

Effect of Drought Stress on Photosynthetic Efficiency of *Glycine max* L. Plants

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ABSTRACT

The current study is carried out to investigate the response of photosynthetic efficiency of soybean plant to drought stress extended for six days. Plants exposed to water deficit, growth inhibition and chlorophyll content losses increased. All drought treatments resulted in distinct physiological and biochemical effects on photosynthesis in a duration dependent manner. Relatively high inhibitory effect was observed throughout the experimental period, six days drought almost totally crushed the dynamics of chlorophyll accumulation in soybean leaves. A marked decrease in the ratio of chlorophyll a/b occurred in 6 days drought treated soybean leaves. Thus, a significant reduction in content of aminolaevulinic acid (ALA) was observed after 6 days drought treatment compared with relatively less decrease after 2 and 4 days drought treatments. The maximal photochemical efficiency of soybean leaves subjected to drought treatment, decreased sharply in Fv/Fm values to approximately half of the corresponding control at the end of drought duration. The photosynthetic strength of the plant indicated by Fv/Fo values showed that, soybean leaves exposed to drought were significantly decreased especially at the end of experimental period. Drought stressed soybean leaves showed a highly significant increase in H₂O₂ and MDA contents reached about 4.5 fold increase relative to control after 6 days drought treatment. This means that drought caused depression of photosynthetic efficiency of soybean leaves in a period-dependent manner.

Key words: Photosynthesis, drought, soybean, photosynthetic pigments.

INTRODUCTION

Water stress is considered as one of the most important environmental factors that causes osmotic stress and limiting plant growth and development as well as crop productivity. At the whole plant level, the effect of water stress is usually perceived as a decrease in photosynthesis and growth, and is associated with alterations in C and N metabolism. Furthermore, the imposition of biotic and abiotic stress conditions can give rise to excess concentrations of active oxygen species, resulting in oxidative damage at the cellular level. Therefore, a consequence of water stress is the limitation of photosynthesis and usually accompanied by the formation of active oxygen species (AOS) in the chloroplasts (Smirnoff, 1993) such as the superoxide radical, H₂O₂, and the hydroxyl radical (Foyer *et al.*, 1994; Asada, 1997).

In chloroplasts, the superoxide anion radical (O₂⁻) is produced by photoreduction of O₂ at PSI and PSII, and singlet oxygen (¹O₂) is formed by energy transfer to O₂ from triplet excited state chlorophyll through the ferredoxin/ferredoxin NADP⁺ oxidoreductase system (Asada *et al.*, 1998), and eventually the highly toxic hydroxyl radical (·OH) produced from the non-enzymatic chemical reaction between superoxide and H₂O₂. Hydrogen peroxide is especially toxic in the chloroplasts because even at low concentrations it inhibits the Calvin-cycle enzymes, hence reducing the photosynthetic carbon dioxide assimilation (Takeda *et al.*, 1995).

The 5-aminolevulinic acid (ALA) is a key precursor in the biosynthesis of porphyrins, such as chlorophyll and heme. The effect of abiotic stress on photosynthetic process was secondary as compared to chlorophyll synthesis in tomato and in maize respectively by interacting with protochlorophyllide reductase and

5-aminolevulinic acid (ALA) formation (Stobart and Ameen-Bukhari, 1984; Watanabe *et al.* 2000).

Abiotic stress including drought is also play an important role in regulation of PSII-mediated electron transport (Ruley *et al.*, 2007). Therefore, chlorophyll fluorescence has been widely used to study the effect of stress on: the electron transport chain associated with photosystem II (PSII) and the course and mechanism(s) of drought-induced damage to the photosynthetic apparatus. The fluorescence yield of chlorophyll a is dependent on the micro-environment in the thylakoid and is used as an indicator of structural or organizational changes of the chloroplastic membranes and also for the physiological and biochemical changes in the photosynthetic apparatus (Van Heerden *et al.*, 2003). Furthermore, Fv/Fm is often used as an indicator of cellular stress and can indicates the overall health of photosynthetic machinery and the photochemical efficiency of the PSII in higher plants (Maxwell and Johnson, 2000). Also Fv/Fo value indicates the size and number of active photosynthetic centers in the chloroplast, and therefore the photosynthetic strength of the plant (Dan *et al.*, 2000).

The objective of the present study was to evaluate the response of photosynthetic apparatus of soybean plant as one of the most important economic food crops to various drought stress.

MATERIALS AND METHODS

Plant material and growth conditions

Seeds of soybean (*Glycine max* L.) were surface sterilized by immersion for 2 min in 0.1% HgCl₂, then washed with five changes of sterile distilled water. Seeds were soaked in continuously aerated distilled water for 24 h in darkness. At the end of soaking period,

seeds were planted in plastic pots (15 cm diameter × 20 cm height), filled with acid washed pure quartz sand. All pots were placed in a growth chamber under 70-80% relative humidity with 16/8h day/night cycle and controlled temperature of 28/26°C. Light intensity was 420 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at the top of plants supplied by a mixture of fluorescent and incandescent lamps. Each pot was irrigated with 250 ml of distilled water at first, then occasionally with a certain amount of water in order to keep the soil water content constant. After seven days, all plants were watered on alternate days with half strength of Hoagland solution. After 15 days from sowing one-half of the plants were subjected to water stress by withholding water for 6 days and sampled in regular intervals (2 days) for analyses. Just after harvest, the whole plants or dissected organs were blotted dry and weighed carefully for fresh weight determination, then dried in a hot-air oven at 65°C until a constant weight to obtain dry weight. For biochemical analyses, the second leaves were harvested and used either immediately for extractions or were stored at -20°C until analysis.

Photosynthetic pigments were extracted from fresh samples ground at a low light intensity in 10 ml 80% acetone at 4°C. Absorbance of centrifuged extracts was measured with a spectrophotometer (Jenway 6305 UV/Vis, UK) at specified wavelengths required for computation of chlorophyll a, b and total carotenoids from published formula (Lichtenthaler, 1987). Pellets remaining after centrifugations were dried at 60 °C and weighed. Photosynthetic pigments contents were expressed as $\text{mg g}^{-1} \text{DM}$.

Aminolaevulinic acid (ALA) was extracted and estimated by the method of Stobart and Ameen-Bukhari (1984). The concentration of ALA was determined using the calculated extension coefficient $7.24 \cdot 10^4$ nmol.

Chlorophyll fluorescence measurements were monitored in fully-expanded and young leaves using a PAM 101 Chlorophyll Fluorometer (Watz, Effelrich, Germany). Stressed and control leaves were pre-darkened for 40 min before starting the measurements. The conceptual approach and detailed analytical derivation of the various parameters have been described by Maxwell and Johnson, 2000.

The level of lipid peroxidation was expressed as MDA content and was assayed by the method of Hodgson and Raison (1991) as 2-thiobarbituric acid (TBA) reactive metabolites. Plant fresh tissues (0.2 g) were homogenized and extracted in 10mL of 0.25% TBA made in 10% trichloroacetic acid (TCA). Extract was heated at 95 °C for 30 min and then quickly cooled on ice. After centrifugation at 10,000×g for 10 min, the absorbance of the supernatant was measured at 532 nm. Correction of non-specific turbidity was made by subtracting the absorbance value taken at 600 nm. The level of lipid peroxidation was expressed as mM g^{-1} fresh weight by using an extinction coefficient of $155 \text{mM}^{-1} \text{cm}^{-1}$.

Hydrogen peroxide was measured colorimetrically as described by Jana and Choudhuri (1982). Leaves (1 g fresh weight) frozen in liquid N₂ were ground using a pestle and mortar with 1 ml of 3% (v/v) HClO₄ containing 2.5mM EDTA. The homogenate was centrifuged at 12,000g for 5 min at 4°C. The supernatant was neutralized with 2.5 M KOH to pH 7.5, and then centrifuged at 12,000g for 5 min at 4°C. The supernatant was then passed through an anion-exchange column (Okuda *et al.*, 1991). The elute was used for the determination of H₂O₂. The intensity of the yellow color of the supernatant was measured at 410nm. H₂O₂ level was calculated using the extinction coefficient $0.28 \text{mM}^{-1} \text{cm}^{-1}$.

Statistical analysis

The experiment was conducted in completely randomized design. Fluorescence results were the mean of ten independent measurements for each treatment. Pigments, H₂O₂, MDA and ALA content results were the mean of four measurements per treatment. The significance difference between mean values was determined by one-way analysis of variance. Duncan's multiple range test was used to compare the means.

RESULTS

Changes in chlorophyll contents were proportional to the relative duration of treatment (Table 1). Relatively high inhibitory effect was observed throughout the experimental period, 6 days drought almost totally crushed the dynamics of chlorophyll accumulation in

Table (1): Changes in chlorophyll a and b contents, chlorophyll a/b ratio and total carotenoids in leaves of soybean plant subjected to drought stress for 6 days. Data are means ± SE (n=5). Values in parentheses were expressed as the percent of reduction relative to the control.

Time (days)	$\text{mg g}^{-1} \text{DW}$							
	Chl a		Chl b		Chl a/b ratio		carotenoids	
	Cont.	Dr.	Cont.	Dr.	Cont.	Dr.	Cont.	Dr.
2	5.9±0.66	4.45±0.40	2.1±0.10	1.87±0.06	2.81±0.20	2.4±0.10	1.72±0.09	2.2±0.20
	100%	(75%)	100%	(89%)	100%	(85%)	100%	(128%)
4	6.63±0.70	2.00±0.40	2.4±0.10	1.09±0.05	2.76±0.23	1.83±0.06	1.8±0.10	0.9±0.011
	100%	(30%)	100%	(45%)	100%	(66%)	100%	(50%)
6	7.01±0.80	0.9±0.01	2.81±0.12	0.63±0.04	2.5±0.28	1.42±0.04	1.84±0.13	0.2±0.009
	100%	(13%)	100%	(22%)	100%	(57%)	100%	(11%)

soybean leaves. At 2, 4 and 6 days drought treatment, chlorophyll a content revealed a reduction equal to 25, 70 and 87 %, respectively in comparison with control. The corresponding values for chlorophyll b were 11, 55 and 78 %, respectively. As a consequence, the Chl a/b ratio decreased significantly during the drought treatments (Table 1). A marked decrease in the ratio of chlorophyll a/b, from 2.5 to 1.42 occurred in 6 d drought treated soybean leaves. A similar relationship was also observed for the total carotenoids pool (Table 1), a sharp decrease in total carotenoids was observed parallel to prolongation of drought treatments especially after completion of the experimental period, which was 89% less than the corresponding control (Table 1). Furthermore, drought stress inhibits the light mediated production of 5-aminolaevulinic acid (ALA) in leaves. The results given in Figure (1) showed that drought decreased the content of ALA in a duration dependent manner. Thus, about 62 % reduction in content of ALA was observed after 6 days drought treatment compared with 40 and 20%, respectively after 2 and 4 days drought treatments. The quantum yield efficiency of PSII expressed as F_v/F_m ratio (Fig. 2). The maximal photochemical efficiency of soybean leaves subjected to drought treatment, decreased sharply in F_v/F_m values to 0.47 (about 59% of the corresponding control) at the end of drought duration, in comparison the seedlings

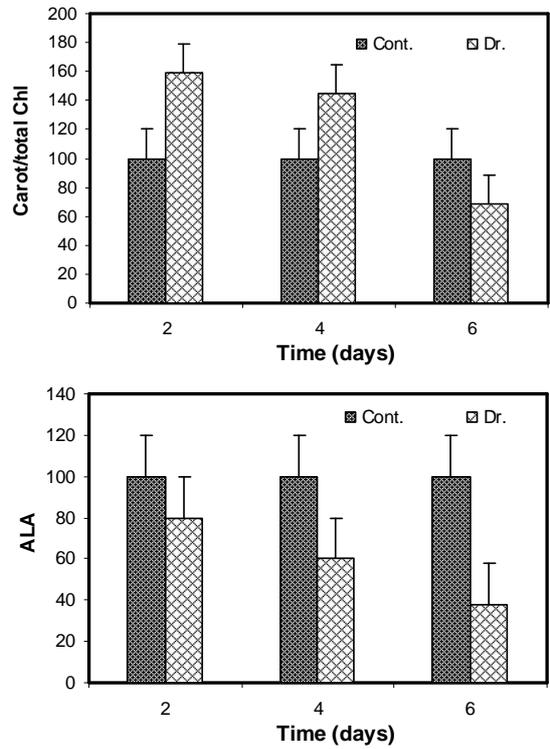


Figure (1): Change in Carotenoids to total chlorophyll pigments and aminolaevulinic acid (ALA) percentage in leaves of control and drought stressed soybean leaves for 6 days. Each value represents mean \pm SE of five replicates.

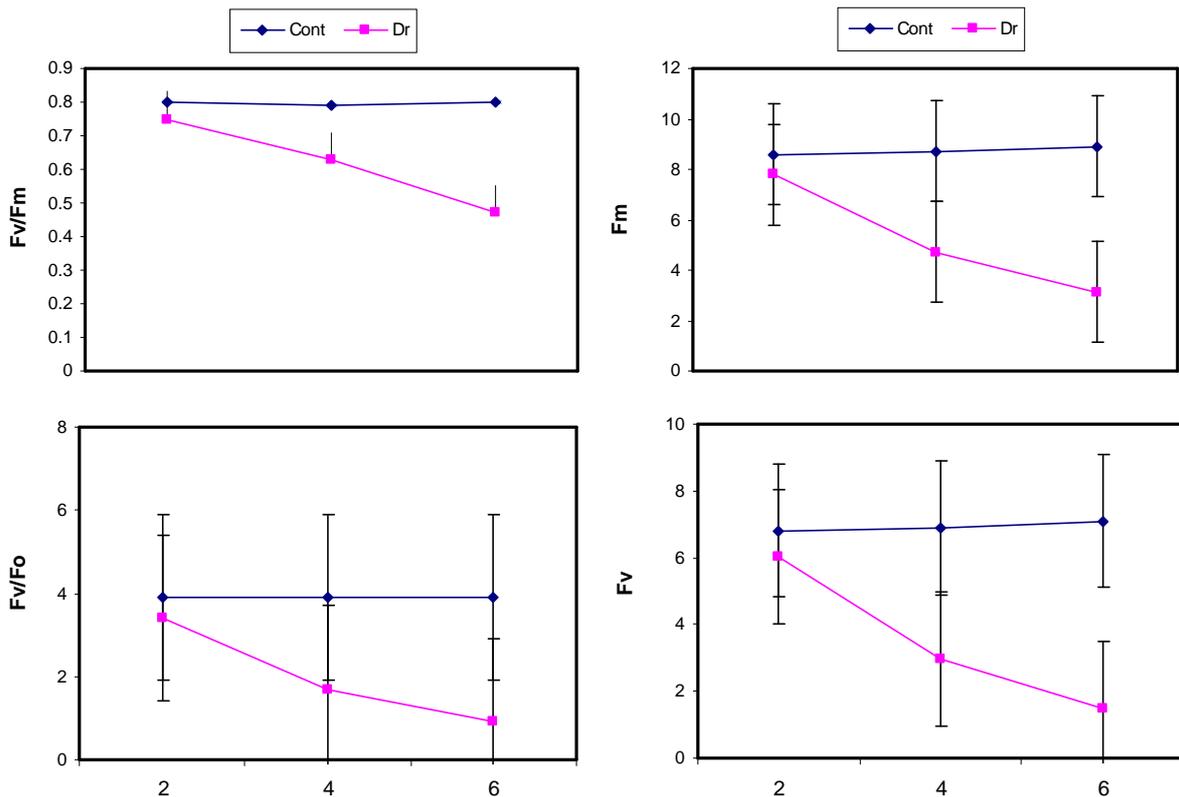


Figure (2): Maximum, variable fluorescence and the ratio of variable to maximum fluorescence and variable to initial fluorescence of chlorophyll.

subjected to 2d drought exhibited a slight decline (about 11% of the control). The drastic effect of drought on photochemical efficiency increased with increased drought duration treatment (Fig. 2D). This shows that leaves exposed to the drought for 6 days were most heavily affected with respect to F_v/F_m . Also the F_v/F_0 showed the same manner throughout the experimental period. F_v/F_0 ratio in soybean drought treated leaves reached to 0.9 (about 77% decrease) compared with approximately 4 in control at the end of drought treatment (Fig. 2C and D).

MDA content increased significantly throughout the experimental period. Significant stimulation (445%) of MDA appeared after 6 days drought treatment (Fig. 3A).

A significant increase in the generation H_2O_2 was observed parallel to increment of drought period treatment. Drought stressed soybean leaves showed a highly significant increase in H_2O_2 content reached about 4.5 fold increase relative to control after 6 days drought treatment (Fig. 3B).

DISCUSSION

It is well known that photosynthetic systems in higher plants are most sensitive to water stress treatment (Falk *et al.*, 1996). Previous studies showed that water stress induced changes in the photosynthetic apparatus and the membrane permeability properties of chloroplasts. This fact may be the result in chlorophyll degradation and/or synthesis deficiency together with a decrease of thylakoid membrane integrity (Dean *et al.*, 1993). Water stress-induced reduction in pigment contents (Table 1). The decline in the chlorophyll content under water stress may be explained by the earlier structural loss of the chloroplast stroma lamellae, containing photosystem I and most of the chlorophyll a (Loggini *et al.*, 1999). Photoinhibition and photodestruction of pigments may contribute to such changes. In addition, the photosynthetic apparatus may show acclimation responses such as changes in the relative proportion of stacked and unstacked membrane domains (Anderson and Aro, 1994). The present results showed that leaves exposed to drought stress not only showed drastic reduction in total chlorophyll content, but also the Chl a/b ratio decreased (Table 1). The ratio of chlorophyll a/b was more sensitive to the water stress treatment, showing that Chl a was more susceptible to water stress, being degraded at a higher rate than Chl b. This can be explained by the fact that part of the decrease in chlorophyll a could be accounted by conversion to chlorophyll b by the oxidation of the methyl group on ring II to the aldehyde (Chettri *et al.*, 1998 and Fang *et al.*, 1998). However, in the present study, the observed decrease in leaf chlorophyll content in response to water stress was in accordance with the findings of Eija *et al.* (2002) and Ibrahim (2004). The decrease in the chlorophyll content may also be a phytotoxic

consequence of lipid peroxidation indicated by the significant increase in MDA content throughout the experiment and is associated with a decrease in photochemical efficiency. This could be achieved through the activation of toxic O_2 molecules that can then attack fatty acids chains in thylakoid membrane resulted in an increase of the membrane damage with a corresponding increase in the formation of MDA in soybean leaves (Fig. 3A).

Ibrahim (2004) has reported that the reduction in the Chl a/b ratio in maize plant might be due to a direct effect of water stress on the light harvesting complex of photosystem II (LHCII), suggesting that water stress treatment induced a lower rate of synthesis and accumulation of chlorophyll a indicated by the great reduction of ALA content, the key precursor in the biosynthesis of chlorophyll, throughout the drought treatment. A reduction of Chl a/b ratio in stressed plants is also due to a reduction of F_m that may reflect increased energy dissipation due to a destruction of photosynthetic apparatus. Hydrogen peroxide act as a redox signal molecules in plants exposed to water stress (Mehdy 1994; Ibrahim 2004) and inhibits chloroplast sulfhydryl-containing enzymes by readily oxidizing their sulfhydryl groups. Therefore, the results suggest that soybean leaves suffered from significant increase in H_2O_2 content which may be responsible for reduction in photochemical efficiency of the chloroplast during drought treatment (Fig. 3B).

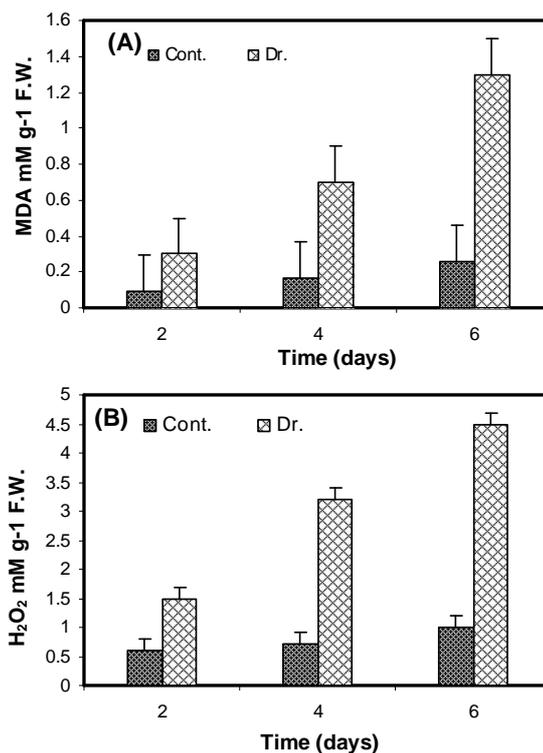


Figure (3): changes in hydrogen peroxide and malondialdehyde contents in soybean leaves in response to drought stress for six days. Each value represents mean \pm SE for five values.

The F_v/F_m value is an indicator of the photosynthetic efficiency of the plant. F_v/F_0 value indicates the size and number of active photosynthetic centers in the chloroplast, and therefore the photosynthetic strength of the plant. An F_v/F_m ratio of 0.8 or above and the F_v/F_0 value of 4.0 or higher indicates the plant is healthy and not suffering photosynthetic stress (Dan *et al.*, 2000). The F_v/F_0 values of soybean plants exposed to drought were significantly less than 4.0 especially at the end of experimental period. It is therefore reasonable to conclude that exposure to drought affect greatly the photosynthetic efficiency of *Glycine max* and thereafter, soybean plants showed some signs of photosynthetic stress when subjected to drought. (Figs. 2C and D). These results are in agreement with Ruley *et al.* (2007), who reported that F_v/F_m and F_v/F_0 values were significantly affected in response to stress. This means that drought caused depression of photosynthetic efficiency of soybean leaves in a period-dependent manner.

REFERENCES

- ANDERSON, J.M., AND E.M. ARO. 1994. Grana stacking and protection of photosystem II in thylakoid membranes of higher plant leaves under high irradiance: an hypothesis. *Photosynthetic Research* **41**: 315-326.
- ASADA, K. 1997. The role of ascorbate peroxidase and monodehydroascorbate reductase in H_2O_2 scavenging in plants. In JG Scandalios (Ed.), *Oxidative Stress and the Molecular Biology of Antioxidant Defenses*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- ASADA, K., T. ENDO, J. MANO, AND C. MIYAKE. 1998. Molecular mechanism for relaxation of and protection from light stress. In K. Sato, N. Murata (Eds.), *Stress Responses of Photosynthetic Organisms*. Elsevier Science Publishing, Amsterdam.
- CHEETRI, M., C.M. COOK, E. VARDAKA, T. SAWIDIS, AND T. LANARAS. 1998. The effect of Cu, Zn and Pb on the chlorophyll content of the lichens *Cladonia convoluta* and *Cladonia rangiformis*. *Environmental and Experimental Botany* **39**: 1-10.
- CISCATO, M., R. VALCKE, K. VAN LOVEN, H. CLUISTERS, AND F. NAVARI-IZZO. 1997. Effect of in vivo copper treatment on the photosynthetic apparatus of two *Triticum durum* cultivars with different stress sensitivity. *Physiologia Plantarum* **100**: 901-908.
- DAN, T.V., S. KRISHNARAJ, AND P.K. SAXENA. 2000. Metal tolerance of scented geranium (*Pelargonium sp.* 'Frensham'): effects of cadmium and nickel on chlorophyll fluorescence kinetics, *International Journal of Biochemistry* **2**: 91-104.
- DEAN, M.A., C.A. LETNER, AND J.H. ELEY. 1993. Effect of autumn foliar senescence on chlorophyll a:b ratio and respiratory enzymes of *Populus tremuloides*. *Bull. Torrey Botany Club* **120**: 269-274.
- EIJA, P., M. KAIRAVUO, S. FRANTISEK, E. ARO, AND T. ESA. 2002. Excess copper predisposes photosystem II to photoinhibition in vivo by outcompeting iron and causing decrease in chlorophyll. *Plant Physiology* **129**: 1359-1367.
- FALK, S., D.P. MAXWELL, D.E. LAUDENBACH, AND N.P.A. HUNER. 1996. In *Advances in Photosynthesis, V.5, Photosynthesis and the Environment* (Neil R. Baker, Ed.), pp. 367-385. Kluwer Academic Publishers, Dordrecht / Boston / London.
- FANG, Z., J.C. BOUWKAMP, AND T. SOLOMOS. 1998. Chlorophyllase activities and chlorophyll degradation during leaf senescence in non-yellowing mutant and wild type of *Phaseolus vulgaris* L. *Journal of Experimental Botany* **49**: 503-510.
- FOYER, C.H., P. DESCOURVIERES, AND K.J. KUNERT. 1994. Protection against oxygen radicals: an important defense mechanism studied in transgenic plants. *Plant, Cell Environ* **17**: 507-523.
- HODGSON, R.A.J., AND J.K. RAISON. 1991. Lipid peroxidation and superoxide dismutase activity in relation to photoinhibition induced by chilling in moderate light *Planta* **185**: 215-219.
- IBRAHIM, M.M. 2004. Antioxidative Defense System, Photosynthetic Efficiency and Some Biochemical Changes in *Zea mays* L. Leaves Subjected to Water Stress. *Journal of Union Arab Biologists, Cairo, Physiology and algae* **15 (B)**: 23-48.
- JANA, S., AND M.A. CHOUDHURI. 1982. Senescence in submerged aquatic angiosperms: Effects of heavy metals. *New Phytologist* **90**: 477-484.
- LICHTENTHALER, H.K. 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Methods in Enz.* **148**: 350-382.
- LOGGINI, B., A. SCARTAZZA, E. BRUGNOLI, AND F. NAVARI-IZZO. 1999. Antioxidative defense system, pigment composition, and photosynthetic efficiency in two wheat cultivar subjected to drought. *Plant Physiology* **119**: 1091-1099.
- MAXWELL, K., AND G.N. JOHNSON. 2000. Chlorophyll fluorescence-a practical guide. *Journal of Experimental Botany* **51**: 659-668.
- MEHDY, M.C. 1994. Active oxygen species in plant defense against pathogens. *Plant Physiology* **105**: 467-472.
- OKUDA, T., Y. MATSUDA, A. YAMANAKA, AND S. SAGISAKA. 1991. Abrupt increase in the level of hydrogen peroxide in leaves of winter wheat is caused by cold treatment. *Plant Physiology* **97**: 1265-1267.
- RULEY, T., A. NILESH, C. SHARMA, AND SHIVENDRA V. SAHI. 2007. Antioxidant defense in a lead accumulating plant *Sesbania drummondii*. *Plant Physiology and Biochemistry* **42 (11)**: 899-906.
- SMIRNOFF, N. 1993. The role of active oxygen in the response of plants to water deficit and desiccation.

- New Phytol **125**: 27-58.
- STOBART, A.K., AND I. AMEEN-BUKHARI. 1984. The regulation of 5-aminolaevulinic acid synthesis and protochlorophyllide regeneration in the leaves of dark-grown barley seedlings. *Biochemistry Journal* **222**: 419-426.
- TAKEDA, T., A. YOKOTA, AND S. SHIGEOKA. 1995. Resistance of photosynthesis to hydrogen peroxide in algae. *Plant Cell Physiology* **36**: 1089-1095.
- VAN HEERDEN, P.D., G.H. KRUGER, M. TSMILLI-MICHAEL, R.J. STRASSER. 2003. Dark chilling effects on soybean cultivars during vegetative development: parallel studies of CO₂ assimilation, chlorophyll a fluorescence kinetics O-J-I-P and nitrogen fixation. *Physiologia Plantarum* **117**: 476-491.
- WATANABE, K., T. TANAKA, H. KURAMOCHI, Y. TAKEUCHI. 2000. Improving salt tolerance of cotton seedling with 5-aminolevulinic acid. *Plant Growth Regulation* **32**: 97-101.
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تأثير الإجهاد بالجفاف على كفاءة عملية البناء الضوئي في نبات فول الصويا

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الملخص العربي

توضح هذه الدراسة تأثير كفاءة عملية البناء الضوئي في بادرات نبات فول الصويا بعد تعرضها للجفاف المستمر لمدة ستة أيام. معدلات النمو والمحتوى الصبغى نقصت بشكل معنوي يزداد مع استمرارية وقت الجفاف. ظهرت تأثيرات سلبية واضحة على عديد من العمليات الفسيولوجية والكيموحيوية وبالأخص عملية البناء الضوئي وازداد هذا التأثير السلبي مع استمرارية تعرض نبات فول الصويا للجفاف. انخفضت نسبة يخضور أ/ ب بنسبة عالية المعنوية خلال فترات الجفاف ويرجع هذا الانخفاض إلى نقص كمية حمض الأمينوليفيولونيك ذو الدور الهام كمؤهل لتكوين الخضور.

كما تأثرت بشكل كبير الفسفرة اليخضورية لأوراق نبات فول الصويا في فترة الجفاف ووصل معدل الانخفاض إلى حوالي 50% مقارنة بالنباتات الغير معرضة للجفاف. أيضا أدى تعرض بادرات فول الصويا للجفاف إلى تكون كميات كبيرة من فوق أكسيد الهيدروجين والمالونداى ألدهيد وصلت إلى حوالي 4.5 ضعف الكمية الموجودة في النباتات الغير معرضة للجفاف. نستخلص من هذه النتائج أن تعرض نبات فول الصويا للجفاف له تأثير سلبي على عملية البناء الضوئي ويعتمد هذا التأثير على المدة التي يتعرض فيها النبات للإجهاد.