



RESEARCH ARTICLE

Unravelling genetic diversity in root system architecture of tomato at vegetative, flowering and harvesting stages

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Abstract

Research on root system architecture (RSA) of tomato plant is meagre and yet not fully exploited for crop improvement programme. In the present investigation forty-four tomato genotypes were initially assessed under field condition to reveal variability in their root traits at harvesting stage. These genotypes showed variation in their root length, number of roots, root volume, fresh and dry root weight and were classified as high, moderate and low root performance group in field evaluation. Fifteen of the forty four genotypes were assessed in pot culture to study divergence in root system architectural (RSA) traits at vegetative, flowering and harvesting stages. The genotypes exhibited significant variation for all RSA traits at different growth stages. Average genotypic coefficient of variation (GCV) and genetic advance as percent of mean of RSA traits were the highest at vegetative stage (46.44 & 89.55) followed by harvesting (34.76 & 67.89) and flowering stage (25.72 & 51.06). D² analysis of RSA traits at vegetative, flowering and harvesting stages grouped the genotypes into 4, 4 and 5 numbers of clusters. PC1 & PC2 together explained 91.65 %, 94.92 % & 79.57 % of total variability at vegetative, flowering and harvesting stages. Total root length had the maximum contribution to the diversity of genotypes both in D² and PCA analysis for all growth stages. This study reveals that root system architecture of tomato varies at different developmental stages.

Keywords: genetic advance; genetic diversity; PCA; root system architecture; tomato

Introduction

The often-overlooked root system of plants plays a crucial role in growth and productivity by transporting essential resources from the soil. However, its contribution to overall plant growth remains underexplored. In crop breeding, the focus on above ground traits for increased yield and stress resistance has led to the neglect of roots' importance. With the global need to produce more with fewer resources on limited land, the next phase of plant breeding must prioritize root systems (1). Roots possess a unique ability to adapt to varying nutrient levels, water availability and other environmental factors.

Root system architecture (RSA) is a critical trait for plant adaptation, especially in water scarce environments (2). Plants demonstrate remarkable adaptability in their roots to thrive in diverse soil conditions, enhancing resource use efficiency and sustaining productivity, even when resources are scarce (3). RSA traits vary across different crops, such as rice, wheat and maize (4 & 5). Manipulating RSA traits can enhance water and nutrient

capture, making it crucial for sustainable agriculture (3). In light of the growing importance of sustainable agriculture and food security, exploring the genetic variability of RSA traits in tomatoes presents a promising avenue for crop improvement programs. Understanding the connection between root traits and fruit yield is also crucial for achieving increased productivity. Yet, there is a dearth of literature on genetic divergence studies of RSA traits in tomatoes, making this endeavour a valuable and timely contribution to the field of plant breeding. The present study seeks to unravel the genetic variability of RSA traits associated with fruit yield.

Materials and Methods

In the present investigation root morphology of tomato genotypes was studied in both field and pot culture condition. Based on field information, pot culture evaluation was undertaken to get detailed information on root system architecture of selected tomato genotypes at vegetative, flowering and harvesting stages.

Field evaluation

In the field experiment, forty-four tomato genotypes (as listed in Table 1) were evaluated and characterised for their root attributes at peak harvesting stage. This experiment followed a randomized block design with two replications during the years 2018-19 and 2019-20. From each genotype, five plants were randomly selected for observation, which included primary root length, number of secondary roots, fresh roots weight, dry roots weight and volume of fresh root.

Pot culture evaluation

Divergence analysis in root system architecture of tomato genotypes was carried out at vegetative, peak flowering and peak harvesting stages, during the year 2020-21. Seeds of 15 randomly selected genotypes (V1:Utkal Pallavi; V2: Utkal Deepti; V3: Utkal Kumari; V4: Utkal Raja; V5: BT-101; V6: BT-136; V7: BT-317; V8: BT 12-2; V9: BT 112-1; V10: BT 442-2; V11: BT 442-2; V12: BT 506-1; V13: BT 22-4-1; V14: BT 19-1-1-1; V15: Arka Vikash) were sown in nursery bed and were transplanted into the poly pots

filled with sandy loam soil, FYM, DAP and MOP as basal dose. Three seedlings were transplanted into each pot. During growth, the pots were watered regularly and water-soluble fertilizer (19:19:19) was applied at a concentration of 0.3 % (3 g per litre of water) at vegetative stage and flower initiation stages. The pots were arranged in completely randomised design with three replications.

RSA trait analysis

The extracted and cleaned root samples at vegetative, peak flowering and harvesting stages were placed onto a transparent, clean plastic tray containing deionised water for complete immersion of root samples. Then the tray was positioned on an EPSON professional scanner for image capture. The Win Rhizo Pro - 2016a Root Analysis System (Regent Instrument Inc., Quebec, Canada) was employed to estimate the component traits of root system architecture based on the scanned images (400 DPI) (6). For the study, observations were recorded on 13 RSA traits.

Table 1. Root traits of forty-four tomato genotypes in field condition (Average of two years data)

SL. NO.	Genotype	Primary root length (cm)	No. of secondary roots	Fresh root weight (g)	Root volume (cc)	Dry root weight (g)	Root performance index (RPI)
V1	BT 1(Utkal Pallavi)	21.0 (1)	14.5(0)	18.25(0)	13.50(0)	3.60(0)	1.0
V2	BT 2 (Utkal Deepti)	18.25 (0)	8.50(0)	11.75(0)	12.50(0)	3.28(0)	0.0
V3	BT 10 (Utkal Kumari)	20.50 (0)	14.00(0)	25.75(0)	24.50(0)	4.63(0)	0.0
V4	BT 12 (Utkal Urbasi)	16.15(0)	27.50(2)	27.00(1)	25.50(1)	4.34(0)	4.0
V5	U. Raja	31.50(2)	11.50(0)	27.75(1)	27.00(1)	4.88(1)	5.0
V6	U. Pragyan	36.00(2)	21.00(1)	24.00(0)	22.00(0)	4.39(0)	3.0
V7	Arka Vikash	29.50(1)	22.00(1)	31.00(1)	30.00(1)	6.39(1)	5.0
V8	Megha tomato	33.00(2)	25.00(2)	34.75(2)	30.00(1)	8.12(2)	9.0
V9	BT 3	25.00(1)	20.00(1)	38.50(1)	32.50(1)	6.06(1)	5.0
V10	BT17	29.50(1)	18.50(1)	45.50(2)	44.50(2)	7.90(2)	8.0
V11	BT 18	32.00(2)	25.50(2)	48.00(2)	49.50(2)	6.97(2)	10.0
V12	BT 101	26.25(1)	14.00(0)	27.25(1)	22.50(0)	4.90(1)	3.0
V13	BT 106	21.00(1)	15.50(0)	18.50 (0)	17.50(0)	3.13(0)	1.0
V14	BT 136	27.00(1)	17.00(0)	37.25 (1)	37.50(2)	5.72(1)	5.0
V15	BT 218	20.25(0)	23.00(1)	18.25 (0)	17.50(0)	3.85(0)	1.0
V16	BT 317	17.75(0)	19.00(1)	28.50(1)	29.50(1)	4.62(0)	3.0
V17	BMZ21	25.00(1)	36.00(2)	26.75(1)	29.00(1)	3.00(0)	5.0
V18	BT 12-2	40.70(2)	44.00(2)	64.00(2)	52.50(2)	6.43(1)	7.0
V19	BT17-2	20.00(0)	16.00(0)	36.25(1)	38.50(2)	5.59(1)	4.0
V20	BT 112-1	30.75(2)	25.00(2)	41.00(2)	35.00(1)	8.78(2)	9.0
V21	BT 207-2	27.50(1)	24.50(1)	51.00(2)	50.00(2)	4.56(0)	6.0
V22	BT 428-3	20.25(0)	22.00(1)	27.50(1)	25.00(1)	5.90(1)	4.0
V23	BT 442-2	33.00(2)	19.00(1)	39.50(2)	41.00(2)	6.50(2)	9.0
V24	BT 506-1	20.50(0)	19.00(1)	38.00(1)	39.00(2)	5.94(1)	5.0
V25	BT 12-3-2	25.50(1)	17.00(0)	32.50(1)	32.50(1)	4.42(0)	3.0
V26	BT 17-2-5	22.75(1)	13.50(0)	14.25(0)	13.50(0)	7.91(2)	3.0
V27	BT 22-4-1	25.00(1)	22.50(1)	47.75(2)	42.50(2)	6.83(2)	8.0
V28	BT 224-3-1	30.25(1)	21.50(1)	30.75(1)	30.00(1)	5.30(1)	5.0
V29	BT 306-1-2	28.25(1)	16.50(0)	34.25(1)	32.50(1)	3.65(0)	3.0
V30	BT 413-1-2	22.75(1)	22.00(1)	24.00(0)	22.50(0)	6.92(2)	4.0
V31	BT 429-1-1	24.75(1)	20.50(1)	29.00(1)	30.00(1)	5.94(1)	5.0
V32	BT 429-2-2	28.50(1)	17.50(1)	34.25(1)	30.00(1)	11.55(2)	6.0
V33	BT 433-2-1	28.25(1)	34.00(2)	41.75(2)	69.50(2)	5.74(1)	8.0
V34	BT 433-2-3	21.00(1)	19.00(1)	61.00(2)	34.50(1)	5.19(1)	6.0
V35	BT 433-1-2	20.50(0)	23.00(1)	36.75(1)	30.00(1)	4.09(0)	3.0
V36	BT 507-2-2	17.50(0)	27.50(2)	24.00(1)	22.50(0)	6.55(2)	5.0
V37	BT 508-1-1	21.00(1)	24.50(1)	33.25(1)	35.00(1)	3.65(0)	4.0
V38	BT 19-1-1-1	18.50(0)	14.00(0)	32.50(1)	22.50(0)	3.87(0)	1.0
V39	BT 215-3-3-1	31.25(2)	14.50(0)	16.50(0)	20.00(0)	7.07(2)	4.0
V40	BT305-2-4-2	23.25(1)	33.00(2)	39.50 (2)	40.00(2)	5.25(1)	8.0
V41	IIVR Selection2	30.50(2)	26.50(2)	28.75 (1)	30.00(1)	4.91(1)	7.0
V42	11/TOBW-3	31.50(2)	21.00(1)	39.25(2)	32.50(1)	5.79(1)	7.0
V43	BT 17-2-5X1	17.75(0)	18.00(1)	25.75(0)	28.50(1)	2.96(0)	2.0
V44	BT 413-1-2X1	35.50(2)	16.00(0)	28.25 (1)	25.00(1)	8.21(2)	6.0
Grand mean		25.60	21.00	32.73	31.13	5.57	4.77
CD (0.05)		13.31	11.16	18.36	17.56	2.59	
CV		25.84	26.43	27.88	28.05	23.13	
SE(m)±		4.68	3.92	6.45	6.17	0.91	

*0, 1 & 2 are the coded value representing low, medium and high value for each root trait

Statistical analysis

Root performance of each genotype in field condition was expressed in terms of root performance index (RPI) following the methodology of (7). The mean field data collected for each root trait were subjected to analysis of variance (ANOVA) using procedures of SAS version 9.3.

Analysis of variance for pot culture was done with the Windostat software, version 9.3 for 13 root traits. D² analysis was done using Windostat software, version 9.3. Principal component analysis (PCA) was performed using XLSTAT software.

Results and Discussion

Data concerning the root traits of forty-four tomato genotypes, derived from preliminary field assessments, were outlined in Table 1. The analysis of variance revealed significant variations in different root traits (Table 1). Primary root length ranged from 16.15 to 40.70 cm, with an average of 25.60 cm. The number of secondary roots varied from 8.50 to 44.00. The lowest and highest fresh root weights were observed in BT 2 (11.75 g) and BT 12-2 (64.00 g), respectively. BT 433-2-1 recorded the maximum root volume of 69.50 cc, while the minimum was noted in BT 2 (12.50 cc). Dry root weight ranged from 3.28 g (BT 2) to 11.55 g (BT 429-2-2). The root performance index (RPI) of the genotypes varied from 0.0 to 10.00. Based on RPI value, it was observed that 14 genotypes had low root performance (RPI value between 0.00 to 3.33), 19 genotypes had moderate root performance (RPI value between 3.34 to 6.67) and 11 had high root performance (RPI value > 6.67). These results prompted a comprehensive investigation into the root system architecture of tomato genotypes.

Pot culture performance

At different growth stages the genotypes in pot culture showed significant variations for different RSA traits. The pot culture performance of tomato genotypes showing variation in respect of some RSA traits was compared at vegetative, flowering and harvesting stages (Fig. 1 to Fig. 7). Total root length (TRL) was found to increase gradually from vegetative to harvesting stage (Fig.1). The genotypic mean value for TRL was 139.51, 1200.91 and 2796.79 cm at vegetative, flowering and harvesting stages,

respectively. The range in TRL was the highest at harvesting stage (2310.21 cm) and the lowest at vegetative stage (235.10 cm). This result indicates a wide spectrum of variation in TRL at harvesting stage. The range in TRL of fine roots (root diameter < 0.5 mm) was found to be the highest also at harvesting stage (2270.78 cm) followed by flowering (673.38 cm) and vegetative stage (147.53 cm; Fig.2). For other RSA traits (surface area of roots, root volume, volume of fine roots and root diameter) the maximum variation was observed at harvesting stage compared to flowering and vegetative stages. Range as a simple measure of variability indicates that the maximum variation in RSA traits occurred at harvesting stage. Significant differences in a large number of root traits of two related tomato species were observed during early growth (8). Extensive variation in the root system architecture related traits of nine wild species of tomato and four cultivated tomato varieties during early growth in a controlled environment was reported (9). Variation in total root length, average root diameter, total root surface area and total root volume of 335 watermelon genotypes at four days old seedlings was revealed by scientist (10).

Genotypic coefficient of variation (GCV), broad sense of heritability (%) and genetic advance as percent of mean of different RSA traits at different growth stages are presented in Table 2. The mean GCV value was the highest (46.44) at vegetative stage followed by harvesting stage (34.76) and it was the lowest at flowering stage (25.72). Volume of fine roots recorded the highest heritability of 97.56 % at the vegetative stage. At flowering stage, TRL (93.57 %), root volume (94.31 %), root forks (87.17 %) and root crossings (92.34 %) showed higher heritability and rest RSA traits had the high heritability at the harvesting stage. High heritability for RSA traits like total root length, root diameter and root length density were reported in wheat (11). The mean heritability of RSA traits was the minimum at vegetative stage (83.34 %) and the maximum (89.92 %) at harvesting stage (Table 2). The mean GA (as percent of mean) was the highest (89.55) at vegetative stage followed by harvesting stage (67.89) and it was the lowest at flowering stage (51.06). Selection of RSA traits having high heritability along with high GA as percent of mean would be rewarding as they have additive gene action. Root images were depicted in plate 1 to 3.

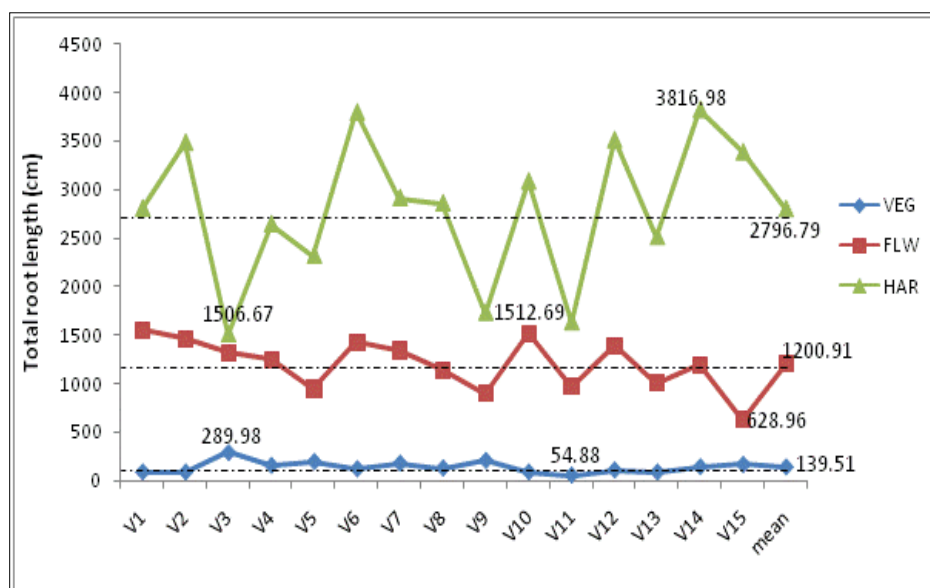


Fig. 1. Total root length.

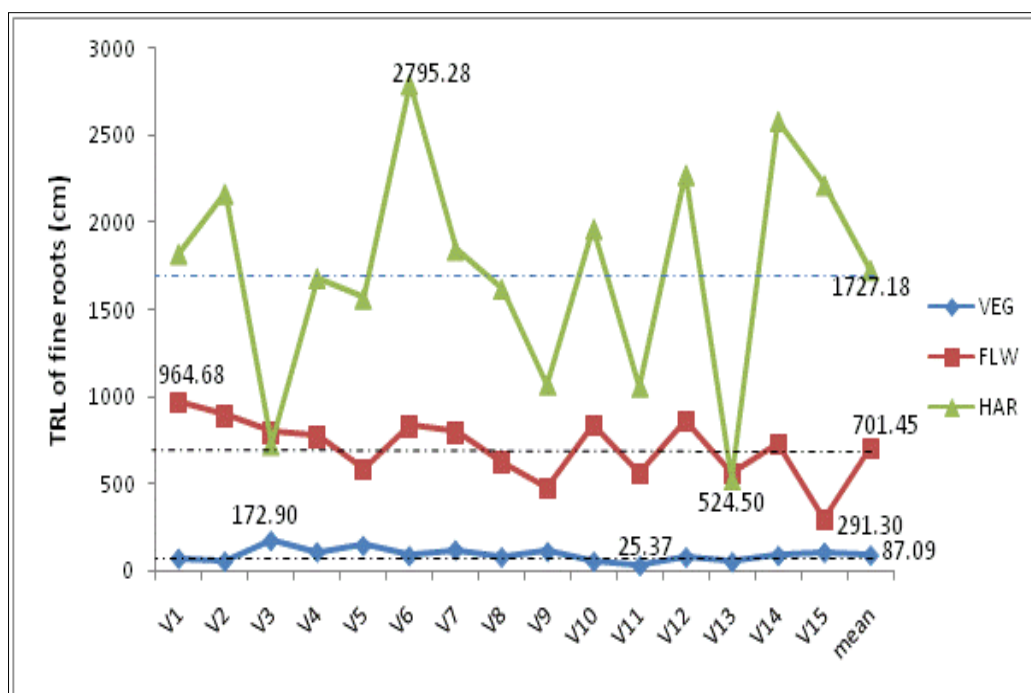


Fig. 2. Total root length of fine roots.

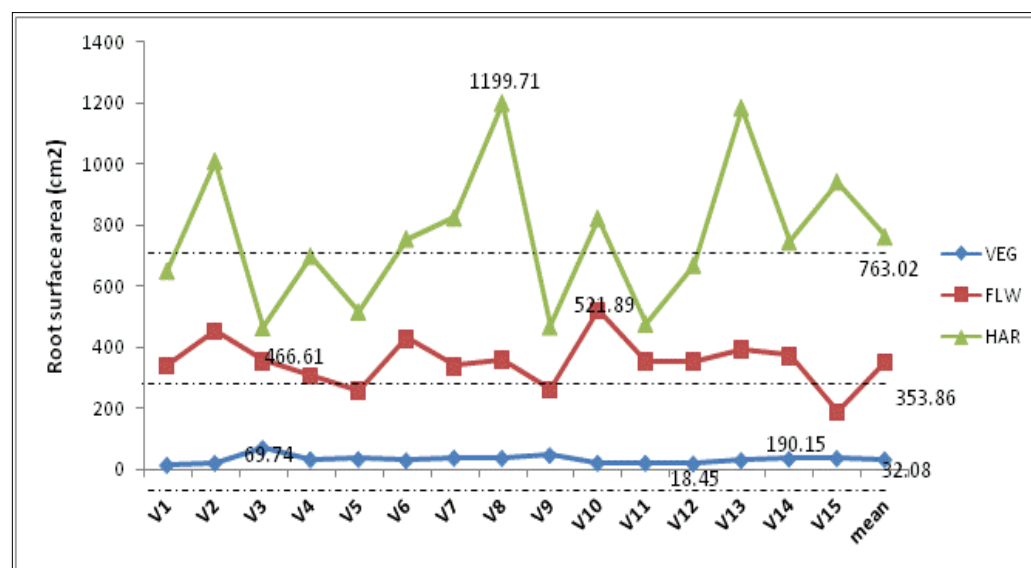


Fig. 3. Surface area of roots.

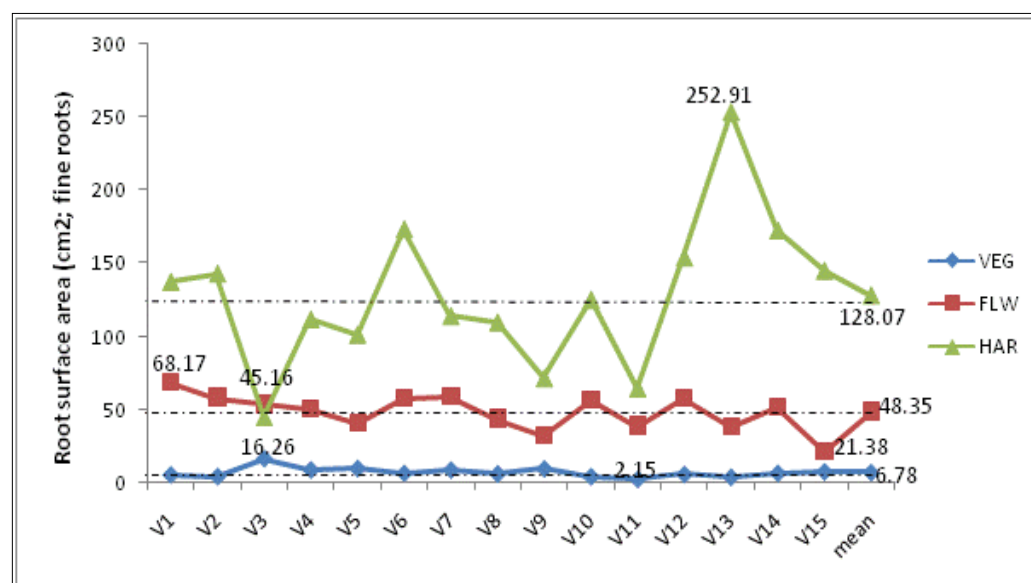


Fig. 4. Surface area of fine roots.

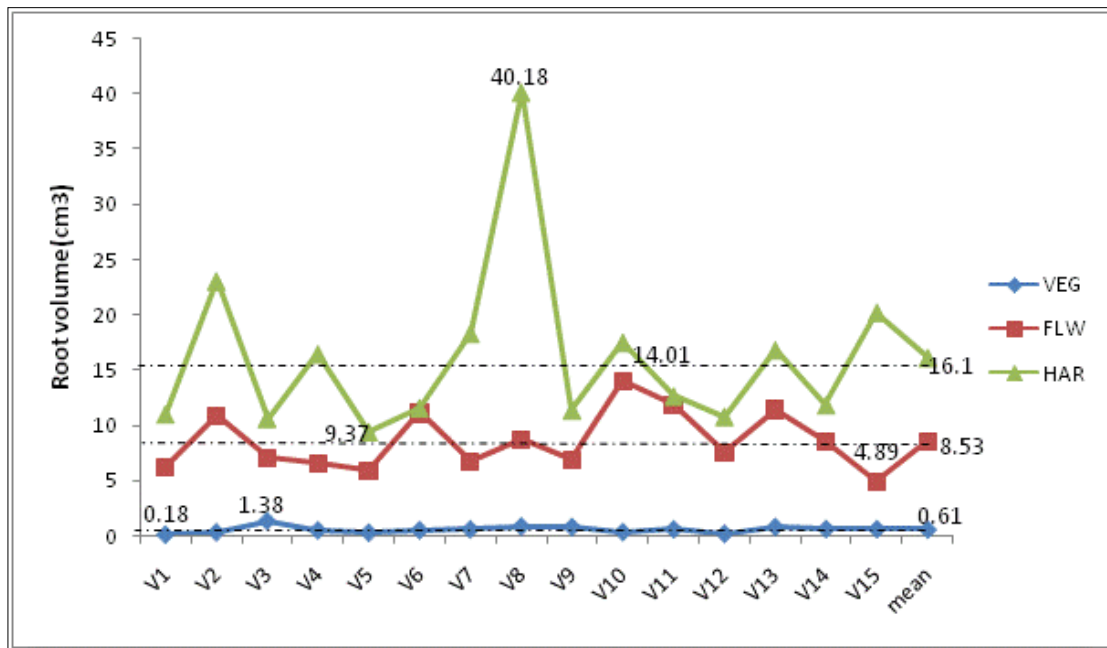


Fig. 5. Root volume.

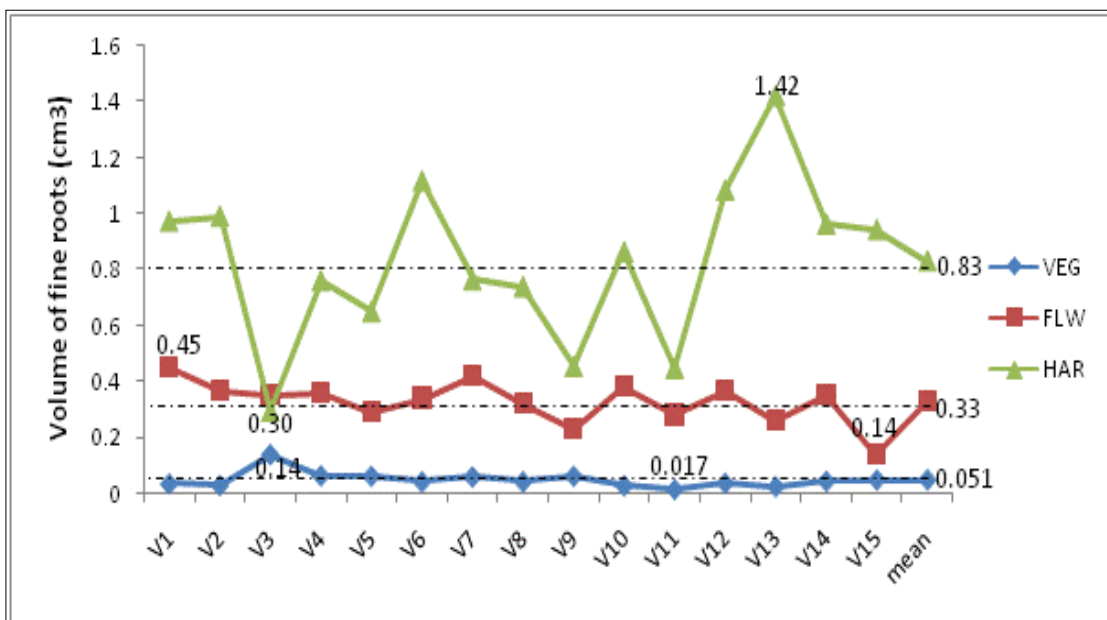


Fig. 6. Root volume of fine roots.

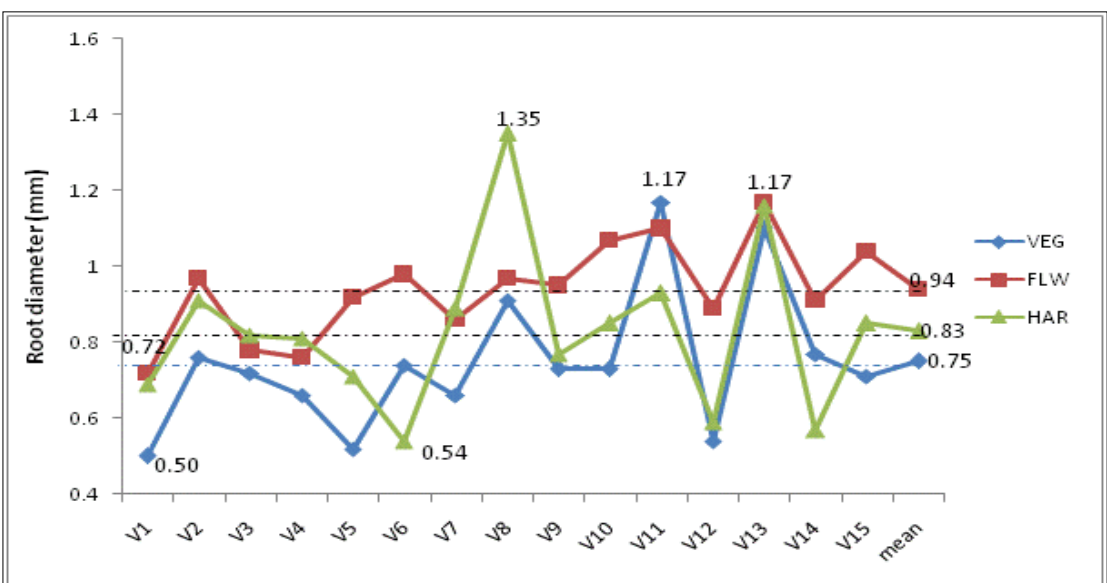


Fig. 7. Root diameter.

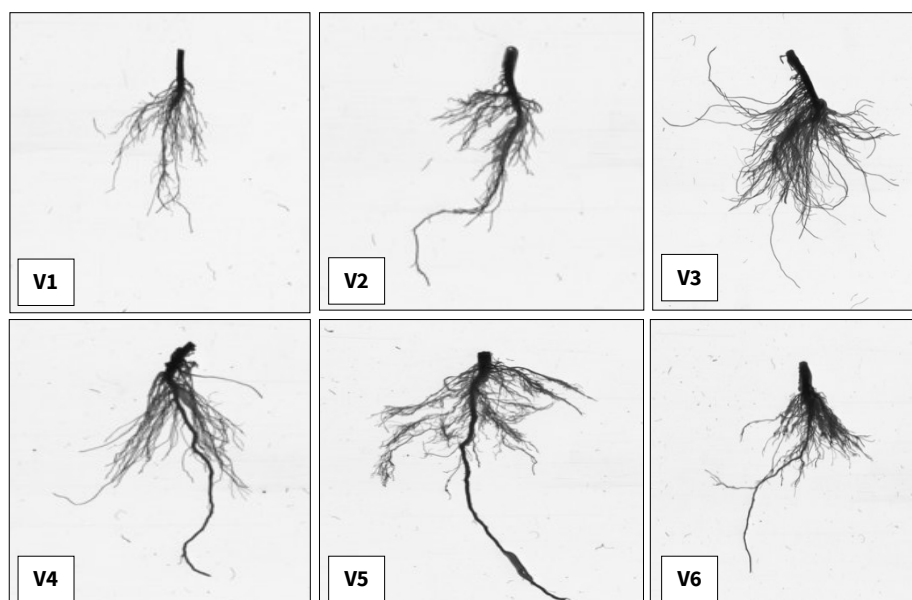


Plate 1. Scanned root images of some tomato genotypes grown in pot culture at vegetative stage

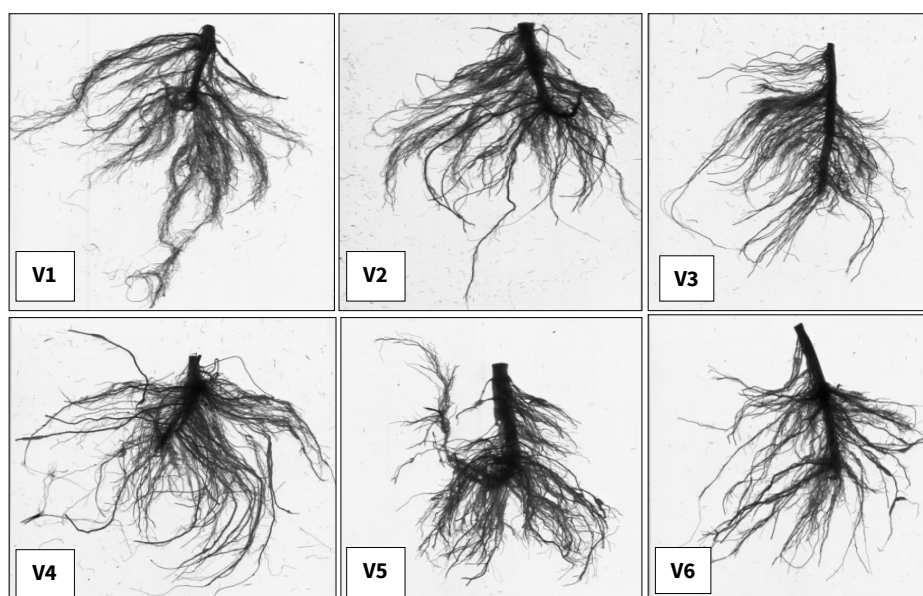


Plate 2. Scanned root images of some tomato genotypes grown in pot culture at flowering stage

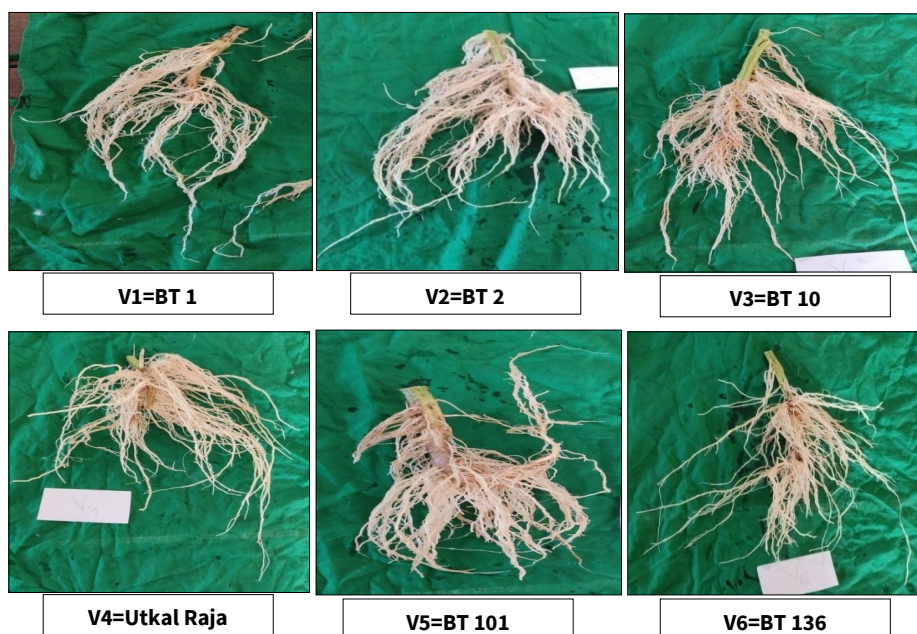


Plate 3. Root images of tomato genotypes grown in pot culture at harvesting stage

Table 2. Estimates of genetic parameters of RSA traits at vegetative, flowering and harvesting stages

RSA trait	GCV			Heritability (%)			GA as % of mean		
	Veg.	Flw.	Har.	Veg.	Flw.	Har.	Veg.	Flw.	Har.
Total root length	43.87	21.88	26.74	82.99	93.57	91.58	82.34	43.79	52.96
TRL of fine roots	44.50	25.84	37.99	74.19	81.21	88.41	78.76	47.84	73.25
Surface area of roots	42.72	22.27	31.39	93.88	91.56	94.50	85.37	45.09	63.20
SA of fine roots	50.18	25.17	39.68	78.59	79.37	82.34	92.08	45.98	73.86
Root volume	48.99	31.30	48.64	81.82	94.31	92.78	91.84	62.42	96.74
RV of fine roots	56.74	23.88	34.96	97.56	91.85	95.51	141.6	66.73	71.08
Root diameter	25.14	13.05	25.59	80.00	83.33	95.74	49.13	24.40	51.65
Root length density	52.12	29.95	44.32	75.49	78.67	93.08	92.68	54.95	88.01
Root tips	45.80	26.61	22.47	94.17	89.27	96.91	91.40	51.63	45.60
Root forks	42.71	27.63	26.25	76.30	87.17	80.00	76.55	53.03	48.37
Root crossings	59.35	32.75	34.45	82.95	92.34	77.81	111.4	64.58	62.75
RGRV	48.99	31.30	48.64	81.05	86.04	90.00	90.80	59.77	95.06
Projected root area	42.55	22.22	30.80	84.37	89.52	90.38	80.20	43.54	60.06
Genotypic Mean	46.44	25.72	34.76	83.34	87.55	89.92	89.55	51.06	67.89

Divergence analysis

Divergence among tomato genotypes for RSA traits was evaluated following D^2 statistics and principal component analysis. Based on D^2 analysis the 15 genotypes were grouped into four clusters both at vegetative and flowering stages but five clusters at harvesting stage (Table 3). Cluster I contained 12 numbers of genotypes both at vegetative and flowering stages and 9 genotypes at harvesting stage. The genotypes included in cluster I of vegetative and flowering stages were not exactly the same. Only four genotypes like V2 (Utkal Deepti), V4 (Utkal Raja), V7 (BT-317) and V10 (BT 442-2) were commonly included in cluster 1 of vegetative, flowering and harvesting stages. Therefore, these four genotypes were supposed to be closely related in their root system architecture. Cluster II was found to contain only one genotype at vegetative and flowering stages and three genotypes at harvesting stage. Rest of the clusters formed at vegetative, flowering and harvesting stages were monotypic. TRL had maximum contribution towards divergence at vegetative (18.0%), flowering (19.0 %) and harvesting stage (23.00 %). The maximum inter cluster distance at vegetative, flowering and harvesting stage was 2040.47, 1637.91 and 2456.28. Genetic diversity in root traits of 111 *Lupinus angustifolius* accession was reported (12).

Principal component analysis

Results of principal component analysis was explained through PC1 & PC2 bi-plot graph and depicted in Fig. 8-10 for vegetative, flowering and harvesting stages respectively. Contribution of PC1 towards the total variation at vegetative, flowering and harvesting stage was 65.32 %, 62.38 % & 45.15 % whereas PC2

at vegetative, flowering and harvesting stages accounted for 26.33 %, 32.55 % & 34.42 % of the total variation. These results reveal that the contribution of PC1 towards total variation gradually decreases with the increase in age and contribution of PC2 gradually increases from vegetative to harvesting stage. At vegetative and flowering stage all the RSA traits had positive influence on PC1 except root diameter (Fig. 8, 9) whereas at harvesting stage all the RSA traits had positive influence on PC1 (Fig.10). At vegetative and flowering stage the RSA traits like root diameter, projected root area, surface area of roots, root volume, root growth rate in volume (RGRV) had negative effect on PC2 but at harvesting stage these five traits had positive influence on PC2. Amongst the RSA traits, TRL had the maximum positive influence (11.56 %) on PC1 followed by surface area of fine roots (10.95 %) at vegetative stage; TRL had the maximum positive influence (11.98 %) on PC1 followed by root forks (11.63 %) at flowering stage; and TRL had the maximum positive influence (12.69 %) on PC1 followed by root surface area (12.11 %) at harvesting stage (Table 4). At all the three stages TRL had maximum contribution towards divergence (Table 4). The scattered distribution of tomato genotypes in four quadrants of the PC1 and PC2 bi-plot at vegetative (Fig. 8), flowering (Fig. 9) and harvesting stages (Fig. 10) indicated a high level of genotypic variation at all the growth stages. The genotypes present in Quadrant-I (QI) were contributed towards the divergence of RSA traits present in Q-I at different growth stages. The genotypes present in Q-II of vegetative, flowering and harvesting stages were not closely related to any RSA traits.

Table 3. Clusters formed by Tocher's method at different growth stages

Cluster	Vegetative stage	Flowering stage	Harvesting stage
Cluster 1	V1, V2, V4, V6, V7, V8, V10, V11, V12, V13, V14, V15	V2, V3, V4, V5, V6, V7, V8, V9, V10, V12, V13, V14	V1, V2, V4, V5, V7, V9, V10, V11, V15
Cluster 2	V9	V11	V6, V12, V14
Cluster 3	V5	V15	V3
Cluster 4	V3	V1	V8
Cluster 5	-	-	V13
Trait with maximum contribution to divergence	TRL (18.0 %)	TRL (19.0 %)	TRL (23.0 %)
Maximum Inter cluster distance	2040.47(Cl 3 & 4)	1637.91(Cl 3 & 4)	2456.28 (Cl 4 & 5)

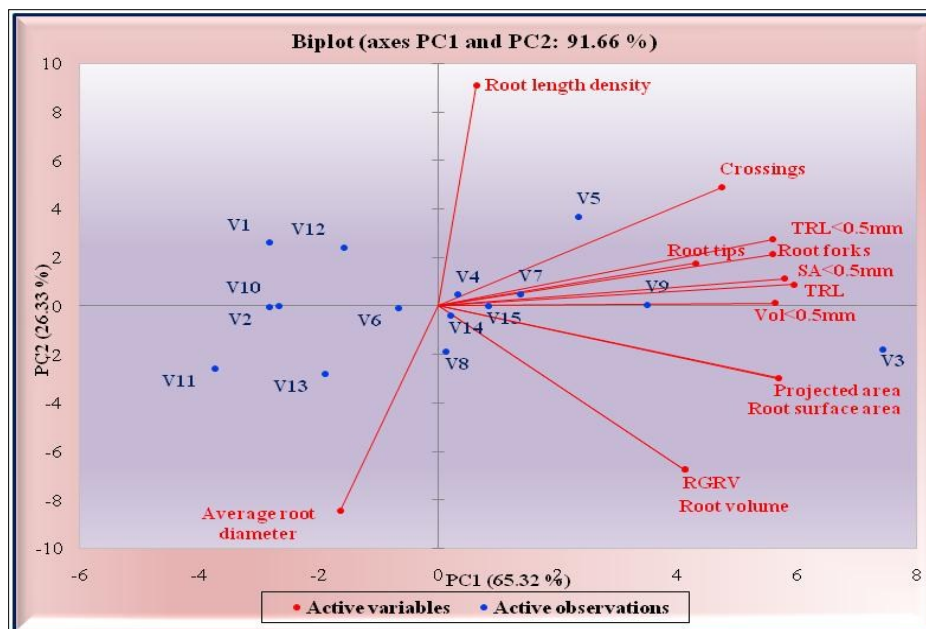


Fig. 8. PCA bi-plot for root traits at vegetative stage.

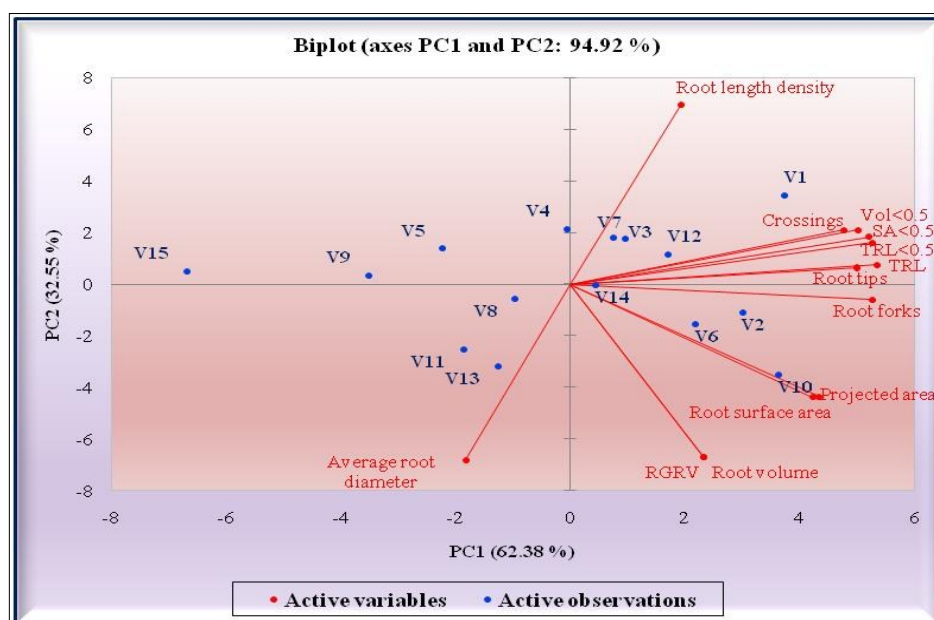


Fig. 9. PCA bi-plot for root traits at flowering stage.

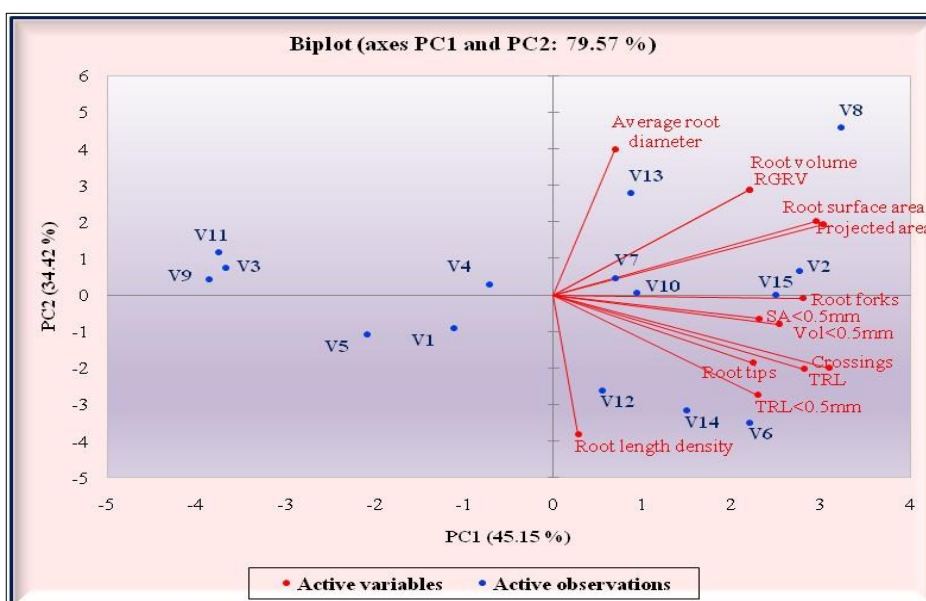


Fig. 10. PCA bi-plot for root traits at harvesting stage.

Table 4. Principal component analysis showing relative contribution of characters towards divergence at different stages

RSA trait	Contribution of the variables (%)					
	Vegetative		Flowering		Harvesting	
	PC1	PC2	PC1	PC2	PC1	PC2
Total root length (cm)	11.56	0.25	11.98	0.24	12.69	5.29
TRL of fine roots (cm; diameter<0.5 mm)	10.24	2.48	11.65	1.07	6.97	10.05
Surface Area of roots (cm ²)	10.57	2.93	7.89	7.97	12.11	5.01
SA of fine roots (cm ² ; diameter<0.5 mm)	10.95	0.41	11.38	1.43	7.07	0.56
Root volume (cm ³)	5.56	15.08	2.26	18.79	6.44	11.06
RV of fine roots (cm ³ ; diameter<0.5 mm)	10.34	0.004	10.53	1.86	8.51	0.88
Root diameter (mm)	0.89	23.51	1.40	19.43	0.63	21.11
Root length density (cm/cm ³)	0.13	27.09	1.54	20.22	1.10	19.51
Root tips	6.03	1.00	10.45	0.17	6.65	4.58
Root forks	10.25	1.46	11.63	0.15	10.39	0.01
Root crossings	7.35	7.81	9.55	1.85	10.47	5.51
Root growth rate in volume (mm ³ /day)	5.56	15.08	2.26	18.78	6.44	11.06
Projected root area (cm ²)	10.56	2.91	7.49	8.03	11.52	5.38
Eigen value	8.49	3.42	8.11	4.23	5.870	4.475
Variability (%)	65.32	26.33	62.38	32.54	45.15	34.42
Cumulative %	65.32	91.65	62.38	94.92	45.15	79.57

Conclusion

This investigation reveals existence of considerable genetic variability among tomato genotypes at vegetative, flowering and harvesting stages for various RSA related traits. GCV was the maximum at vegetative stage followed by harvesting stage for different RSA traits. Among the different root traits total root length had maximum contribution towards divergence. Research on RSA traits would offer valuable insights for future root trait breeding, particularly in the context of changing climate scenarios, by delving into the genetic diversity.

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Authors' contributions

SD carried out manuscript drafting, experimental design formulation. BP carried out the experimental work, data recording, PS provided the facility of using root win-rhizo scanner. MB¹ assisted in taking root data from the software and MB² helped in field work. SS helped in checking of the manuscript. JSS helped in data analysis [MB¹-Madhusmita Barik; MB²-Mamata Behera].

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

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During the preparation of this work the author(s) used Chatgpt-4 to improve language. After using this tool, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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