53 *Egypt. J. Bot*., **Vol. 56**, No. **3,** pp. 895- 912 (2016)

# Physiological and Biochemical Responses of *Medicagotruncatula* to Drought Stress

**M.M. Hegab**
*Department of Botany and Microbiology, Faculty of Science, Beni-Suef University, Egypt.*

It is frequently observed that drought stress decreased plant growth decreased plant growth and induced cellular damages. However, the damages. However, the underlying physiological and biochemical mechanisms are not well understood. *Medicagotruncatula*wassubjected to drought stress by water withdrawing at mature stage and for one week. Drought stress reduced plant growth, inhibited photosynthesis, stomatal conductance and induced oxidative stress. Water stress induced changes *e.g*. it increased osmo-protectants (proline, glycine betaine), and the level of oxidative stress parameters ($H_2O_2$ and lipid peroxidation). In parallel with higher levels of $H_2O_2$ and MDA, there were increase in NADPH oxidase and lipoxygenase (LOX) activities in *Medicagotruncatula*. Presumably as a consequence of the induction of $H_2O_2$ production, activation in some antioxidant defense components was observed (e.g. increased superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), peroxidase (POX) and glutathione reductase (GR). Other antioxidant component were also significantly increased by drought stress.

**Keyword:** Drought, Legume, *Medicagotruncatula*, Oxidative stress, Plant redox network.

Drought is a worldwide threat to plant growth and production, inducing many plant biochemical, molecular, and physiological perturbations. For instance, it down regulates rates of photosynthetic $CO_2$ assimilation and transpiration and decreases stomatal and mesophyll conductance (Lawlor and Tezara, 2009). Moreover, the combination of elevated temperature with drought could exacerbate the stress effect as some studies indicate a higher detrimental effect on plant growth and productivity than when each stress was applied individually (Savin and Nicolas, 1996). Stress-induced alterations in plant metabolism, generally increase reactive oxygen species levels (ROS) (Gill and Tuteja, 2010), ROS accumulation can damage plant cells at the level of nucleic acids, membrane lipids, chlorophyll and proteins (Foyer and Noctor, 2005). One ROS, hydrogen peroxide ($H_2O_2$) is not only an indicative of a harmful process, but also participates in many signaling,resistance and regulation mechanisms including, reinforcement of the cell wall (Dempsey and Klessig 1995), stomatal movement (Bright *et al*. 2006), photorespiration and photosynthesis (Foyer *et al*., 2009),

*IRO momtaz@science.bsu.edu.eg momtazyehya@hotmail.com)

growth and development (Foreman *et al.* 2003) and hormonal signaling (Kwak *et al.*, 2006). Plants often minimize free radical damage by enhanced antioxidant defensive systems (Le Martret *et al.* 2011).

Accumulation of osmolytes, including polyols, free amino acids and soluble sugars, is a well-known plant response to water deficit (Szabados and Savourè, 2010). Osmolytes are compatible solutes that adjust cellular osmotic potential, function as low molecular-weight chaperones, protect membranes and proteins, stabilize photosystem II, and protect against oxidative damage by scavenging ROS (Chen and Murata, 2008; Szabados and Savourè, 2010). Increasing photosynthetic carbon assimilation and ATP synthesis (Johnson *et al*, 2002). On the other hand, a significant reduction in proline and soluble sugar content was reported for wheat in combinations of drought and UV-C radiation.

Understanding and predicting future climate plant responses, is further complicated by significant 'species x future climate' interactions, *i.e.* species react differently to climate changes.For example, a competitive advantage of Legume-species over other species, under climate change was observed (Wand et al., 1999), similarly, Hebeisen *et al.* (1997) has suggested that white clover could profit more than ryegrass. The competitive advantage of legumes arises from their ability to fix atmospheric N (Rogers et al 2006).

The aim of the current research was, therefore, to analyze the drought response a legume species (*Medicagotruncatula*), at the physiological (photosynthesis, stomatal conductance), and molecular (osmolytes, antioxidants, redox network) level. In addition, studying molecular mechanisms to drought stress, in a modelspecies, may shed new light on some defense pathways.

## Materials and Methods

*Experimental set-up and plant harvest*

A greenhouse experiment was conducted in 2014, at the University of Beni-Suef, Faculty of Science, Botany Department. Anitrogen fixing dicot (*Medicagotruncatula*L.) was grown for 4 months in controlled growth chambers. The plants seeds were grown in pots filled with sandy soil (93.2% sand, 4.6% silt, 2.2% clay; field capacity 0.13 $m^3$ $m^3$; pH 7.6; total Kjeldahl-N 0.42 g kg-1; 1% C in humus).During vegetative stage, water-deficit stress was induced by withdrawal of irrigation, and, depending upon how fast target species responded to drought (by observing yellowing, wilting or curling of leaves), it was applied for one week in *M. truncatula*. Aboveground biomass (fresh weight, FW) was collected by cutting plants 4 cm above the soil surface.

*Photosynthesis, stomatal conductance and chlorophyll a*

Light saturated photosynthetic rate ($A_{sat}$, µmol $CO_2$ $m^{-2}$ $s^{-1}$) and stomatal conductance ($g_s$, µmol $H_2O$ $m^{-2}$ $s^{-1}$) were determined (LI-COR LI-6400, LI-COR Inc., Lincoln, NE, USA) on the youngest fully expanded leaves. LI-COR leaf

chamber conditions were set at 385 or 630 ppm $CO_2$, and 23.5 or 26.5 ºC (block temperature), according to the climate treatments, saturated photon flux density (1500 µmol.$m^{-2}$.$s^{-1}$), and ambient relative humidity. Sixteen replications per species were randomly measured.Chlorophyll "a" and carotenoid contentwere determined after acetone extractionand calculated according to Porra et al. (1989).

*Proline and free amino acids*

Plant shoots (200 mg FW) were homogenized in 2 ml of 3% (v/v) aqueous sulfosalicylic acid and the homogenate was centrifuged at 10,000 rpm for 20 min. Proline content was measured after toluene extraction using the ninhydrin reagent (Bates *et al*. 1973). Free amino acids (FAA) were determined in ethanolic extracts (0.1 ml 80% (v/v) ethanol, per 200 mg FW), and extraction in a ninhydrin-citrate-glycerol mixture (20 min, 100°C)(Lee and Takahashi 1966). Proline and glycine were used as standards respectively.

*Glycine betaine*

Plant material (200 mg FW) was ground by a MagNALyser in liquid nitrogen and extracted in deionized water (5 ml, 48 h, 25 ºC). Quaternary ammonium compounds were measured according to Grieve and Grattan (1983), glycine betaine was used as the standard.

*Soluble sugars*

Plant shoots (200 mg FW) were ground by a MagNALyser in liquid nitrogen and interfering pigments were removed by extraction with 100% acetone. Sugar content was estimated with the anthrone reagent (Leyva et al., 2008), using a glucose standard curve.

*Lipid peroxidation*

Lipid peroxidation was determined on frozen shoot tissues, homogenized in 80% ethanol by mortar and pestle, using a thiobarbituric acid-malondialdehyde (TBA-MDA) assay (Hodges et al., 1999).

*$H_2O_2$ concentration*

$H_2O_2$ concentration was measured by the FOX1 method (Jiang et al., 1990), based on the peroxide-mediated oxidation of $Fe^{2+}$, followed by reaction of $Fe^{3+}$, with xylenol orange. Specificity for $H_2O_2$ was tested by eliminating $H_2O_2$ from the reaction mixture with catalase.

*Total antioxidant capacity*

Plant tissues (200 mg FW) were ground in liquid nitrogen and the antioxidants were extracted in 2ml of ice cold 80% ethanol. FRAP (ferric reducing/antioxidant power assay) reagent (0.3 M acetate buffer (pH3.6), 0.01 mM TPTZ in 0.04 mMHCl and 0.02 M $FeCl_3.6H_2O$) was mixed with the extract and measured at 600 nm using a microplate reader (Benzie & Strain, 1996). Troloxwas used as standard.

*Ascorbate, glutathione, homo-glutathione and their redox status*

Ascorbate (ASC), glutathione (GSH) and homo-glutathione (hGSH) were determined by HPLC analysis. The redox status was calculated as the ratio of the reduced form to the total concentration of the antioxidant. In *M. truncatula*, GSH is largely replaced by hGSH, and the added concentrations (hGSH+GSH) are used throughout.

*Tocopherols*

Tocopherols were extracted with hexane using the MagNALyser. The dried extract (CentriVap concentrator, Labconco, Kansas, USA) was re-suspended in hexane, and tocopherols were separated and quantified by HPLC (Shimadzu, s Hertogenbosch, The Netherlands) (normal phase conditions, Particil Pac 5 µm column material, length 250 mm, i.d. 4.6 mm). Dimethyl tocol (DMT) was used as internal standard (5 ppm). Data were analyzed with Shimadzu Class VP 6.14 software.

*Polyphenols and flavonoids*

Polyphenols and flavonoids were extracted in 80% ethanol (v/v) and determined according to Zhang et al. (2006), and Chang *et al*. (2002), and with gallic acid and quercetin respectively as standards.

*Enzyme assays*

Lipoxygenase (LOX) was extracted in 50mMpotassium phosphate buffer (pH 7.0) and its activity was measured according to Axelrod et al. (1981). The standard assay mixture (0.2mL) consisted of 160 µL of sodium phosphate buffer (50 mM, pH 7.0); 20 µL of crude extract and 20 µL of a substrate emulsion (10 mM linoleic acid emulsified in 0.36% Tween-20). The formation of hydroperoxides was calculated by using extinction coefficient 25,000 M-1cm-1. One unit of enzyme was defined as the quantity that generates 1 mmol of conjugate diene per minute at 25$^{o}$C.

NADPH oxidase was assayed according to Van Gestelen *et al*. (1997) and Sarath et al. (2007), afterextraction in 50mMpotassium phosphate buffer (pH 7.0). Monoformazan concentrations were calculated using an extinction coefficient of 12.8 $mM^{-1}$ $cm^{-1}$.

Ascorbate peroxidase (APX), mono-dehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR) and glutathione reductase (GR) were determined in microplates (Synergy Mx, Biotek Instruments Inc., Vermont, USA) (Murshed *et al*., 2008). Peroxidase (POX) activity was determined by the oxidation of pyrogallol ($\varepsilon 430 = 2.47$ $mM^{-1}cm^{-1}$) (Kumar and Khan, 1982). Superoxide dismutase (SOD) activity was determined by Dhindsa *et al*. (1981) by measuring the inhibition of NBT reduction at 560 nm. Catalase activity was assayed according to Aebi (1984) by monitoring the rate of decomposition of $H_2O_2$ at 240 nm.For all activities 100 mg frozen tissue was extracted with a MagNALyser, in 1 mL of extraction buffer (50 mM MES/KOH (pH 6.0), 4 mMKCl, 2 mM $CaCl_2$ and 1 mM ascorbic acid). In addition, measurements were scaled down for semi-high throughput using a micro-plate reader, and optimized

to obtain linear time and protein-concentration dependence.

*Statistical analysis*

Results were analyzed by one-way ANOVA, using SPSS 16.0 statistical software (COMPANY, CITY, COUNTRY), and significant differences between the means were determined by using the Duncan test ($P < 0.05$) (n=16).

## Results

*Effect of droughton plantgrowth, photosynthesis, stomatal conductance and chlorophyll content*

Drought treatment reduced plant biomass, at the DW and FW level (Fig.1 A&B). Light saturated photosynthesis ($A_{sat}$) was strongly inhibited ($P<0.001$ compared to controls) (Fig. 2A). Similarly, strong reductions of stomatal conductance ($g_s$) were observed (Fig. 2B). Chlorophyll a content was also reduced by droughtin *Medicagotruncatula* (Fig. 2C).

*Oxidative stress indicators*

Oxidative stress levels were measured by $H_2O_2$ levels and lipid peroxidation. $H_2O_2$ levels were increased by drought (Fig. 3A). In parallel with the increase in $H_2O_2$ levels, also the NADPH oxidase activity increased under drought stress(Fig. 3B). Increases in MDA levels demonstrate that the drought treatment increases lipid peroxidation (Fig. 3C). The increases in MDA levels are largely matchedby elevated levels of lipid peroxidase (LOX) activity (Fig. 3D).

*Changes in osmolytes*

Drought caused strong increases of proline in *Medicagotruncatula*(Fig. 4A). In parallel to the proline levels, we investigated changes in overall free amino acid concentrations (Fig. 4B). Compared to changes in free amino acids, prolinewas less induced by drought. Glycine betaine concentrations increased under drought *Medicagotruncatula* (Fig. 4C).

*Antioxidant parameters: molecular antioxidant levels*

As a tool to asses overall antioxidant changes, the measurement of Total Antioxidant Capacity (TAC) is commonly used. TAC increases as a consequence of drought stress(Fig. 5A). The TAC assay does not allow discrimination between changes in specific groups of antioxidant molecules.We therefore separately analyzed polyphenol, flavonoid and totaltocopherol levels (Fig. 5B-D). In *Medicagotruncatula*, drought increased the levels of polyphenols, flavonoids and tocopherols, although to different extends, (Fig. 5B-D).

*Antioxidant parameters: ROS scavenging enzymes SOD, POX, APX, GPX and CAT*

Superoxide dismutase (SOD) activitywas induced by drought in *Medicagotruncatula* (Fig. 6A).The responses of hydrogen peroxide scavenging enzymes to stress conditions were recorded. Peroxidase and Catalase (CAT) activity increased under drought stress (Fig. 6B). General peroxidase (POX) and ascorbate oxidase (APX) activity increases were observed (Fig. 6C and D). Similar

to CAT, glutathione peroxidase (GPX) activity also increased in drought (Fig. 6E).

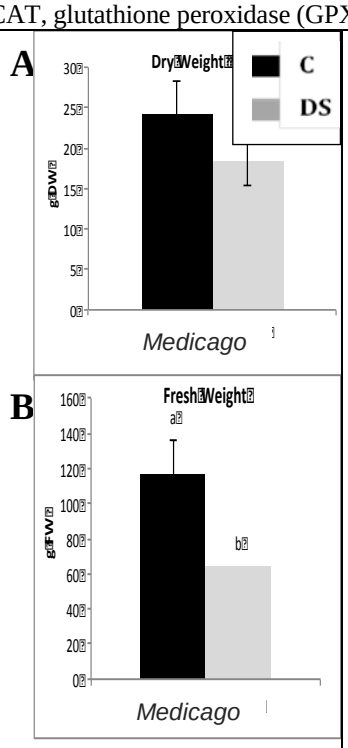


**Fig. 1. Above ground biomass of *Medicago truncatula* under drought stress (Ds) compared with control (C); (A) dry weight, (B) fresh weight. Different letters in the graph represents the significant differences between the four treatments (Duncan test; P < 0.05; (n=4).**

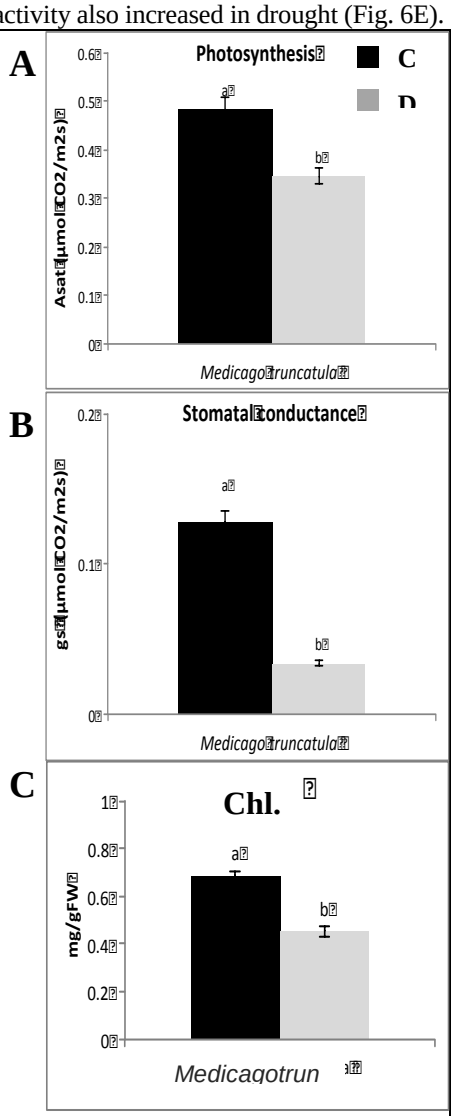


**Fig. 2. Photosynthesis, stomatal conductance and chlorophyll "a" content of *Medicago truncatula* under drought stress (Ds) compared with control (C); (A) light saturated net CO2 assimilation rate (Asat), (B) stomatal conductance (gs), (C) chlorophyll "a" content. Different letters in the graph represents the significant differences between the four treatments (Duncan test; P < 0.05; (n=4).**

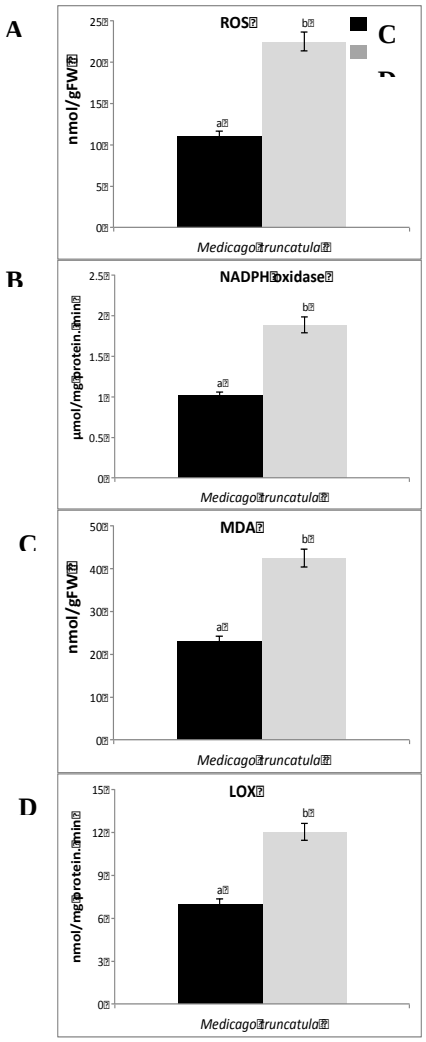


**Fig. 3. Oxidative stress indicators of *Medicago truncatula* under drought stress (Ds) compared with control (C); (A) hydrogen peroxide, (B) NADPH oxidase activity, (C) lipid peroxidation detected as malondialdehyde (MDA), (D) lipoxygenase activity (LOX). Different letters in the graph represents the significant differences between the four treatments (Duncan test; P < 0.05; (n=4).**

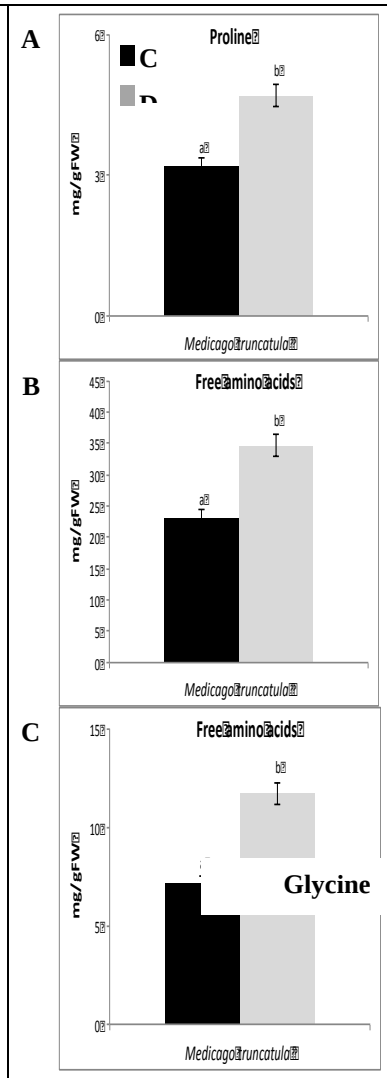


**Fig. 4. Osmolytes and glycine serine ratio of *Medicago truncatula* under drought stress (Ds) compared with control (C); (A) proline, (B) free amino acids, (C) glycine betaine.**

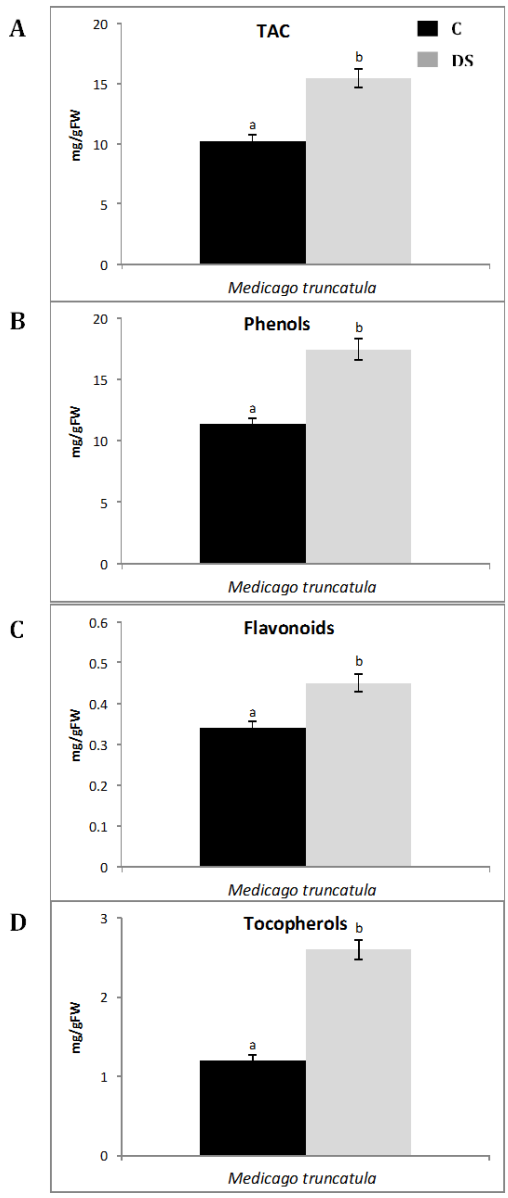


**Fig. 5. Molecular antioxidant levels of *Medicagotruncatula* under drought stress (Ds) compared with control (C); (A) antioxidant capacity (FRAP), (B) polyphenols level, (C) flavonoids, (D) tocopherols.**

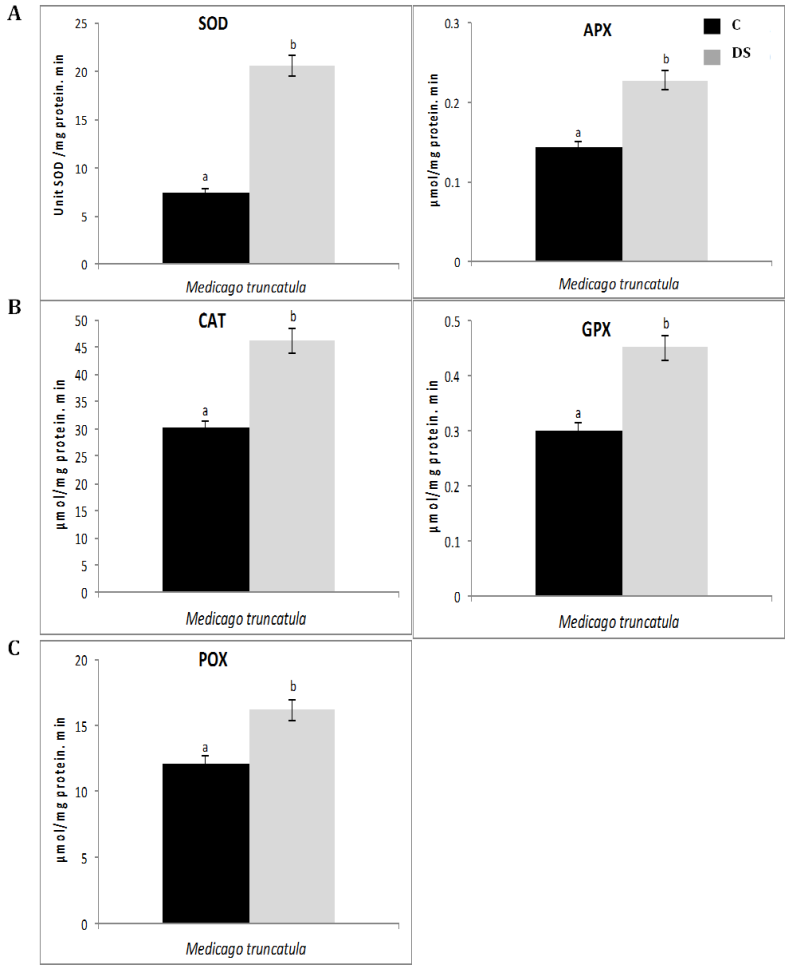


**Fig. 6. ROS scavenging enzymesof *Medicagotruncatula*under drought stress (Ds) compared with control (C);(A) superoxide dismutase (SOD), (B) catalase (CAT), (C) peroxidase, (D) ascorbate peroxidase (APX), (E) glutathione peroxidase (GPX).Different letters in the graph represents the significant differences between the four treatments (Duncan test; P < 0.05; (n=4).**

*Antioxidant parameters: ascorbate-glutathione cycle*

One well-documented antioxidant defense system involves reduced ascorbate (ASC) and glutathione (GSH) as principal electron donors, hydrogen peroxide scavenging enzymes (APX, GPX), and reductant recycling enzymes (GSH-dependent DHA reductase (DHAR), MDHAR, glutathione reductase (GR) (Foyer and Noctor 2005; Potters *et al* 2004). The redox status of ASC and GSH, *i.e.* the relative balance of the reduced and oxidized molecules, is often considered an indicator for oxidative stress conditions.

Levels of ASC as well as the ASC redox status, were hardly affected by the drought, (Figure 7A and B). There was a tendency of ASC redox status to decrease. The ascorbate redox status was generally high (70-90%), in control conditions, and decreased (strongly) to about 40% under drought.GSH levelsincreased by the drought in *Medicagotruncatula*. The glutathione redox status in stressedconditions was low in *Medicagotruncatula*(27%) (Figure 7D). The activity of the GSH-dependent ASC recycling enzyme DHAR increased under drought, (Figure 7C).Changes in GSH recycling GR activity, induced by stress were similar to the changes in GSH levels (Figure 7F).

## Discussion

*Photosynthesis and stomatal conductance*

The present study clearly showed the inhibitory effect of drought on $A_{sat}$ and $g_s$. In the same way, Shah and Paulsen 2003, Xu and Zhou 2006, stated that drought application resulted in more detrimental effect comparing with its effect separately. Although some studies stated that the limitations of photosynthesis under most drought conditionsare mainly related to mesophyll conductance (Flexas et al., 2006). But others suggested that the changes in photosynthesis under stress are not directly related to stomatal conductance (Lodge *et al.,* 2001), particularly with severely combined stresses (Chaves *et al.* 2003). Similarly, we found unparalleled changes in $A_{sat}$ and $g_s$ condition which proposed the non-stomatal limitations pf the photosynthesis ($A_{sat}$). Since, alteration in photosynthetic capacity can be attributed to photo-inhibition, oxidative damage to protein and Chls. and alteration in Rubisco activity or reallocation of leaf nitrogen content (Smirnoff 1998, Flexas and Medrano, 2002).

*Changes in osmotic protectants*

Proline and glycine betaine are known to be stress-related compounds (Chen & Murata, 2008) and their extent of induction depended on plant species and the severity of stress (Goyal and Asthir 2010, Chen & Murata 2011). In our study, overall drought caused considerable inductions of proline and glycine betaine. Accumulation of large amounts of osmolytes is an adaptive response and a protection mechanism against drought promoted cellular damage, by detoxification of reactive oxygen species, stabilization of proteins and protein complexes, protecting chloroplast and photosynthesis system II (PSII), and indirectly interacting with phosphatidylcholine moieties of membranes to alter their thermodynamic properties and as signaling/regulatory molecules (Chen & Murata, 2008; Szabados and Savourè, 2010). For example, *Lotus corniculatus* cv. San Gabriel under drought and salt (Borsani *et al.* 1999) and sugarcane under heat stress (Wahid and Close 2007) were reported to accumulate large amounts of proline or GB.

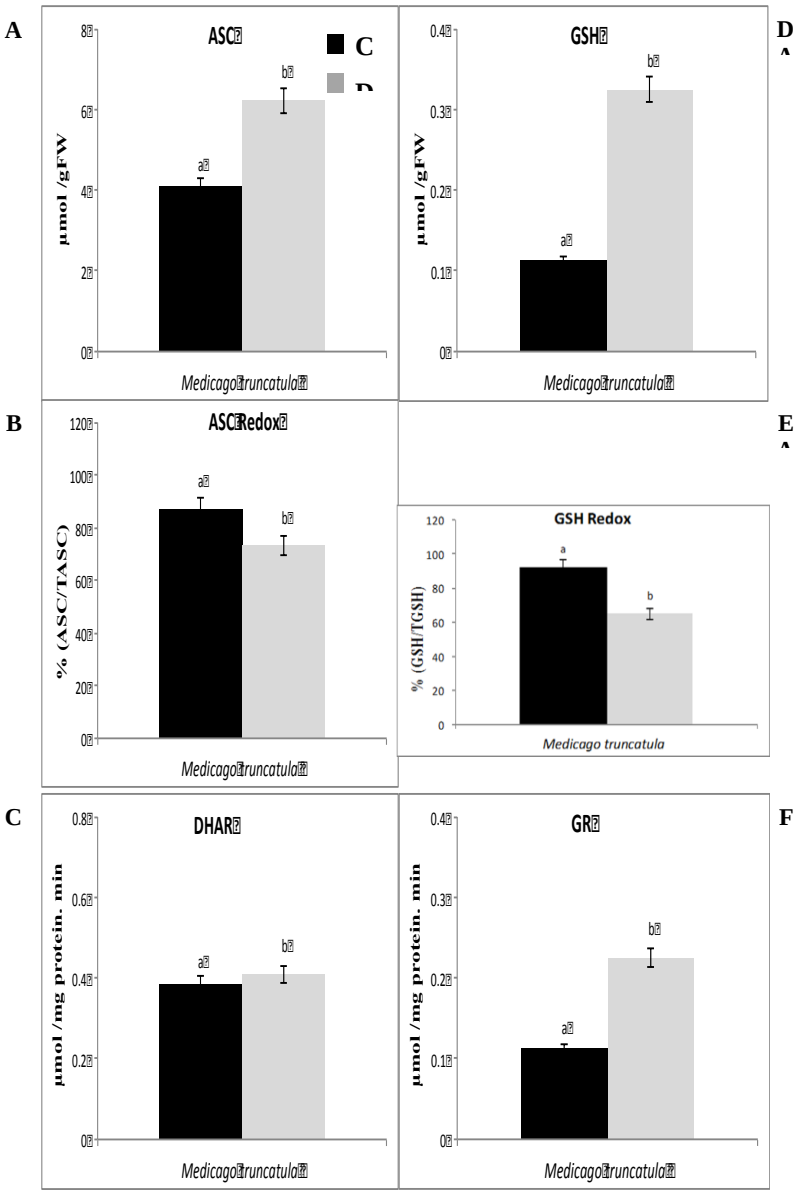


**Fig. 7. Ascorbate-glutathione cycle of Medicagotruncatula under drought stress (Ds) compared with control (C); (A) ascorbate, (B) ascorbate redox status (ASC/totalASC), (C) dehydroascorbate reductase (DHAR), (D) glutathione, (E) glutathione redox status (GSH/totalGSH), (F) glutathione reductase (GR). Different letters in the graph represents the significant differences between the four treatments (Duncan test; $P < 0.05$; (n=4).**

*Oxidative stress / antioxidants*

Apart from changes in osmo-protectants, drought typically also results in oxidative stress responses (Erice *et al.* 2007; Cruz de Carvalho 2008). Generally, these results confirm the occurrence of oxidative cellular damage, as well as the induction of antioxidant responses by drought demonstrating that plants experience oxidative stress. Lipid peroxidation (MDA) as one of stress damage markers was increased with drought. The increases of MDA matched well with rise of $H_2O_2$ by drought condition which could be an explanation for high destructive oxidative processes (eg., MDA) (Salazar-Parra *et al* 2012). Moreover, Schwanz and Polle (1998) and Erice *et al* (2007) attributed the low rate of oxidative damage to the decrease in ROS formation which resulting from an enhanced use of reluctant for assimilation in photosynthesis and a reduced photorespiration.

Similar increases by drought are observed in total antioxidant capacity, in prominent classes of antioxidant molecules, the flavonoids and polyphenols, and in the lipid-phase antioxidants, the tocopherols. Induction of ROS scavenging as an adaptive response to stressful condition to minimize free radicals damage was also observed in many studies (Mittler *et al.* 2004; Gill and Tuteja 2010). The content of the water soluble and non soluble antioxidants increased under drought stresses and also increases under elevated-temperature conditions (Borsand Michel 2002), which depend strongly on the species, and plant growth stages (Munné-Bosch *et al.* 2013). Remarkably, despite increases in various antioxidant molecules, including membrane-associated tocopherols, the increased level of lipid peroxidation demonstrates that these increases are insufficient to effectively protect the plant membranes where ROS production exceeded the capacity of the antioxidative systems to remove them (Smirnoff 1993; Gill &Tuteja, 2010)

*ASC / GSH - cycle*

The ascorbate-glutathione cycle, constitutes an important antioxidant system in plants. Reduced ascorbate (ASC) is oxidized by APX-mediated $H_2O_2$-scavengingreactions, and regenerated by mono-dehydroascorbate reductase (MDHAR) and/or dehydroascorbate reductase (DHAR), at the expense of NADPH and reduced glutathione respectively (Potters *et al* 2004; Foyer and Noctor 2005). These enzymes are therefore believed to determine cellular ASC levels as well as the ascorbate redox status.

However, quite in contrast with the other parameters we studied, the patterns of changes induced by drought in ASC, APX, MDHAR and DHAR. For example, in *Medicagolupulina*, the decreasing ASC levels with drought correlate to increased APX levels (consumption), and decreasing MDHAR activities (regeneration). Yet, at the same time, DHAR activities (regeneration) are also increased, which should result in higher ASC levels As a result of this heterogeneity, it is very difficult to correlate the changes in ASC levels to changes in particular enzymes.There was no correlation between ASC/GSH

content and their enzymes also was recorded in other studies for instance, Erice *et al* (2007), stated that GSH increased and ASC decreased while GR decreased and APX increased. The increase in ASC content also combined by increased in APX. Jime´nez *et al* (1998) also found the same uncorrelated pattern in pea leaves, where APX and MDHAR strongly decreased but ASC also decreased and GR decreased while GSH increased. Hernandez *et al* (1999) observed the same state in pea under High $CO_2$ (Decreasing the ASC with increasing DHAR and MDHAR while APX slightly up regulated).

## Conclusion

Drought stress significantly reduced photosynthesis and hence less biomass production of *Medicagotruncatula* . It also induced the antioxidant defense system, however oxidative stress was still increased.Induced oxidative stress was mirrored by high $H_2O_2$levels and that was accompanied by a significant induction in the most of antioxidants e.g., SOD, CAT, GPX, POX and GR and ASC and GSH.

## References

**AEBI H**., (1984) Catalase *in vitro*. Methods Enzymol., **105**, 121-126

**Bates, L.E., Waldren, RP Teare, I.D. (1973)** Rapid determination of free proline for water stress studies.*Plant and Soil***, 39**, 205-207.

**Benzie, I.F.F. & Strain J.J. (1996)** Ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. *Anal Biochem*, **239**: 70-76.

**Borsani, O. D.´ıaz P, Monza J. (1999)** Proline is involved in water stress responses of L. corniculatus nitrogen fixing and nitrate fed plants. *Journal of Plant Physiology*, **155**, 269-273.

**Bors, W. & Michel, C. (2002)** Chemistry of the antioxidant effect of polyphenols. Annals of the New York *Academy of Sciences*, **957**, 57-69.

**Booker, F.L., Reid, C.D., BrunschonHarti, S., Fiscus, E.L., & Miller, J.E. (1997)** Photosynthesis and photorespiration in soybean Glycine max (L.) Merr. chronically exposed to elevated carbon dioxide and ozone. *Journal of Experimental Botany*, **48**, 1843-1852.

**Bright, Jo., Radhika, Desikan, John, T. Hancock, Iain, S. Weir and Steven, J. Neill1 (2006)**ABA-induced NO generation and stomatal closure in Arabidopsis are dependent on H2O2 synthesis. *The Plant Journal*, **45**, 113-122

**Chang, C.-C., Yang, M.-H., Wen, H.-M. and Chern, J.-C. (2002)** Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis*, **10**, 178-182.

**Chaves, M.M., Maroco, J.P. and Pereira, J.S. (2003)** Understanding plant responses to drought — from genes to the whole plant. *Functional Plant Biology*, **30**, 239.

**Chen, T. H.H. and Murata, N. (2008)** Glycinebetaine: an effective protectant against abiotic stress in plants. *Trends in Plant Science,*Volume, **13,** Issue 9, Pages 499-505

**Chen, T.H.H. & Murata, N. (2011)** Glycinebetaine protects plants against abiotic stress : mechanisms and biotechnological applications. *Plant, Cell and Environment*, **34**, 1-20.

**Cruz, de Carvalho, M.H. (2008)** Drought stress and reactive oxygen species. *Plant Signaling & Behavior*, **3**, 156-165.

**Dempsey, D. A. and Klessig, D. F. (1995)** Signals in plant disease resistance. *Bulletin de I'Institut Pasteur*, **93**:167-186.

**DHINDSA, R.S., PLUMB-DHINDSA, P. and THORPE, T.A. (1981)** Leaf Senescence: Correlated with Increased Levels of Membrane Permeability and Lipid Peroxidation, and Decreased Levels of Superoxide Dismutase and Catalase. *Journal of Experimental Botany*, **32**, 93-101.

**Erice, G., Aranjuelo, I., Irigoyen, J.J. and Sánchez-Díaz, M. (2007)** Effect of elevated CO 2 , temperature and limited water supply on antioxidant status during regrowth of nodulated alfalfa. *Physiologia Plantarum*, **130**, 33-45.

**Flexas, J. and Medrano, H. (2002)** Drought-inhibition of photosynthesis in C3 plants: stomatal and non-stomatal limitations revisited. *Annals of Botany*, **89**, 183-189.

**Flexas, J., Ribas-Carbó, M., Hanson, D.T., Bota, J., Otto, B., Cifre, J., McDowell, N., Medrano, H. and Kaldenhoff, R. (2006)** Tobacco aquaporin NtAQP1 is involved in mesophyll conductance to CO2 in vivo. *The Plant journal : for cell and molecular biology*, **48**, 427-39.

**Foreman, Julia , Vadim, Demidchik, John, H. F. Bothwell, Panagiota, Mylona, Henk, Miedema, Miguel Angel Torres, Paul Linstead, Silvia Costa, Colin Brownlee, Jonathan, D. G. Jones, Julia M. Davies & Liam, Dolan (2003)** Reactive oxygen species produced by NADPH oxidase regulate plant cell growth. *Nature* **422**, 442-446

**Foyer, C.H. and Noctor, G. (2005)** Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. *The Plant cell*, **17**, 1866-1875.

**Foyer, C.H., Bloom, A.J., Queval, G. and Noctor, G. (2009)** Photorespiratory metabolism: genes, mutants, energetics, and redox signaling. *Annual Review of Plant Biology* **60**, 455-84.

**Gill, S.S. and Tuteja, N. (2010)** Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant physiology and biochemistry*, **48**, 909-930.

**Goyal, M. and Asthir, B. (2010)** Polyamine catabolism influences antioxidative defense mechanism in shoots and roots of five wheat genotypes under high temperature stress. *Plant Growth Regulation*, **60**, 13-25.

**Grieve, C.M. and S.R. Grattan. (1983)** Rapid assay for the determination of water soluble quaternary ammonium compounds. *Plant Soil*, **70**: 303-307.

**Hebeisen, T., Lüscher, A., Zanetti, S., Fischer, B.U., Hartwig, U. a, Frehner, M., Hendrey, G.R., Blum, H. and Nösberger, J. (1997)** Growth response of Trifolium repens L . and Lolium perenne L . as monocultures and bi-species mixture to free air CO 2 enrichment and management. *Global Change Biology*, **3**, 149-160.

**Hernández, J.A, Campillo, A, Jiménez, A, Alarcón, J.J. & Sevilla, F. (1999)** Response of antioxidant systems and leaf water relations to NaCl stress in pea plants. *New Phytologist*, **141**, 241-251.

**Hodges, D.M., DeLong, J.M., Forney, C.F. & Prange, R.K. (1999)** Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. *Planta*, **207**, 604-611.

**Jiang, Z.Y., Woollard, A.C. and Wolff, S.P. (1990)** Hydrogen peroxide production during experimental protein glycation. *FEBS letters*, **268**, 69-71.

**Jiménez, A., Hernández, JA., Pastori, G., del Río LA. and Sevilla, F. (1998)** Role of the ascorbate ‑ glutathione cycle of mitochondria and peroxisomes in the senescence of pea leaves. *Plant Physiology*, **118**,1327-1335.

**Johnson, J.D., Tognetti, R., & Paris, P. (2002)** Water relations and gas exchange in poplar and willow under water stress and elevated atmospheric CO2. *Physiologia plantarum*, **115**, 93-100.

**Kumar, K.B. and Khan, P.A. (1982)** Peroxidase in excised ragi (Eleusine coracana cv. PR 202) leaves during senescence. *Indian Journal of Experimental Botany*, **20**, 412-416.

**Kwak, June M., Vinh, Nguyen and Julian, I. Schroeder (2006)** The Role of Reactive Oxygen Species in Hormonal Responses. *Plant Physiology* June 2006 vol. 141 no. 2 323-329

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

**Le Martret, B., Poage, M., Shiel, K., Nugent, G.D. and Dix, PJ. (2011)** Tobacco chloroplast transformants expressing genes encoding dehydroascorbate reductase, glutathione reductase, and glutathione-S-transferase, exhibit altered anti-oxidant metabolism and improved abiotic stress tolerance. *Plant Biotechnology Journal* **9**, 661-673.

**Lee, Y.P. and Takahashi, T. (1966)** *Anal. Biochem.*, **14**, pp 71-77.

**Leyva, M., Vicedo, B., Finiti, I., Flors, V., Del Amo, G. and Real, M. (2008)** Preventive and post-infection control of Botrytis cinerea in tomato plants by hexanoic acid. *Plant Pathol.***57,** 1038-1046.

**Lodge, R.J., Dijkstra, P., Drake, B.G. and Morison, J.I.L. (2001)** Stomatal acclimation to increased CO2 concentration in a Florida scrub oak species, Quercus myrtifolia Willd. *Plant, Cell & Environment,* **24**: 77- 88.

**Mittler, R. (2002)** Oxidative stress, antioxidants and stress tolerance. *Trends in plant science*, **7**, 405-410.

**Mittler, R., Vanderauwera, S., Gollery, M., Breusegem, F. Van and Van Breusegem, F. (2004)** Reactive oxygen gene network of plants. *Trends in plant science*, **9**, 490-498.

**Munné-Bosch, S., Queval, G. and Foyer, C.H. (2013)** The impact of global change factors on redox signaling underpinning stress tolerance. *Plant Physiology*, **161**, 5-19.

**Murshed, S.M.S., Leong, K.C. and Yang, C. (2008)** Thermophysical and electrokinetics properties of nanofluids - A Critical Review. *Applied Thermal Engineering*, **28**, 2109-25.

**Porra, R.J., Thompson, W.a. and Kriedemann, P.E. (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. *Biochimica et Biophysica Acta* (BBA) - *Bioenergetics*, **975**, 384-394.

**Potters, G., Horemans, N., Bellone, S., Caubergs, R.J., Trost, P., Guisez, Y.& Asard, H. (2004)** Dehydroascorbate influences the plant cell cycle through a glutathione-independent reduction mechanism. *Plant physiology*, **134**, 1479-1487.

**Rogers, Alistair, (2006)** "The Effect of Elevated CO2 on Carbon and Nitrogen Metabolism in Soybean Under FACE ASA-CSSA-SSSA Annual Meeting Abstracts. Vol. 22990. ASA-CSSA-SSSA Annual Meeting Abstracts. Available: http://crops/cpmfex. com/crops/2006am/techprogram, 2006.

**Schwanz, P. and Polle, A. (1998)** Antioxidative systems, pigment and protein contents in leaves of adult mediterranean oak species (Quercus pubescens and Q. ilex) with lifetime exposure to elevated CO2. *New Phytologist* **140**, 411-423.

**Salazar-Parra, C., Aguirreolea, J., Sánchez-Díaz, M., Irigoyen J.J. and Morales, F. (2012)** Climate change (elevated $CO_2$, elevated temperature and moderate drought) triggers the antioxidant enzymes' response of grapevine cv. Tempranillo, avoiding oxidative damage. *Physiologia plantarum*, **144**, 99-110.

**Sarath, G., Hou, G., Baird, L.M. and Mitchell, R.B. (2007)** Reactive oxygen species, ABA and nitric oxide interactions on the germination of warm-season C4-grasses. *Planta*, 226, 697-708.

**Savin, R. and Nicolas, M. (1996)** Effects of Short Periods of Drought and High Temperature on Grain Growth and Starch Accumulation of Two Malting Barley Cultivars. *Australian Journal of Plant Physiology*, **23**, 201.

**Shah, N.H. and Paulsen, G.M. (2003)** Interaction of drought and high temperature on photosynthesis and grain-filling of wheat. *Plant and Soil*, **257**, 219-226.

**Smirnoff, N. (1993)** The role of active oxygen in the response of plants to water deficit and desiccation. New Phytologist, Tansley re, 27-58.

**Szabados, L. & Savouré, A. (2010)** Proline: a multifunctional amino acid. *Trends in plant science*, **15**, 89-97.

**Van Gestelen, P., Asard, H. and Caubergs, R.J. (1997)** Solubilization and separation of a plant plasma membrane NADPH-O 2 synthase from other 2 NAD(P)H oxidoreductases. *Plant Physiol*, **115**: 543-550

**Wahid, A., Gelani, S., Ashraf, M. & Foolad, MR. (2007)** Heat tolerance in plants : An overview. **61**, 199-223.

**Wand Stephanie, J. E., GuY. F. Midgley, Michael, H. Jones, and Peter, S. Curtis (1999)** Responses of wild C4 and C3 grass (Poaceae) species to elevated atmospheric CO2 concentration: a meta-analytic test of current theories and perceptions. *Global Change Biology* **5**(6), 723-741.

**Xu Z.Z. and Zhou G.S. (2006)** Combined effects of water stress and high temperature on photosynthesis, nitrogen metabolism and lipid peroxidation of a perennial grass Leymus chinensis. *Planta*, **224**, 1080-1090.

**Zhang, Q., Zhang, J., Shen, J., Silva, A., Dennis, DA. and Barrow, C.J. (2006)** A Simple 96-Well Microplate Method for Estimation of Total Polyphenol Content in Seaweeds. *Journal of Applied Phycology*, **18**, 445-450.

(*Received* 27/6/2016;
*accepted* 6 /3/2017)

# الاستجابات الفسيولوجية والبيوكيميائية للفصة البرميلية تجاه إجهاد الجفاف

**ممتاز محمد يحيى حجاب**
قسم النبات و الميكروبيولوجي - كلية العلوم - جامعة بني سويف -جمهورية مصر العربية.

كثيراً ما يلاحظ إن إجهاد الجفافيؤدي إلىانخفاض نمو النبات و العديد من الأضرار الخلوية الناتجة عنه. ومع ذلك، لا يتضح الفهم الكاملللآليات الفسيولوجية والبيوكيميائية الأساسية المرتبطة به. في هذا البحث، تم تعريض نبات الفصةالبرميلية (الفصيلة البقولية) لإجهاد الجفاف عن طريق سحب المياه في مرحلة النضج ولمدة أسبوع واحد. وقد أدى إجهاد الجفاف لانخفاض نمو النبات، مع تثبيط التمثيل الضوئي،و حركة الغازات من و إلى الثغور والاكسدة التي سببها. ومن التغيرات الناجمة أيضاً عنالإجهاد المائي، على سبيل المثالزيادة الوقاية من التناضح عن طريق زيادة المحتوى من البرولين و البيتادين ومستوى المعلمات الاكسدة( أول أكسيد الهيدروجين وبيروكسيد الدهون). و بالتوازي،يصاحبالمستويات العالية منأول أكسيد الهيدروجينو MDA، نشاط واضح لكلاً من أكسيجيناز الدهني و NADPH. و نتيجة لزيادة إنتاج أول أكسيد الهيدروجين، لوحظ ظهور نشاط واضح في بعض مكونات الدفاع لمضادات الأكسدة (على سبيل المثال زيادة السوبر أكسي ديس ميتويز(SOD)، الكاتاليز (CAT)، الجلوتاثيون بيروكسيديز (GPX)، البيروكسيداز (POX) و الجلوتاثيون المختزل(GR) . وتبع ذلك زيادة ملحوظة في مكونات أخرى لمضادات الأكسدةنتيجة لإجهاد الجفاف(دورة أسكوربات الجلوتاثيون).