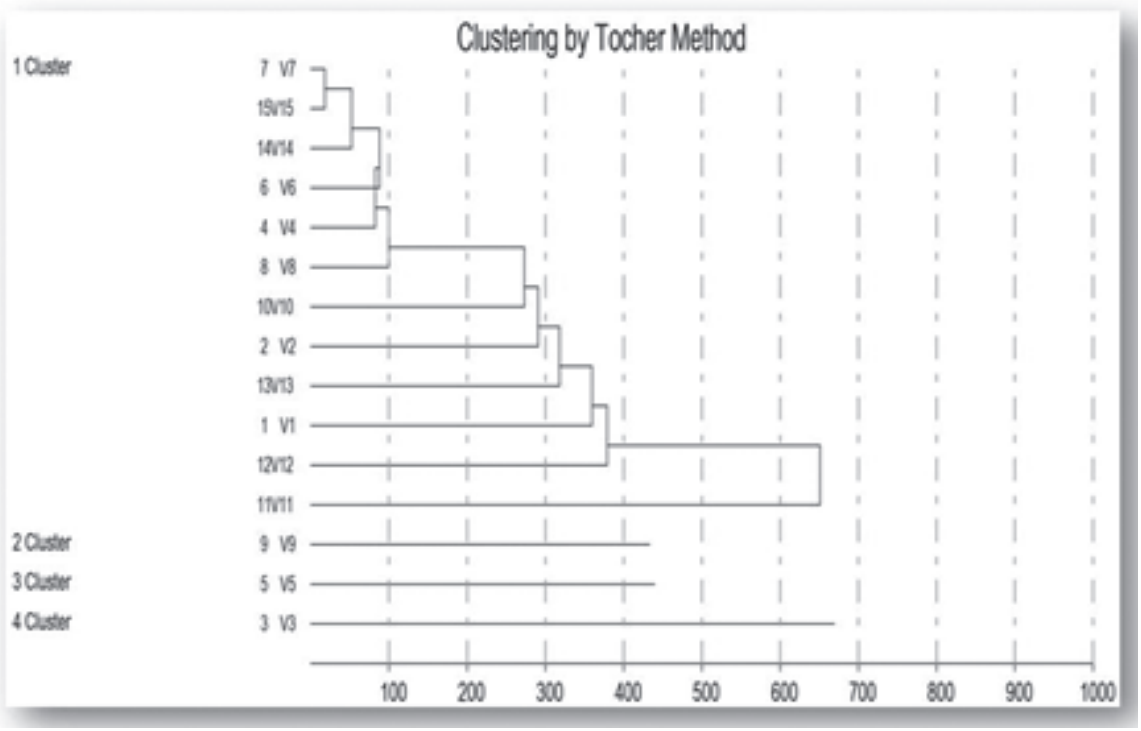


Fig. 1: **Clustering of tomato genotypes at vegetative stage**

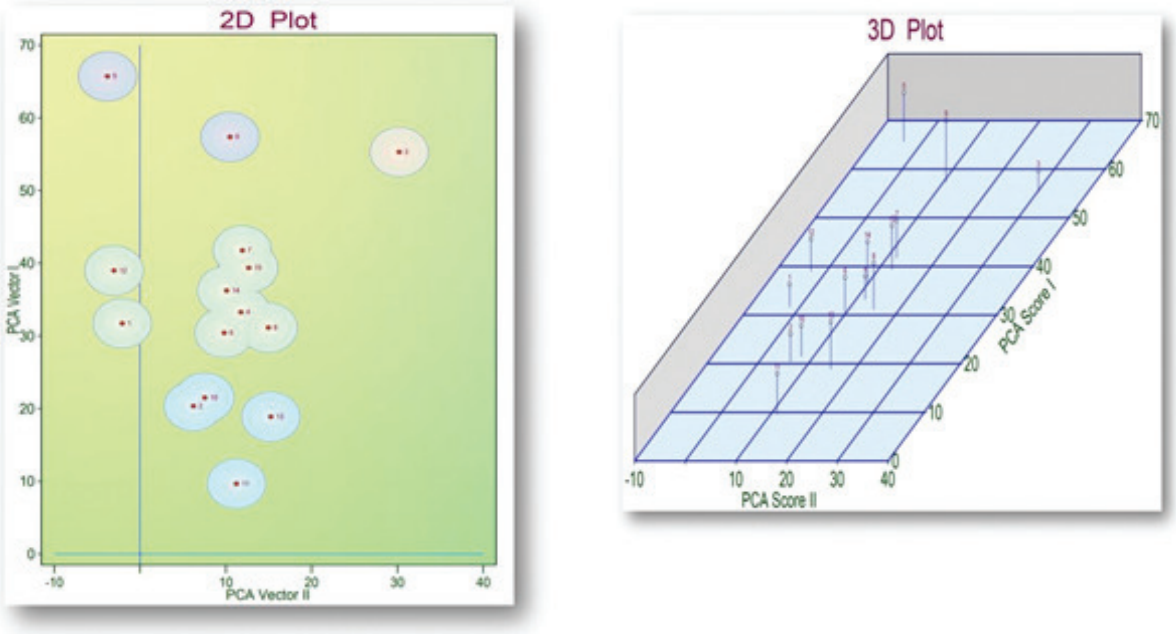


Fig. 2 & 3:Clustering of tomato genotypes at vegetative stage (2D & 3D plot)

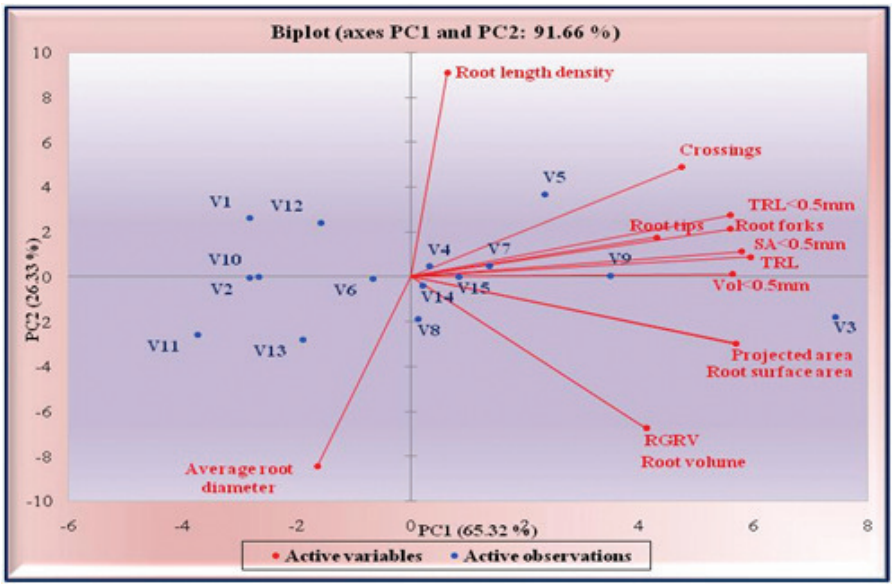


Fig. 4: PCA bi-plot for root traits at vegetative stage

variability present in the population in respect of RSA traits of tomato genotypes could be exploited to improve the root traits that are associated with yield in tomato. Variability study in root traits was also performed by different researchers in different crops (Chen *et al*., 2011; Atta *et al*., 2013; Nasir *et al*., 2017; Dennis *et al*., 2020).

CONCLUSION

At vegetative stage, most of the characters exhibited high heritability and high genetic advance because of the presence of high genetic variability. Divergence study through D^2 analysis revealed that fifteen tomato genotypes were grouped into 4 numbers of clusters at the higher Euclidean distance level (700). In principal component analysis, PC1 and PC2 together contributed 91.66 % of total variation. PC1 was highly positively influenced by total root length with loading factor of 0.991 followed by surface area of fine roots with loading factor of 0.964. The scatter plot of PC1 and PC2 showed

that the tomato genotypes were dispersed in all four quarters, indicating a high level of genotypic variation.

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