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HIGHER ANTIOXIDANT CAPACITY PROTECTS PHOTOSYNTHETIC ACTIVITIES AS REVEALED BY CHL *A* FLUORESCENCE IN DROUGHT TOLERANT TOMATO GENOTYPES

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Abstract

Drought is the most important factor limiting growth and yield of tomato. Genetic improvement in tomato for water stress tolerance is of prime importance for economically and efficient utilization of arid area land resources. Since photosynthetic efficiency and antioxidant capacity are associated with degree of water stress tolerance in tomato genotypes, experiment was conducted to assess relationship between plant antioxidant capacity and activity of photosynthetic apparatus. Fifteen tomato genotypes differing in their drought tolerance were subjected to different levels of PEG_{8000} (Control, 5%, 10% & 15%) at the seedling stage. It was concluded that water stress tolerant tomato genotypes (CLN-1767 and Lyallpur-1) also maintain relatively higher photosynthetic efficiency as assessed through *A/Ci* curve or PSII efficiency. Chlorophyll fluorescence measurements revealed that NPQ increased whereas the electron transport rate decreased under waters stress. Water stress tolerant tomato genotypes down regulate ETR with increase in NPQ to avoid photoinhibition and photodamge. Protection of photosynthetic machinery in water stress tolerant genotypes might have been due to higher antioxidant capacity. Water stress tolerant cultivars exhibited much lower lipid peroxidation, and showed increased activities of the enzymes involved in the ROS scavenging system. Up-regulation of the antioxidant system plays a role in water stress tolerance of tomato.

Introduction

Water stress is one of the most important abiotic stresses reducing crop productivity in the world (Reichstein *et al*., 2013). Water stress greatly affects the photosynthetic capacity of plants; a vital physiological process controlling growth of plants (Athar & Ashraf, 2005; 2009). Assessment of photosynthesis could help in understanding the mechanism of water stress tolerance in plants as other drought tolerance indices are being used such as carbon isotope discrimination, osmotic adjustment and plant water status (Kauser *et al*., 2006; Baker, 2008). It has been documented that stomatal limitation resulted in lower availability of CO_2 (low *Ci*) in order to accomplish metabolism. Hence, decrease in photosynthetic rate is due to decreases in CO_2 concentration in intracellular spaces of leaf. Increased CO_2 ultimately results in increased RuBP carboxylation at the expense of oxygenation so photosynthetic rate is increased. In contrast non-stomatal limitation is metabolic impairment/limitation due to loss of ATP (decrease ATP synthesis by the enzyme ATP synthase) in chloroplast (Lawlor & Cornic, 2002). Responses of photosynthetic rate to CO_2 can be Rubisco limited (photosynthetic ETR increases with increase in CO_2), RuBP regeneration limited (photosynthetic ETR did not change with CO_2) or TPU (Triose phosphate use) limited (if fluorescence indicated ETR fell with increased CO_2) (Sharkey *et al*., 2007). It has been well reported that under water stress PSI and PSII exhibit dissimilar response. Genty *et al*. (1990) found that decrease in the efficacy of PSII is directly coupled with decline in assimilation of carbondioxide. Reduced PSII efficiency due to decrease in electron transport chain safeguards plant from damages that may likely happen at PSII reaction centers. Besides non-photochemical quenching, a photoprotective energy dissipation process activates as an alternate strategy due to declining of PSII. Under water deficit conditions carbon fixation is hampered resulting in down regulation of electron transport chain in order to meet the reduced need of electrons. For plants protection from ROS at this phase cyclic electron transport switches on due to increase in Δ pH of thylakoid membranes as well as increase in NPQ (Golding & Johnson, 2003).

Derivation of reactive oxygen species which is also energy demanding may have been through three sites. Firstly via water-water cycle (Mehler reaction) that is found to be linked with PSI. In case of drop in electron acceptors of PSI this route is favored. Second route of ROS formation is through contact of triplet excited chlorophyll with molecular oxygen in the PSII antenna (Golding & Johnson, 2003). Lastly, ROS might originate at the oxidizing side of PSII due to splitting of water. Besides plant scavenge and protect them from oxidative damage by producing various enzymes and antioxidants. Hence, plants lean towards an alternative strategy which is less energy demanding as well as safeguard for ROS. Stepien and Johnson (2009) provided ample evidence that plants achieve this target by regulation of electron transport chain which impedes ROS generation. They also opined that plastid terminal oxidase (PTOX) works as alternate electron sink for the regulation of electron transport chain in stress tolerant plants. Nevertheless, in view of the evidence from some recent reports it is also suggested that production of ROS is also hampered in stress tolerant plants by regulating carbondioxide assimilation and stomatal conductance. Plant scientists and physiologists have suggested various selection criteria for drought tolerance but direct and better usage of chlorophyll fluorescence technique for screening and selection of stress tolerant crops has been reported by many workers (Genty *et al*., 1989; Maxwell & Johnson, 2000; Baker & Rosengvist, 2004; Oguntimehin, 2010; Roostaei *et al*., 2011). This technique has been used to estimate quantum efficiency of electron transport chain

through PSII in leaves because PSII is related to assimilation of carbondioxide (Genty *et al*., 1990; Maxwell & Johnson, 2000). Similarly, another very attractive tool is analysis of *A/Ci* curve for the determination of photosynthetic rate under specific set of experimental and environmental conditions (Farquhar *et al*., 1980; Manter & Kerrigan, 2004). Behind many physiological models of plants, the response function stands for mechanistic basis (Harley *et al*., 1992; Manter *et al*., 2003). Hence, *A/Ci* curves during water stress have proved a practical tool to evaluate the relative photosynthetic limitations (Farquhar & Sharkey, 1982; Sharkey *et al*., 2007). In vision of this cited information, the idea of the present experiment was to explore main stomatal and metabolic factors involved in photosynthetic activities under water stress in tomato genotypes differing in water stress tolerance.

Materials and Methods

Plant growth and tolerance: Tomato (*Lycopersicon esculentum* L.) germplasm was supplied by TGRC, USA. Plants were grown at 20°C in growth chamber and 150 µmol $m^{-2}s^{-1}$ light with 16 hour photoperiod. Seeds germination was done by standing in moderate light in plastic trays with full-strength Hoagland's nutrient solution (Hoagland & Arnon, 1950). After 2 weeks, the seedlings were treated with various levels of water stress 0 (control), 5%, 10% and 15% PEG_{8000} in Hoagland's nutrient solution for 2 weeks. Chlorophyll fluorescence and gas exchange were measured on expanded and youngest leaves as follows:

Gas exchange measurements: An infra-red gas analyzer (IRGA) (CIRAS-1; PP Systems, Herts., UK) was used for gas exchange measurements. The first leaves of the three individual plants were fixed into the cuvette of IRGA. Controlled external CO_2 concentration was supplied to the leaf and assimilation rate *A* (µmol m^2 s^{-1}), concentration of internal CO_2 and *Ci* (2000 ppm) were estimated as describe by von Caemmerer and Farquhar (1981).

Chlorophyll fluorescence measurements: A PAM 101 fluorometer along with a 101-ED emitter-detector unit (Walz) was used for chlorophyll fluorescence measurements. Saturating pulses of light were provided by a Luxeon III red LED in a laboratory built lamp. The same light was used to provide actinic light. Lights were controlled and data recorded using software written using Labview (National Instrument, USA). Maxwell and Johnson (2000) protocol was used for the measurement of fluorescence parameters.

Malondialdehyde (MDA): Water stress-induced oxidative damage was recorded by measuring the malondialdehyde amount in tissue as illustrated by Carmak & Horst (1991). Homogenized leaf sample weighing 1.0 g was prepared in 3 mL of 0.1% (w/v) trichloroacetic acid (TCA) solution. This homogenate was centrifuged for 15 min at 20000 x *g*. To 0.5 mL of the supernatant, three mL of 0.5% thiobarbituric acid (TBA) prepared in 20% trichloroacetic acid (TCA) was mixed. Heating of the mixture was done at 95°C in a water bath for 50 min. The reaction was stopped by cooling the tubes in an ice water bath. Later on, these samples were centrifuged at 10,000 × *g* for 10 min, and the absorbance of the supernatant was recorded at 532 and 600 nm. Absorption coefficient for calculating MDA is 156 $mmol^{-1}cm^{-1}$. The MDA concentration was measured as difference in absorbance at 600 and 532 nm.

$$\text{MDA concentration (nmol)} = \Delta A\ (A_{532\text{-}600})/1.56 \times 10^5$$

Leaf chlorophyll determination: The amount of chlorophylls '*a*' and '*b*' was calculated with appropriate coefficient as describe by Porra *et al*. (1989). Fully expanded leaves of control as well as different concentrations (0, 5%, 10% and 15%) of PEG_{8000} induced water stress treated leaves were harvested. Leaves were homogenized by adding 80% (v/v) acetone by a mortar and pestle. The volume was raised up to 10 mL out of which 1.0 mL of the sample was centrifuged at 2000 rpm in a microfuge. Absorbance was read by using a USB2000 spectrophotometer.

$$\textbf{Chl } \boldsymbol{a} = 13.71 \times A_{(663.6\text{-}750)} - 2.85 \times A_{(646.6\text{-}750)}$$

$$\textbf{Chl } \boldsymbol{b} = 22.39 \times A_{(646.6\text{-}750)} - 5.42 \times A_{(663.6\text{-}750)}$$

Statistical analysis of data: Gas exchange characteristics were computed following von Caemmerer and Farquhar (1981). Polynomial regression equations for various chlorophyll fluorescence characteristics were drawn using MS-Excel 2010.

Results

Water stress tolerance is the ability of a plant to withstand low water. Leaf changes (e.g., leaf rolling, curling or folding, shape, size, angle, cuticular waxing and reflectance) enable the plant to cope with water stress (Fig. 1). Such changes help plants to slow down the transpiration rate as in *L. pennellii* whose transpiration rate is slower due to waxy blooms with respect to drought sensitive cultivated tomato (Fig. 2).

Photosynthesis is one of the main physiological processes which support plant development. Thus, the relationship between *A* and C_i measured at varying PEG_{8000}-induced water stress level had significant curvature, and the shape was highly conserved across the 15 tomato genotypes (Fig. 3), though significant differences were among genotypes in the elevation of the curve at varying levels of water stress. As in this analysis (A/C_i curve) logarithmic regression was used to predict limitations on *A*. Genotypic differences in *A* for a given C_i were significant at varying level of water stress, which are largely due to variation in non-stomatal limitations (rubisco, RuBP stress regeneration, Triose Phosphate limitation). Comparative analysis of A/C_i curves revealed that effect of highest water stress on non-stomatal limitations (metabolic limitations) was greater on moderately water stress sensitive genotypes Roma, Condine Red, Penheart, Moneymaker, Ailsa Craig, whereas least effective genotypes were Lyallpur-1, *L. chilense* Flordade, *L. pimpinellifolium* and *L. pennellii*.

Fig. 1. Leaf difference among three genotypes Lyallpur-1, *L. Chilense* and *L. Pennellii* (left to right) under control conditions.

Fig. 2. Effect of PEG_{8000} induced water stress on leaf rolling of different genotypes of tomato.

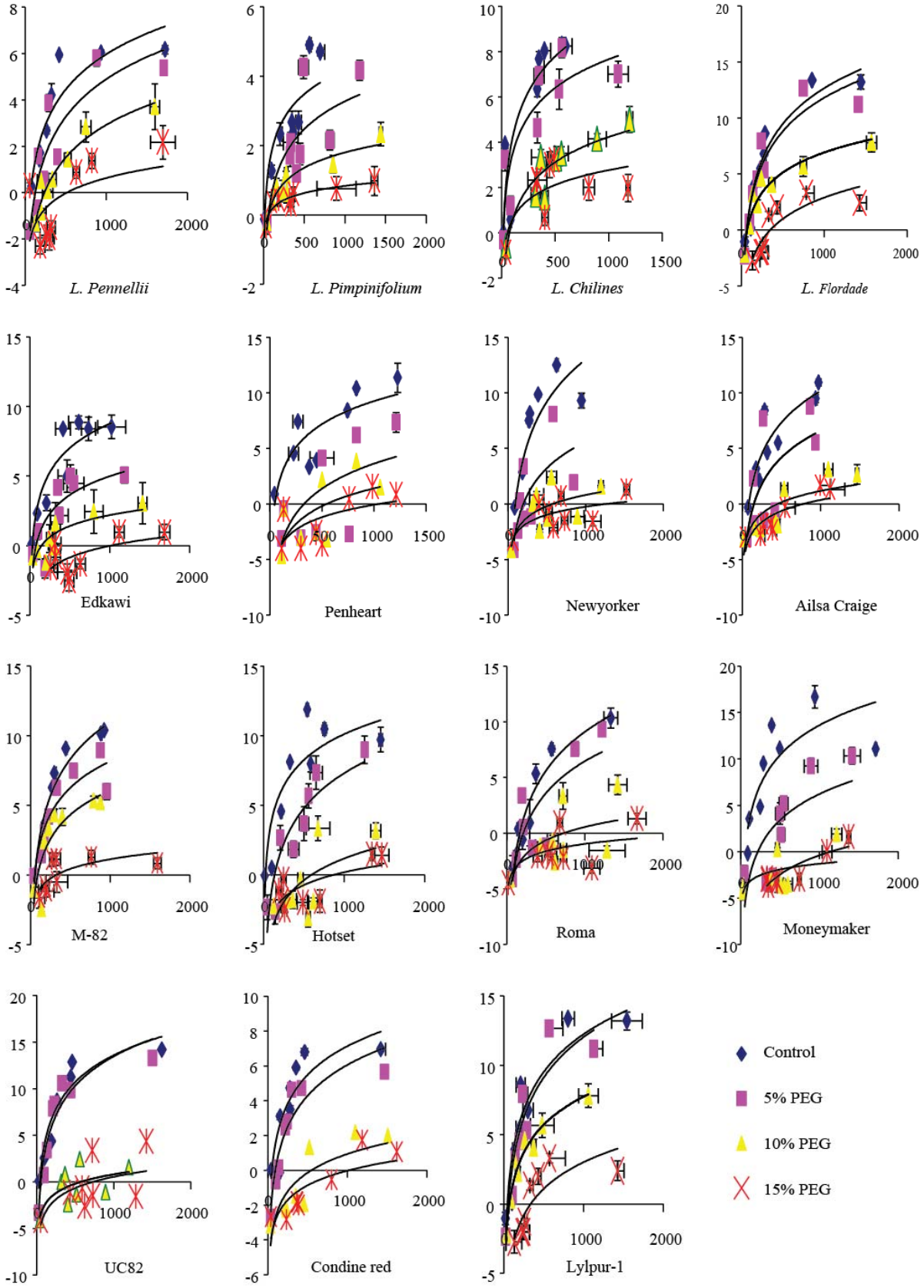


Fig. 3. Effect of varying levels (0, 5%, 10% and 15%) of PEG_{8000} induced water stress on *A/Ci* curves on 4 weeks old seedlings of tomato genotypes.

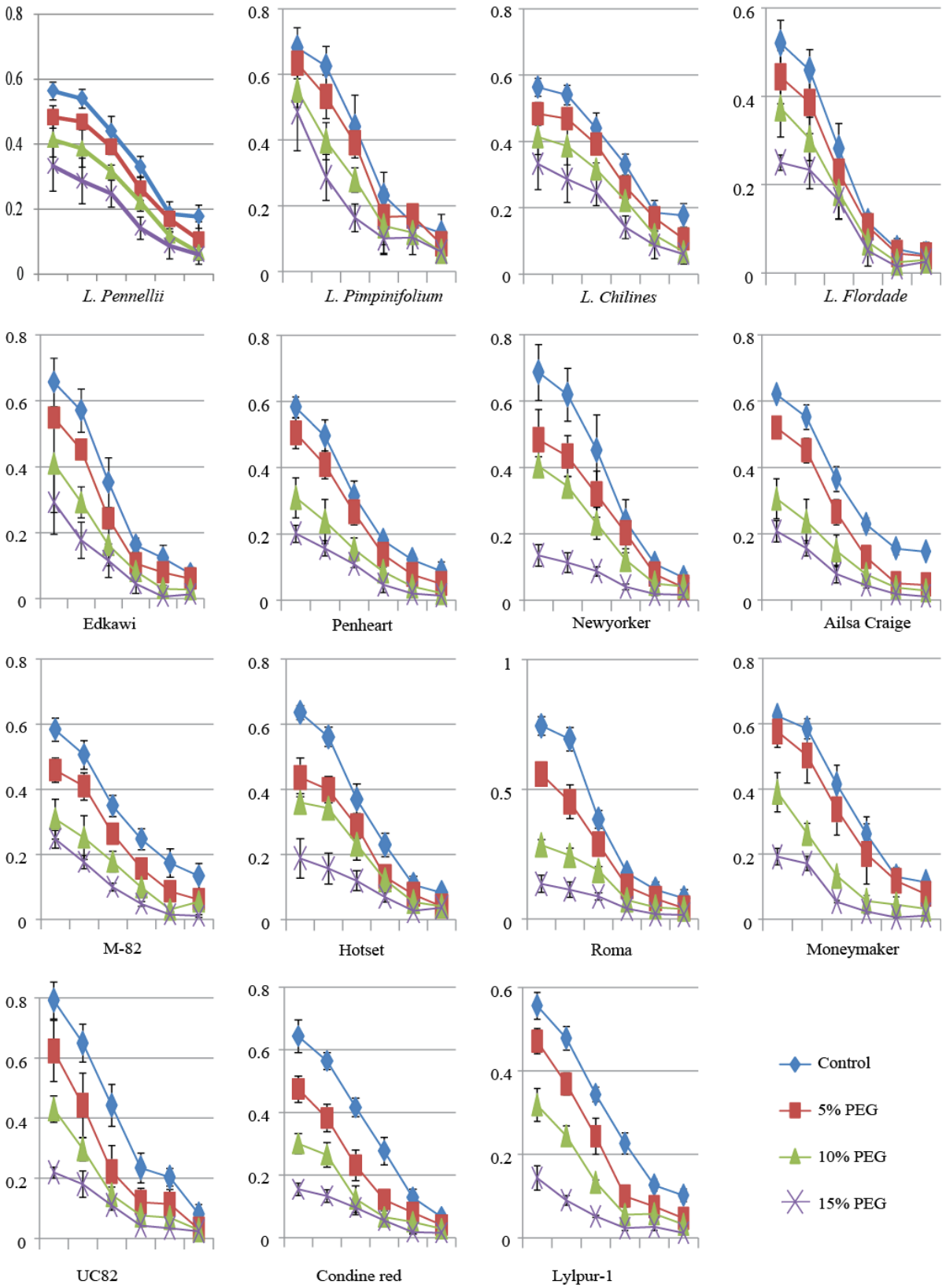
From the results of quantum yield of PSII, it is obvious that Φ PSII of the leaves of all tomato genotypes decreased considerably due to water stress (Fig. 4). Moreover, Φ PSII of the leaves of all tomato genotypes decreased progressively as PPFD increased. Decreasing effect of water stress and increasing PPFD was observed on Roma, New-Yorker and Lyallpur-1, whilst minimum effect observed on *L. pimpinellifolium* followed by *L. pennellii, L. chilense* and Edkawi. Rate of electron transport chain increased considerably as PPFD increased in water stressed and non-stressed conditions (Fig. 5). This increasing effect with increasing PPFD on ETR decreased in all tomato genotypes due to increase in moisture stress level. Moreover, genotypes differed significantly. Increasing irradiance did not increase rate of ETR in most of the tomato genotypes at the highest level of water stress. However, increasing PPFD caused maximum increase in ETR was found in genotypes *L. pennellii, L. pimpinellifolium* and *L. chilense* whereas genotypes Flordade and UC-82 were intermediated in ETR at the highest level of moisture stress. Non-photochemical quenching efficiency (NPQ) increased considerably due to both water stress and increasing irradiance level. Tomato genotypes differed significantly in this physiological attribute. NPQ remained almost constant in all tomato genotypes at all water stress levels when assessed at lower irradiance level (>100 μmol $m^{-2}s^{-1}$). At the highest water stress level, NPQ was minimal in *L. Pennellii, L. chiliness* followed by *L. Pimpinellifolium* (Fig. 6). Maximum increase in NPQ found in water stressed plants of Lyallpur-1 and Condine Red.

A significant reduction due to water stress was noticed in leaf chlorophyll '*a*' and chlorophyll '*b*' for all 15 tomato genotypes. Maximum chlorophyll '*a*' was observed in genotypes *L. pennellii, L. pimpinellifolium* than the other genotypes under water deficit conditions, whereas genotype Roma, M-82 and Condine Red were the lowest in having leaf chlorophyll '*a*' under drought conditions (Fig. 7). Leaf chlorophyll '*b*' was higher in *L. pennellii, L. pimpinellifolium, L. chiliness* and Flordade under non-stress and PEG-imposed water stress conditions, whereas under stress genotypes: Roma, M-82 and Condine Red were the lowest (Fig. 8). Ratio of chlorophyll *a*/*b* increased in leaves of stressed seedlings of *L. pennellii*, and *L. pimpinellifolium* and remained almost unaffected in most of the genotypes. However, leaf chlorophyll *a*/*b* ratio slightly decreased in Condine Red, Flordade and Moneymaker (Fig. 9). Noteworthy increase in concentration of leaf MDA was noted in tomato genotypes/cultivars with increase in PEG-induced water stress. Effect of drought on MDA was dissimilar in different tomato genotypes. Maximum increase in MDA was recorded in water stressed leaves of Roma and Edkawi followed by Ailsa Craig, M-82 and Condine Red. The least adverse effect of water stress in increasing MDA was observed in *L. pennellii* followed by *L. pimpinellifolium* (Fig. 10).

Discussion

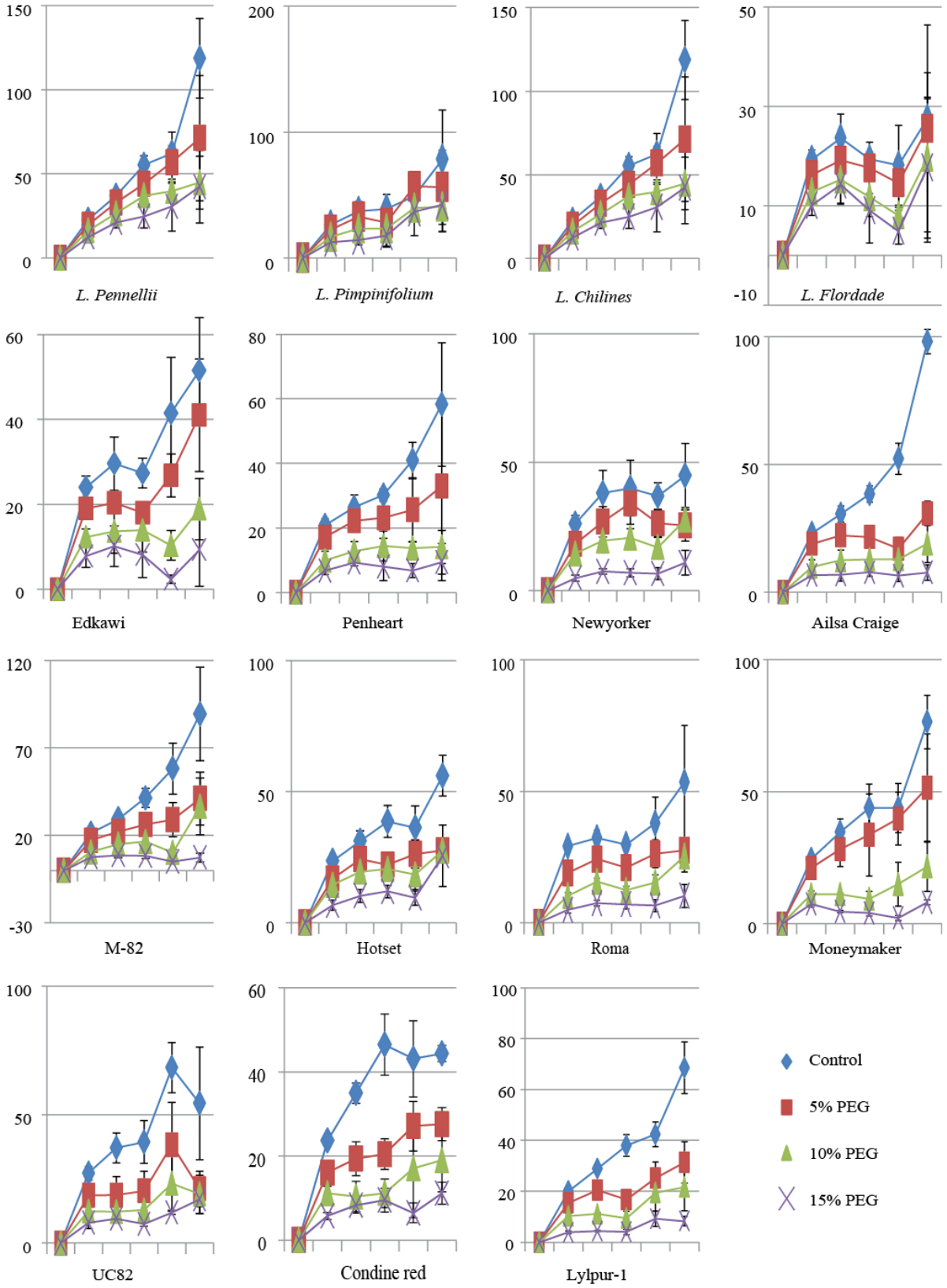
Photosynthesis in crops including tomato is highly affected by water deficits, via metabolic constraints and decreased CO_2 diffusion to the chloroplast (Makela *et al*., 1999). A pre-requisite under water stress is leaf stomatal closing through reduced uptake of carbondioxide from atmosphere. Whereas, photosynthetic machinery is sensitivity to less accessibility of carbondioxide and photodamage is most likely to be occur (Cornic & Massacci, 1996; Carvalho *et al*., 2010). As a consequence photosynthesis is reduced which results in reduced crop yield due impairment of photosynthetic machinery and destruction of Calvin cycle enzymes (von Caemmerer & Farquhar, 1999; Monakhova & Chernyadev, 2002; Anjum *et al*., 2003b). The impact of those limitations diverges with the stress intensity, and these processes can be expressed mathematically. Determination of leaf photosynthesis and gas exchange via *A/Ci* curve regression analysis lead us to conclude that at which point *A/Ci* curve switches between the Rubisco and electron transport limited portions of the curve. The aim of the present study was to review specific parameters, which are involved with gas exchange and leaf photosynthesis measurements to optimize *A/Ci* analysis and assessment of related procedures. It is evident from the result of present study that in most genotypes of tomato, photosynthesis was on the portion of the CO_2 response limited by V_{cmax} (rubisco activity) across the observed range of C_i. This finding suggests that photosynthetic advantages across a broad range of C_i is partially due to the lack of limitation by electron transport chain (J_{max}) that would be associated with transitions to V_{cmax} limitation under drought. Thus, non-stomatal (metabolic limitations) limitations appear to be the major source of variation in photosynthetic rates between tomato genotypes for a given C_i. However, Rubisco-limited photosynthesis in the current study is consistent with Bernacchi *et al*., (2005) who found that field-grown soybeans were largely Rubisco-limited during most of growing period. Similar arguments were also given by a number of scientists that variation in *A* is due to shifts from stomatal to metabolic limitations of photosynthesis under mild to high water stress (Medrano *et al*., 2002; Ennahli & Earl, 2005; Lawlor & Tezara, 2009). The magnitude of metabolic limitations increased with decreasing stomatal conductance and C_i and significant differences were observed in tomato genotypes. Metabolic limitations are likely to become important under severe drought: a state in which the water-saving genotypes are better able to avoid. However, conclusions based on *A/Ci* curves could be incorrect due to errors in *Ci* measurements (Daniel *et al*., 2004) because of stomatal patchiness i.e., non-uniform distribution of stomata (Flexas & Medrano, 2002).

An alternative to measuring A/C_i curve, measuring F_v/F_m is easier way to detect drought induced damage to the light harvesting system (Oukarroum *et al*., 2009). This measurement has been shown to be a sensitive method for ranking drought tolerance in the early vegetative growth of barley cultivars (Oukarroum *et al*., 2009). It is clear from the results that drought had adverse effects on PSII photochemistry and electron transport chain. However, this adverse effect was less on water stress tolerant genotype *L. pennellii, L. pimpinellifolium.* From these results, it is suggested that the genotypes which are tolerant to water stress, avoid the deleterious effects of water stress by electron transfer between PSII and other components of electron transport as well as due to regulation of energy transfer from antenna to reaction center and electron transfer between PSII and other components of electron transport as reflected by quantum yield of PSII and ETR at varying levels of irradiance.



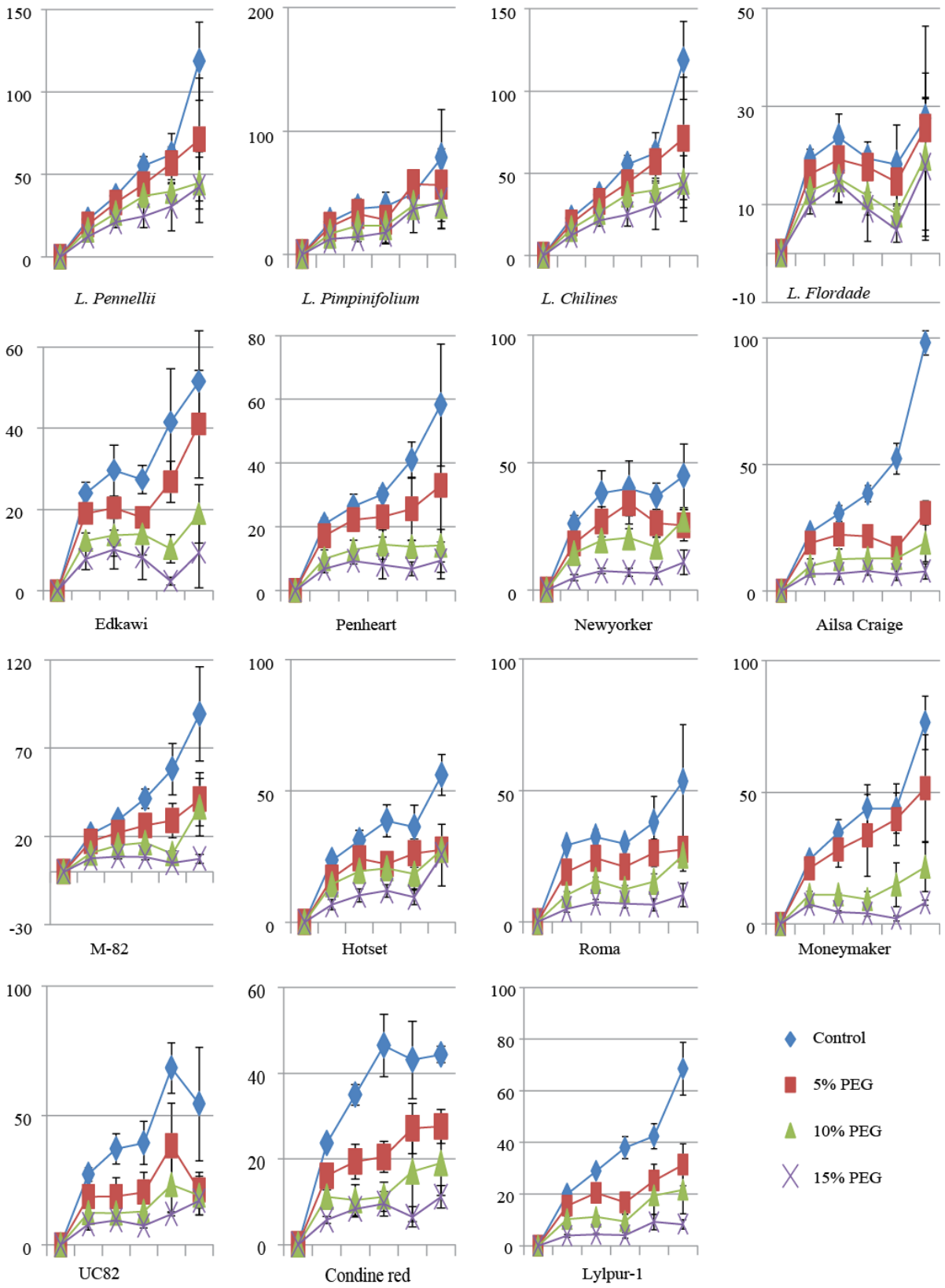
X-Axis (PDF: µmolm^{-2}s^{-1}): 0, 100, 200, 400, 800, 1600 Y-Axis (□ PSII): 0, 0.2, 0.4, 0.6 and 0.8

Fig. 4. Effect of varying levels (0, 5%, 10% and 15%) of PEG_{8000} induced water stress on □ PSII on 4 week old seedlings of tomato genotypes.



X-Axis (PDF: µmolm^{-2}s^{-1}): 0, 100, 200, 400, 800, 1600 Y-Axis (ETR): 0, 20, 40, 60, 80

Fig. 5. Effect of varying levels (0, 5%, 10% and 15%) of PEG_{8000} induced water stress on electron transport rate (ETR) on 4 weeks old seedlings of tomato genotypes.



X-Axis (PDF: µmolm^{-2}s^{-1}): 0, 100, 200, 400, 800, 1600 Y-Axis (NPQ): 0, 1, 2, 3 or 0, 0.5, 1, 1.5, 2.0, 2.5

Fig. 6. Effect of varying levels (0, 5%, 10% and 15%) of PEG_{8000} induced water stress on non-photochemical quenching (NPQ) on 4 weeks old seedlings of tomato genotypes.

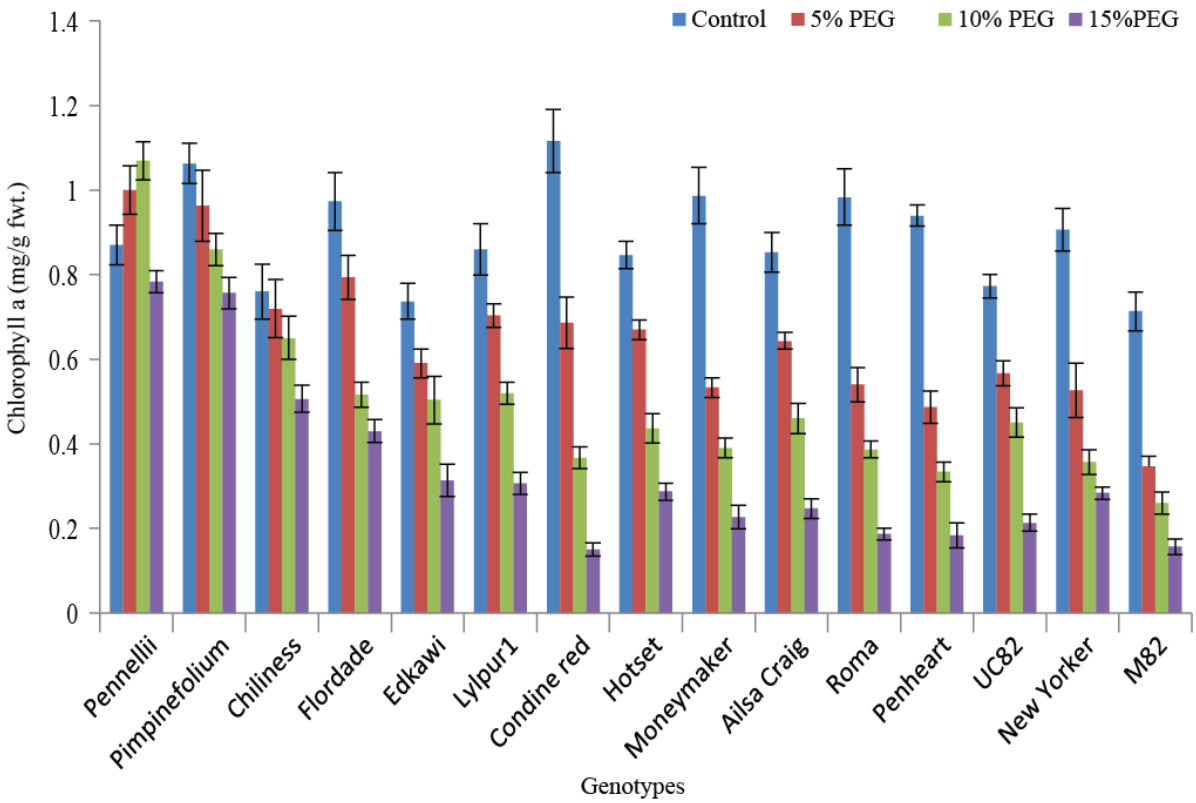


Fig. 7. Effect of varying levels (0, 5%, 10% and 15%) of PEG_{8000} induced water stress on chlorophyll *a* (mg/g fwt.) on 4 weeks old seedlings of tomato genotypes.

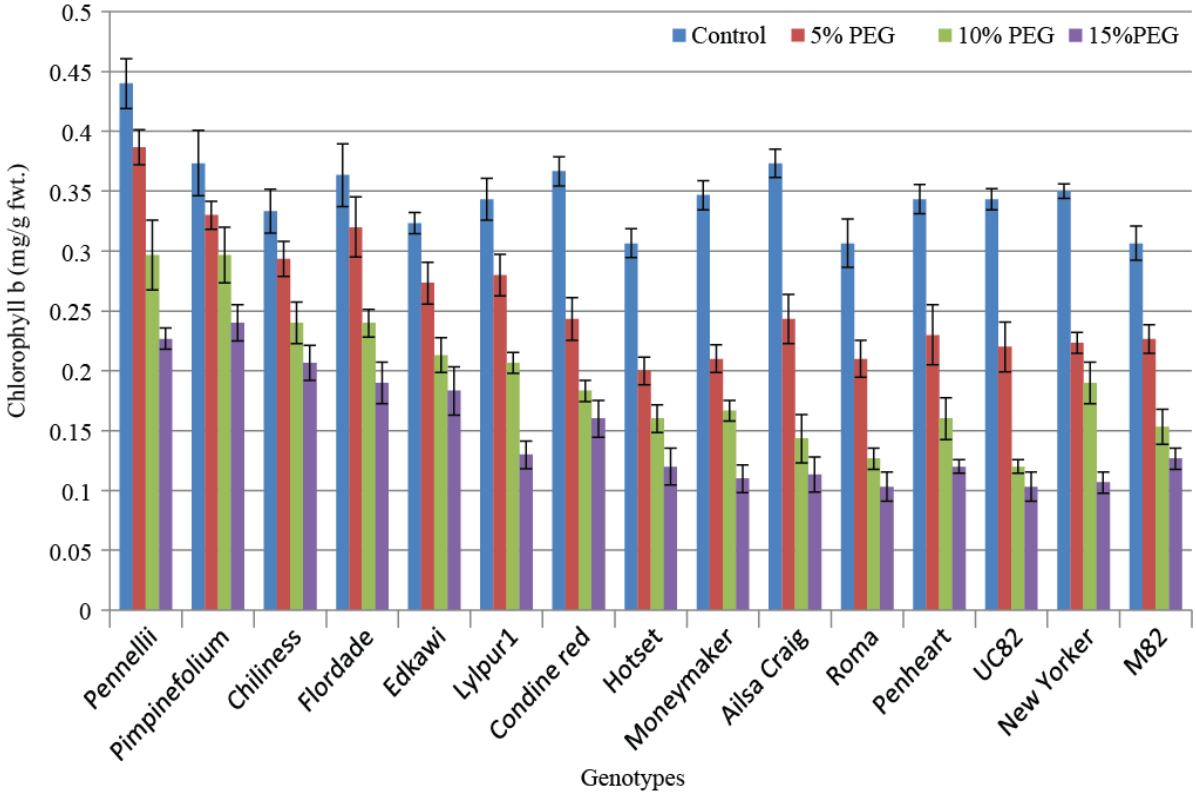


Fig. 8. Effect of varying levels (0, 5%, 10% and 15%) of PEG_{8000} induced water stress on chlorophyll *b* (mg/g fwt.) on 4 weeks old seedlings of tomato genotypes.

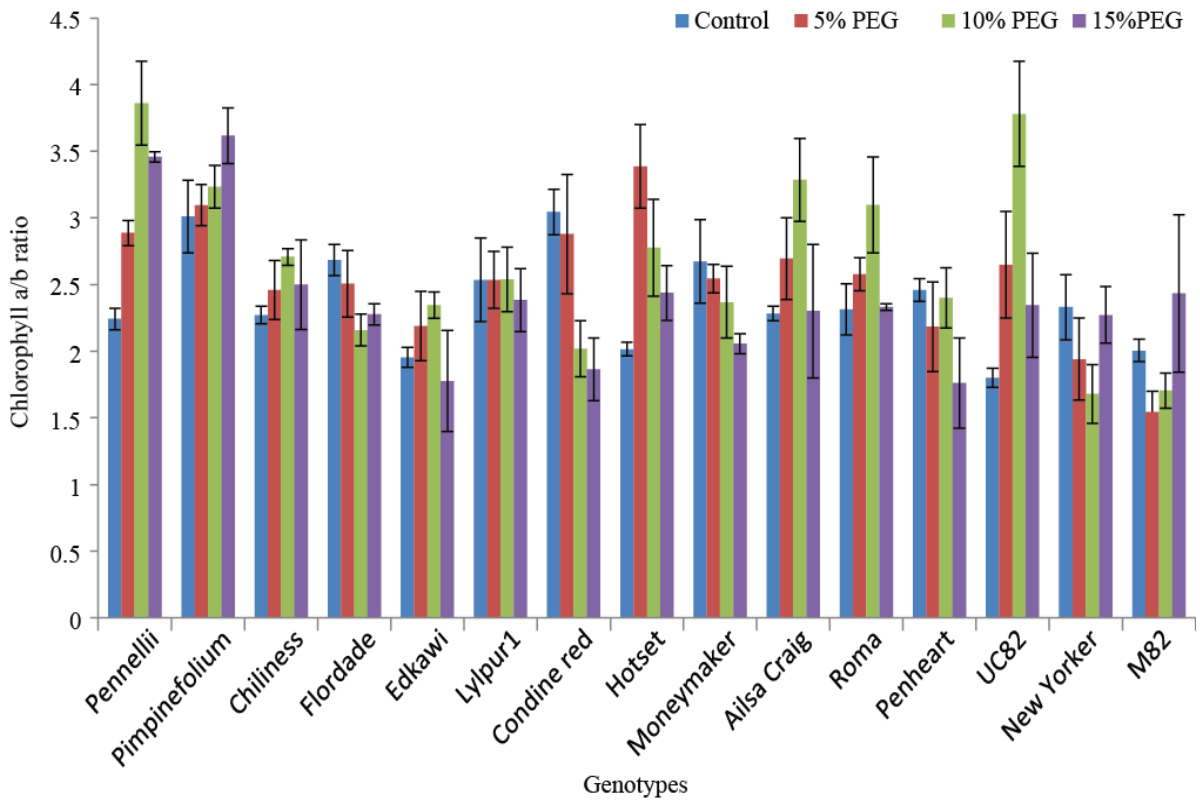


Fig. 9. Effect of varying levels (0, 5%, 10% and 15%) of PEG_{8000} induced water stress on chlorophyll *a/b* ratio on 4 weeks old seedlings of tomato genotypes.

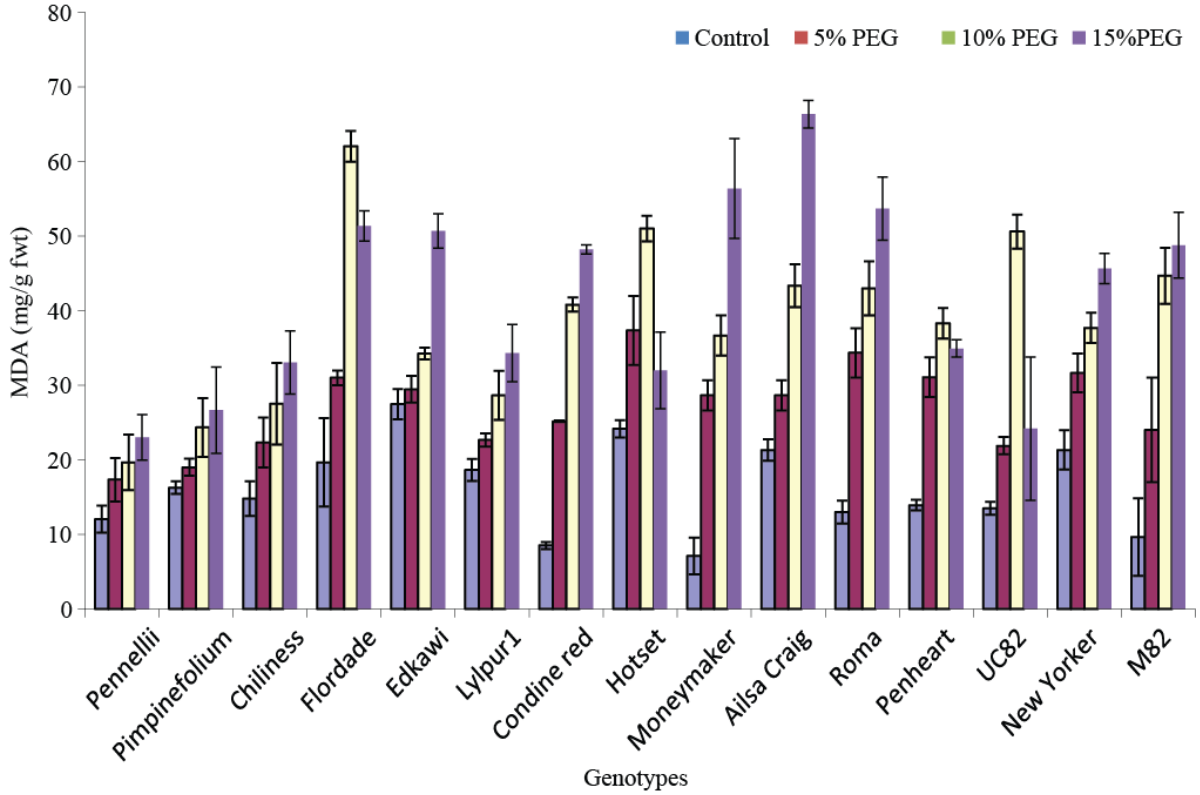


Fig. 10. Effect of varying levels (0, 5%, 10% and 15%) of PEG_{8000} induced water stress on malanodialdehyde (mg/g fwt.) on 4 weeks old seedlings of tomato genotypes.

Keeping these above reports in mind, it is evident that due to water stress photosynthetic efficiency reduces owing to various factors like **(1)** gas exchange attributes **(2)** impairment of electron transport chain (Zhou *et al*., 2007; Delatorre *et al*., 2008; Sofo *et al*., 2009) **(3)** disproportion of PSII activities which may have been due to disorganization of extrinsic proteins (Miyao & Murata, 1983; Murata *et al*., 1992). It is also apparent from current results that NPQ of all tomato genotypes increased considerably on increasing moisture stress and irradiance. However, this increase in NPQ was minimal in *L. Pennellii, L. chilense* followed by *L. Pimpinellifolium*. Reduction in quantum yield of PSII and ETR due to water stress and concomitant increase in NPQ of all tomato genotypes suggested that all plants of all genotypes try to acclimate to the water stress conditions. These results are similar with those of Gulias *et al*., (2002) who found that photochemistry of leaves was down regulated with an increase in NPQ in response to drought in grapevines. Now it is vibrant that ETR remains largely unpretentious. But a further down regulation of ETR occurs when stomata are closed. Also, an increase in thermal dissipation (NPQ) compensates the down regulation of ETR. It is suggested by these findings that increased thermal dissipation and drought-induced down regulation of ETR may directly respond to low availability of CO_2 in the chloroplast due to closure of stomata, hence being independent of the acclimation to drought and rate of drought imposition.

However, according to Reddy *et al*. (2004) imbalanced antioxidant system due to water stress is another crucial facet which has hostile effects on photosynthesis and hampers photosynthetic process. As ROS generation has been reported in different cell organelles including mitochondria, chloroplast and peroxisome hence ROS interacts with lipids of membranes instigating lipid peroxidation. Therefore, malondialdehyde (MDA) accumulates in cells under stressful environment. Accretion of MDA is a significant phenomenon responsible for stress tolerance in plants because it is a measure of oxidative stress-induced membrane destruction (Farooq *et al*., 2010). In the current exploration, water stress conditions substantially increased oxidative stress as reflected by leaf MDA contents in tomato genotypes. Minimum increase in MDA contents found in water stressed plants of *L. pennellii* was followed by *L. pimpinellifolium*. However, maximum increase in MDA was recorded in the leaves of water stressed plants of Roma and Edkawi followed by Ailsa Craig, M-82 and Condine Red. The less adverse effect on tolerant tomato genotypes might have been due to increased activities of antioxidant enzymes or relatively higher ability to utilize absorbed light. Because over-production of ROS in chloroplast deters the photosynthetic rate due to water stress which has been further found to be associated with degree of imbalance in the utilization of absorbed light (Reddy *et al*., 2004). Such imbalance was also noted in all tomato genotypes at varying water stress level in the present study as reflected from reduced values of quantum yield of PSII and ETR as well as increased values of MDA. These results can be interpreted in view of the argument of Peltzer *et al*. (2002) who also corroborated that under water deficit conditions ROS production occurred due to imbalance in utilization of electrons at PSII core and antenna center which lead to indulgence of surplus light energy. From the above results and discussion presented here it can be concluded that a considerable genetic variation exist in tomato germplasm for drought tolerance. Moreover, drought tolerance in tomato was found to be linked with their ability to maintain crop water status and by enhancing some chief antioxidant enzymes activities which has direct effects on photosynthetic activity and growth. Nevertheless, accumulation of proline and soluble sugars were effective in osmotic adjustment in tomato plants.

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References

Anjum, F., M. Yaseen, E. Rasul, A. Wahid and S. Anjum. 2003b. Water stress in barley (*Hordeum vulgare* L.). II. Effect on chemical composition and chlorophyll contents. *Pak. J. Agric. Sci.,* 40: 45-49.

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.

Athar, H.R. and M. Ashraf. 2009. Strategies for crop improvement against salt and water stress: An overview. In: *Salinity and water stress: Improving crop efficiency*. (Eds.): M. Ashraf, M. Ozturk and H. R. Athar. Springer-Verlag, The Netherlands, 1-16.

Baker, N.R. and E. Rosengvist. 2004. Applications of chlorophyll fluorescence can improve crop production strategies: an examination of future possibilities. *J. Exp. Bot*., 55: 1607-1621.

Baker, N.R. 2008. Chlorophyll fluorescence: A probe of photosynthesis *In vivo*. Annu. Rev. *Plant Biol*., 59: 89-113.

Bernacchi, C.J., P.B. Morgan, D.R. Ort and S.P. Long. 2005. The growth of soybean under free air CO_2 enrichment (FACE) stimulates photosynthesis while decreasing *in vivo* Rubisco capacity. *Planta*, 220: 434-446.

Carmak, P. and J.H. Horst. 1991. Effects of aluminum on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*). *Plant Physiol*., 83: 463-468.

Carvalho, M.C., K. Hayashizaki and H. Ogawa. 2010. Effect of pH on the carbon stable isotope fractionation in photosynthesis by the kelp *Undaria pinnatifida*. Coast. *Mar. Sci*., 34: 135-139.

Cornic, C. and A. Massacci. 1996. Leaf photosynthesis under drought stress. In: Photosynthesis and Environment. Ed. N. R. Baker, Kluwer Acad. Publs., 347-366.

Daniel, K., D.K. Manter and J. Kerrigan. 2004. A/C_i curve analysis across a range of woody plant species: influence of regression analysis parameters and mesophyll conductance. *Plant Sci*., 165: 2581-2588.

Delatorre, J., M. Pinto and L. Cardemil. 2008. Effects of water stress and high temperature on photosynthetic rates of two species of *Prosopis*. *J. Photochem. Photobiol. B. Biol*., 92: 67-76.

Ennahli, S. and H.J. Earl. 2005. Physiological limitations to photosynthetic carbon assimilation in cotton under water stress. *Crop Sci*., 45: 2374-2382.

Farooq, J., I. Khaliq, A.S. Khan and M.A. Pervez. 2010. Studying the genetic mechanism of some yield contributing traits in wheat (*Triticum aestivum*). *Int. J. Agri. Biol*. 12: 241-246.

Farquhar, G.D. and T.D. Sharkey. 1982. Stomatal conductance and photosynthesis. Ann. Rev. *Plant Physiol*., 33: 317-345.

Farquhar, G.D., S. von Caemmerer and J.A. Berry. 1980. A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. *Planta*, 149: 78-90.

Flexas, J. and H. Medrano. 2002. Energy dissipation in C_3 plants under drought. *Func. Plant Biol*., 29(10): 1209-1215.

Genty, B., J. Harbinson, J.M. Briantais and N.R. Baker. 1990. The relationship between non-photochemical quenching of chlorophyll fluorescence and the rate of photosystem II photochemistry in leaves. *Photosynth. Res*., 25: 249-257.

Genty, B., J.M. Briantais and N.R. Baker. 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochem. biophys. Acta*., 990: 87-92.

Golding, A.J. and G.N. Johnson. 2003. Down regulation of linear and activation of cyclic electron transport during drought. *Planta*, 218: 107-114+erratum.

Gulias, J., J. Flexas, A. Abadia and H. Medrano. 2002. Photosynthetic responses to water deficit in six Mediterranean sclerophyll species: possible factors explaining the declining distribution of *Rhamnus ludovici-salvatoris*, an endemic Balearic species. *Tree physiol*., 22: 687-697.

Harley, C.B., H. Vaziri, C.M. Counter and R.C. Allsopp. 1992. The telomere hypothesis of cellular aging. *Exp. Gerontol*., 27: 375-382.

Hoagland, D.R. and D.I. Arnon. 1950. The water culture for growing plants without soil. *Calif. Agic. Expt. Station Circ*., 347: 1-32.

Lawlor, D.W. and W. Tezara. 2009. Causes of decreased photosynthetic rate and metabolic capacity in water-deficient leaf cells: a critical evaluation of mechanisms and integration of processes. *Ann. Bot*., 103: 561-579.

Lawlor, D.W. and G. Cornic. 2002. Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. *Plant Cell Environ*., 25: 275-294.

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.

Manter, D.K. and J. Kerrigan. 2004. A/Ci curve analysis across a range of woody plant species: Influence of regression analysis parameters and mesophyll conductance. *J. Exp. Bot*., 55: 2581-2588.

Manter, D. K., B.J. Bond, K.L. Kavanagh, J.K. Stone and G.M. Filip. 2003. Modelling the impacts of the foliar pathogen, *Phaeocryptopus gaeumannii*, on Douglas-fir physiology: net canopy carbon assimilation, needle abscission and growth. *Ecol. Model*., 164: 211-226.

Maxwell, K. and G.N. Johnson. 2000. Chlorophyll fluorescence: a beginner's guide. *J. Exp. Bot*., 51: 659-668.

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.

Miyao, M. and N. Murata. 1983. Partial disintegration and reconstitution of the photosynthetic oxygen evolution system: binding of 24 kDa and 18 kDa polypeptides. *Biochim. Biophys. Acta*., 725: 87-93.

Monakhova, O.F. and I.I. Chernyadev. 2002. Protective role of kartolin-4 in wheat plants exposed in soil drought. *Appl. Biochem. Micro*., 38: 373-380.

Murata, N., P.S Mohanty, H. Hayashi and G.C. Papageorgiou. 1992. Glycine betaine stabilizes the association of extrinsic proteins with the photosynthetic oxygen-evolving complex. *FEBS Lett*., 296: 187-189.

Oguntimehin, I., F. Eissa and H. sakugawa. 2010. Simultaneous ozone fumigation and fluoranthene sprayed at mists negatively affected cherry tomato *(Lycopersicon esculentum* Mill). *Ecotoxicol. Environ. Saf*., 73: 1028-1033.

Oukarroum, A., G. Schansker and R.J. Strasser. 2009. Drought stress effects on photosystem I content and photosystem II thermotolerance analyzed using Chl *a* fluorescence kinetics in barley varieties differing in their drought tolerance. *Physiol. Plant*., 137: 188-199.

Peltzer, D., E. Dreyer and A. Polle. 2002. Temperature dependencies of antioxidative enzymes in two contrasting species. *Plant Physiol. Biochem*., 40(2): 141-150.

Porra, R. J., W.A. Thompson and P.E. Kriedemann. 1989. Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. *Biochim. Biophys. Acta*., 975: 384-394.

Reddy, K.R., G.K. Vigaya, Z. Duli, K. Saliaja and G. Wei. 2004. Interactive effects of ultraviolet-B radiation and temperature on cotton physiology, growth, development and hyperspectral reflectance. *Photochem. Photobiol*., 79(5): 416-427.

Reichstein, M., M. Bahn, P. Ciais, D. Frank, M.D. Mahecha, I. Sonia, W. Martin ± *et al*. 2013. Climate extremes and the carbon cycle. *Nature*, 500(5): 287-295.

Roostaei, M., S.A. Mohammadi, A. Amri, E. Majidid, M. Nachit and R. Haghparast. 2011. Chlorophyll fluorescence parameters and drought tolerance in a mapping population of winter bread wheat in the highlands of Iran. *Russ. J. Plant Physiol*., 58(2): 351-358.

Sharkey, T.D., C.J. Bernacchi, G.D. Farquhar and E.L. Singsaas. 2007. Fitting photosynthetic carbondioxide response curves for C_3 leaves. *Plant Cell Environ*., 30(9): 1035-1040.

Sofo, A., B. Dichio, G. Montanaro and C. Xiloyannis. 2009. Shade effect on photosynthesis and photoinhibition in olive during drought and rewatering. *Agric. Water Manag*., 96: 1201-1206.

Stepien, P. and G.N. Johnson. 2009. Contrasting responses of photosynthesis to salt stress in the glycophyte *Arabidopsis thaliana* and the halophyte *Thellungiella halophila*. Role of the plastid terminal oxidase as an alternative electron sink. *Plant Physiol*., 149: 1154-1165.

von Caemmerer, S. and G.D. Farquhar. 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta*, 159: 376-387.

von Caemmerer, S. and G.D. Farquhar. 1999. Plants in Action 1.1 Adaptation in nature, performance in cultivation. Ed. 1, Development of A/Ci curves, CASE STUDY 1.1. *Macmillan Education Australia Pvt. Ltd*., Melbourne, Australia.

Zhou, Y., H.M. Lam and J. Zhang. 2007. Inhibition of photosynthesis and energy dissipation induced by water and high light stresses in rice. *J. Exp. Bot*., 58: 1207-1217.

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