Mysore J. Agric. Sci., 50 (2) : 312-315, 2016

Identification of Contrasting Germplasm Accessions of Tomato (*Lycopersicum esculentum* L.) for Root Architecture and Intrinsic Tolerance at Cellular Level

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Abstract

Due to limited availability of aerable land and the high market demand for vegetables around the world, solanaceous (eggplant, pepper and tomato) crops are cultivated under unfavourable soil and environmental conditions leading to reduced quality and productivity of crops. One way to avoid or to reduce losses in production and productivity of vegetable crops from environmental stresses (drought) would be to graft them onto rootstocks capable of reducing the effect of external stresses on the shoot. In order to achieve this, identification of suitable root stock having superior root characteristics with higher level of root cellular level tolerance (CLT) is highly relevant under drought stress condition. In this context, 100 tomato accessions were evaluated in specially constructed root structures following augmented block design under normal conditions to study the genetic variability for root architecture *viz.,* root length (cm), root volume (cm^3), root dry weight (g) and intrinsic tolerance at cellular level (TTC). Study indicated a wide and significant variation among the accessions for all the root traits, intrinsic tolerance and also for ancillary traits. Based on root dry weight, total biomass and intrinsic tolerance, 6 contrasting lines of tomato were identified and further evaluated under different levels of moisture stress and finally root stock donors were identified for grafting approach.

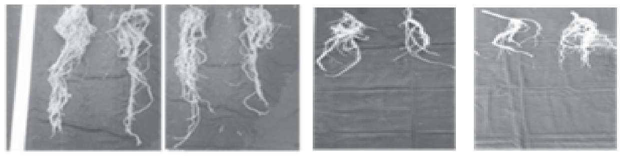
Tomato a member of solanaceous family is one of the most popular and widely grown vegetables after potato in the world (FAOSTAT Database, 2015). This crop has become an important vegetable of the world in view of the increasing demand for fresh consumption as well as processing industry. Abiotic stresses like drought, heat, cold, heavy metal and salinity represent the most limiting conditions for horticultural crop productivity and plant exploitation worldwide. Hence, this warrants the development of stress tolerant horticultural crops. Improving stress tolerance through conventional breeding approaches takes longer time. Therefore, an alternative and efficient method is required to improve stress tolerance of horticultural crops. A special method of adapting plants to stresses is by grafting elite, commercial cultivars onto selected vigorous rootstocks gives fruitful results (Lee and Oda, 2003). Grafting is nowadays regarded as a rapid and alternative method to the relatively slow breeding methodology aimed at increasing environmental-stress tolerance of fruits and vegetables (Flores *et al.,* 2010). In vegetable production, grafting is used for more than 50 years in the world. Grafting considered being an environment-friendly since it is not associated with the input of agrochemicals to the crops and is therefore operation of substantial and sustainable relevance crop management systems (Rivard and Louws, 2008). Grafting the scion above the rootstock using tube grafting method was made using soilless medium. After grafting performance, seedlings were placed in a high humidity (80-90%) chamber with controlled temperature (21–23 °C) to heal the graft union (Rivard and Louws, 2008). After one week, seedlings were removed from the chamber and placed in a greenhouse for 10–14 days before transplanting. Since the grafting was made below the rootstock, care was taken at planting to maintain the graft union above the soil and transplanted into pots in a commercial growth media. The cultivated area of grafted Solanaceae and Cucurbitaceae has increased in recent years because of grafting success in these families (Lee *et al.*, 2010).

Taken together, we have hypothesised that; rootstock having superior root characteristics with superior CLT mechanism would improve the stress tolerance of the scion. While the root traits improve the water uptake and hence, maintain tissue water status, the CLT help to withstand stress and helps in recovery growth after stress alleviation. Therefore in this study, experiments were planned to identify tomato accessions with efficient root traits and CLT for using them as root stocks to generate plants for moisture stress conditions.

Genetic variability for root traits: One hundred accessions of tomato (*Solanum lycopersicum* L.) along with five check varieties (ArkaVikas,Arka Alok, Arka abha, Arka Ahuthi and Arka Meghali) were evaluated for root traits under field conditions in root structure. Twenty five days old seedlings raised in nursery beds were transplanted in an augmented design in root structures by maintaining a spacing of 45 x 90 cm between plants. A basal dose of 100:100:100 Kg NPK ha^{-1} was applied to the root structure and the recommended management practices were followed during the crop growth period to raise a healthy crop (Plate 1).

Data were recorded on three randomly tagged plants on root, shoot and its ancillary traits such as root length (cm), shoot length (cm), root volume (cm^3), (Table I) number of branches, SPAD chlorophyll meter reading, specific leaf area (cm^2/g), root dry weight (g), leaf dry weight (g), stem dry weight (g) and shoot dry weight (g). When plants reached the flower initiation stage, the brick walls of root structures were dismantled and root was washed off carefully using a jet of water as shown in Plate 1. The entire root was then carefully removed and root length, root volume and shoot length was determined (Raju, 2009).

(a) Root excavation



Arka Ashish IIHR 2617 IIHR 2615 IIHR 2613

(b) Variation in root architecture among germplasm accession

Plate. 1: Phenotyping tomato accessions in root structures

Viability test (TTC) for CLT: In order to study the intrinsic tolerance at cellular level, the root samples of all the accessions were checked for viability following 2, 3, 5-Tri phenyl tetrazolium chloride (TTC) assay after subjecting the root tissues to different stresses. Chopped root samples were floated on -1 bar PEG (Poly Ethylene Glycol-8000) and 150mM NaCl (Sodium chloride) solution to simulate moisture stress and salinity stress, respectively for 48 hours. The extent of cell viability in root tissue was quantified by accessing the extent of TTC reduction to formazan. The formazan was extracted in ethanol (Towill and mazur, 1975) and OD

TABLE I

Analysis of variance of tomato germplasm accessions for root, shoot and its ancillary traits (50 days after transplanting)

Source of variation	df	Mean sum of squares									
		Shoot length (cm)	Root length (cm)	Root volume (cm^3)	Number of branches	SPAD Meter reading	Specific leaf area (cm^2/g)	Root dry weight (g)	Leaf dry weight (g)	Stem dry weight (g)	Shoot dry weight (g)
Blocks	03	12.39	15.56	1.15	0.93	7.24	307.04	0.13	28.11	0.83	32.28
Entries (Accessions + Checks)	104	201.10**	302.76**	233.93**	2.43**	121.66**	4630.04**	2.82**	170.28**	107.89**	449.42**
Checks	04	198.43**	247.27**	852.81**	0.55	238.77**	524.47	5.76**	258.33**	55.00**	400.27**
Accessions	99	200.59**	293.37**	183.21**	2.03**	118.15**	4827.10**	2.30**	163.56**	109.21**	443.07**
Checks vs. Accessions	01	262.91**	1454.95**	2779.74**	49.31**	0.71	1543.37	42.41**	483.55**	188.17**	1275.08**
Error	12	4.55	16.42	11.56	0.52	18.09	400.01	0.19	15.87	7.08	25.56

*Significant @ p=0.05; ** Significant @ p=0.01 SPAD = Soil Plant Analysis Development read at 485nm. Based on the OD value, contrasting lines differing in root cell viability were selected. The OD value, an indication of root viability ranged from 1.176 to 0.355 with a mean of 0.829 in control, while under stress conditions, it ranged from 0.972 to 0.1375 with a mean of 0.6108. Similar results were documented in wheat under different stress (Yamauchi, 2014).

Based on root traits, TDM and root viability (intrinsic tolerance of roots), six contrasting lines were identified and used further to study their response under stress condition. The list of contrasting lines with root, TDM and CLT data were given in Table II.

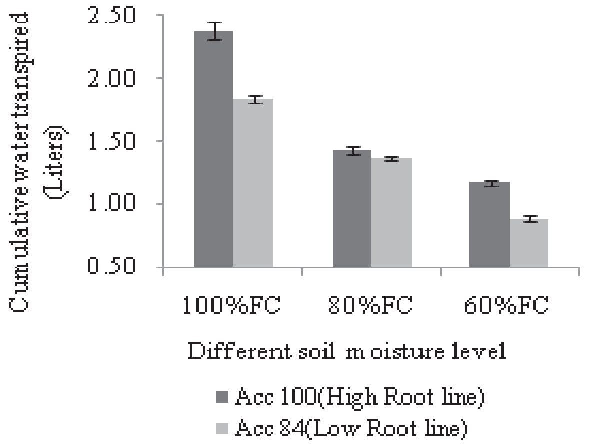


Fig.1: Variation in cumulative water loss of contrasting lines of tomato under different levels of moisture stress

TABLE II

Contrasting tomato germplasm accessions differing in root traits, TDM and root viability assay (Intrinsic tolerance)

Accessions	TTC			Root length (cm)	Root dry weight(g)	Root volume (cm^3)	TDM
	Control	Stress	% Reduction				
Low root viability, root traits and TDM							
Arka ashish	0.98	0.95	3.061	83.67	8.33	21.67	108.33
IIHR 2617	0.90	0.89	1.941	50.67	6.60	8.33	111.00
IIHR 2621	1.00	0.97	2.488	55.00	5.13	20.00	74.30
IIHR 2624	0.90	0.87	3.541	53.67	5.97	28.33	71.80
Low root viability, root traits and TDM							
IIHR 2613	0.41	0.19	54.112	44.33	2.00	16.67	68.67
IIHR 2615	0.83	0.57	32.297	45.33	1.33	23.33	67.33

Relevance of root traits and root viability under stress

In order to check the relevance of root traits and root cell viability under stress condition, the contrasting lines were subjected for different levels of moisture stress. Seedlings of all the six lines ((Four high root + high CLT) and (two low root + low CLT)) were transplanted to pots. Three levels of soil moisture status were maintained *viz.,* 100, 80, 60 per cent Field capacity (FC) from 15 days of transplanting to 35 days of transplanting. The pots were irrigated every day in the morning hours to bring back the soil moisture status to the desired field capacity by gravimetric approach. The water losses by evaporation from the exposed soil in the pots were checked by covering the soil surface by plastic pieces. The total amount of water transpired by the plants for a total duration of 20 days was computed and presented in the Fig. 1. Accordingly high root lines showed high water loss compared to low root lines particularly in 100 per cent FC. However, under 80 per cent and 60 per cent FC, there is slight increase in high roots over low root types. Similar results were documented by Karaba *et al.,* 2007.

Based on the preliminary data generated, the accession no. IIHR2617, IIHR2621, IIHR2624 and Arka Ashish are found to be promising root trait donor lines which can be used as root stocks to improve stress tolerance of scion by grafting approach.

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(Received : May, 2016 **Accepted :** June, 2016**)**