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Genetic variability for root and shoot traits in germplasm accessions of tomato

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Abstract

Solanaceous family are the most cultivated plants across the world, where tomato is one of amenable Solanaceous model crop plant extensively grown because of its nutritive value. The plants are sessile in nature therefore they experiences many adverse environmental conditions. Due to some erratic environmental conditions, situation become harsh and leads to abiotic and biotic stresses. To overcome this undesired condition, plants have adapted several mechanisms at cellular level, molecular and physiological and also at whole plant level. Amongst them, root characters play a major role during stress condition. Under these premises, 100 tomato accessions were evaluated using augmented block design in root structure under normal conditions to study genetic variability for root characters such as root length (cm), root volume (cm³) and root dry weight (g). There is a large variation among the accessions for all the root traits and also for ancillary traits like shoots length, SPAD, specific leaf area (cm²), leaf dry weight (g), stem dry weight (g), shoot dry weight (g). Based on root dry weight contrast lines were identified and can be used for crop improvement approach.

Keywords: Root dry weight, SPAD, specific leaf area, trait-specific accessions, 2, 3, 5 - Tri phenyl tetrazolium chloride

Introduction

The Solanaceous family is one of the biggest families which include potato, tobacco, tomato and pepper. Tomato is one of the largely cultivable popularly grown vegetables in the world after potato. In terms of human health, tomato fruit provide significant quantities of beta carotene, a pro vitamin-A carotenoid and ascorbic acid. Lycopene is the major carotenoid in tomato fruit, act as a powerful anti-oxidant and is associated with reduced risk of certain cancers, heart diseases and age-related diseases (Heber and Lu, 2002). Fresh fruits of tomato are in great demand round the year and throughout the country. Presently the average production, area and productivity of tomato in the India are 19697 million tonnes, 809 hectare and 24.34 million tons per hectare, respectively (Horticulture statistics at glance, 2017). From these data we can conclude that tomato production is an important aspect of the agricultural economy in the India and other nations.

Although these species have adequate adaptation plasticity, only some cultivars within each species display moderate tolerance to abiotic stress such as salinity, flooding, heat and water stress. But, often exposure to different abiotic stresses leads to reduction of production and thus cause severe constrains to growth. Being a sessile organism, plants have adapted several specific mechanisms that allow them to detect precise environmental changes and respond to complex stress conditions, minimizing damage while conserving valuable resources for growth and reproduction at the transcriptome, cellular, and physiological levels. In this mechanism roots are one of the most important adaptations of land plants, because they provide anchorage, facilitate absorption of water and minerals, and also aid in speciality functions, such as storage of food, water and vegetative reproduction in some plant species.

Environmental stresses represent the most limiting conditions for horticultural productivity and plant exploitation worldwide. One direction out of these problems is to develop crops that are more tolerant to such stresses. This is carried out with tremendous efforts particularly at breeding companies; however, due to a lack of practical selection tools like genetic markers, it is a slow and inefficient process so far. A special method of adapting plants to counteract environmental stresses is by grafting elite, commercial cultivars onto selected vigorous rootstocks (Lee and Oda, 2003) ^[11]. Grafting is now a day's regarded as a rapid alternative tool to the relatively slow breeding methodology aimed at increasing environmental-stress tolerance of fruit vegetables (Flores *et al.*, 2010) ^[12].

Fruit yield of tomato depends upon the extent and nature of genetic variability present in the population. Genetic variability is the material from which superior genotypes can be evolved after selection. Higher the amount of variability in the population, greater is the scope for its improvement by selection. Knowledge of association of fruit yield with its contributing traits helps in breeding and other programmes. Wide range of variability provides better scope of selecting desirable genotypes. Effective selection depends upon existence of genetic variability in the population. Therefore, the present experiment was conducted to determine genetic variability under normal condition and selection of contrast lines for root trait.

Materials and methods

The material for the study comprises of 100 germplasm accessions of tomato and five check entries (ArkaVikas, Arka Alok, Arka abha, Arka Ahuthi and Arka Meghali) procured from Indian Institute of Horticultural Research (IIHR), Bengaluru. Most of the accessions are of originated mainly from Taiwan, IIHR and USA. The germplasm accessions along with five check entries were sown in Augmented design (Federer, 1956) [2] in four compact blocks, which consists 25 germplasm accessions and five checks, during 2014 Rabi seasons at the experimental plots of Dept. of Crop Physiology, UAS, Bengaluru. The seeds of 100 tomato accessions were sown in raised bed nursery, after 25 days from sowing seedlings were transplanted to root structure by maintaining a spacing of 45 cm between plants and 60cm between the rows. A basal dose of 200:100:100Kg NPK per ha was applied to the plants by following package of practice.

Morphological and physiological parameters

Data were recorded from the three randomly tagged plants. Root, shoot and its ancillary traits such as root length (cm), shoot length (cm), root volume (cm³), number of branches, SPAD, specific leaf area (cm²/g), root dry weight (g), leaf dry weight (g), stem dry weight (g) and shoot dry weight (g).

Specific Leaf area

A sample of 5 representative leaves from all canopy positions was harvested and the leaf area was determined by measuring the area by using leaf area meter WINdias5.2. The same leaves were oven dried and their dry weight was determined. The remaining leaves of the plant were harvested separately at the time of final harvest and their oven dry weight was recorded. Finally the total plant leaf area was estimated using following the formula:

$$SLA = \text{Area of 5 leaves (cm}^2\text{)} / \text{Weight of 5 leaves (g)}$$

$$\text{Total leaf area (cm}^2\text{/pl)} = \text{total leaf dry weight} \times \text{SLA (cm}^2\text{/g)}$$

SPAD chlorophyll meter reading (SCMR)

Leaf nitrogen status is normally manifested with the leaf chlorophyll content. The unit less value measured by the chlorophyll meter (SPAD-502) is termed as SCMR (SPAD Chlorophyll Meter Reading) and is a good estimate of chlorophyll content and hence N content. SCMR values were recorded in the 100 contrasting germplasm accessions grown in root structure at 45 DAS. Several measurements were recorded from different canopy leaves and averaged to make an approximate estimate of the whole plant N status.

After 50 days from transplanting plants roots were excavated by dismantling the brick walls and soil was washed off carefully using a jet of water. The entire root was then carefully removed and root length, root volume and shoot

length was determined and each samples were collected separately and oven dried at 80°C for one week. Finally weights of oven dried samples were recorded.

Statistical analysis

Traits mean values were used for estimating descriptive statistics such as trait mean, range, variance, skewness, kurtosis, heritability in broad sense, phenotypic (PCV) and genotypic co-efficient of variation (GCV) and genetic advance as percent of mean (GAM). The skewness and kurtosis were estimated using 'SPSS' software program to understand the nature of genetic control of target traits. The 100 accessions were classified into four clusters following model-based 'K means' clustering (Mac Queen, 1967) approach to unravel organization of variability. To examine this, the significance of difference in traits means and variances of the accessions distributed among the clusters was examined using 'one-way ANOVA' and Levene's (Levene, 1960) [4] tests, respectively.

Identification of trait-specific accessions

Based on root dry weight (Between 8.33-1.23g) and TDM the accessions promising for each of the trait were identified.

Results and discussion

Variability in population

Analysis of variance revealed highly significant mean squares attributable to 'germplasm accessions' and 'check varieties' for all traits. Mean squares due to 'accessions vs check varieties' were significant for all traits except for SPAD. These results suggested significant differences among the accessions and they differed from the checks.

Detection of genetic variability is a prerequisite for quantifying variability and assessing relative contribution of genetic and non-genetic sources on the traits variability which is in turn useful in formulating appropriate selection strategies for improvement of tomato cultivars for root adaptive traits. The estimates of standardized range provide clues about the occurrence of accessions with extreme expression which varied with the trait. However, standardized range per se does not reflect variability in the expression of all the accessions. The estimates of GCV and PCV which reflect average inter-accession differences are more useful statistics to understand variability among the germplasm accessions. The traits standardized range of the accessions were relatively higher, amply reflected by the estimates of PCV and GCV for most of the traits (Table 4). The accessions were highly variable for root length, Root volume, Number of branches, Specific leaf area, Root dry weight, leaf dry weight, stem dry weight and shoot dry weight as indicated by the estimates of PCV (>20%). The accessions were moderately variable (10.1% < PCV < 19.9%) for shoot length and SPAD. Relatively narrow difference between PCV and GCV estimates for these traits has amply reflected in higher broad-sense heritability estimates. Broad-sense heritability was higher (>60%) for all the traits (Table 2). Since, the germplasm accessions used in the study are a mixture of pure lines whose expression predominantly determined by additive genetic effects and additive x additive type of epistasis. Thus selection of desired accessions for any of the traits considered in the present investigation would be effective as all the traits. It is proposed that an interdependent relationship exists between root and shoot: i.e., active shoots that ensure a sufficient supply of carbohydrates to roots can develop and maintain active root functions; the activation of root functions can, in turn,

improve shoot characteristics by supplying a sufficient amount of nutrients, water, and phytohormones to shoots, thus ensures increases biomass productivity.

Variability in the performance of different tomato genotypes was also reported by Saikia *et al.*, (1990) [13]. Selection of the genotypes based on the variability parameters of the traits is more relevant and effective. The traits with high values of variance, coefficients of variation, heritability and genetic advance could be utilized in indirect means of selection.

The major application of heritability and the other genetic parameters that compose the heritability estimate is to compare the expected genetic gains from selection based on alternative selection strategies. The information elicited from such comparisons could be used to design optimal breeding strategies. The estimates of GAM were higher for all the traits studied (Table 1). Narrow difference between PCV and GCV estimates also suggested stable expression of accessions for these traits and their variability could be attributed largely due to genetic causes, a pre-requisite for effectiveness of selection for these traits.

The distribution of germplasm accessions for all traits was near normal (Fig.1). Positively skewed distribution of accessions suggested the prevalence of large number of accessions with high trait means. The platy-kurtic distribution of accessions indicated the involvement of large number of genes in trait expression.

Organization of variability

The efficiency and pace of tomato improvement programmes hinges on the precise information on the relative magnitudes of fixable (additive and additive based epistasis) and non-fixable components of genetic variability, genotype (g) × environment (e) (both spatial and temporal) interaction, and DNA marker-assisted chromosomal localization and unravelling mode of action of genes controlling traits of economic importance. The identification of accessions contrasting for traits of economic importance is a prelude to eliciting such information. Cluster analysis helps in grouping of and to identify genetically diverse and desirable genotypes for use in generating variability to identify pure-lines with desirable combination of traits. The traits mean differences among the clusters were significant for all the traits except number of branches and stem dry weight (Table 2). The trait variances among the four clusters were significant for all the traits (Table 3) except shoot length, number of branches.

These results suggested effectiveness of K-means clustering approach to minimise 'within-cluster' variance and maximise 'between-cluster' variance as a result of inclusion of diverse accessions into different clusters. The estimates of the means of the traits such as shoot length, root length, number of branches, SPAD, specific leaf area, root dry weight, leaf dry weight, stem dry weight and shoot dry weight were highest among the accessions included in clusters I and II and were least among the accessions included in cluster IV. It is desirable to choose germplasm accessions from among those included in cluster I and cluster II for various applications in tomato breeding research.

Significant variability among the accessions for shoot and root traits was expected as they are wild accessions which have evolved over millennia through a combination of natural and human selection on the variation originated by mutations and distributed and reshuffled by recombination (Allard, 1999) [5]. These wild accessions possess combination of traits that enable them better adapted to different production environments and/or a combination of production environments (Haussmann *et al.* 2004) [6].

Traits-specific accessions

Progress in crop genetic improvement depends on identification of new sources of genetic variation for economically important traits. Germplasm accessions with drought-avoidance root traits have been identified from germplasm collections in chickpea (Kashiwagi *et al.* 2005) [7], with traits related to drought resistance in pigeonpea (Upadhyaya *et al.* 2007) [8], with resistance to multiple disease in pigeonpea (Sharma *et al.* 2012) [9] and with traits related to drought adaptation in faba bean (Khazaei *et al.* 2013) [10] were reported but not in case of tomato.

In the present study, some of the germplasm accessions are comparable to or superior for root dry weight and TDM are mentioned in Table 4. The accessions such as IIHR2343, IIHR 2359, IIHR2360, IIHR237, IIHR 2380, IIHR2397, IIHR 2621, IIHR 2624, IIHR2745, IIHR 2613, IIHR 2615, IIHR 2617, Arka alok and Arka ashish were promising for multiple traits. These genotypes are suggested for preferential use in multiple crossing programmes to generate variability for root traits. To enhance the pace of tomato grafting, these genotypes may be recombined with elite advanced high yielding cultivar and used as drought donor lines/as rootstock for economically important traits.

Table 1: Descriptive statistics for root, shoot and its ancillary traits in tomato germplasm accessions

Traits	Mean ± SE	Range		Standardized range	Coefficient of variability		Broad sense h ²	GAM (%)
		Minimum	Maximum		PCV (%)	GCV (%)		
Shoot length (cm)	70.87 ± 1.33	17.00	123.33	1.50	18.55	18.30	0.97	37.19
Root length (cm)	56.57 ± 1.68	8.67	184.00	3.10	28.61	27.66	0.94	55.11
Root volume (cm ³)	28.07 ± 1.35	3.33	83.33	2.85	45.78	44.08	0.93	87.41
Number of branches	5 ± 0.13	1.00	10.00	1.80	30.02	25.37	0.71	44.16
SPAD (nmol/cm ²)	58.09 ± 1.03	24.30	87.10	1.08	17.51	15.91	0.83	29.76
Specific leaf area (cm ² /g)	231.5 ± 6.47	83.70	485.20	1.73	27.86	26.50	0.90	51.90
Root dry weight (g)	3.91 ± 0.14	1.23	9.03	1.99	36.75	34.99	0.91	68.61
Leaf dry weight (g)	39.29 ± 1.22	13.33	85.00	1.82	30.52	28.76	0.89	55.83
Stem dry weight (g)	27.29 ± 1.00	11.67	70.00	2.14	35.77	34.40	0.92	68.15
Shoot dry weight (g)	123.03 ± 1.98	31.67	155.00	1.00	29.53	28.52	0.93	56.76

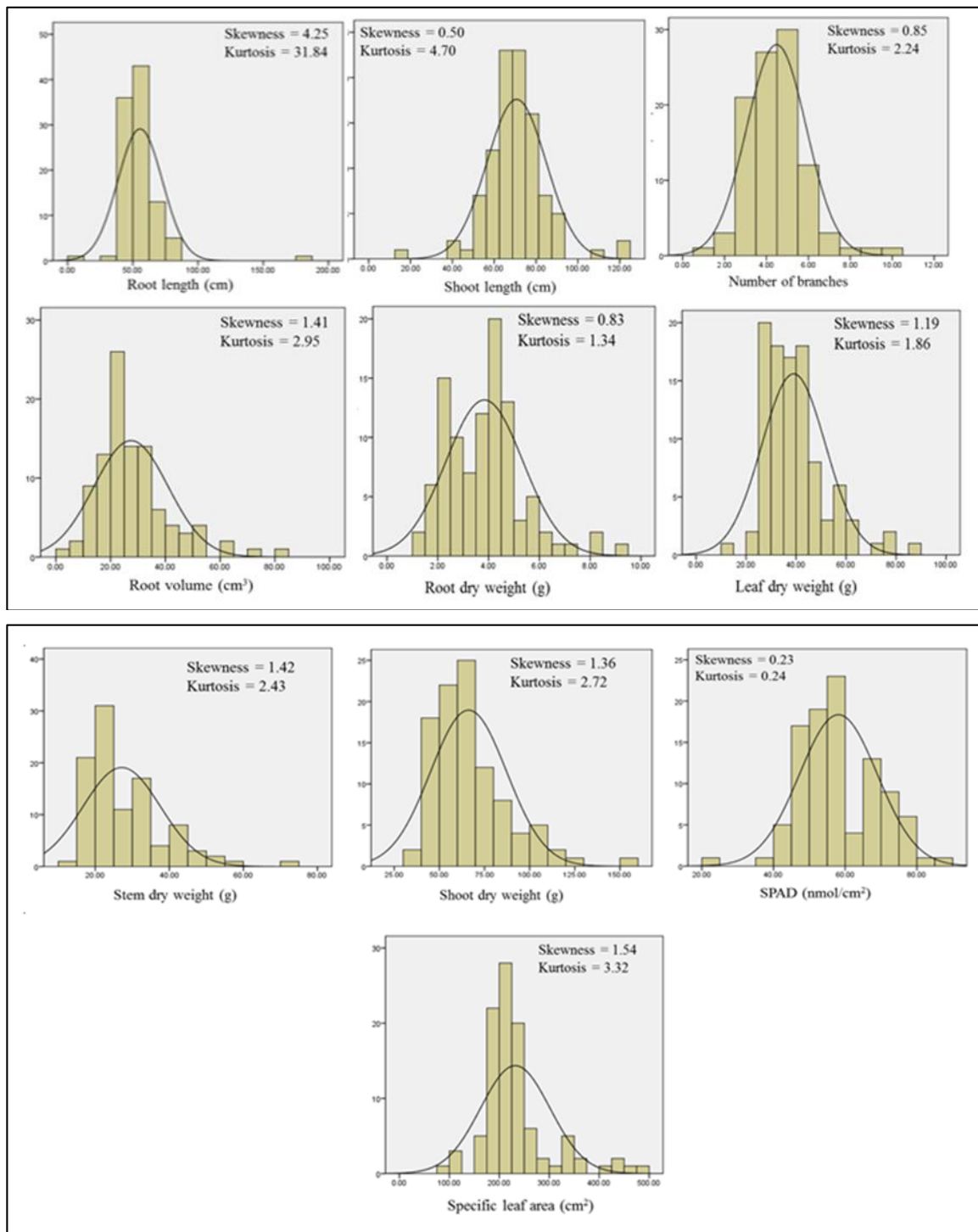


Fig 1: Frequency distribution of tomato germplasm accessions for root, shoot and its ancillary traits

Table 2: Estimates of traits means of tomato germplasm accessions belonging to different clusters (C_i)

Sl. No	Traits	Means of clusters				'F' Statistic	Probability
		C ₁	C ₂	C ₃	C ₄		
	Size of the cluster →	12	13	43	32		
1	Shoot length (cm)	77.16	85.09	69.52	63.98	9.91	<.0001
2	Root length (cm)	73.09	51.54	51.81	55.70	5.87	0.001
3	Root volume (cm ³)	40.56	22.56	27.66	24.27	5.59	0.0014
4	Number of branches	4.64	5.18	4.13	4.45	2.01	0.1179
5	SPAD (nmol/cm ²)	5.10	4.49	3.38	3.71	5.64	0.0013
6	Specific leaf area (cm ² /g)	58.44	46.85	35.27	33.66	23.46	<.0001
7	Root dry weight (g)	38.50	39.54	24.11	21.92	25.69	<.0001
8	Leaf dry weight (g)	96.94	86.38	59.38	55.57	32.96	<.0001
9	Stem dry weight (g)	57.00	60.28	56.38	59.95	0.88	0.4546
10	Shoot dry weight (g)	209.83	379.70	234.13	177.40	132.82	<.0001

Table 3: Estimates of quantitative traits variances among tomato germplasm accessions belonging to different clusters (Ci)

Sl. No	Traits	Variance of clusters				'F' Statistic	Probability
		C ₁	C ₂	C ₃	C ₄		
	Size of the cluster →	12	13	43	32		
1	Shoot length (cm)	34.96	399.01	158.31	107.77	2.25	0.0875
2	Root length (cm)	1346.81	45.79	133.15	115.59	3.08	0.0310
3	Root volume (cm ³)	448.65	97.97	135.25	117.77	4.90	0.0033
4	Number of branches	2.74	2.59	2.16	1.14	0.52	0.6692
5	SPAD (nmol/cm ²)	1.80	5.48	1.21	1.83	5.89	0.0010
6	Specific leaf area (cm ² /g)	85.70	310.68	67.69	58.96	6.10	0.0008
7	Root dry weight (g)	94.88	170.27	39.34	40.58	4.25	0.0073
8	Leaf dry weight (g)	239.03	741.22	146.66	126.58	4.89	0.0033
9	Stem dry weight (g)	92.32	58.54	144.13	116.18	1.08	0.3601
10	Shoot dry weight (g)	653.07	3445.16	387.47	902.79	11.33	<.0001

Table 4: Genetic variability in few biometric parameters among the selected contrast tomato germplasm accession

Traits	High root types				Low root types			
	Mean	Max	Min	Standard Range	Mean	Max	Min	Standard Range
Root dry weight (g)	5.50	9.03	5.10	0.71	1.55	2.18	1.23	0.61
Leaf dry weight (g)	44.52	85.00	31.67	1.20	27.18	42.00	13.33	1.05
Stem dry weight (g)	34.91	70.00	20.00	1.43	20.05	41.67	11.67	1.50
Shoot dry weight (g)	79.72	155.00	56.66	1.23	46.68	72.00	31.67	0.86
SPAD	53.92	71.00	47.83	0.43	48.46	87.10	24.33	1.30
Specific leaf area (cm ² /g)	217.48	485.16	83.66	1.85	232.88	358.10	187.70	0.73
Shoot length	71.71	123.30	39.00	1.18	55.97	74.33	17.00	1.02
Root length	59.52	184.00	44.00	2.35	39.24	83.67	8.67	1.91
Root volume	28.36	50.00	15.00	1.23	15.58	26.67	3.33	1.50
No. of branches	4.97	9.00	2.00	1.41	3.61	6.00	1.00	1.38
Total dry matter	87.96	164.00	98.40	0.75	48.22	73.33	34.23	0.81

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