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In vitro Evaluation of Physiological and Biochemical Changes under Drought Conditions in Tolerant and Susceptible Genotypes on Tomato

Pratibha Pandey[1], Ashutosh Rai[2], Major Singh[3] and Sudhakar Prasad Mishra[1*]

[1]Mahatma Gandhi Chitrakoot Gramodaya Vishwavidyalay, Chitrakoot, M. P. - 485334, India
[2]Department of Crop Improvement, Indian Institute of Vegetable Research, Shahanshahpur Jakhini, Varanasi U.P. -221305, India
[3]Directorate of Onion and Garlic Research, Rajguru Nagar, Pune-410505, India

**Corresponding author*

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Drought is the major stress limiting crop productivity and development in arid and semi-arid regions. Tomato genotypes have wide range of sensitivity to drought, two genotypes of tomato H-86 and EC-520061 were used in two different types of field experiment in 2016-2017 at Indian Institute of Vegetable Research, Varanasi. Each experiment was conducted in randomized complete block with three replications. Moisture level of one field was 80% and of another field was reduced to 40%. Several biochemical analyses consisting of various enzymatic and non-enzymatic antioxidant defense system and some factors of oxidative damage were analyzed in two experiment setups. The enzymatic activities Proline content, H_2O_2 and LPO increased in drought stressed condition of tomato plant at flowering stage of plant whereas Carotenoid content and MSI (Membrane Stability Index) decreased in both the genotypes. In drought tolerant genotypes enzymatic and non-enzymatic antioxidants were higher, whereas LPO and H_2O_2 content was low, but in case of drought susceptible genotype trend was reverse.

Introduction

Environmental abiotic stresses, such as drought, extreme temperature, cold, heavy metals, or high salinity, severely impair plant growth and productivity worldwide. Drought being one of the most important abiotic stresses impairs plant growth, development and limits crop productivity (Umezawa *et al.,* 2006). Drought is one of the most significant environmental stress on world agricultural production (Tuberosa and Salvi 2006; Cattivelli *et al.,* 2008). Severe water stress results in the arrest of photosynthesis,

disturbance of metabolism and finally the death of plant (Jaleel *et al.,* 2009). However, in certain tolerant crop plants morpho-physiological and metabolic changes occur in response to drought, which contribute towards adaptation to such unavoidable environmental constraints (Sairam and Sirvastava, 2001, Agarwal *et al.,* 2006). The cultivated tomato (*Solanum lycopersicum* L.) is an important vegetable crop, ranking second to potato in world's vegetables production (Bakht and Khan, 2014). In spite of being grown successfully round the year, the growth and development of tomato crops are affected by number of environmental stresses, such as drought, salinity, and extreme temperatures especially drought has a profound effect on production of tomato around the world (Foolad, 2007). Tomato is sensitive to drought stress at all stages of plant growth and development, but seed germination and early seedling growth are the most sensitive stages to drought and other extreme environmental conditions. Sufficient genetic variation for abiotic stress exists within the cultivated tomato *(S. lycopersicum*) as well as in its related wild species such as *S. habrochaitis*, *S. pimpinellifolium*, and *S. penellii* (Wolf *et al.,*1986) Despite their vast natural variation, rather limited efforts have been devoted to the physiological, genetical and molecular characterization of this variation in tomato to warrant its use for developing drought-tolerant cultivars (Khan *et al.,* 2012; Martin *et al.,* 1999). This contrasts with the considerable amount of research that has been conducted on abiotic stress in relation to other crop species, including rice (*Oryza sativa* L) (Zhang *et al.,* 2001) and lettuce (Johnson *et al.,* 2000).

Drought stress results in stomata closure, which limits CO^{2-} concentration in leaf mesophyll tissue and reduces NADP+ regeneration by the Calvin Cycle. These adverse conditions increase the rate of reactivated oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide (O^{2-}), singlet oxygen ($^1O_2$) and hydroxyl (OH) radicals by enhanced leakage of electrons toward molecular oxygen during photosynthetic and respirator processes (Foyer *et al.,* 1994). These ROS can cause damage to membrane lipids, proteins and DNA leading to cell death (Cadenas, 1989). Plants process very efficient enzymatic (superoxide distumase, SOD; catalase, CAT; Ascorbate Peroxidase, APX; Peroxidase, POD and glutathione reductase, GR) and non-enzymatic (carotenoids, ascorbic acid, glutathione, and proline) antioxidant defense systems which protect cell and sub cellular systems against oxidative damages by scavenging of ROS (Dhindsa *et al.,* 1981; Mittler, 2002) SOD catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide (Alscher *et al.,* 2002). Hydrogen Peroxide can be eliminated by CAT, APX and POD (Asada, 1999; Ramachandra *et al.,* 2004). Carotenoid a lipid soluble antioxidant plays a multitude of functions in plant metabolism including oxidative stress tolerance (Sarvajeet and Narendra, 2010). Accumulation of protective solutes like proline and glycine betaine is a unique plant response to drought stress. Also proline is considered as a potent antioxidant and potential inhibitor of programmed cell death (Bates *et al.,* 1973; Pireivatloum *et al.,* 2010). The objective of the present study was to understand the influence of drought stress on oxidative damage, enzymatic and non-enzymatic antioxidant systems in tolerant and susceptible tomato genotypes and also identify the effective biochemical traits in the screening tolerant genotypes to drought.

Materials and Methods

Plant material and experimental conditions

The tomato genotypes Arka Harit (H-86) and EC-520061 were selected for the present study. EC-520061 was identified as drought

tolerant and genotypes Arka Harit (H-86) as drought susceptible according to preliminary field screening. The genotypes selected for present experiments are widely cultivated in Uttar Pradesh, Bihar, Gujarat, Andhra Pradesh, Rajasthan and Karnataka, States of India.

Experimental design and drought stress treatments

The resistant seedlings of the EC-520061 as well as the susceptible Seedlings of Arka Harit (H-86) (30 ds old) were transplanted to individual earthen pots (22 cm upper diameter, 12 cm lower diameter, and 18 cm high; 5 L volume) filled with a 5:1 soil: farmyard manure mixture at experimental field of Indian Institute of Vegetable Research (IIVR) Varanasi, in 4 sets with 4 pots for each genotype for each set. IIVR is situated at 25.10º N, 82.52º E and 76.1 m exceeding mean sea level in the Eastern Indo-Gangetic plains of India. The soil used in the pot was mixture of sand, loamy clay, and farmyard manure (1:2:1) with bulk density of 1.34 gm cm-3 and to some extent of basic pH (6.8). Soil had 0.39% organic carbon content, 0.30% total nitrogen content, 0.51 mg g-1 and 0.35 mg g-1 available phosphorus and potassium content, respectively. The plants grew in a cultivation chamber under controlled conditions with relative humidity of 50 %, at 25°C/15°C (day/night), and a 16 h/8 h photoperiod with a photon flux density of 350 μmol m−2 s−1. Under these conditions, progressive water-deficit stress treatments began after 50 d of germination when plants were at the late vegetative stage (before flowering), in triplicate, by withholding water for 7, 14, 21, or 28 d. The control treatment (well-watered:0d) was watered daily to receive approximately 80 % field capacity irrigation; whereas 7, 14, 21, or 28 d drought stress corresponded to about 40, 25, 15, or 10 % field capacity soil moisture, respectively (Fig.1 and Fig. 2).

The plants received 1 L water on release of the drought stress. All the experiments were carried out in three replications. Soil moisture was measured according to the formula: SWC (%) = [(FW-DW)/DW] × 100. Where, FW is the fresh weight of the soil portion taken from the pot and DW is the dry weight of the soil portion after drying in hot air oven at 85°C for 4 days (Coombs *et al.,* 1987).

Enzymatic Antioxidant Assays-

Superoxide Dismutase

Superoxide Dismutase (SOD, EC 1.15.1.1) activity was assayed according to Misra and Fridovich (1972). About 200 mg fresh leaf samples were taken from control and drought stressed tomato plants and homogenized in 5 ml of 100 mM potassium-phosphate buffer (pH 7.8), containing 0.1 mM EDTA, 0.1% (v/v) Triton X-100 and 2% (w/v) of soluble polyvinyl pyrrolidone (PVP) using pre-chilled mortar and pestle. Extracts were centrifuged at 22,000 x *g* for 10 min at 4°C and SOD activity was assayed in the supernatant.

The assay mixture contained 50 mM sodium carbonate-bicarbonate buffer (pH 9.8), containing 0.1 mM EDTA, 0.6 mM epinephrine and enzyme in a total volume of 3 ml. Epinephrine was the last component to be added. The adrenochrome formation during the next 5 min was recorded at 470 nm in a UV–Vis spectrophotometer (ELICO, SL-159; India). One unit of SOD activity is defined as the amount of enzyme required to cause 50% inhibition of epinephrine oxidation under the experimental conditions.

Ascorbate peroxidase

Ascorbate Peroxidase (APX; EC 1.11.1.11) activity was measured using the method of Nakano and Asada (1981). The assay mixture contained of 50 mM potassium phosphate buffer (pH 7.0) containing 0.5 mM ascorbic

acid, 0.15 mM H_2O_2, 0.1 mM EDTA, and 50 µL of enzyme extract (supernatant). Ascorbate Peroxidase was spectrophotometrically assayed following a decrease in the absorbance at 290 nm. One unit of APX oxidises 1 mM ascorbic acid in 1 min at 25°C.

Catalase

Catalase (CAT, EC 1.11.1.6) activity was measured by following the reduction of H_2O_2 (ε = 39.4 $mM^{-1}cm^{-1}$) at 240 nm according to the method of Beers and Sizers (1952). The assay solution contained 50 mM potassium phosphate buffer (pH 7.0) and 15 mM H_2O_2. The reaction was started by the addition of 100 µl enzyme extract to the reaction mixture and the change in absorbance was followed 1 min after the start of the reaction. One unit of activity was considered as the amount of enzyme which decomposes 1 mM of H_2O_2 in one minute.

Guaiacol Peroxidase

Guaiacol Peroxidase (GPX, EC 1.11.1.7) activity was assayed according to (Egley *et al.,* 1983). Leaf samples (~200 mg) were homogenized using chilled mortar and pestle in 5 ml of 60 mM sodium phosphate buffer (pH 7.0) at 4°C.

The homogenates were centrifuged at 22,000×g for 10 min and after dialysis in cellophane membrane tubings, the supernatant served as enzyme preparation. Assay mixture in a total volume of 2.5 ml contained 40 mM sodium phosphate buffer (pH 6.0), 2 mM H_2O_2, 9 mM guaiacol and the reaction was initiated by adding 50 µl enzyme extract. Increase in absorbance was measured at 470 nm (extinction coefficient of 26.6 mM^{-1} cm^{-1}) up to 5 min and enzyme specific activity was expressed as µmol of H_2O_2 reduced min^{-1} mg^{-1} protein.

Non-enzymatic Antioxidants Assay

Proline

The content of Proline was extracted and determined by the method of (Bates *et al.,* 1973). Leaf tissues (0.5 g) were homogenized in 3 % sulfosalicylic acid and the homogenate was centrifuged at 3,000×g for 10 min. The supernatant was treated with acetic acid and ninhydrin, boiled for 1 h, and then the absorbance was determined at 520 nm. Proline concentration was calculated with a standard curve and expressed as $\mu molg^{-1}$ fresh mass.

Carotenoid

The amount of carotenoid (Car) was determined according to (Lichtenthaler and Wellburn.,1983). Leaf tissues (0.5g) were homogenized in acetone (80%). Extract was centrifuged at 3,000×g and absorbance was recorded at 646.8 nm and 663.2 nm for chlorophyll assay and 470 nm for Car determine by spectrophotometer. Car and Pigments content were calculated due to the following formulae:

Chl a = (12.25 A663.2– 2.79 A646.8)

Chl b = (21.21 A646.8 – 5.1 A663.2)

Car = (1000 A470 – 1.8 Chl a – 85.02Chl b)/198

Oxidative Damage Assay

Hydrogen peroxide (H_2O_2) measurement

The H_2O_2 levels in leaf samples from NT and T tomato plants were measured in leaf samples from plants exposed to heat stress at 42^oC for 1 hour under controlled conditions of heat stress (Jana and Choudhari., 1982). For extraction of H_2O_2 about 200 mg tissues were homogenized in 5 ml of 50 mM phosphate

buffer (pH 6.5) and centrifuged at 7000 x *g* for 20 minutes. To 3 ml of the supernatant, 1 ml of 0.1% titanium sulphate in 20% H_2SO_4 was added. The mixture was then centrifuged at 7000 x *g* for 15 minutes. The intensity of the yellow color developed was measured spectrophotometrically (ELICO, SL-159, India) at 410 nm. The amount of H_2O_2 was calculated using an extinction coefficient of 0.28 μM^{-1} cm^{-1} and expressed as µmol g^{-1} fresh weight.

Lipid peroxides (malondialdehyde) measurement

Lipid peroxidation was measured as malondialdehyde content by Thiobarbituric acid (TBA) reaction according to the method given by (Heath and Packer., 1968). Approximately 400 mg of leaf sample was crushed in in 0.25% 2-thiobarbituric acid (TBA) in 10% TCA using a mortar and pestle. After heating at 95°C for 30 min, the mixture was quickly cooled in an ice bath and centrifuged at 10,000xg for 10 min. The absorbance of supernatant at 532 nm was noted and the correction for unspecific turbidity was done by subtracting the absorbance of the same at 600 nm. The blank was 0.25% TBA in 10% TCA. The concentration of lipid peroxides together with oxidatively modified proteins of leaf tissue were thus quantified in terms of MDA content using an extinction coefficient (ϵ) of 155 mM^{-1} cm^{-1} and expressed as nmol g^{-1} fresh weight.

Membrane Stability Index measurement

Electrolyte leakage was determined by using a conductivity meter (CM-180, Elico, India) according to the operating instructions. Ten leaf discs of equal size from fully expanded fresh leaf were placed in 25 ml deionized water. The conductivity of the water was assessed after keeping for a 15-min vacuum filtration (VF), and thereafter autoclaving at 121°C for 30 min (AW). The EL was calculated by the equation: EL (%) = (VF/AW) ×100.

Results and Discussion

Enzymatic Antioxidants Defense Response

The results of the present study showed that considerable variations among genotypes for antioxidant activity were observed when grown under drought stress and non-stress conditions (Table 3). Peroxidase (POD) activity increased significantly ($P<0.01$) under water stress condition. POX is one of the major enzymes that have a role in the biosynthesis of lignin and defense against water stress by scavenges H_2O_2 in chloroplasts (Mittler, 2002; Sarvajeet and Narendra., 2010). The highest POD activity were observed in genotypes EC-520061 (drought tolerant), and the lowest activity in H-86 (susceptible) under water stress condition. The result clearly shows that POD is the suitable indicator for drought resistance. Water stress tolerance is closely related with POD activities, the similar activity was observed in wheat genotypes by (Shao *et al.,* 2005).

Catalase

Catalase enzyme has the highest turnover rate among all the enzyme.CAT is a heme containing enzyme with potential to directly dismutase H_2O_2 into H_2 and O_2 (Gill and Tuteja; 2010) and is indispensable for ROS detoxification during stressed conditions (Asada 1999; Sairam and Srivastava 2001) The activity of catalase enzyme CAT increased up to 14 days of water deficit in the both H-86 as well EC-520061 plants, and thereafter a gradual decrease was observed (Fig. 2). At 7 day of water deficit stress, as compared to WT plants, a 1.5-fold ($P \leq 0.05$) increase was noted in transgenic tomato lines which later increased to 2 folds ($P \leq 0.01$) at

14 d of stress. (Sánchez-Rodríguez *et al.,*2010) reported increased CAT activity in cherry tomato under drought stress, especially in tolerant varieties. (Hsieh *et al.,*2002) characterized higher expression of *CATALASE1* (*CAT1*) gene in response to heterologous over-expression of *AtCBF1* in transgenic tomato plants. Due to the *CAT1* expression, catalase activity increased, and H_2O_2 concentration decreased in constitutively over-expressing *AtCBF1*/*DREB1B* transgenic tomato plants compared with the wild-type plants with or without water deficit stress (Hsieh *et al.,*2002). In the case of *B. campestris*, a simultaneous activation of SOD and CAT is required for enhanced oxidative stress tolerance and enhancement of either SOD or CAT activity had only a minor effect (Tseng *et al.,* 2007). In addition, (Fazeli *et al.,*2007) observed that the SOD and CAT activities were higher in water stress tolerant sesame cultivars.

APX (Ascorbate Peroxidase)

APX is thought to play most significant role in scavenging of ROS and prokaryotic cells in all plants (Nakano and Asada; 1981). APX has a higher affinity for H_2O_2 than CAT and POD and plays a crucial role in management of ROS during stress (Gill and Tuteja 2010). Both drought tolerant and drought susceptible genotypes under well-watered conditions did not differ significantly in APX specific activity. The activity of enzyme APX in H-86 and EC-520061 increased significantly up to 14 d of water-deficit, and thereafter a sharp decline was noted. At 7 days of water deficit EC-520061 plant showed a (85%) 7 days and (260%) 14 days increase in comparison to this H-86 at 7 days shows (71%) which goes up to (195%) at 14 days of stress. Enhanced expression of APX in many plants has been demonstrated during different stress conditions (Sharma and Dubey 2005; Carvalho 2008) including the drought tolerant tomato lines (Sánchez-Rodríguez *et al.,* 2010; Rai *et al.,* 2012b) as well as *AtDREB1A* over expressing transgenic peanut plants (Bathnagar-mathur *et al.,* 2009).

Superoxide Dismutase (SOD)

The activity was influenced by water stress. SOD activity increased in the H-86 and EC-520061 plants of tomato were comparable under well-watered conditions. But under water deficit, the EC-520061 lines exhibited significantly higher SOD activities than those of WT plants (Fig. 3); the magnitude of this increase became progressively higher with the duration of moisture stress (57% at 7 d, 141% at 14 d, and 326% at 21 d in resistant lines). The SOD activity was the highest on 14 d of stress in EC-520061 plants. Efficient destruction of O_2^- and H_2O_2 in plant cells requires the highest concentrated action of antioxidants. SOD detoxifies superoxide anion which is harmful to the membrane, chloroplasts, nucleic acid and proteins. CAT, POD and APX eliminate H_2O_2 (Alscher *et al.,* 2002; Amjad *et al.,* 2011). Thus higher SOD activity in tolerant genotype compared to susceptible can be explained that susceptible genotypes had less efficient system in O_2^- scavenging under drought condition.

Non- Enzymatic Defence Response

The chlorophyll and carotenoid pigments are involved in harvesting light energy in plants (Tzvetkova-Chevolleau *et al.,* 2007) and their content is related to plant drought tolerance (Saglam *et al.,* 2011). The carotenoid play fundamental roles and help plants to resist drought stress (Jaleel *et al.,* 2009).

During stress condition in tomato, EC-520061 genotypes have higher carotenoid content than the H-86 genotype in all drought treatments as well as well watered plants, in EC-520061 the carotenoid percentage range 92.8-79.5%, after

21 days the value goes to 74.5%, while in H-86 the carotenoid value is 71.2-66.0% in 7 to 21 days of water withdrawal condition. Water deficit stress reduces the tissue concentrations of chlorophylls and carotenoids (Havaux 1998; Kiani *et al.,* 2008), primarily due to the production of ROS in the thylakoids (Reddy *et al.,* 2004).

Proline

Accumulation of Proline solutes in cytoplasm improves uptake of water during drying of soil and thus it plays a significant role in maintaining leaf turgidity in plant (Alonso *et al.,* 2001). Proline is the most widely studied (Singh *et al.,* 2011) because of its considerable importance in the stress tolerance in many species including tomato (Sánchez-Rodríguez *et al.,* 2010; Shukla *et al.,* 2012; Chandra *et al.,* 2004).

Proline can also have various other functions under stress conditions, such as an eliminator of free radicals, scavenger of ROS, a buffer of the cellular redox potential, an important component of cell-wall proteins (Nanjo *et al.,* 1999; Hare and Cress 1997; Singh *et al.,* 2011). In the present experiment, we analyzed concentration of proline in the susceptible and tolerant tomato plants subjected to the drought treatments. In our study, accumulation of proline was significantly increased under drought stress conditions. (Fig. 4) shows that the EC-520061 tolerant tomato plants kept accumulating the proline with increasing severity of drought stress, and presented 2–6 fold higher values of this amino acid in comparison to the corresponding WT plants during the drought stress treatments.

The elevated proline level in drought resistant EC-520061 tomato lines contributed to cellular osmotic balance and enhanced drought tolerance. These data substantiated with those authors who indicate that the expression of *AtCBF3*/*DREB1A* gene in *Arabidopsis* transgenic (Gilmour *et al.,* 2000), *AtCBF1*/*DREB1B* gene in tomato (Singh *et al.,* 2011; Hsieh *et al.,* 2002), and *OsDREB2A* in rice (Cui *et al.,*2011) results in elevated proline accumulation and thus greater water-deficit tolerance. (Hsieh *et al.,* 2002a) reported up to seven–fold increases in proline concentration under water-deficit stress in *CBF1* over-expressing transgenic tomato plants. (Geravandi *et al.,* 2011) reported accumulation of proline varies with degree of drought tolerance in wheat. (Pireivatlum *et al.,*; 2010) reported that proline is an important osmolytes to adjust the plant under drought condition.

Oxidative Damage

In drought stressed plant, overproduction of Reactive Oxygen Species leads to oxidative damage in plants. (Cadenas *et al.,*; 1989) Sairam and Sirvastava (2001) observed that much of the injury to plants caused by water stress exposure is associated with oxidative damage at the membrane cell. In the experiment conducted plants showed a higher accumulation of H_2O_2 under water stressed condition.

During well-watered condition ROS value of Drought resistant and drought susceptible were almost similar. H_2O_2 concentration increased in both susceptible and resistant during water stressed condition. In H-86 H_2O_2 concentration was comparatively higher than EC-520061.

The value increased to during 7-21 days of stress. In EC-520061 a gradual increase of (121.01 %) at 7days to (146.5 %) at 21d over the well-watered condition was recorded for drought resistant plant in comparison to this higher increase of (149.24%) at 7d to (240.4%) at 21d was registered in drought susceptible plants.

Fig.1 Tolerant plants (EC-520061) in 0, 7, 14, 28 days of drought stress.

Fig.2 Susceptible plants (Arka Harit) in 0, 7, 14 and 28 days of drought stress

Fig.3 Effect of water stress on Catalase activity in tomato genotype. The data are mean of three replicates ±SE. Asterisks represent significantly different values in transgenic lines relative to WT plants; *P≤ 0.05, and **P ≤ 0.01.

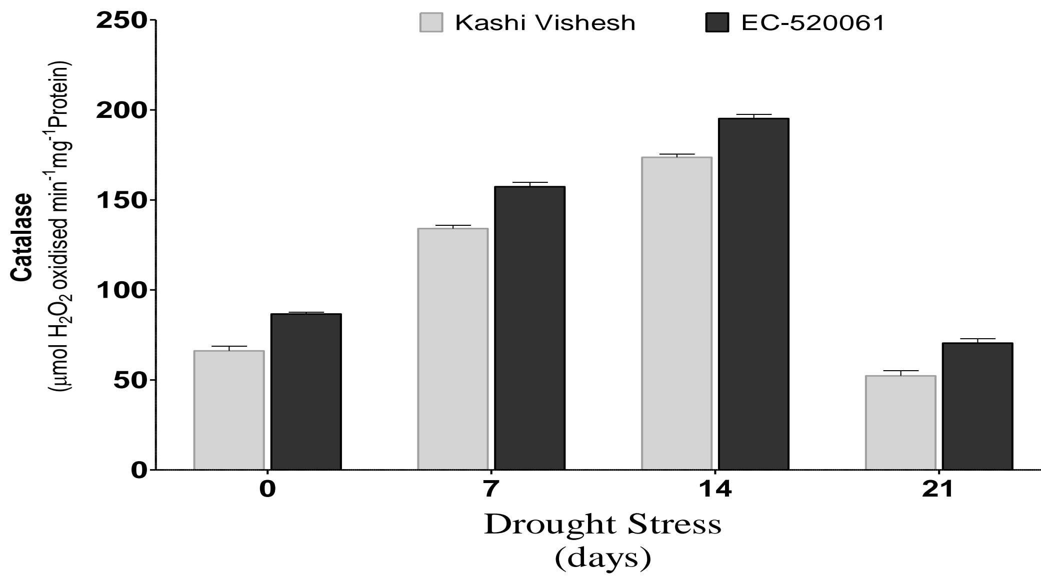


Fig.4 Effect of Water Stress on Superoxidise Dismutase Activity in tomato genotype. The data are mean of three replicates ±SE. Asterisks represent significantly different values in transgenic lines relative to WT plants; *P≤ 0.05, and **P ≤ 0.01.

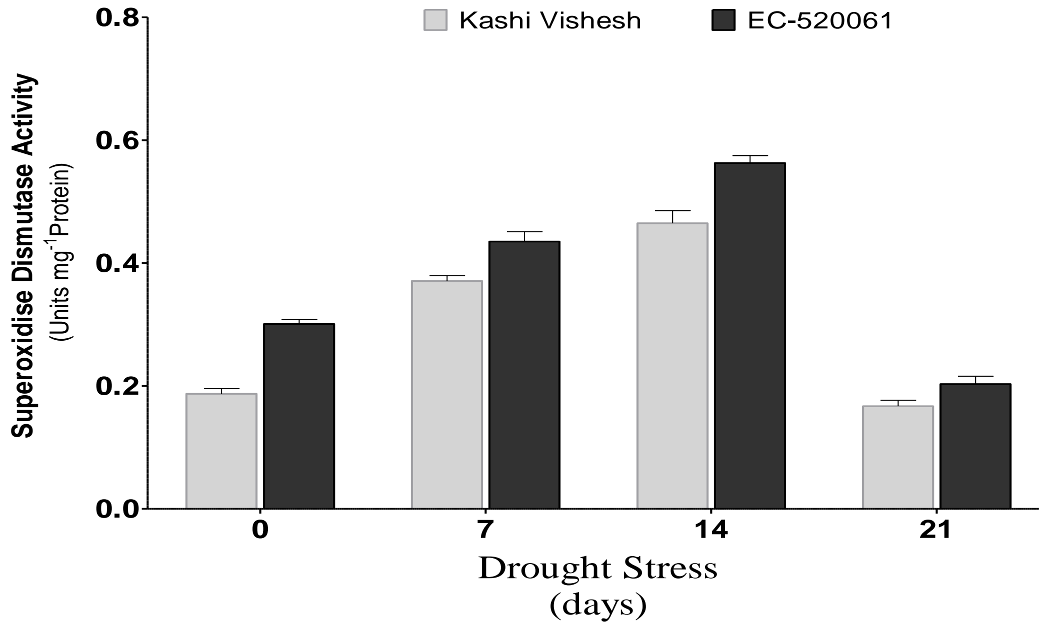


Fig.5 Effect of Drought stress on Proline Activity in tomato genotype The data are mean of three replicates ±SE. Asterisks represent significantly different values in transgenic lines relative to WT plants; *P≤ 0.05, and **P ≤ 0.01.

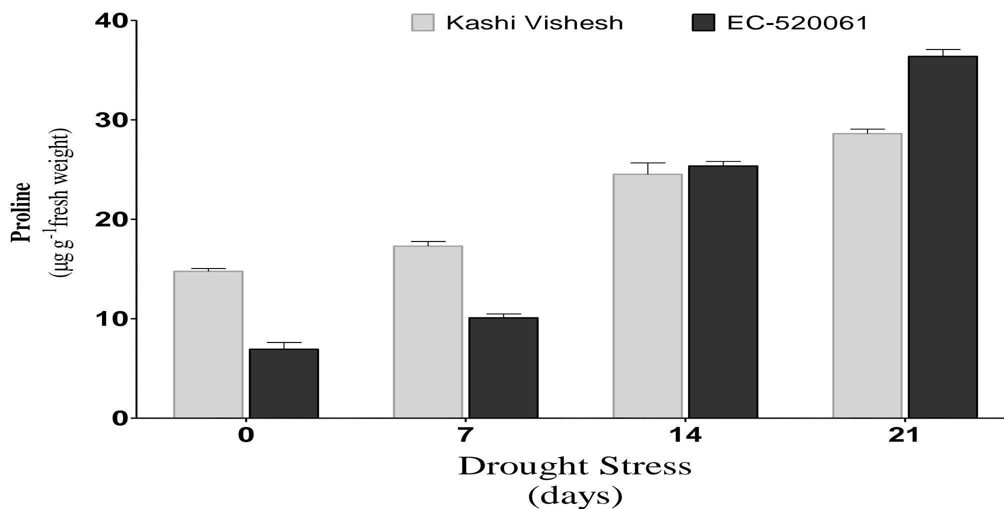
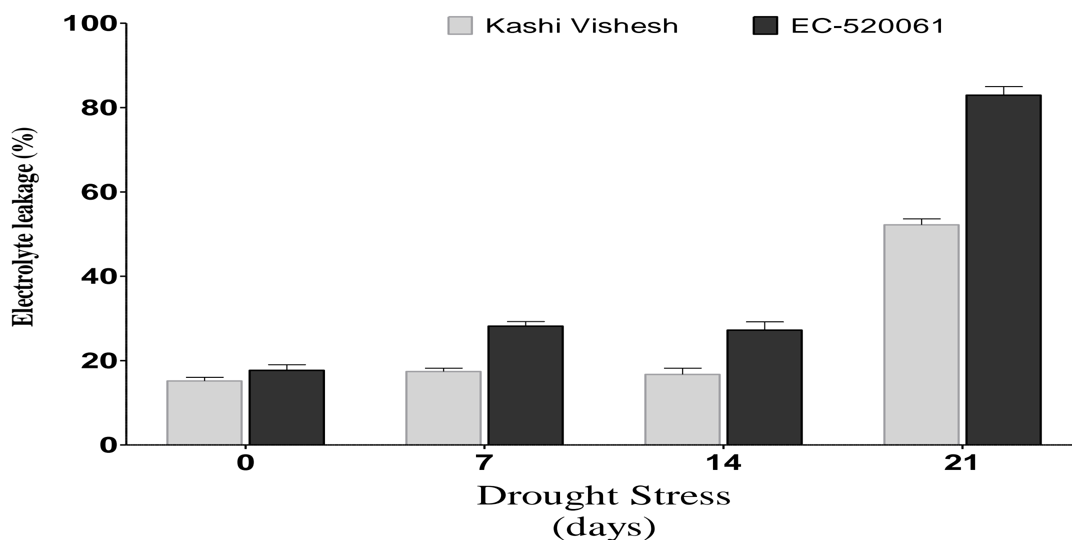


Fig.6 Effect of Drought stress on Membrane Stability Index in tomato genotype The data are mean of three replicates ±SE. Asterisks represent significantly different values in transgenic lines relative to WT plants; *P≤ 0.05, and **P ≤ 0.01.



Lipid Peroxidation (LPO)

Lipid Peroxidation (LPO) is considered as the most damaging factor in all organisms in various stress condition (Renu and Devarishi; 2007). LPO is determined as malondialdehyde content. LPO has been associated with damages provoked by a variety of environmental stresses (Hernandez *et al.*,; 2003). Poly unsaturated fatty acids (PUFA) are the main membrane lipid components susceptible to peroxidation and degradation (Hernandez *et al.,* 2001). It has been observed that during water stress condition LPO level increased significantly in all genotypes. In general, during the increasing days of drought stress treatments, a strong increase in MDA production was observed in H-86 plants, but only a slight increase was seen in the EC-520061 plants. The significant increase was observed at 21 days of stress in H-86 i.e., drought susceptible plant. The increase in LPO can be correlated with the accumulation of ions and active oxygen species (AOS) production under salt stress (Smirnoff *et al.*,; 2000). Increased LPO as a result of oxidative stress and consequent cell injury have been reported by several workers (Zlatev et a.,2006; Turkan *et al.,* 2005; Amjad *et al.,* 2011) The level of LPO, in case, indicates the extent of stress tolerance as reported by (Bor *et al.,* 2003) in sugar beet and wild beet under NaCl treatment.

Membrane stability index

Membrane damage is considered as the most important parameter for estimating the level of lipid destruction during various stress conditions. The extent of cell membrane damage caused by water stress can be simply anticipated through measurements of electrolyte leakage from the cell (Sarvajeet and Narendra, 2010; Ahmadizadeh *et al.,* 2011).Membrane stability index (MSI) decreased significantly during water logged condition it also show a foremost decline in susceptible genotype i.e. H-86 (Figure 4). A decrease in membrane stability reflected the extent of lipid peroxidation caused by reactive oxygen species (Sairam and Sirvastava, 2001). In Figure 5, the lowest MSI were observed in

H-86 and highest in EC-520061. In this link it has been reported that drought stress tolerant genotypes were superior to susceptible ones in maintaining membrane stability and lower LPO under drought stress condition (Amjad *et al.,* 2011). Higher MSI and antioxidant activity, and lower LPO and H_2O_2 have been reported in drought tolerant genotypes of wheat (Renu and Devarshi, 2007), bean (Zlatev *et al.,* 2006) and Mulberry (Ramachandra *et al.,* 2004) (Fig. 6).

The result showed that genotypes have different responses towards the oxidative damage due to variation in their defense mechanism. During water stressed conditions CAT, POD, APX, SOD, Proline content, H_2O_2 and LPO increased significantly while carotenoid decreased.

Drought tolerant genotypes having lowest membrane damage and H_2O_2 content have highest membrane stability index (MSI) and have highest enzymatic antioxidant activity CAT, POD, APX and SOD and non-enzymatic antioxidants Proline and Carotenoid. While drought susceptible genotype showed the lowest antioxidants defends and MSI and highest H_2O_2 and MDA content. From the experiment conducted it has been found that enzymatic antioxidants had played more significant role then non enzymatic antioxidants in plant protection against oxidative damage.

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