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Deciphering the Morphological, Physiological and Biochemical Mechanism Associated with Drought Stress Tolerance in Tomato Genotypes

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ABSTRACT

Abiotic stresses are one of the key limitations to global crop production and food security. Among the abiotic stresses, drought is one of the most vital factors that causes change in morphological, biochemical and physiological characteristics in plants, and consequently affects the growth and productivity of crops. The main purpose of the present study was to evaluate the effect of drought on morphological [Plant height, root length (cm), shoot length (cm), number of branches, yield attributing traits], physiological ratio of root/shoot length, leaf area (cm²), relative water content (%), and electrolyte leakage (% conductivity) and biochemical traits [ascorbic acid content (mg/100g), total carotenoids (mg/100g), total chlorophyll content, proline, sugar content] in 15 tomato genotypes and to identify drought stress tolerant genotypes. The results confirmed that there are significant variations in agronomic, physiological and biochemical parameters among 15 tomato genotypes under drought and irrigated conditions. Among the 15 genotypes, EC-317-6-1 and WIR-4360 were found highly tolerant to drought in comparison to others while Kashi Amrit and Kashi Sharad were found susceptible to drought conditions. The performance of tomato genotypes used in the study showed significant differences in all studied traits, suggesting that they could be taken into account when selecting for drought tolerance. EC-317-6-171 and WIR-4360 had good yield performance under deficit irrigation treatment. Moreover, results indicate that biochemical and physiological parameters are more useful for the screening of drought tolerant tomato genotypes.

Keywords

Tomato, Drought,
Morphology,
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Ascorbic acid,
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Introduction

Tomato are a major source of antioxidants such as carotenoids, including lycopene and beta carotene, vitamin-C, vitamin-E and polyphenols such as Kaempferol and quercetin, a notable capacity to eliminate active oxygen species (AOS) (Rao *et al.*, 1998; Rai *et al.*, 2012). Ascorbic acid being an antioxidant directly eliminates superoxide, hydroxyl radicals, oxygen singlet radicals and reduces hydrogen peroxide (Rai *et al.*, 2012). Lycopene is a carotenoid that accounts for the reddening of tomato due to the differentiation of the chloroplasts and chromoplasts, so lycopene contributes to the nutritional and marketable quality of this plant product (Dumas *et al.*, 2003). Various research findings have demonstrated the direct correlation of quality of the tomato with its lycopene content (Singh *et al.*, 2004; Singh *et al.*, 2007; George *et al.*, 2004). Moreover, it is also well known that the mixture of antioxidants exert positive effect on health benefits, related to consumption of fresh fruits and vegetables. Due to its high consumption rates, tomato can provide the total intake of these components significantly (Abushita *et al.*, 1997; Beecher, 1998).

Tomato is a popular and economically important vegetable species worldwide. Tomato consumption as well as production is permanently increasing because of its anti-oxidative and anti-cancerous properties (Raiola *et al.*, 2014). In 2015, Tomato was 7th globally in terms of production, accomplishing a world production of approximately 164,000,000.00 million tones on an area of nearly 4.8 million hectares (Gerszberg *et al.*, 2016). Tomato is the second most important vegetable in terms of production worldwide due to its intense breeding programs (FOASTAT, 2014). In India, the tomato is cultivated on 0.458M ha area with 7.277 M mt production and

15.9mt/ha productivity and the major tomato producing states in the country are Andhra Pradesh, Madhya Pradesh, Karnataka, Gujarat, Odisha, West Bengal, Chhattisgarh, Maharashtra, Bihar, Haryana, Uttar Pradesh, Telangana and Tamil Nadu (State Departments of Agriculture and Horticulture, 2018). Being agriculturally and economically important crop and due its complete genome sequencing, tomato is considered to be a pre-eminent model for genetics, breeding and genomics studies (Choudhary *et al.*, 2018). Feeding the world is a most important challenge under climate change scenario and increased water scarcity, which is further exacerbated due to growing global population (Lesk *et al.*, 2016). Moreover, various environmental stresses especially extreme temperature, drought, salinity and inadequate moisture impaired global crop productivity. Temperature between 25 to 30°C during the day and 20°C at night is the optimum for tomato cultivation. Average global temperatures are rising by approximately 0.3°C per decade. Due to a 2-4°C increase over the optimal (25°C) temperature, plant growth, embryo development, flowering, gamete development, seed germination, fruit ripening, ability of pollinated flowers to develop into seeded fruit, and consequently the yield are adversely affected (Solankey *et al.*, 2015). Drought, an another important natural disaster for tomato production and its quality, resulting from prolonged shortage in rainfall, often accompanied by quite high temperature affects photosynthesis and ultimately reduces crop productivity.

Drought often causes adaptive changes in plant growth and physio-biochemical processes such as changes in plant structure, growth rate, issue osmotic potential and antioxidant defenses (Kusvuran and Dasgan, 2017). Plants develop a wide range of strategies to avoid or tolerate water deficit. In order to circumvent water deficit condition,

plants maintain high water status either by efficient water absorption from roots or by reducing evapo-transpiration from aerial parts. In case of drought tolerance, plants maintain turgor and continue metabolism even at low water potential through synthesis of osmoprotectants, osmolytes or compatible solutes or by protoplasmic tolerance (Mishra *et al.*, 2012). Initially, drought stress closes stomata to diminish water loss by abscisic acid mediated process. Drought causes the excessive generation of reactive oxygen species (ROS) resulting into progressive oxidative damage and ultimately cell death (Rai *et al.*, 2018). Tomatoes contribute to antioxidants, such as carotenoids (especially β -carotene and lycopene), phenolics, ascorbic acid (vitamin C) and small amounts of vitamin E on a daily basis. Scavenging of excessive ROS is accomplished by a competent antioxidative defense system consisting of the non-enzymic as well as enzymic antioxidants (Baxter *et al.*, 2012). Various studies have reported that various environmental stresses induce increased amount of antioxidant phytochemicals and osmolytes against oxidative stress. The maintenance of high antioxidant capacity to detoxify the toxic ROS directly results into increased tolerance to environmental stresses (Kusvuran *et al.*, 2016). In this perspective, it is supposed to obtain an increase in the plant protective mechanisms with simultaneous increase in several components of antioxidative defense system (Sanchez-Rodriguez *et al.*, 2010).

Plant secondary metabolites are often considered to as compounds that play central role in plant-environment interaction for adaptation and defense (Ramakrishna and Ravishankar, 2011). When subjected to various stresses including different elicitors or signal molecules, plants often tend to accumulate secondary metabolites (Bennet and Wallsgrave, 1994). Various studies

demonstrated that drought often causes oxidative stress and leads to enhance amount of flavonoids and phenolic acids in willow leaves. Polyphenolic compounds are widely present in plants and are known for their overall antioxidant activity (Ramakrishna and Ravishankar, 2011). Environmental stress often induces production of phenolic metabolites such as flavonoids, lignin, tannins, hydroxycinnamate esters that serve specific roles in plant protection (Hernandez *et al.*, 2004).

The purpose of this study was to assess morphological, physiological and biochemical mechanisms adapted by fifteen tomato genotypes which differing to tolerate drought and assess whether a certain degree of drought stress could enhance the antioxidant phytochemicals, carotenoids, chlorophyll, proline and sugar contents of tolerant and sensitive tomato genotypes.

Materials and Methods

Fifteen tomato genotypes were used in this research for the identification of tolerant tomato lines. Seed material of all the fifteen tomato genotypes was obtained from IIVR, Varanasi. All the selected genotypes with their source are enlisted in Table 1. Pot experiments were carried out in a controlled conditions (temperature: $25^{\circ}\text{C}\pm 2$ and relative humidity: $55\% \pm 5$) at School of Biotechnology, Sher-e-Kashmir University of Agricultural Sciences and Technology. The plants were subjected to drought stress at growth stage (45 days) till temporary wilting point and irrigated plants were grown under non-stress conditions for the same period of time.

Responses of the genotypes to drought were evaluated using some plant morphological (Plant height, root length (cm), shoot length (cm) number of branches, and yield

attributing characters), physiological (ratio of root/shoot length, leaf area (cm²), relative water content (%), and electrolyte leakage (% conductivity) and biochemical parameters such as ascorbic acid content (mg/100g), total carotenoids (mg/100g), total chlorophyll content and proline. Observations were recorded on 11 economic traits from five randomly selected competitive plants of 15 genotypes in 5 replications and their mean were worked out for statistical analysis. Plant height was measured in centimeters from the base of the plant to tip of the main shoot at the time of final picking and average plant height of each genotype was worked out. Number of branches originating from the main stem was counted and has been determined as an average number of productive primary branches from ten randomly sampled plants at maturity. Root length was measured from the bottom of the shoot to top of the root. Number of flowers per cluster was counted manually starting from bottom to top in each cluster and on every branch of a plant. Number of cluster per plant was counted manually starting from bottom to top on each branch plant. Number of fruits per plant was counted manually starting from the down to up in each cluster and on every branch of a plant. Fruit setting percentage was calculated by dividing number of fruits per cluster with number of flowers per cluster and then multiplying the product with 100. Root/shoot length ratio was calculated by dividing the root length by shoot length. Leaf area was calculated with the help of graph paper. Leaves to be measured were layered on a 1 cm grid and outline was traced. The number of partial squares was estimated and partial squares that are less than half covered were not counted. The area of stem (petiole) was not included in the calculations. Relative water content (RWC) in the leaves was calculated according to the formula (Bars and Weatherly, 1962): $RWC (\%) = [(fresh\ weight - dry\ weight) / (saturated\ weight - dry\ weight)]$. The leaf dry

weight was measured after oven drying at 105°C for 24 h, and the saturated weight was measured after incubating the leaves in moist filter paper for 24h in petri dishes at room temperature. The total ions leaked out of the leaf were estimated by the method described by Ben (Hamed *et al.*, 2007). The electrolyte leakage was calculated using formula: $Electrolyte\ leakage\% = (EC_b - EC_c / EC_a) 100$ (EC_a-electrical conductivity of distilled water, EC_b-electrical conductivity at 45⁰C, EC_c-electrical conductivity at 100⁰C).

Leaf membrane stability index (MSI) was determined according to the method of Premchandra *et al.*, (1990) as modified by Sairam (1994). The MSI was calculated as: $Membrane\ stability\ index\ (MSI) = [1 - (C1 / C2)] \times 100$

The estimation of chlorophyll content was done by SPAD method (SPAD-502 plus). Fresh leaves were taken and cut into round discs. Readings in triplicate were taken with the help of SPAD. The ascorbic acid content was estimated titrimetrically, using 2,6-dichlorophenol indophenols (2,6-DCPIP) dye, as per the method of (Rangana1977). Ascorbic acid content was calculated as ascorbic acid mg/100g leaf sample. The total carotenoids were extracted and partitioned in acetone and petroleum ether, as described by (Thimmaiah1999). Absorbance measured at 452nm and total carotenoid content (mg/100g) was calculated using a calibration curve prepared against a high purity β carotene. Proline was extracted and estimated according to (Bates *et al.*, 1973). 100 mg of leaf tissues were homogenized in 2 ml of 3% sulfosalicylic acid solution using tissue homogenizer. The homogenate was centrifuged at 13,000 g for 10 minutes. 1 ml of the supernatant was then added into a test tube to which 1 ml of glacial acetic acid and 1 ml of freshly prepared acid Ninhydrin solution were added. Tubes were incubated in

a water bath for 1 h at 100⁰C, allowed to cool to room temperature and then 2 ml of toluene was added and vortexed for 20 seconds. The test tubes were allowed to stand for at least 10 minutes to allow the separation of toluene and aqueous phase. The absorbance of toluene phase was measured at 520 nm in a spectrophotometer. The concentration of proline was calculated from proline standard curve. The concentration of proline was expressed as $\mu\text{mol/g FW}$. Reducing sugars other than starch were extracted from fresh leaf material according to the procedure of (Cerning and Guilhot, 1973). Total soluble sugars were determined spectrometrically using 0.2 % anthrone in concentrated sulphuric acid as reagent following the method of (Yemm and Willis, 1954).

All the experimental data are the mean of five replicates. The mean values are shown with the critical difference (CD) in the tables and in the figures.

A Student's t-test was performed to determine significant differences between control and drought treatment and differences among genotypes under both conditions (irrigated as well as drought stress) were analysed by one-way analysis of variance (ANOVA). The dendrogram representing agglomerative hierarchical clustering was constructed using UPGMA method. All these statistical analyses were done using Microsoft EXCEL 2007 software package (Microsoft Corp.; Redlands, WA, USA).

Results and Discussion

Present study investigated the morphological, physiological and biochemical performance of the fifteen tomato genotypes exposed to drought stress at growth stage (45 days) and irrigated plants were grown under non-stress conditions for the same period of time. Results showed that drought stress

considerably reduced the growth of tomato genotypes in terms of plant height, number of branches, root length, shoot length, yield and their attributing characters as well as physiological traits. Drought stress unfavorably affects the meristematic activity, cell elongation, causes premature abscission of leaves and roots, reduces the accumulation of dry matter and the photosynthetic activity (Latif *et al.*, 2016).

Plant height data ranged from 39.62 cm to 99.55 cm amongst the 15 tomato genotypes in irrigated condition (Table 2). The maximum plant height in irrigated condition was recorded in EC-317-6-1 (99.55 cm) followed by WIR-13706 and minimum was recorded in Money Maker (39.62 cm). EC-317-6-1 genotypes showed significantly higher value than other genotypes. Under drought stress condition, height varied from 22.61 cm to 54.51 cm amongst the tomato genotypes. The maximum plant height in drought stress condition was recorded in F-7012 (54.51 cm) followed by Roma and however, minimum plant height was recorded in Money Maker followed by C-26-1 under drought stress condition. The genotypes Azad T-5, Roma, F-7012, WIR-13706 are significantly at par. The results showed decreasing trend in drought stress as compared to irrigated condition (Fig. 1).

The number of branches ranged from 7.33 cm to 19.00 cm amongst 15 tomato genotypes in irrigated condition (Table 2). The maximum number of branches in irrigated condition was recorded in Kashi Amrit (19.00 cm) followed by EC-317-6-1 and minimum was recorded in Azad T-5 (7.33). EC-317-6-1 genotype showed significantly higher than other genotypes. Under drought stress condition, number of branches varied from 5.33-14.33 amongst the tomato genotypes. The maximum of branches in drought stress condition was recorded in Kashi Amrit (14.33) followed by

Roma and EC-317-6-1 and minimum number of branches in drought condition was recorded in Azad T-5 (5.33) followed by VRT-32 and Kashi Sharad. The genotypes Kashi Anupam, WIR-13706 and Roma are significantly at par. The results showed decreasing trend in drought stress as compared to irrigated condition (Fig. 2).

The maximum root length was recorded in F-7012 (13.36) in irrigated condition followed by VRT-32 and WIR-4360. The minimum root length was recorded in Kashi Sharad (3.45), followed by Money Maker, C-26-1 and F-7028 in irrigated condition. The maximum root length in drought stress condition was recorded in EC-317-6-1 (21.22), followed by VRT-32 and minimum root length in drought condition was recorded in C-26-1 (5.60) followed by Kashi Amrit and F-7012 and Kashi Sharad (Table 2). The results showed Increasing trend in drought stress as compared to irrigated condition (Fig. 3).

The maximum shoot length was recorded in EC-317-6-1(57.46cm) in irrigated condition followed by F-7028. The minimum root length was recorded in Money maker(29.96) in irrigated condition which is followed by VRT-32.Under drought stress shoot length varied from 18.60cm- 43.12cm.The maximum shoot length in drought condition was recorded in F-7028 (43.12) followed by Roma and Kashi Sharad. The minimum shoot length in drought condition was recorded in Money maker (18.60) followed by VRT-32 and Kashi Anupam (Table 2). The results showed decreasing trend in drought stress as compared to irrigated condition (Fig. 4).

The number of cluster /plant ranged from 2.00-12.67 amongst the 15 tomato genotypes in irrigated condition. The maximum number of cluster /plant in irrigated condition was recorded in EC-317-6-1 (12.67) followed by

Swaran Naveen and minimum was recorded in Kashi Amrit (2.00). EC-316-6-1 genotypes showed significantly higher than other genotypes. Under drought stress condition, number of cluster /plant varied from 1.00-8.67amongst the tomato genotypes The maximum number of cluster /plant in drought stress condition was recorded in EC-317-6-1 (8.67) followed by Swaran Naveen and minimum number of cluster /plant in drought condition was recorded in Kashi Amrit (1.00) followed by VRT-32 under drought stress. The genotypes Swaran Naveen and Kashi Anupam are significantly at par (Table 3). The results showed decreasing trend in drought stress as compared to irrigated condition (Fig. 5).

The maximum Number of flower/cluster in irrigated condition was recorded in Azad T-5 (7.33), followed by VRT-32 and minimum was recorded in F-7012 (4.33). Under drought stress condition, number of flower/cluster varied from 2.00-4.33. amongst the tomato genotypes. The maximum number of flower/cluster in drought stress condition was recorded in Money Maker (4.33) followed by Azad T-5 and minimum number of flower/cluster in drought condition was recorded in F-7012(1.00) followed by VRT-32 under drought stress. The genotypes Swaran Naveen and VRT-32 are significantly at par (Table 3). The results showed decreasing trend in drought stress as compared to irrigated condition (Fig. 6).

The maximum number of flowers per plant in irrigated condition was recorded in EC-317-6-1(61.00) followed by WIR-4360 and minimum was recorded in C-26-1(10.67). EC-317-6-1 genotype showed significantly higher number of flowers per plant than other genotypes under irrigated conditions. Under drought stress condition number of flower per plant varied from (10.00-51.00) amongst the tomato genotypes. The maximum number of

flower per plant in drought stress condition was recorded in EC-317-6-1 (51.00) followed by Kashi Anupam and minimum number of flower per plant in drought condition was recorded in C-26-1(10.00) followed by Kashi Amrit under drought stress (Table 3). The results showed decreasing trend in drought stress as compared to irrigated condition (Fig. 7).

The maximum number of fruits per plant was recorded in Money Maker (12.33) and minimum was recorded in Kashi Sharad (2.33). Under drought stress, range varied from 1.00-5.67 (Table 3). The maximum number of fruits per plant was recorded in Money Maker (5.67) and minimum number of fruits per plant was recorded in Kashi Sharad (1.00) in drought stress conditions. The results showed decreasing trend in drought stress as compared to irrigated condition (Fig. 8).

The Fruit setting (%) ranged from 7.01-48.14 amongst the 15 tomato genotypes in control condition and in drought condition 2.86-43.85. The maximum Fruit setting (%) was recorded in Money Maker in both the conditions. The minimum Fruit setting (%) was recorded in Kashi Sharad (7.01) in control condition and in drought condition in KashiAnupam (2.86) (Table 3).The results showed decreasing trend in drought stress as compared to irrigated condition (Fig. 9).

The Ratio of Root length/Shoot length ranged from 0.16-0.54 amongst the 15 tomato genotypes in irrigated condition. The maximum Ratio of Root length/Shoot length was recorded in VRT-32 (0.542) in irrigated condition followed by WIR-4360. The minimum Ratio of Root length/Shoot length was recorded in C-26-1 (0.16) in control condition. Under drought stress condition range varied from 0.07-0.41.The maximum ratio of Root length/Shoot length in drought

condition was recorded in WIR-4360 (0.41). The minimum Ratio of Root length/Shoot length was recorded in F-7028(0.07) followed by C-26-1 (Table 3). The results showed decreasing trend in drought stress as compared to irrigated condition (Fig. 10).

The Leaf Area (cm²) ranged from 67.30-204.75 amongst the 15 tomato genotypes in irrigated condition. Under drought stress, leaf area varied from 60.88-152.60. The maximum Leaf Area (cm²) was recorded in Kashi Sharad in both the conditions followed by Kashi Amrit and Kashi Anupam and the minimum Leaf Area (cm²) was recorded in EC-317-6-1 in both the conditions followed by Kashi Anupam. The genotypes Swaran Naveen, Money Maker and VRT-32 are statistically at par (Table 3). The results showed decreasing trend in drought stress as compared to irrigated condition (Fig. 11).

The maximum Relative water content (%) was recorded in F-7028 (95.43) in irrigated condition followed by Azad T-5. The minimum Relative water content (%) was recorded in Kashi Amrit (52.11) in control condition followed by F-7012. Under drought stress range varied from 47.43-74.83 (Table 3). The maximum Relative water content (%) was recorded in Kashi Amrit (74.83) in drought condition followed by Swaran Naveen. The minimum relative water content (%) was recorded in drought condition in WIR-4360 (47.43) genotypes followed by F-7012 (Fig. 12).

The maximum Electrolyte Leakage (% conductivity) was recorded in Kashi Amrit (96.83) in irrigated condition followed by F-7012. The minimum Electrolyte Leakage (% conductivity) was recorded in WIR-4360(73.64) in irrigated condition followed by Money Maker. Under drought condition range varied from 75.78-94.69. The maximum Electrolyte Leakage (%)

conductivity) was recorded in WIR-4360 (94.69) in drought condition followed by WIR-13706. The minimum Electrolyte Leakage (% conductivity) was recorded in drought condition in C-26-1(75.78) followed by F-7028 (Table 3). The results showed decreasing trend in drought stress as compared to irrigated condition (Fig. 13).

Titrimetric analysis of ascorbic acid showed significant variation in vitamin-C levels estimated in freshly harvested fruits of 15 tomato genotypes. In this study, the vitamin-C concentration ranged from (14.94-32.54mg/100g) in irrigated condition and in drought condition (15.66-25.51 mg/100g). The maximum ascorbic acid content was recorded in F-7028 (32.54 mg/100g) in irrigated condition and in Money Maker (25.51 mg/100g) in drought condition. The minimum ascorbic acid content was recorded in WIR-13706 (14.91 mg/100g) in irrigated condition and in drought condition in Kashi Sharad (15.66 mg/100g) (Table 4). The results showed increasing trend in drought stress as compared to irrigated condition (Fig. 14).

Maximum carotenoid content was recorded in Swaran Naveen (8.92 mg/100g) in irrigated condition and in drought condition in EC-317-6-1 (3.04 mg/100g) respectively. The minimum total carotenoids content was noted in F-7028 (3.42mg/100g) in irrigated condition and in drought condition in WIR-4360(0.58 mg/100g) (Table 4). The results showed increasing trend in drought stress as compared to irrigated condition (Fig.15).

Maximum Total Chlorophyll content was recorded in Azad T-5 (57.36) in irrigated condition and in Kashi Anupam (49.73) in drought condition respectively. The minimum Total Chlorophyll content was noted in Swaran Naveen (23.38) in control condition

and in drought condition in Kashi Sharad (10.11) (Table 4). The results showed decreasing trend in drought stress as compared to irrigated condition (Fig. 16).

Proline level was increased significantly ($P \leq 0.05$) in all genotypes under drought. Significant variation was recorded in the Total Chlorophyll content amongst the 15 tomato genotypes. Under drought stress, WIR-4360 showed increase in proline level as compared to control. Minimum increase was found in Roma as compared to control (Table 5). The results showed increasing trend in drought stress as compared to irrigated condition (Fig. 17).

Sugar level was increased significantly ($P \leq 0.01$ and 0.05) in all genotypes under drought. Significant variation was recorded in the Total Chlorophyll content amongst the 15 tomato genotypes. Under drought stress condition, Roma showed increase in Sugar level as compared to control. Minimum increase was found in Kashi Amrit compared to control (Table 5). The results showed increasing trend in drought stress as compared to irrigated condition (Fig. 18). Descriptive statistics for plant growth parameters in 15 tomato genotypes under drought stress is given in Table 6.

Based on the above investigation of morphological characters, yield attributing characters, physiological characters, antioxidant phytochemicals and osmolytes, the hierarchical cluster was formed which distinguished the 15 genotypes and were classified into 2 groups, one cluster contain only cultivated genotypes and another cluster contain other 12 mixed wild and cultivated genotypes. Now from the 2 clusters, 2 wild and 2 other genotypes were selected for the Antioxidant isozymes (Fig. 19).

Table.1 List of tomato genotypes used in the study along with their source

S.No.	Genotypes	Source
1.	Azad T-5 (<i>Solanum lycopersicum</i> L.)	IIVR, Varanasi
2.	Kashi Sharad (<i>Solanum lycopersicum</i>)	IIVR, Varanasi
3.	Roma (<i>Solanum lycopersicum</i>)	IIVR, Varanasi
4.	Kashi Amrit (<i>Solanum lycopersicum</i>)	IIVR, Varanasi
5.	EC-317-6-1 (<i>Solanum lycopersicum</i>)	IIVR, Varanasi
6.	Hisar Arun (<i>Solanum lycopersicum</i>)	IIVR, Varanasi
7.	Swaran Naveen (<i>Solanum lycopersicum</i>)	IIVR, Varanasi
8.	Money Maker (<i>Solanum lycopersicum</i>)	IIVR, Varanasi
9.	WIR-4360 (<i>Solanum peruvianum</i>)	IIVR, Varanasi
10.	VRT-32 (<i>Solanum lycopersicum</i>)	IIVR, Varanasi
11.	F-7012 (<i>Solanum lycopersicum</i>)	IIVR, Varanasi
12.	WIR-13706 (<i>Solanum L. ceresiforme</i>)	IIVR, Varanasi
13.	Kashi Anupam (<i>Solanum lycopersicum</i>)	IIVR, Varanasi
14.	C-26-1 (<i>Solanum lycopersicum</i>)	IIVR, Varanasi
15.	F-7028 (<i>Solanum lycopersicum</i>)	IIVR, Varanasi

Table.2 Changes in morphological parameters of fifteen tomato genotypes exposed to drought stress (N= normal condition, D= drought condition)

Genotypes	Plant height (cm)		Root length (cm)		Shoot length (cm)		No. of branches	
	N	D	N	D	N	D	N	D
Azad T-5	65.53	38.35	4.54	14.56	50.64	33.75	7.33	5.33
Kashi Sharad	51.09	43.59	3.45	7.30	43.67	40.14	12.33	6.67
Roma	70.11	51.04	9.39	17.42	52.85	41.65	16.33	11.33
Kashi Amrit	49.24	41.34	6.28	6.98	38.85	35.05	19.00	14.33
EC-317-6-1	99.55	48.52	8.69	21.17	57.46	36.32	18.33	11.33
HisarArun	44.69	42.32	8.66	9.01	43.67	31.55	11.67	7.67
SwaranNaveen	51.82	43.67	10.65	12.94	48.62	34.78	13.00	12.67
Money Maker	39.62	22.61	3.64	9.48	29.96	18.60	14.33	9.33
WIR-4360	50.67	38.62	11.20	16.13	34.54	27.43	11.00	7.00
VRT-32	53.49	42.92	11.26	18.26	33.72	28.71	11.00	6.00
F-7012	71.34	50.03	13.36	7.30	42.32	36.48	12.00	10.00
WIR-13706	68.06	47.68	9.48	12.22	51.68	37.61	16.00	12.67
Kashi Anupam	44.71	40.08	9.55	10.50	34.03	31.48	15.00	10.00
C-26-1	43.67	40.87	3.56	5.60	40.15	35.04	11.33	7.00
F-7028	57.22	54.51	3.62	10.73	53.37	43.12	11.33	9.00
Range	39.62-	22.61-	3.45-	5.60-	29.96-	18.60-	7.33-	5.33-
C.D@5%	99.55	51.04	13.36	21.17	57.46	43.12	19.00	14.33
	9.38	9.31	2.25	6.65	8.75	9.02	3.48	3.66

Table.3 Changes in yield attributing parameters of fifteen tomato genotypes exposed to drought stress (N= normal condition, D= drought condition)

Genotypes	Clusters /plant		Flower /cluster		Flowers /plant(N)		Fruit/Plant		Fruit setting%	
	N	D	N	D	N	D	N	D	N	D
Azad T-5	5.67	2.33	7.33	3.67	43.00	34.67	5.33	2.00	12.51	5.84
Kashi Sharad	.33	6.00	6.33	3.00	33.33	27.67	2.33	1.00	7.01	3.71
Roma	5.00	3.00	5.00	2.67	26.00	15.33	2.67	2	17.59	7.69
Kashi Amrit	2.00	1.00	4.67	2.67	15.33	10.33	4.00	3.00	39.09	20.73
EC-317-6-1	12.67	8.67	4.67	3.00	61.00	51.00	11.00	3.00	18.08	5.89
Hisar Arun	3.67	2.00	5.33	2.33	20.67	13.67	4.00	2.67	32.47	12.27
Swaran Naveen	8.00	8.00	6.33	2.67	38.67	33.67	9.00	1.67	23.33	5.69
Money Maker	6.00	4.00	5.33	4.33	25.67	14.00	12.33	5.67	48.14	43.85
WIR-4360	7.00	3.00	6.33	2.00	47.00	33.67	5.00	1.00	10.64	2.97
VRT-32	4.67	2.00	6.67	3.00	32.00	26.33	3.00	2.67	13.44	11.36
F-7012	6.33	3.33	4.33	2.00	24.00	13.33	3.67	2.33	15.46	14.91
WIR-13706	6.00	3.00	6.00	3.00	28.67	18.67	4.00	2.33	14.05	13.21
KashiAnupam	7.33	7.33	5.67	2.00	38.67	36.33	3.33	1.33	8.86	2.86
C-26-1	2.00	2.00	6.33	2.67	10.67	10.00	4.33	1.67	41.11	20.00
F-7028	4.00	2.00	6.67	3.33	24.67	15.33	4.33	1.67	17.55	6.55
Range	2.00- 12.67	1.00- 8.67	4.33- 7.33	2.00- 4.33	10.67- 61.00	10.00- 51.00	2.33- 12.33	1.00- 5.67	7.01- 48.14	2.86- 43.85
C.D@5%	1.13	1.28	2.15	1.44	5.26	6.87	1.18	1.93	10.80	10.86

Table.4 Changes in Physiological characters parameters of fifteen tomato genotypes exposed to drought stress (N= normal condition, D= drought condition)

GENOTYPES	Ratio Root/ Shoot length		Leaf area		RWC		Electrolyte leakage	
	N	D	N	D	N	D	N	D
	Azad T-5	0.29	0.134	98.18	80.05	84.14	64.57	91.43
Kashi Sharad	0.17	0.086	204.75	152.60	70.22	48.82	82.47	92.29
Roma	0.330	0.23	96.33	76.67	71.80	69.82	86.79	89.89
KashiAmrit	0.179	0.14	192.55	88.43	52.11	74.83	96.83	91.51
EC-317-6-1	0.33	0.284	67.30	60.88	64.67	65.50	88.11	88.61
HisarArun	0.29	0.239	95.07	64.64	68.96	66.07	94.56	88.93
Swaran Naveen	0.269	0.25	88.21	69.94	64.83	72.43	96.23	90.85
Money Maker	0.32	0.20	85.26	72.75	74.13	53.47	82.8	84.41
WIR-4360	0.467	0.41	79.51	65.57	72.11	47.43	73.64	94.69
VRT-32	0.542	0.39	89.27	72.61	71.85	53.70	92.69	85.67
F-7012	0.366	0.11	103.51	86.24	60.23	52.43	96.37	84.13
WIR-13706	0.252	0.22	75.45	64.41	84.07	61.83	87.4	92.4
KashiAnupam	0.303	0.31	185.72	95.57	75.93	68.37	91.24	88.7
C-26-1	0.16	0.09	87.96	72.37	67.86	50.07	91.63	75.78
F-7028	0.246	0.07	109.27	85.84	95.43	63.17	85.8	79.51
Range	0.16- 0.542	0.07- 0.41	67.30- 204.75	60.88- 152.60	52.11- 95.43	47.43- 74.83	73.64- 96.83	75.78- 94.69
C.D@5%	0.100	0.182	12.23	13.95	3.715	7.381	0.576	0.880

Table.5 Changes in Antioxidant phytochemicals and osmolytes of fifteen tomato genotypes exposed to drought stress (N= normal condition, D= drought condition)

Genotypes	Ascorbic Acid		Chlorophyll content		Carotenoid(mg/100g)		Proline ($\mu\text{mol g}^{-1}$ of fresh leaf wt.)		Reducing Sugar content (mg/g of fresh wt.)	
	N	D	N	D	N	D	N	D	N	D
Azad T-5	18.41	17.67	57.36	26.35	7.57	0.97	11.34	12.21	16.34	19.74
Kashi Sharad	18.60	15.66	25.47	10.11	4.67	2.58	9.04	10.43	15.98	18.17
Roma	22.14	20.10	53.80	46.51	7.92	2.64	4.94	6.32	16.56	20.89
Kashi Amrit	25.48	22.51	47.93	42.25	8.54	2.73	6.38	7.14	16.68	16.57
EC-317-6-1	27.33	25.12	53.40	45.37	6.83	3.04	4.46	8.85	16.82	20.36
Hisar Arun	23.53	15.71	26.47	17.14	7.9	1.83	8.08	10.43	15.86	17.93
Swaran Naveen	21.56	19.49	23.38	15.11	8.92	1.85	11.33	12.43	16.69	18.42
Money Maker	25.48	25.51	45.33	40.72	8.82	0.81	7.05	9.75	14.92	18.28
WIR-4360	24.78	19.13	51.46	47.52	6.73	0.58	10.02	12.85	15.38	19.58
VRT-32	27.26	21.74	56.40	39.48	5.21	1.83	5.09	8.65	16.83	17.42
F-7012	23.20	23.88	39.27	23.45	8.73	1.74	8.88	9.75	15.48	19.81
WIR-13706	14.94	21.76	39.60	34.62	5.34	1.89	6.32	8.79	16.18	18.59
Kashi Anupam	23.54	20.15	54.57	49.73	7.62	3.01	7.89	8.23	16.94	17.85
C-26-1	26.27	23.74	46.50	39.76	8.29	2.39	7.7	11.97	14.27	19.36
F-7028	32.54	23.39	40.53	27.17	3.42	2.91	7.79	10.34	15.48	19.62
Range	14.94- 32.54	15.66- 25.51	23.38- 57.36	10.11 49.73	3.42- 8.92	0.58- 3.04	4.46- 11.34	6.32- 12.85	14.27- 16.94	16.57- 20.89
C.D@5%	1.99	3.515	3.47	3.924	0.880	0.574	1.64	3.23	3.41	3.94

Table.6 Descriptive statistics for plant growth parameters in 15 tomato genotypes under drought stress (N= normal condition, D= drought condition)

Parameters	Minimum value	Maximum value	Mean
Plant Height(N)(cm)	32.51	78.49	50.53
Plant Height(D)(cm)	2.54	23.31	10.45
Root length(N)(cm)	2.54	22.86	9.29
Root length(D)(cm)	12.19	84.58	39.46
Shoot length(N)(cm)	21.34	67.82	40.86
Shoot length(D)(cm)	0.06	0.63	0.27
Ratio R/S(N)(cm)	0.05	0.56	0.24
Ratio R/S(D)(cm)	6.0	21.0	13.33
Number of branches(N)	2.0	17.0	9.36
Number of branches(D)	3.0	9.0	5.80
Clusters per plant(N)	1.0	5.0	2.82
Clusters per plant(D)	1.0	5.0	2.82
Flowers per plant(N)	1.0	9.0	3.84
Flowers per plant(D)	10.0	63.0	30.49
Flowers per cluster(N)	10.0	54.0	24.40
Flowers per cluster(D)	2.0	13.0	5.18
Fruits per plant(N)	1.0	7.0	2.31
Fruits per plant(D)	5.55	50.0	20.63
Fruit setting (%) (N)	2.17	58.33	12.50
Fruit setting (%) (D)	14.79	32.98	23.67
Ascorbic Acid(mg/100g)(N)	12.82	27.31	21.04
Ascorbic Acid(mg/100g)(D)	22.0	59.54	44.10
Chlorophyll content(N)ug /cm ²	7.7	52.3	33.69
Chlorophyll content(D)ug /cm ²	51.0	96.58	71.89
Relative water content Percentage(N)	42.9	76.75	60.83
Relative water content Percentage (D)	65.0	224.86	110.56
Leaf Area (cm)(N)	67.30	204.75	1.10
Leaf Area(cm)(D)	60.88	152.60	80.57
Percent conductivity(N)	73.64	96.83	89.19
Percent conductivity(D)	75.78	94.69	87.56
Total Carotenoid (mg/100g)(N)	3.42	8.92	7.100
Total Carotenoid (mg/100g)(D)	.58	3.04	2.053

Fig.1 Plant height of tomato genotypes

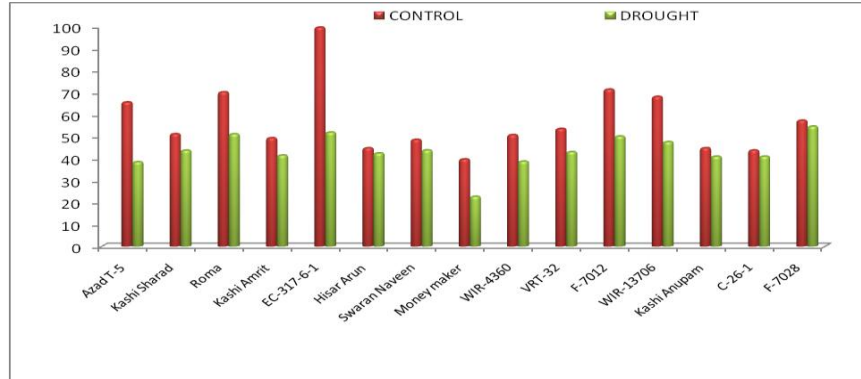


Fig.2 Number of branches of tomato genotypes

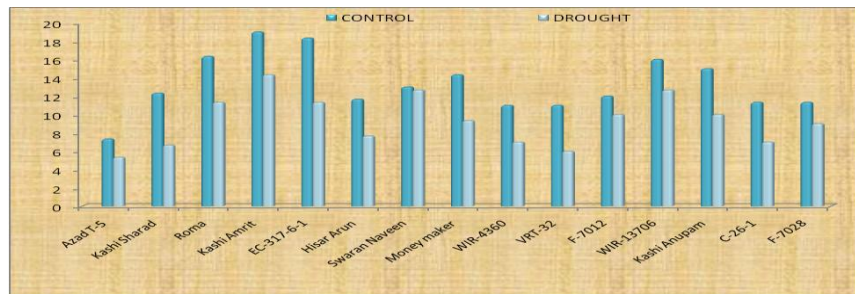


Fig.3 Root length of tomato genotypes

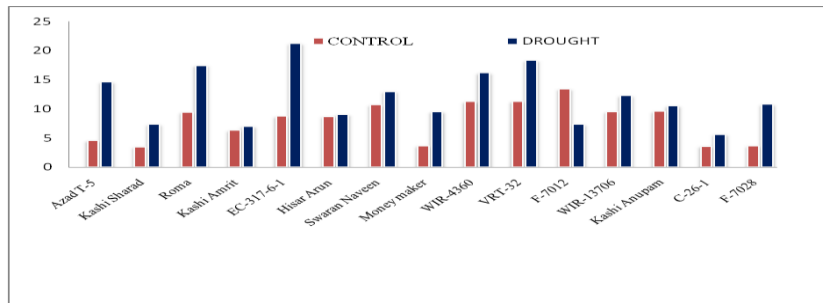


Fig.4 Shoot length of Tomato genotypes

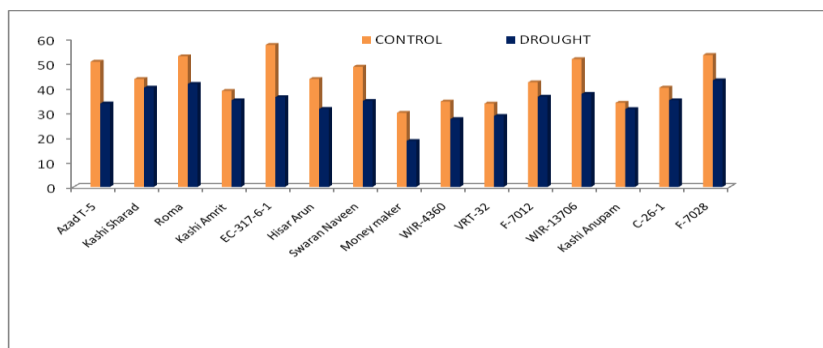


Fig.5 Number of cluster/Plant of tomato genotypes

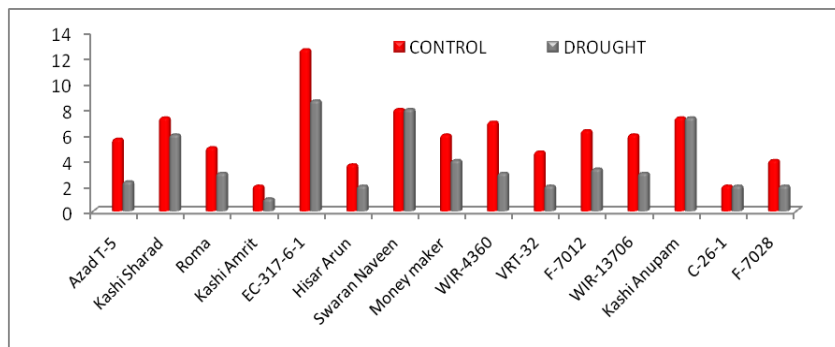


Fig.6 Number of Flower/cluster of tomato genotypes

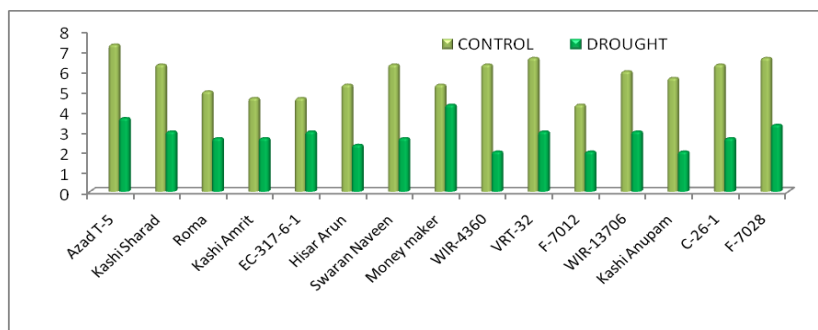


Fig.7 Number of Flower/plant of tomato



Fig.8 Number of Fruits/plant of tomato genotypes

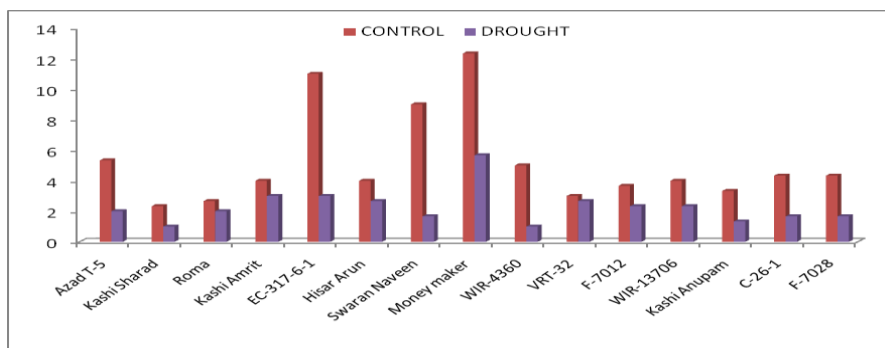


Fig.9 Fruit setting percent of tomato genotypes

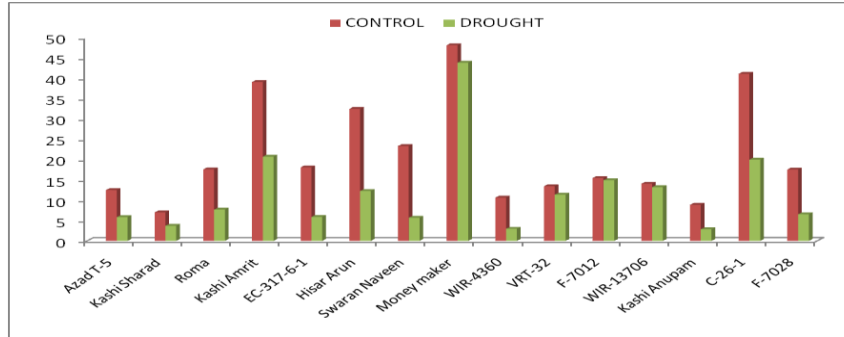


Fig.10 Ratio root length /shoot length of tomato genotypes

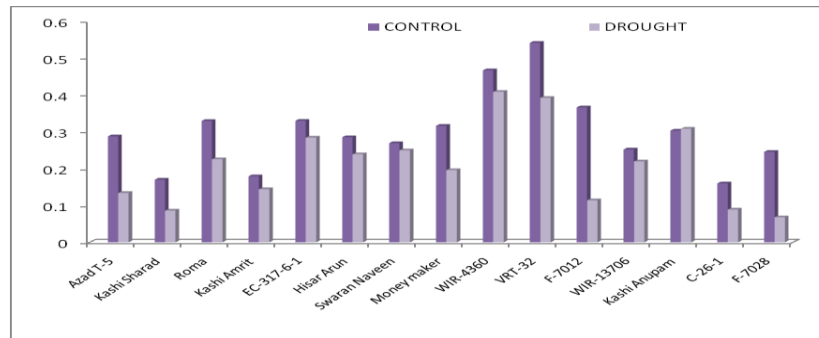


Fig.11 Leaf area of tomato genotypes

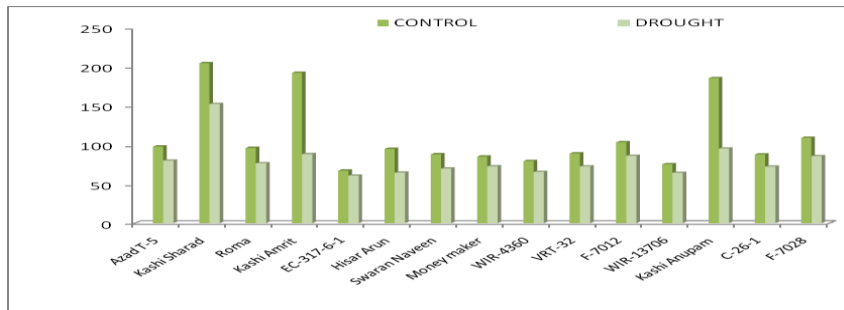


Fig.12 Relative water content (%) of tomato genotypes

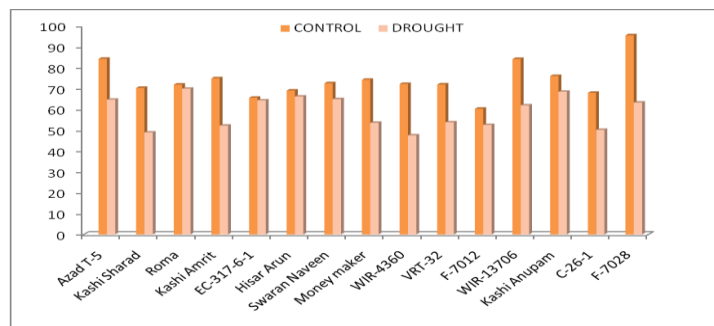


Fig.13 Electrolyte leakage of tomato genotypes

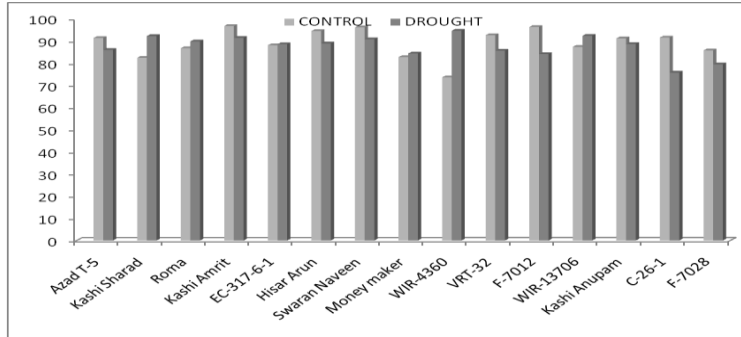


Fig.14 Ascorbic acid content of tomato genotypes

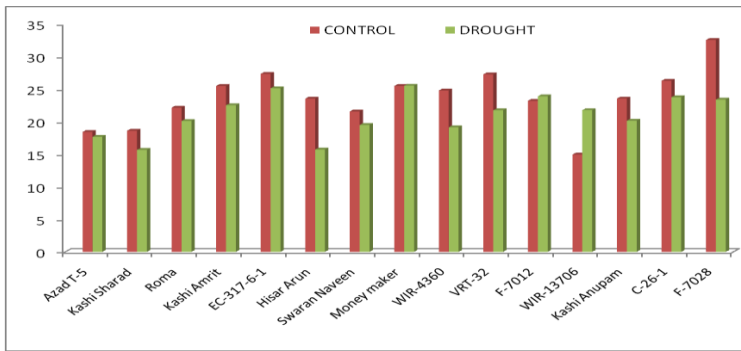


Fig.15 Carotenoid content of tomato genotypes

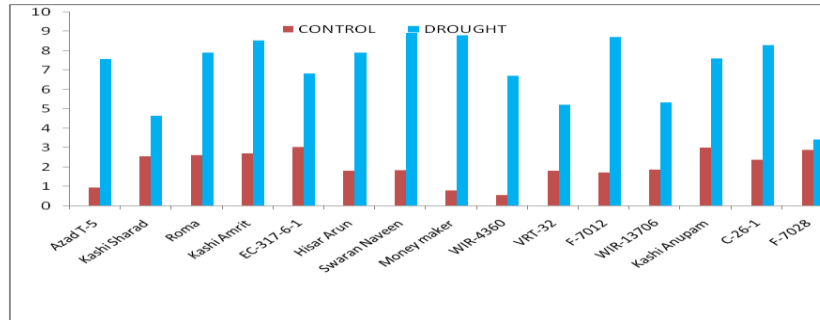


Fig.16 Chlorophyll content of tomato genotypes

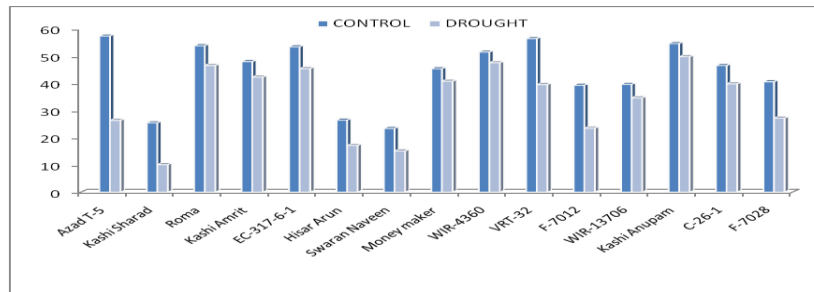


Fig.17 Proline content of tomato genotypes

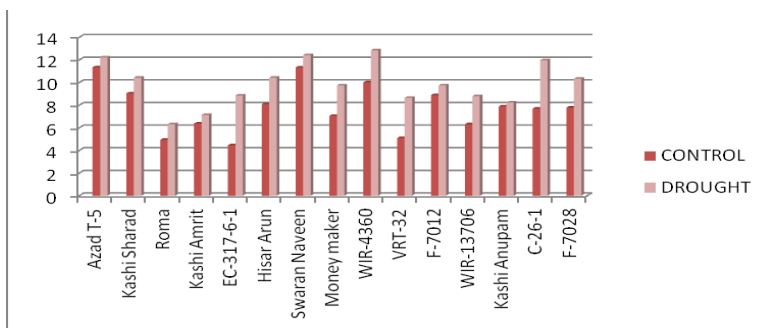


Fig.18 Sugar content of tomato genotypes under drought stress

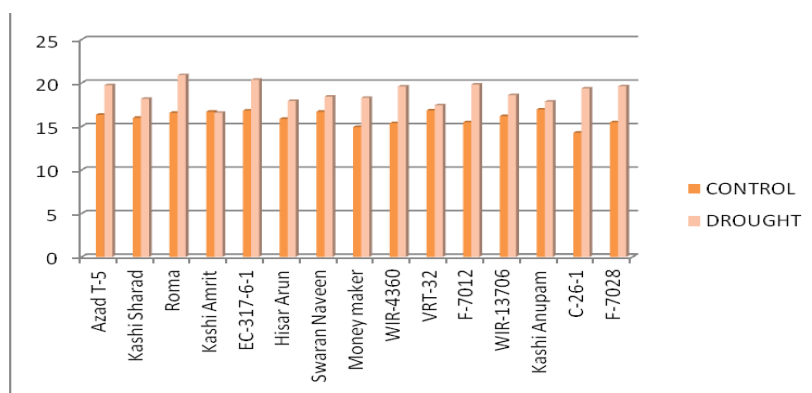
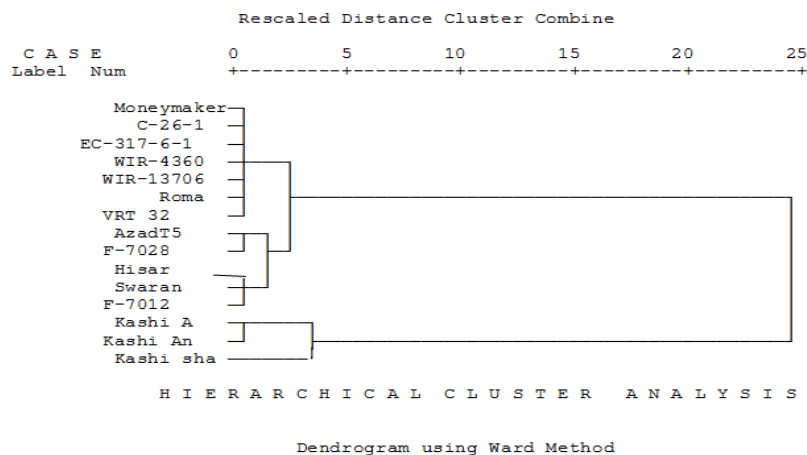


Fig.19 Dendrogram of tomato genotypes under drought stress using ward method (Kashi A = Kashi Amrit, Kashi An= Kashi Anupam, Kashi sha= Kashi Sharad)



Present study was carried out to screen 15 tomato genotypes with respect to 14 characters. Significant variations /differences for all the 14 characters in 15 tomato genotypes were

observed which were used for screening the genotypes of tomato undertaken for the study. Among the morphological characters, under drought stress the widest range (22.61-99.55cm)

was recorded for plant height followed by shoot length (18.60-78.62 cm), root length (3.45-21.17 cm), and number of branches (7.33-19.00) respectively. Photosynthesis and growth are the most significant processes affected by drought (Sapeta *et al.*, 2012). Drought affects plant growth by diminishing cell division, enlargement and ultimately reduces transport to the root surface which further leads to decrease in plant growth. An early morphological response to drought stress is the avoidance mechanism through adjustment of plant growth rate such as a reduction in shoot height, basal diameter, and total fresh mass in 15 tomato genotypes used in our study. Root length increases as plant was going for adaptation mechanism. Among the yield attributing characters under drought stress the widest range (2.86-43.85) was recorded for number of flowers/plant followed by number of clusters/plant (1.00-8.67), number of fruits/plant (1.00-5.67), and number of flowers/cluster (2.00-4.33). The range of the mean values defines the distance of the potential of different genotypes for various characters studied. The results showed that there was enormous genetic distance between the genotypes for some of the characters like yield and its attributing traits. These results further indicate that if the genotypes are having larger value for range of variability for various characters, there will be better chance to improve the existing cultivars by different breeding procedures. It can be used in selection or hybridization programme for the respective characters. The result regarding mean and range values obtained in the present investigation are in accordance with the results obtained by (Manna and Paul, 2012; Naz *et al.*, 2013; Nwosu *et al.*, 2014; Biswas *et al.*, 2015).

Optimal leaf area is considered as an important factor to photosynthesis and dry matter yield. Water deficit stress mostly reduced leaf growth and leaf area (Jaleel *et al.*, 2009). Drought stress results into decrease in relative water content, closes stomata and after blocking of stomata will reduce photosynthesis rate. It has been demonstrated that high relative water content is because of more osmotic regulation or less

elasticity of tissue cell wall and is a resistant mechanism to drought (Keyvan, 2010). Among the physiological changes in tomato under drought stress, the widest range (60.88-152.60) was recorded for Leaf Area (cm²) followed by relative water content (RWC) (47.43-74.83%), Electrolyte Leakage (conductivity) (75.78-92.29%) and Ratio root shoot length (0.07-0.41), respectively. Plants tolerate 80% relative water content which is a good criteria for the screening of drought tolerant genotypes. Electrolyte leakage increases imbalance because moisture level was reduced.

Among the antioxidant phytochemicals and osmolyte changes under drought stress the range varied from 10.11 to 49.73 µg/cm² was recorded for chlorophyll content followed by number of Ascorbic Acid 15.66-25.5 mg/100g FLwt, and Carotenoid (58-3.04 mg/100g FLwt), respectively. In osmolytes the widest range was recorded in proline content i.e. 6.32-12.85 µg/g of fresh leaf wt. followed by reducing sugar content i.e. 16.57-20.89 mg/g fresh wt.

Earlier study by Reddy *et al.*, (2013) evaluated nineteen genotypes of tomato composing of sixteen exotic collections and three varieties of tomato, reporting similar findings in respect to ascorbic acid content. Singh *et al.*, (2003) has shown that ascorbic acid content in 11.21 to 53.29 mg/100g in fifteen cultivars of tomato. Similar findings, Sharma *et al.*, (1996) demonstrated that ascorbic acid content ranged from 11.21 to 53.29 mg/100g in 53 genotypes of tomato. The findings are in agreement with Rai *et al.*, (2012).

In mammalian cells, vitamin-C acts as a co-factor for reactions requiring reduced iron and copper metallo-enzymes (Tsao 1997). Considerably high cellular levels of vitamin-C are responsible for antioxidant protection against photosynthetically generated free radicals (Delamere 1996). Moreover, vitamin C plays a role in regenerating other biologically important antioxidants such as glutathione and vitamin E into their reduced state.

The carotenoid content in tomato mainly determines its vitamin activity, thus the tomato cultivars were also evaluated for total carotenoids. Significant variation was recorded in the total carotenoid content amongst the 15 tomato genotypes. The total carotenoid content values recorded in this study confirms those reported by Rai *et al.*, (2012) and Singh *et al.*, (2007) who reported that the total carotenoids values varied from 1.00 to 9.47mg/100g in 40 tomato genotypes. Raffo *et al.*, (2002) reported that the carotenoids content of tomato were very low at the breaker stage (1.08mg/100g), which increased ≥ 10 -fold during ripening and reached 12.705mg/100g at full ripening stage. Abdul-Hammed *et al.*, (2015) demonstrated that the pro-vitamin A index (β -carotene) contents ranged between 0.86 and 4.09 $\mu\text{g/g}$ in Cherry-*Nasmata* while *Var-10* tomatoes showed lower range in terms of pro-vitamin A index (β -carotene).

Dark red pigment of tomato fruits also contains lesser amount of β carotene and other carotenoids. Abdul-Hammed *et al.*, (2015) studied the accumulation pattern of lycopene and beta-carotene in tomato and noted that the maximum concentrations of lycopene (antioxidant index) of 9.42 and 6.68 $\mu\text{g/g}$ were obtained at the Light-red and fully red stages of Cherry-*Nasmata* and *Var-10* tomato cultivars respectively.

Based on the above investigation of morphological characters, yield attributing characters, physiological characters, Antioxidant phytochemicals and osmolytes, the hierarchical cluster was formed which distinguish the 15 genotypes.

Hierarchical cluster analysis was conducted for morphological as well as biochemical traits. Distance between all pairs of genotypes was calculated using Squard Euclidean distance method and genotypes were clustered based on ward's method. Since these clusters are group of individuals possessing similar characters mathematically gathered into the same cluster, these individuals are supposed to exhibit higher

external heterozygosity. Cluster analysis based on morphological and biochemical traits were classified into 2 groups one cluster contain only cultivated genotypes and another cluster contain other 12 mixed wild and cultivated genotypes. Now from the 2 clusters 2 wild and 2 other genotypes were selected for the further experiments. The information obtained through clustering may well assist tomato breeders in identifying a limited number of highly differentiated genotypes to be selected for further use in developing suitable variety. Singh *et al.*, (2007) reported that the similar findings in tomato genotypes on basis of morphological and biochemical traits. Rai *et al.*, (2012) were also reported hierarchical cluster analysis in exotic introductions of chilli (*Capsicum annuum* L.). Hierarchical cluster analysis on the basis of morphological and biochemical variation in pea (*Pisum sativum* L.) was reported by Kalloo *et al.*, (2005).

Using morphological, physiological and biochemical characters, fifteen tomato genotypes were screened against drought for identification of tolerant genotype. Among the 15 genotypes, two genotypes i.e. EC-317-6-1 and WIR-4360 were found tolerant to drought in comparison to others. Kashi Amrit and Kashi Sharad were noted susceptible to drought conditions.

It was found that higher proline level, sugar content, carotenoid, and ascorbic acid maintains optimum photosynthesis and RWC under drought, and allowed plants to grow and maintain optimum physiological activity in water stressed condition.

Conclusively, we found that tomato genotypes could differently enhance their ability to struggle their drought. It is possible that proline, chlorophyll, ascorbic acid, caroteniod and sugar content could be used as the effective mechanisms for drought tolerance in tomato genotypes. Furthermore, results demonstrated that the biochemical and physiological parameters are more useful for the screening of drought tolerance of tomato genotypes.

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Author's Contribution

G.K. Rai has contributed to the experimental conception and design, to the acquisition of data, to the analysis and interpretation of data. Abida Parveena and G.K. Rai have performed most of the experiments and in drafting and revising the manuscript. Abida Parveen, G. K. Rai and Muntazir Mushtaq have contributed to the experiment related to the preparation of phylogenetic tree by using software. G.K. Rai, Abida Parveen, Monica Singh, Muntazir Mushtaq and Ajaz Ahmad Kundoo involved in drafting the manuscript and interpretation of phylogenetic tree. P.K. Rai, R.R. Kumar and S.K. Rai contributed to all related works for data analysis and interpretation of data including statistical analysis. All authors have read and approved the final manuscript.

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