Pak. J. Bot., 47(4): 1231-1239, 2015.

PROTECTION OF PHOTOSYNTHETIC MACHINERY BY UP-REGULATION OF ANTIOXIDANT ENZYMES IN CONTRASTING TOMATO GENOTYPES UNDER DROUGHT

FAKHRA SHAMIM[1*], KHALIDA KHAN[2], HABIB-UR-REHMAN ATHAR[3] AND ABDUL WAHEED[4]

[1,2]*Centre for Integrated Mountain Research, University of the Punjab, Lahore, Pakistan*
[3]*Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan, Pakistan*
[4]*COMSATS Institutes of Information Technology, Sahiwal, Pakistan*
[*]*Corresponding author e-mail: fakhra_dr@yahoo.co.uk*

Abstract

Current study was designed to evaluate the drought effect on some physiological and biochemical properties of tomato plants. Some native and exotic tomato genotypes were subjected to drought stress to investigate the effect on antioxidant enzymes and photosynthetic machinery. The tomato genotypes were exposed to different water regimes viz: 80, 60 and 40% of field capacity. Statistical analysis revealed significant interactions in some physiological parameters including transpiration rate (*E*), photosynthetic rate (*A*) and stomatal conductance (g_s). Drought stress enhanced the above properties in tolerant varieties like '*L. pennellii*', '*L. chilense*', 'Lyallpur-1' and 'CLN1767' in contrast to rest of the water stress sensitive genotypes. Moreover, same type of significant elevations were also observed when antioxidant enzymes like catalase (CAT), superoxide dismutase (SOD) and peroxidase (POD) were caloremitrcially quantified in drought tolerant tomato varieties. Overall, it was found that some tomato genotypes maintained their degree of water stress tolerance during their growth but with varying mechanism of water stress tolerance. Moreover, the above mentioned physiological and biochemical characteristics can act as valuable markers for selection and breeding programs for development of drought tolerant tomato genotypes.

Key words: Drought tolerance, *Solanum lycopersicum*, Stomatal conductance, Antioxidant enzymes.

Introduction

Plants tend to set a plethora of adaptation strategies such as longer duration physiological and metabolic alterations for sustainable growth and to cope with a variety of environmental stresses like drought (Farooq *et al*., 2009; Ashraf, 2010; Suzuki *et al*., 2014). However, photosynthesis is one of the key phenomenon, which contributes substantially to plant development and growth but this process, in fact, can be adversely affected by water deficit conditions which may have been caused by **1)** reduce light harvesting efficiency or less utilization efficacy of harvested light, **2)** decrease in activity or concentration of the enzyme; Rubisco, or **3)** due to reduction in stomatal conductance (g_s) or less availability of CO_2 at its fixation site (Athar & Ashraf, 2005; Ashraf, 2010; Foyer & Shigeoka, 2011; Ashraf & Harris, 2013). Hence, one of the prime factors assumed to decrease photosynthetic rate (*A*) is less availability of CO_2 in the mesophyll tissues due to stomatal closure thus leakage of electrons during photosynthesis (Fig. 1) ultimately leads to generation of reactive oxygen species (ROS) like singlet oxygen and hydrogen peroxide (Golding & Johnson, 2003; Johnson, 2011). However, plants have developed a complex shielding mechanism to ameliorate the damage initiated by ROS whereas, antioxidant enzymes system like peroxidase (POX), catalase (CAT), superoxide dismutase (SOD) breaks down the cellular concentration of ROS (Smirnoff, 1993; Carvalho, 2008; Foyer & Shigeoka, 2011). It has been reported that under water deficit conditions most of the injury is linked to oxidative damage at the cellular level while, SOD, POX and CAT are considered as the most effective antioxidant enzymes in averting cellular damage. Higher activities of these enzymes have also been reported in wild and cultivated tomato genotypes which suggest that the drought tolerant or wild tomato genotypes like *L. pennellii* are better protected against ROS than the relatively sensitive plants of the cultivated species (Shalata & Tal, 1998; Barbagallo *et al*., 2012; Shamim *et al*., 2013a; Martinez *et al*., 2014; Kavitha *et al*., 2014; Mittova *et al*., 2015). Tomato (*Solanum lycopersicum* L., syn. *Lycopersicon esculentum* Mill.) belongs to the family Solanaceae and ranked as second main vegetable crop after potato (Tigchelaar, 1986; Passam, 2008). In Pakistan, tomatoes have been grown over about 53.1 thousand hectares with an average yield of about 10.1 tons ha^{-1} (Fig. 2) but these statistics are far below than average yield being achieved in many other countries of the world (Anon., 2008). Although, considerable research efforts have been made for the production of vegetables in rainfed zones to feed increasing population, but these efforts have been significantly hampered due to complexity of genes under drought. Furthermore, there are many physiological and biochemical characteristics which contribute to the drought tolerance of horticultural crops like tomato (Rahman *et al*., 2004; Nakuja *et al*., 2012; Shamim *et al*., 2013b; Kavitha *et al*., 2014) but in the current scenario of climatic irregularities it is important to screen large number of tomato genotypes for their water stress tolerance or must be exploited for their cultivation under water deficit situation (Shamim *et al*., 2014b; Sivakumar *et al*., 2014). Besides, providing an evidence for the pivotal role of antioxidant enzymes for protection of photosynthetic machinery, the study aims also to screen large number of tomato genotypes to facilitate their screening and selection in breeding program for drought tolerance.

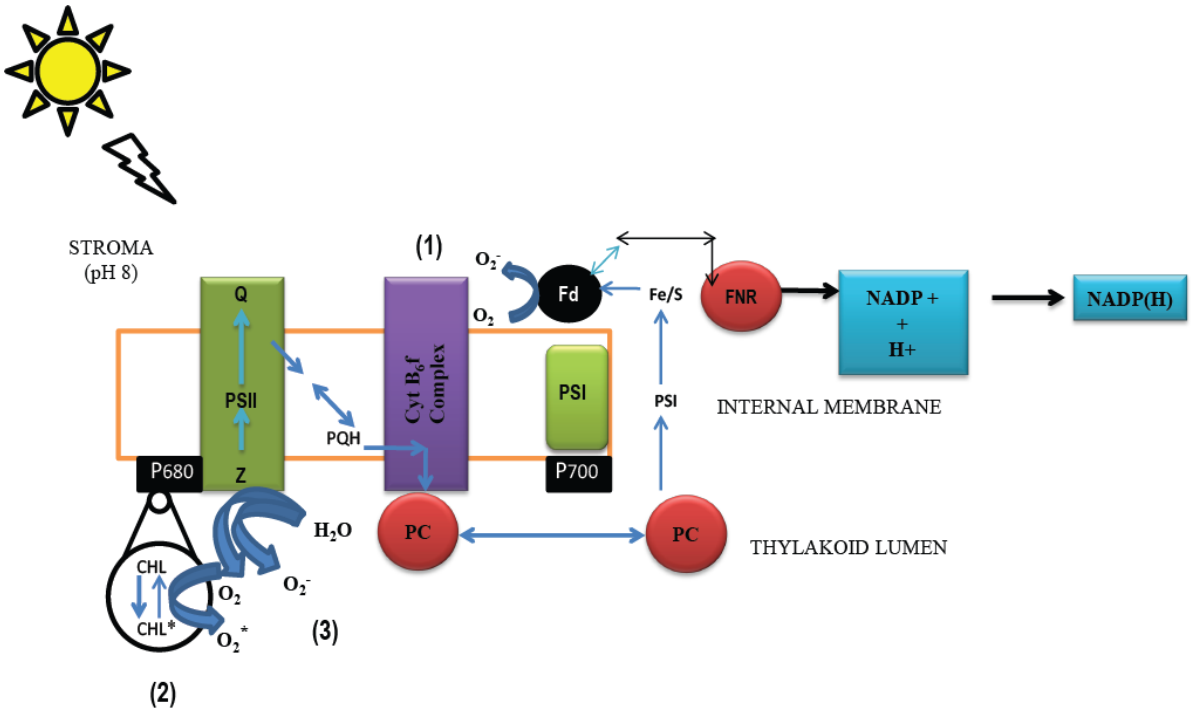


Fig. 1. Schematic representation of the electron transport system in the thylakoid membrane showing three possible sites of activated oxygen production (ROS). **(1)** During Mehler reaction that is linked with PSI. This path is ideal if there is drop in electron acceptors of PSI **(2)** ROS formation is through contact of triplet excited chlorophyll with molecular oxygen in the PSII antenna **(3)** ROS might originate at the oxidizing side of PSII due to splitting of water (PS II = Photosystem II; PS II = Phptosytem I; CHL= chlorophyll; PC= plastocyanin; Fd = Ferrodoxin; FNR= Ferredoxin-$NADP^+$ reductase).

Materials and Methods

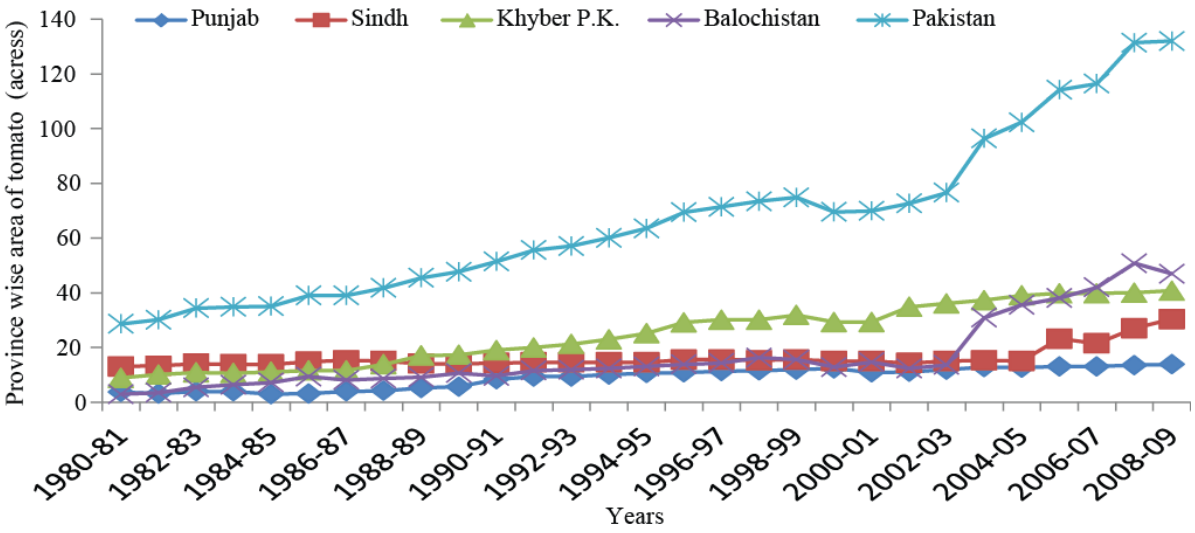
From the set of 120 tomato genotypes used in one of the study (Shamim *et al.*, 2014b) a subset of 11 genotypes was selected to perform the current study (Table 1). Tomato nursery was transplanted in pots containing 6 kg normal potting mixture (sand, well rotten farmyard manure and soil in 1:2:3 ratio) under drought stress (60 & 40% field capacity) and control condition (80% field capacity) and regularly monitored (Mahmood-ul-Hasan, 2001) by employing ThetaProbe (Delta-T Devices, Ltd., England) at the National Agriculture Research Center (NARC), Islamabad, Pakistan (33.40° N and 73.07° E; 683 meter above sea level) under rain shelter to prevent tomato crop from rain water (Fig. 3). Lay out for experiment was complete block design thru randomization (RCBD) with three water stress treatments and three replications. Water stress treatments were started when the seedlings were five weeks old.

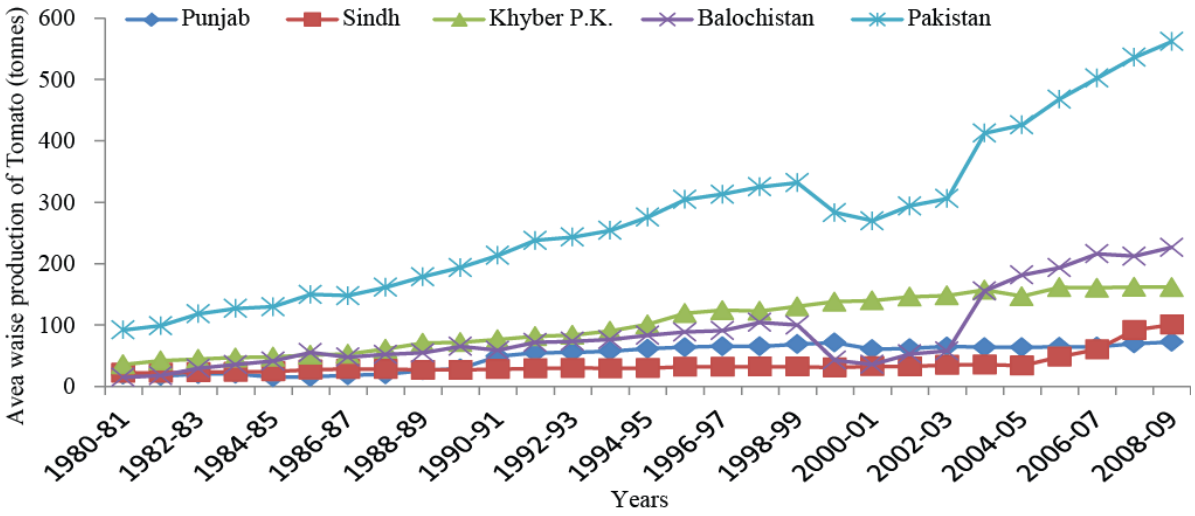
Gas exchange parameters: Measurement of net CO^2 assimilation rate (*A*), transpiration rate (*E*), and stomatal conductance (*gs*), were made on the youngest fully emerged leaf (usually 3rd leaf from top) of each plant by using an open system LCA-4 ADC portable infrared gas analyzer (Analytical Development Company, Hoddeson, England) between 10:00 to 14.00 hours. As a pre-caution the measurements of control plants were immediately noted followed by that of the same cultivated under water stress conditions.

Procedure specified by Bradford (1976) was followed for the measurement of total soluble proteins. Fresh leaves samples (0.2 g) were homogenized in sodium phosphate buffer solution (4 mL) centrifuging followed by homogenization at 6000 × g for 10 minutes. The extract was treated with Bradford reagent and total soluble proteins were analyzed spectrophotometrically at 595 nm. Bovine serum albumin (BSA) worked as a standard.

Extraction of enzyme: Antioxidant enzymes extraction was carried out by grinding the leaves (0.5 g) in (5 mL) cooled phosphate buffer (50 mM) by using pestle and mortar (pH = 7.8) and adding 1.0 g Polyvinylpolypyrrolidone (PVPP) and (0.5%) Triton X-100 in the mixture. The homogenate was centrifuged at 15000 × *g* for 20 min (4°C). For enzymes assay, supernatant was used.

Activity of SOD was estimated by quantifying its capability to inhibit the photoreduction of (NBT) nitroblue tetrazolium (Giannopolitis and Ries, 1977). Reaction solution had 50 µM NBT (3 mL), 13 mM methionine, 1.3 µM riboflavin, 50 mM phosphate buffer (pH 7.8), 75 nM EDTA and 50 µL of enzyme extract. This solution in test tubes was irradiated for 15 min at 78 µmol m^{-2} s^{-1}. The absorbance of solution was measured by using spectrophotometer (Shimadzu, UV-160U Japan) at 560 nm besides, one unit of SOD activity, which inhibits 50% of NBT photoreduction, is equivalent to the quantity of enzyme.





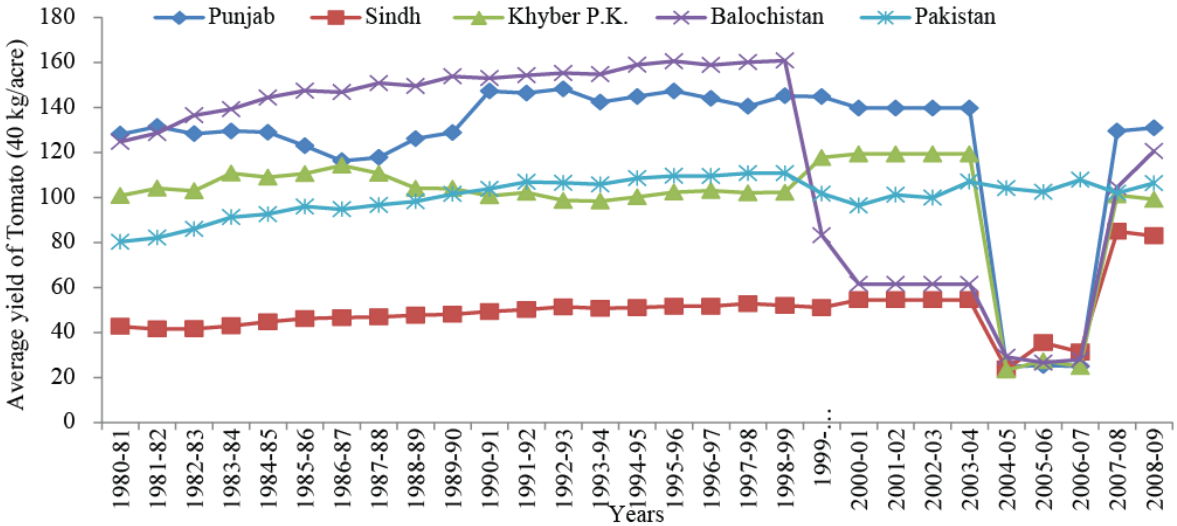


Fig. 2. Province wise (**a**) area of tomato production (acre) (**b**) average production (tonnes) and (**c**) average yield (40 kg/acre) of tomato crop in Pakistan from 1980-81 to 2008-2.

Table 1. Selected 11 tomato genotypes from a subset of 120 genotypes, ranked in four groups viz. tolerant, moderately tolerant, moderately sensitive and sensitive.

Taxon	Accessions	Ranking
L. pennellii	LA0716	Tolerant
L. Chilense	LA0458	Tolerant
Lyallpur-1		Tolerant
CLN1767		Tolerant
10584/G		Moderately tolerant
Punjab Chuhara	017865	Moderately tolerant
Ailsa Craig	LA2711	Moderately sensitive
Pusa Ruby	017860	Moderately sensitive
Roma	006233	Moderately sensitive
Avinash-2	017867	Sensitive
Ratan	017870	Sensitive

Fig. 3. Pots were arranged in triplicate and water stress treatments (80, 60 and 40% of field capacity) were started when the seedlings were five weeks old, stress was maintained throughout the experiment till the fruiting stage at National Agriculture Research Center, Islamabad/Pakistan.

Catalase and peroxidase activities were recorded after some modification in the procedure given by Chance & Maehley (1955). The catalase reaction solution (3 mL) contain enzyme extract (0.1 mL) 50 mM phosphate buffer (pH = 7.0) and 5.9 mM H_2O_2. Enzyme extract was added to initiate the reaction. After 20 second intervals at 240 nm, differences in absorbance of solution were recorded. One unit catalase activity is the absorbance change of 0.01 units per minute. The peroxidase reaction solution (3 mL) had 50 mM phosphate buffer (pH = 5.0), 40 mM H_2O_2, 20 mM guaiacol, and 0.1 mL enzyme extract. After every 20 seconds at 470 nm, variations in absorbance of solution were recorded. Activity for one unit of peroxidase is equivalent to the absorbance change of 0.01 units per minute.

Statistical analysis of the data: Data for each recorded variable was analyzed statistically through analysis of variance using STATISTICA version 7 software (StatSoft, Inc, OK, USA). Comparison of average values was undertaken through least significance difference (*lsd*) test as described by Snedecor and Cochran (1980).

Results

Effect of different levels of water stress on photosynthetic and antioxidant activities of tomato were studied in water stress tolerant and susceptible genotypes. Significant decrease ($p \leq 0.001$) was recorded due to water stress for transpiration rate, photosynthetic rate and stomatal conductance of all genotypes. Water stress tolerant wild genotype *L. pennellii* followed by

water stress tolerant Lyallpur-1 and *L. chilense* showed maximum net CO_2 assimilation rate (*A*) at 40% field capacity moisture regime. Although water stress sensitive genotypes Roma, Avinash-2, and Ratan were the lowest in photosynthetic rate at the highest water stress level, water stress tolerant CLN1767 was also the lower in this gas exchange attribute at the same water stress level but showed more transpiration rate (Table 2). Generally, at the highest water stress level, water stress tolerant genotypes were lower in their transpiration rate whereas, water stress sensitive genotypes were higher in their transpiration rate (Table 2). At the highest water stress (40% field capacity) maximum values for stomatal conductance (g_s) were found in water stress tolerant genotypes (*L. pennellii*, *L. chilense*, Lyallpur-1 and CLN1767 while, water stress sensitive genotypes (Pusa Ruby, Roma, Avinash-2 and Ratan) were found lower in their stomatal conductance (Table 3). Nevertheless, at maximum stress level (40% of field capacity), water stress tolerant genotypes had greater WUE except in CLN1767, where it was lower than the other genotypes. Similarly, at the same water stress level, water stress sensitive genotypes had minimal WUE except in Pusa Ruby who had WUE similar to water stress tolerant genotypes (Table 3). A greater reduction in total soluble proteins was observed in water stress sensitive tomato genotypes. Water stress tolerant *L. pennellii* followed by *L. chilense* and Lyallpur-1 was the highest in total soluble proteins of all genotypes at 40% of field capacity, whereas water stress genotypes sensitive Roma and Avinash-2 show lowest tolerance (Table 4). Significant increase in activities of superoxide dismutase (SOD) was recorded in tomato genotypes at 60% and 40% field capacities except in *L. pennellii*, Avinash-2 and Ratan where it decreased significantly (Table 4). In addition, water stress did not affect the superoxide dismutase (SOD) activity in *L. pennellii*. Genotype Pusa Ruby and *L. chilense* had greater SOD activity in the leaves of water stressed plants than in the other genotypes, whereas the reverse was true for *L. pennellii* at the same moisture stress level. Conversely, *L. chilense*, Lyallpur-1 and CLN1767 exhibited enhanced activity of peroxidase (POD), whereas it decreased in water stress sensitive or moderately water stress tolerant genotypes (Table 5). Genotype CLN1767 and Lyallpur-1 had maximum activities of POD in water stressed plants, whereas the Ailsa Craig was the lowest in POD activity at the highest moisture stress level. Soil water stress induced at adult stage of tomato resulted in significant boost for concentration of catalase. In water stress sensitive genotypes, catalase (CAT) activity only increased at moderate level of water stress and at higher water deficit conditions it decreased considerably (Table 5). The antioxidant activity of CAT was higher in moderately water stress tolerant Punjab Chuhara compared with the other genotypes at 40% field capacity. Although water stress tolerant genotypes had greater activities of CAT but the wild relative of tomato *L. pennellii* was the lowest in CAT activity under water stress conditions. Moreover, water stress sensitive genotypes (Ratan, Roma and Avinsah-2) were also lower in CAT activity values at 40% field capacity.

Table 2. Photosynthetic rate and Transpiration rate of 40 days old plants of 11 selected tomato genotypes at levels of stress.

Genotypes	Photosynthetic rate (*A*)			Transpiration rate (*E*)		
	Moisture stress (fraction of field capacity)					
	80%	60%	40%	80%	60%	40%
	mmol/m^2/s			mmol/m^2/s		
L. pennellii	11.08 a	8.83 a	8.00 a	2.53 d	1.95 d	0.71 ef
L. chilense	8.53 d	6.86 d	5.55 c	1.04 h	0.45 h	0.61 f
Lyallpur-1	9.00 c	7.33 c	6.00 b	1.53 g	0.95 g	0.61 f
CLN1767	5.70 h	4.27 h	3.80 g	3.53 a	4.89 a	1.72 b
10584/G	6.40 g	4.73 g	3.80 g	3.33 b	2.75 b	2.01 a
P. Chuhara	7.00 f	5.33 f	4.10 f	2.52 d	1.95 d	1.25 c
Ailsa Craig	8.50 d	6.83 d	5.00 d	1.72 f	1.15 f	0.82 e
Pusa Ruby	8.00 e	6.33 e	4.55 e	1.42 g	0.88 g	0.62 f
Roma	8.60 d	6.93 d	3.00 i	2.92 c	2.46 c	1.62 b
Avinash-2	9.30 b	7.63 b	3.50 h	1.92 e	1.72 e	1.12 d
Ratan	9.03 c	6.33 e	3.50 h	1.92 e	1.60 e	1.32 c

LSD 5% = 0.12; LSD 5% = 0.126

Means in each column with similar letters (a-g) did not differ significantly at $p< 0.05$ level

Table 3. Stomatal conductance and Water Use Efficiency of 40 days old plants of 11 selected tomato genotypes at levels of stress.

Genotypes	Stomatal conductance			Water use efficiency		
	Moisture stress (fraction of field capacity)					
	80%	60%	40%	80%	60%	40%
	mmol $m^{-2}s^{-1}$			*A/E*		
L. pennellii	300.00 d	250.00 b	225.00 a	4.38 d	4.52 e	11.31 a
L. chilense	300.00 d	225.00 e	200.00 d	8.21 a	15.16 a	9.14 c
Lyallpur-1	288.00 h	225.00 e	200.00 d	5.89 b	7.70 b	9.94 b
CLN1767	300.00 d	248.67 c	223.00 b	1.62 f	1.48 h	2.22 h
10584/G	299.00 f	250.00 b	225.00 a	1.92 f	1.72 h	1.89 h
P. Chuhara	299.33 e	205.00 f	180.00 e	2.78 e	2.73 g	3.47 f
Ailsa Craig	300.00 d	235.00 d	210.00 c	4.95 c	5.93 d	6.12 e
Pusa Ruby	290.00 g	195.00 g	150.00 h	5.64 b	7.15 c	7.42 d
Roma	315.00 c	205.00 f	180.00 e	2.95 e	2.82 g	1.86 h
Avinash-2	330.00 b	195.00 g	170.00 f	4.85 c	4.44 e	3.13 f
Ratan	340.00 a	270.00 a	151.33 g	4.71 cd	3.95 f	2.67 g

LSD 5% = 0.326; LSD 5% = 0.36

Means in each column with similar letters (a-g) did not differ significantly at p< 0.05 level

Table 4. Soluble proteins and Superoxide dismutase of 40 days old plants of 11 selected tomato genotypes at levels of stress.

Genotypes	Soluble proteins			Superoxide dismutase		
	Moisture stress (fraction of field capacity)					
	80%	60%	40%	80%	60%	40%
	mg/g F.wt			units/g F. wt		
L. pennellii	3.59 bc	3.44 a	3.43 a	22.38 h	22.59 g	22.80 i
L. chilense	3.50 c	3.37 a	3.17 b	45.22 a	52.19 b	61.99 b
Lyallpur-1	3.57 bc	3.16 b	3.09 b	39.22 bc	45.19 c	48.99 d
CLN1767	3.80 a	2.90 c	2.49 c	40.12 b	45.09 c	50.80 c
10584/G	3.67 ab	2.70 d	2.10 d	35.42 d	50.89 b	46.80 e
P. Chuhara	3.17 d	2.35 ef	1.89 e	32.42 e	65.23 a	47.03 e
Ailsa Craig	2.37 f	2.50 e	1.69 f	24.22 g	25.23 f	26.03 h
Pusa Ruby	3.17 d	2.28 f	2.00 de	28.22 f	32.23 e	65.03 a
Roma	2.87 e	2.20 fg	1.30 g	27.22 f	63.73 a	38.33 f
Avinash-2	2.37 f	2.11 g	1.28 g	35.72 d	34.42 d	27.03 gh
Ratan	2.97 e	2.19 fg	1.60 f	38.22 c	35.22 d	28.03 g

LSD 5% = 0.16; LSD 5% = 1.58

Means in each column with similar letters (a-g) did not differ significantly at p< 0.05 level

Table 5. Peroxidase and Catalase of 40 days old plants of 11 selected tomato genotypes at levels of stress.

Genotypes	Peroxidase			Catalase		
	Moisture stress (fraction of field capacity)					
	80%	60%	40%	80%	60%	40%
	units/g F. wt			units/g F. wt		
L. pennellii	60.22 g	110.19 c	112.00 e	25.22 e	32.19 i	35.99 h
L. chilense	81.22 e	80.19 e	130.00 c	20.22 f	40.19 g	69.99 c
Lyallpur-1	60.22 g	99.19 d	150 b	40.22 c	60.19 f	74.99 b
CLN1767	71.22 f	77.19 e	165.80 a	42.22 c	70.52 d	68.80 c
10584/G	85.22 e	55.19 f	75.00 h	49.22 ab	65.19 e	65.99 d
P. Chuhara	108.22 c	76.23 e	70.03 h	51.22 a	88.23 a	90.03 a
Ailsa Craig	45.22 h	40.23 g	30.03 j	30.22 d	35.23 h	40.03 fg
Pusa Ruby	150.22 b	113.23 c	89.03 g	48.22 b	78.23 c	46.03 e
Roma	180.22 a	150.23 a	120.0 d	47.22 b	81.23 b	42.03 f
Avinash-2	175.22 a	144.23 b	99.03 f	40.22 c	71.23 d	45.03 e
Ratan	99.22 d	75.23 e	64.03 i	41.22 c	69.23 d	39.03 g

LSD 5% = 5.38; LSD 5% = 4.27

Means in each column with similar letters (a-g) did not differ significantly at p< 0.05 level

Discussion

The most important requisite for water stress tolerance regarding selection and breeding needs genetic variability in gene pool of the species. To achieve this target it is necessary to improve the tolerance of the specie. Hence, in order to devise a selection strategy for stress resilience in species, understanding of genetic variability is of prime importance. Outcomes of the current experiment revealed that considerable genetic variation exist among tomato genotypes. The findings of the current research showed that significant decrease occurred in photosynthetic rate due to water stress in all tomato genotypes which is in conformity with the reports on tomato (Makela *et al*., 1999; Srinivasa *et al*., 2000; Shamim *et al*., 2013a). In one of our previous study (Shamim *et al*., 2014a) we have reported a positive correlation between photosynthetic rate and water stress tolerance in terms of biomass or yield production (shoot dry weight vs *Ar* = 0.617***). This indicates that genotypic difference to under water stress condition might have been due to variations in photosynthetic rate. For instance, at the maximum water stress, all sensitive genotypes exhibited the lowest photosynthetic rate whereas water stress tolerant wild genotype *L. pennellii* and Lyallpur-1 showed greater photosynthetic rate. A strong correlation has also been observed in photosynthetic rate and stomatal conductance (*A* vs. g_s *r* = 0.705***) which depicts that water stress significantly decreased rate of photosynthesis in all 11 genotypes, which occurred predominantly due to stomatal closure. It is in agreement with a number of earlier studies (Medrano *et al*., 2002; Flexas *et al*., 2002; Raza *et al*., 2006; Ulfat *et al*., 2007). Certain genotypes did not bear this relationship under water stress conditions. For instance, water stress tolerant genotype CLN1767 was lower in '*A*', which is similar to water stress sensitive genotypes Roma, Ratan and Avinash-2 but CLN1767 was higher in 'g_s' under water deficit conditions. From these results and findings it can be suggested that under water deficit conditions, stomatal conductance is not the solitary feature triggering variation in photosynthesis among tomato genotypes. Though transpiration rate decreased in tomato genotypes with an increase in levels of moisture stress but most of water stress tolerant genotypes were lower in transpiration rate than in water stress sensitive genotypes hence by considering only gas exchange trait, the tomato genotypes cannot be categorized. This argument can be further supported by the data that water stress tolerant genotype CLN1767 had greater transpiration rate than all water stress sensitive genotypes at the highest moisture stress. The higher water use efficiency of genotypes *L. pennellii, L. chilense* and Lyallpur-1 was generally because of their comparatively lower transpiration rate. Consequently, water stress tolerance in tomato genotypes was observed to be partially linked with WUE (Wahab-Allah *et al*., 2011). It has been suggested earlier that to improve drought tolerance of tomato, WUE is a desirable character (Cao *et al*., 2007). Hence, photosynthetic capacity could be utilized as a good selection criterion for screening tomato germplasm under drought condition (Runkulatile *et al*., 1993; Kiani *et al*., 2007). However, such type of association was not observed when individual tomato genotypes having different level of water stress tolerance were evaluated with respect to their photosynthetic rate. For example, water stress tolerant genotype CLN1767 in terms of high dry biomass and high fruit yield (Shamim *et al*., 2014a) was similar in photosynthetic rate to water stress sensitive genotypes Ratan and Avinash-2. This is clearly suggests that genetic variability in photosynthetic rate of tomato genotypes is not the sole reason for differential water stress tolerance in tomato genotypes, but due to some other biological processes controlling growth and yield (Rahman *et al*., 2004).

Furthermore, the results of current research also indicate a marked difference in total soluble protein content of drought stressed plants as compared to normal irrigated plants. A decreased level of total soluble proteins in water stressed leaf tissues appeared due to more degradation of proteins as well as due to overall inhibition in protein synthesis under drought (Hsiao, 1973) Similar results has also been observed in mung bean (Kumar and Singh, 1991), wild tomato (Kavitha *et al*., 2014) and in field grown tomato (Chamseddine *et al*., 2009; Barbagallo *et al*., 2012). Obtained data revealed that concentrations of SOD, POD and CAT increased in water stress tolerant tomato genotypes, whereas activities of these enzymes in water stress sensitive and moderately water stress tolerant genotype decreased, or remained unchanged. The water stress sensitive Pusa Ruby was the highest in SOD activity at the highest water stress and water stress tolerant *L. pennellii* was the lowest in SOD activity at the highest moisture stress. These results differ to what earlier has been recorded in wheat by Zaefyzadeh *et al*. (2009) who reported that increase in SOD activity is correlated with degree of drought tolerance. Similarly, in present study water stress sensitive genotype Roma had greater or similar POD activity than water stress tolerant genotypes *L. pennellii* and *L. chilense* at the highest moisture stress. Parallel results were also demonstrated by other researchers (Zgallai *et al*., 2006; Zheng *et al*., 2010) that activities of SOD and POD increased with increasing levels of water stress in water stress tolerant species. Likewise, CAT activity was the highest in moderately water stress tolerant Punjab Chuhara compared with the other genotypes at 40% field capacity but water stress tolerant wild genotype *L. pennellii* was the lowest in CAT activity under water deficit conditions. Similarly, drought stress tolerant genotypes of tomato were also reported with greater POD and CAT activity than water stress sensitive genotypes (Zgallai *et al*., 2004; Unyayar, 2005; Ismail and Phizackerley, 2009). Hence, Better protection of photosynthetic machinery in water stress tolerant genotypes may be attributed to higher antioxidant capacity, therefore the tolerance of genotype Lyallpur-1 and CLN1767 under water deficit situation proves that during the scavenging of ROS, the activity of antioxidant enzymes enhanced.

Conclusively, on the basis of the results obtained it has been observed that antioxidative enzymatic system; one of the important components of the mechanism of drought tolerance in crops; not only differs among species but also with in cultivars of a single species. However, considerable genetic variation exists in tomato germpalsm explored in the current study hence genotypes CLN1767, Lyallpur-1 were water stress tolerant due to having better

photosynthetic capacity and partially greater antioxidant enzymes. The obtained result suggest that photosynthetic adeptness with scavenging enzymes could be utilized as a potential indicator selection criterion for screening and breeding as well as for monitoring environmental stresses like drought in field grown horticultural crops like tomato.

Acknowledgements

The support of British Council Pakistan and the financial support of Higher Education Commission (HEC) of Pakistan is gratefully acknowledged. Tomato germplasm was kindly provided by Plant Genetic Resource Institute, NARC, Islamabad, Pakistan and Tomato Genetic Resource Center (TGRC) California, USA which is also highly acknowledged.

References

Anonymous. 2008. Generally accepted agriculture and management practices for irrigation water use. Draft for Public Rev. May. Agric. Irrig. Water Use, GAAMP Task Force, Michigan. *Comm. Agric*.

Ashraf, M. 2010. Inducing drought tolerance in plants: Recent advances. *Biotechnol. Adv*., 28: 169-183.

Ashraf, M. and P.J.C. Harris. 2013. Photosynthesis under stressful environments: an overview. *Photosynthetica*, 51(2): 163-190.

Athar, H.R. and M. Ashraf. 2005. Photosynthesis under drought stress. In: Hand Book Photosynthesis, 2nd (Ed.) by M. Pessarakli. *C. R. C. Press*, New York, USA., 795-810.

Barbagallo, R.N., I.D. Silvestro and C. Patane. 2012. Yield, physicochemical traits, antioxidant pattern, polyphenol oxidase activity and total visual quality of field-grown processing tomato cv. Brigade as affected by water stress in Mediterranean climate. *J. Sci. Food Agric*., 93: 1449-1457.

Bradford, M.M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. *Anal. Biochem*., 72: 248-254.

Cao, H.X., Z.B. Zhang, P. Xu, L.Y. Chu, H.B. Shao, Z.H. Lu, and J.H. Liu. 2007. Mutual physiological genetic mechanism of plant high water use efficiency and nutrition use efficiency. Rev. *Colloids Surf. B. Biointerfaces*., 57: 1-7.

Carvalho, M.H.C. 2008. Drought stress and reactive oxygen species. Production, scavenging and signaling. *Plant Signal Behav*., 3(3): 153-155.

Chamseddine, M., B.A. Wided, H. Guy, C. Marie-Edith and J. Fatma. 2009. Cadmium and copper induction of oxidative stress and antioxidative response in tomato (*Solanum lycopersicon*) leaves. *Plant Growth Regul*., 27: 89-99.

Chance, B., and A. Maehley. 1955. Assay of Catalases and Peroxidases, *Meth. Enzymol*., 2: 764.

electron transport during drought. *Planta*., 218:107-114.

Farooq, M., A. Wahid, N. Kobayashi, D. Fujita and S.M.A. Basra. 2009. Plant drought stress: effects, mechanisms and management. *Agron. Sustain. Dev*., 29: 185-212.

Flexas, J., J. Bota, J.M. Escalona, B. Sampol and H. Medrano. 2002. Effects of drought on photosynthesis in grapevines under field conditions: an evaluation of stomatal and mesophyll limitations. *Funct. Plant Biol*., 29(4): 461-471.

Foyer, C.H. and S. Shigeoka. 2011. Understanding oxidative stress and antioxidant functions to enhance photosynthesis. *Plant Physiol*., 155: 93-100.

Giannopolitis, C.N. and S.K. Ries. 1977. Superoxide dismutase. I. Occurrence in higher plants. *Plant Physiol*., 59: 309-314.

Golding, A.J. and G.N. Johnson. 2003. Down-regulation of linear and activation of cyclic

Hsiao, T.C. 1973. Plant responses to waters stress. Annul. Rev. *Plant Physiol*., 24: 519-570.

Ismail, M.R. and S. Phizackerley. 2009. Effects of partial root zone and controlled deficit irrigation on growth, yield and peroxidase activities of tomatoes (*Lycopersicon esculentum* Mill.). *Inter. J. Agric. Res*., 4(1): 46-52.

Johnson, G.N. 2011. Physiology of PSI cyclic electron transport in higher plants. *Biochim. Biophys. Acta*., 1807: 384-389

Kavitha, P., K.S. Shivashankara, V.K. Rao, A.T. Sadashiva, K.V. Ravishanka and G.J. Sathish. 2014. Genotypic variability for antioxidant and quality parameters among tomato cultivars, hybrids, cherry tomatoes and wild species. *J. Sci. Food Agric*. 94(5): 993-9.

Kiani, S.P., P. Talia, P. Maury, P. Grieu, R. Heinz, A. Perrault, V. Nishinakamasu, E. Hopp, L. Gentzbittel, N. Paniego and A. Sarrafi. 2007. Genetic analysis of plant water status and osmotic adjustment in recombinant inbred lines of sunflower under water treatments. *Plant Sci*., 172: (4)773-787.

Kumar, P.K. and R.A. Singh. 1991. Germination and metabolism in susceptible and tolerant mung bean genotypes under moisture stress. *Indian J. Plant Physi*., 34: 267- 269.

Mahmood-ul-Hasan, M. 2001. Soil moisture measurement by soil impedance measuring meter: laboratory calibration and field evaluation. *Pak. J. Sci. Ind. Res*., 45(3): 145-150.

Makela, P., M. Kontturib, E. Pehua and S. Somersaloa. 1999. Photosynthetic response of drought and salt-stressed tomato and turnip rape plants to foliar-applied glycinebetaine. *Physiol. Plant*., 105: 45-50.

Martinez, J.P, A. Antunez, H. Araya, R. Pertuze, L. Fuentes, X.C. Lizana and S. Lutts. 2014. Salt stress differently affects growth, water status and antioxidant enzyme activities in *Solanum lycopersicum* and its wild relative *Solanum chilense*. *Aust. J. Bot*., 62(5): 359-368.

Medrano, H., J.M. Escalona, J. Bota, J. Gulias and J. Flexas. 2002. Regulation of photosynthesis of C_3 plants in response to progressive drought: stomatal conductance as a reference parameter. *Ann. Bot*., 89: 895-905.

Mittova, V., M. Volokita and M. Guy. 2015. Antioxidative systems and stress tolerance: Insight from wild and cultivated tomato species. *Signal. Commun. plants*., 23: 89-131.

Nakuja, T., D.B. Sarpong, J.K.M. Kuwornu and A. Felix. 2012. Water storage for dry season vegetable farming as an adaptation to climate change in the upper east region of Ghana. *Afric. J. Agric. Res*., 7(2): 298-306.

Passam, H. 2008. The fruiting species of the Solanaceae. *European J. Plant Sci*., 2: (1): 1-2.

Rahman, S.M.L., W.A. Macky, E. Nawata, T. Sakuratani, A.S.M.M. Uddin and B. Quebedeaux. 2004. Superoxide dimutase and stress tolerance of four tomato cultivars. *Hort. Sci*., 39(5): 983-986.

Raza, S.H., H.R. Athar and M. Ashraf. 2006. Influence of exogenously applied glycinebetaine on the photosynthetic capacity of two differently adapted wheat cultivars under salt stress. *Pak. J. Bot*., 38 (2): 341-351.

Runkulatile, H.H.H., J.O. Nyabund and P.O. Ayiecho. 1993. Relationship between leaf water status and variety adaptation of beans to wet and dry agricultural zones of Kenya. *East Afr. Agric. For. J*., 58: 95-104.

Shalata, A. and M. Tal. 1998. The effect of salt stress on lipid peroxidation and antioxidants in the leaf of the cultivated tomato and its wild salt tolerant relative *Lycopersicon pennellii. Physiol. Plantarum*., 104(2): 169-174.

Shamim, F., G.N. Johnson, S.M.S. Naqvi and A. Waheed. 2013a. Higher Antioxidant Capacity Protects Photosynthetic Activities as Revealed by Chl '*a*' Fluorescence in Drought Tolerant Tomato Genotypes. *Pak. J. Bot*., 45(5): 1631-1642.

Shamim, F., H.R. Athar and A. Waheed. 2013b. Role of osmolytes in degree of water stress tolerance in tomato. *Pak. J. Phytopath*., 25(1): 37-42.

Shamim, F., K. Farooq and A. Waheed. 2014a. Effect of different water regimes on biometric traits of some tolerant and sensitive tomato genotypes. *J. Animal and Plant Sci*., 24(4): 1178-1182.

Shamim, F., S.M.S. Naqvi, H.R. Athar and A. Waheed. 2014b. Screening and selection of tomato genotypes/cultivars for drought tolerance using multivariate analysis. *Pak. J. Bot*., 46(4): 1165-1178.

Sivakumar, R., D.D. Devi, C.N. Chandrasekar, R. Santhi and R.M. Vijaykumar. 2014. Impact of drought on gas exchange and physiological parameter and yield in contrasting genotypes of tomato. *Ind. J. Plant Physiol*., 19(1): 1-7.

Smirnoff, N. 1993. The role of active oxygen in the response of plants to water deficit and desiccation. *New Phytol*., 125: 27-58.

Snedecor, G.W. and S. Cochran. 1980. Statistical Methods. 7th edition. Iowa State University Press, Ames, 507 pp.

Srinivasa, R.N.K., R.M. Bhatt and A.T. Sadashiva, 2000: Tolerance to water stress in tomato cultivars. *Photosynthetica*, 38: 465-467.

Suzuki, N., R.M. Rivero, V. Shulaev, E. Blumwald and R. Mittler. 2014. Abiotic and biotic stress combinations. *New Phytol*., 203: 32-43.

Tigchelaar, E.C. 1986. Tomato Breeding. Breeding Vegetable Crop. The AVI Publish. Co. Inc. Westport, USA.

Ulfat, M., H.R. Athar, M. Ashraf., N.A. Akram and A. Jamil. 2007. Appraisal of physiological and biochemical selection criteria for evaluation of salt tolerance in canola (*Brassica napus* L.). *Pak. J. Bot*., 39: 1593-1608.

Unyayar, S., Y. Keles and F.O. Cekic. 2005. The antioxidative response of two tomato species with different tolerances as a result of drought and cadmium stress combinations. *Plant Soil Environ*., 51(2): 57-64.

Wahab-Allah, M.A., A.A. Alsadon and A. Ibrahim. 2011. Drought tolerance of several tomato genotypes under greenhouse conditions. *World Appl. Sci. J*., 15(7): 933-940.

Zaefyzadeh, M., R. Quliyev, S.M. Babayeva and M.A. Abbasov. 2009. The Effect of the interaction between genotypes and drought stress on the superoxide dismutase and chlorophyll content in durum wheat landraces. *Turk. J. Biol*., 33: 1-7.

Zgallai, H., K. Steppe and R. Lemeur. 2004. Water stress induces changes in the proline content and up-regulates the activities of antioxidant enzymes in tomato leaves. *Commun. Agric. Appl. Biol. Sci*., 69(2): 27-28

Zgallai, H., K. Steppe and R. Lemeur. 2006. Effects of different levels of water stress on leaf water potential, stomatal resistance, protein and chlorophyll content and certain antioxidant enzymes in tomato plants. *J. Integ. Plant Biol*., 48(6): 679-685.

Zheng, L., Z. Xin, K.S. Amandeep and G. Liwei. 2010. Effects of exogenous abscisic acid on yield, antioxidant capacities and phytochemical contents of greenhouse grown lettuces. *J. Agric. Food Chem*., 58: 6503-6509.

(Received for publication 5 June 2014)