2011 2nd International Conference on Chemical Engineering and Applications
IPCBEE vol. 23 (2011) © (2011) IACSIT Press, Singapore

Effect of Plant Growth Promoting Rhizobacteria and Foliar Application of Amino Acids and Silicic Acid on Biochemical Biomarkers Activity of Wheat under Drought Stress

Farhad Davoodi Fard [1 +], Davood Habibi [2] and Mahdi Davoodi Fard [3]

[1] Department of Agronomy and Plant Breeding, Science and Research Branch, Islamic Azad University, Tehran, Iran.

[2] Department of Agronomy and Plant Breeding, Karaj Branch, Islamic Azad University, Karaj, Iran.

[3] Department of Agronomy and Plant Breeding, Roudehen Branch, Islamic Azad University, Roudehen, Iran.

Abstract. Dry and the stress resulting from factors that are important to agricultural production in restricted areas and yields of dry reduce. irrigation factor at two levels includes{a1 first level : Control, a2 second level: cut irrigation after flowering stage}anol the second experimental treatment at the five levels includes { b1 first level: control ,b2 second level: seed inoculated with bacteria (Azospirillum+Azotobacter +Pseudomonase), b3third level: seed inoculated with bacteria (Azospirillum +Azotobacter +Pseudomonase) and sprayed silicic acids, b4 fourth level : seed inoculated with bacteria (Azospirillum +Azotobacter + Pseudomonase) and sprayed amino acids,b5fifth level : seed inoculated with bacteria (Azospirillum +Azotobacter +Pseudomonase) and sprayed silicic acids with amino acids} in randomized complete blocks design with four replicates using analysis of split plot.Analysis of variance showed that the biochemical biomarkers in the1 percent level is significant, so that the highest levels of Malondialdehede, Dityrosine and8-Hydroxy-2-Deoxyguanosinerelated to cut irrigation after flowering. Effect second experimental treatment that the biochemical biomarkers in the1 percent level is significant and highest levels of biochemical biomarkers related to control and lowest levels belonged to seed inoculated with bacteria and sprayed silicic acids with amino acids, while the interactions between level irrigation and the second experimental treatment showed that the lowest levels of Malondialdehede and Dityrosine belonged to normal irrigation and seed inoculated with bacteria and sprayed silicic acid with amino acids (77.8 and 21.1 µ /mol g protein) ,respectively, while the lowest levels of 8-Hydroxy-2-Deoxyguanosine (10.33 µ /mol g protein)belonged to normal irrigation and seed inoculated with bacteria and sprayed silicic acid respectively. The results of this experiment can be inferred that the plant growth promoting bacteria associated with spraying silicic acid and amino acids in increased tolerance to drought stress in wheat because of lower production resulting the biochemical biomarkers there by reducing the oxidative damage caused by active oxygen species that are under drought stress .

Keywords: Wheat, Drought stress, Biochemical biomarkers, Plant growth promoting bacteria, Silicic acid, Amino acid.

1. Introduction

Drought stress is one of the most adverse factors for plant growth and productivity [1]. Drought affects different aspect of plant growth, through a series of morphological, physiological and metabolic changes [9] and reduces the yield of plant. Accumulation of reactive oxygen species (ROS) is one of the biochemical changes and occurred when plants are exposed to drought stress condition [15]. ROS include super oxide (O2), hydrogen peroxide (H2O2), hydroxyl (OH) [13, 12]. These ROS are cytotoxic for cells [8] and in high density, hurt cells lipids, proteins and nucleic acids and finally stop the natural metabolism of plant [8, 13].Chloroplast and mitochondria of plant cell are the major intracellular generator of reactive oxygen

+ Corresponding author. Tel.: +989122369651,+982144413185.
E-mail address: Fdavoodi1980@gmail.com.

species [15].When plants suffering from drought stress, they show a series of physiological, morphological and biochemical reactions to resist against the stress condition.

Further damage to fatty acid could then produce small hydrocarbon fragments including malondialdehyde (MDA) [2]. It hypothesized that modulation of the activities of these enzymes at early growth stage may be important in imparting resistance to a plant against environmental stresses. Therefore, in the present investigation the relative significance of anti oxidative enzymes, MDA, H2O2 content, PRO, GB accumulation, photosynthetic activity and membrane permeability has been examined at seedling stage in drought-tolerant and susceptible maize genotypes.

Acclimation of plants to drought is considered to promote antioxidants defense systems to face the increased levels of activated oxygen species (AOS), which in turn, cause membrane damage by lipid peroxidation and indicated by malondialdehyde (MDA) content, which is one main parameter for evaluating membrane oxidation extent and are toxic for the cells [7,14]. This research has been done to investigation the effect of moisture stress on the biochemical biomarkers activity of wheat and studying drought stress effects on membrane leakage /DNA damage and proteins degradation. We also wanted to find the effect of PGPR and Silicic acid and amino acid on decreasing stress in wheat.

2. Material and Methods

2.1. Agricultural practices

This research was carried out in 2009 -2010 in research field of Islamic Azad University-Karaj Branch Iran (longitude 59and $51^0$of east, latitude 48 and $35^0$of north with height of 1313 meter above sea). The soil texture of field was clay-loam with 0.091% nitrogen, 0.93% organic matter, saturated clay acidity 7.9 and 3.76 ds.m-1 electrical conductivity. irrigation factor at two levels includes{ a1 first level: Control, a2 second level: cut irrigation after flowering stage}and the second experimental treatment at the five levels includes {b1 first level: control, b2 second level: seed inoculated with bacteria (Azospirillum+ Azotobacter +Pseudomonase), b3 third level: seed inoculated with bacteria (Azospirillum +Azotobacter + Pseudomonase) and sprayed silicic acids, b4 fourth level: seed inoculated with bacteria (Azospirillum +Azotobacter + Pseudomonase) and sprayed amino acids, b5 fifth level : seed inoculated with bacteria (Azospirillum +Azotobacter + Pseudomonase) and sprayed silicic acids with amino acids} in randomized complete blocks design with four replicates using analysis of split plot.

For measuring grain yield, after physiological ripening, 2 square meter area separated and grains harvested.

2.2. Enzyme assays

2.2.1. Malondialdehyde Analysis

Proteins of tissue homogenate were precipitated with 40% trichloroacetic acid (TCA), w/v. The MDA assay was based on the condensation of one molecule malondialdehyde with two molecules of thiobarbituric acid (TBA) in the presence of reduced reagent volumes to increase sensitivity, generating a chromogen with UV absorbance. The TBA

+ MDA complex was analyzed by HPLC essentially as described by Bird et al [4]. Briefly, the HPL Csystem consisted of a Hewlett + Packard 1050 gradient pump (Avondale, PA) equipped with an automatic injector, a 1050 diode-array absorption detector and a personal computer using Chem Station Software from Hewlett + Packard. Aliquots of the TBA + MDA samples were injected on a 5 mm Supelcosil LC-18 reversed phase column (30 × 4.6 mm). The mobile phase consisted of 15%methanol in double-distilled water degassed by filtering through a 0.5 μm filter (Millipore, Bedford, MA).

The flow rate was 2 ml/min. MDA + TBA standards were prepared using tetraethoxypropane. The absorption spectra of standards and samples were identical with a characteristic peak at 540 nm. Measurements were expressed in terms of malondialdehyde (MDA) normalized to the sample protein content. Protein content was determined by the method of Bradford, with standard curves prepared using BSA [6].

2.2.2. Determination of 8-Hydroxy-2-Deoxyguanosine (8-OH-2-DG) in Urine

8-hydroxy-2-deoxyguanosine levels in tissue extraction were measured essentially as described previously [5]. Briefly, an automated column switching LCEC method for 8-OH-2-DG is based on the unique selectivity of integral porous carbon column for purines. Samples were injected on to a C8 column and the band containing 8-OH-2-DG was then quantitatively trapped one carbon column. The selectivity of the carbon column for8-OH-2-DG allows elimination of interfering peaks by washing the column with a second mobile phase and then eluting 8-OH-2-DG to an analytical C18 column with an identical mobile phase containing adenosine to displace8-OH-2-DG. Detection with series colorimetric electrodes provides qualitative certainty for 8-OH-2-DG peak by response ratios.

2.2.3. Measurement of Dityrosine

1.2 grams of fresh tissue material were homogenized with 5 ml of ice-cold 50mMHEPES-KOH, pH 7.2, containing 10 mM EDTA, 2 mMPMSF, 0.1 mM p-chloromercuribenzoic acid, 0.1 mM DL norleucine and 100 mg polyclar AT. The plan tissue homogenate was centrifuged at 5000 g for 60 min to remove debris. Purification of o,o'_dityrosine in the clear tissue homogenized supernatant fluid was accomplished by preparative HPLC. o,o' dityrosine was recovered by gradient elution from the C-18 column (Econosil C18,250mm × 10 mm) [11]. The composition of eluent varies linearly from acetonitrile-water-TFA (1:99:0.02) to acetonitrile-water- TFA (20:80:0.02) over 25 min. The gradient was started 5 min after the injection. A flow rate of 4 ml/min was used. o,o'_dityrosine was analyzed by reversed-phase HPLC with simultaneous UV-detection(280 nm) and fluorescence-detection (ex. 280 nm, em. 410nm). A phenomenex inertsil ODS2 (150mm × 4.6 mm, 5μm) HPLC column (Bester, Amsterdam, the Netherlands) equipped with a guard column was used for these analyses. A gradient was formed from 10 mM ammonium acetate, adjusted to pH 4.5 with acetic acid and methanol, starting with 1% methanol and increasing to 10% over 30min. The flow rate was 0.8 ml/min. A standard dityrosine sample was prepared according to Amado et al [3]. Dityrosine was quantified by assuming that it's generation from the reaction of tyrosine with horseradish peroxidase in the presence of H O was quantitative (using the extinction coefficient e = 4.5 mMG cmG at pH 7.5).

2.3. Statistical analysis

All data were analyzed using SAS software each treatment was analyzed in three replication .When ANOVA showed significant treatment effects. Duncan's multiple range test was applied to compare the means at $p<0.05$[16].

3. Results

3.1. Grain Yield

Result of analysis of variance (Table 1) showed that there were significant differences between irrigation levels, second experimental treatments and interaction between them (%1). In normal condition obtained higher grain yield (2502.5 kg/ha).There was %62.28 lower grain yield in the stress condition.

Mix of (PGPR + Silicic acid +Amino acid) showed higher grain yield (2443.8 kg/ha) in com pare with control treatment (1606.3 kg/ha).

3.2. Biochemical Biomarkers

3.2.1. Malondialdehyde

Result of analysis of variance (Table 1) showed that there was significant differences between irrigation levels at %1 level of probability. In normal condition lowest activity of MDA observed (121.37 μ / mol g protein).Results also showed significant differences for second experimental treatments .Application of PGPR+ Silicic acid +Amino acid significantly (%1) decreased MDA activity. This shows bacterias prepare a condition in the plant that decrease stress full condition of environment. According to the results of Moslemi et al, bacteria can increase proline in the plants and proline act as an AOS scavenger [10]. Correlation between grain yield and MDA (Fig 1) was negative and significant (R^2=0.69).This shows plants for cope with environmental stress increase this antioxidant enzyme activity, but this action cost decrease of yield in the plant.

3.2.2. Dityrosine

Result of analysis of variance (Table 1) showed that there was significant differences between irrigation levels at %1 level of probability. In normal condition lowest activity of Di-Ty observed (49.7 µ / mol g protein).Results also showed significant differences for second experimental treatments .Application of PGPR + Silicic acid +Amino acid significantly (%1) decreased Di-Ty activity. This shows bacterias prepare a condition in the plant that decrease stress full condition of environment. According to the results of Moslemi et al, bacteria can increase proline in the plants and proline act as an AOS scavenger [10]. Correlation between grain yield and Di-Ty (Fig 2) was negative and significant (R^2=0.57).This shows plants for cope with environmental stress increase this antioxidant enzyme activity, but this action cost decrease of yield in the plant.

3.2.3. 8-Hydroxy-2-Deoxyguanosine

Result of analysis of variance (Table 1) showed that there was significant differences between irrigation levels at %1 level of probability. In normal condition lowest activity of 8-OH-2DG observed (12.7 µ / mol g protein).Results also showed significant differences for second experimental treatments .Application of PGPR + Silicic acid +Amino acid significantly (%1) decreased 8-OH-2DG activity. This shows bacterias prepare a condition in the plant that decrease stress full condition of environment. According to the results of Moslemi et al, bacteria can increase proline in the plants and proline act as an AOS scavenger [10]. Correlation between grain yield and 8-OH-2DG (Fig 3) was negative and significant (R^2=0.57).This shows plants for cope with environmental stress increase this antioxidant enzyme activity, but this action cost decrease of yield in the plant.

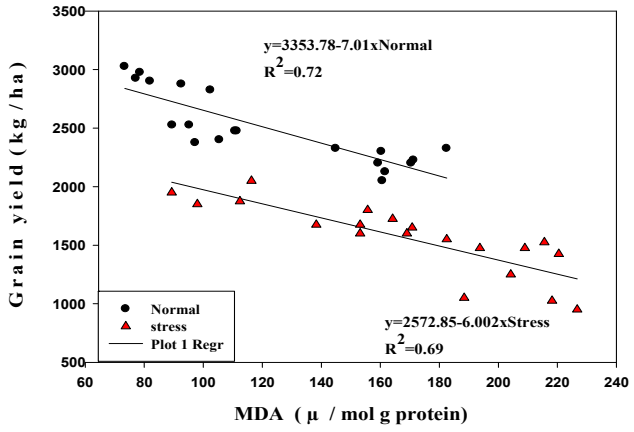

Fig. 1: Regresion Curve for grain yield MDA

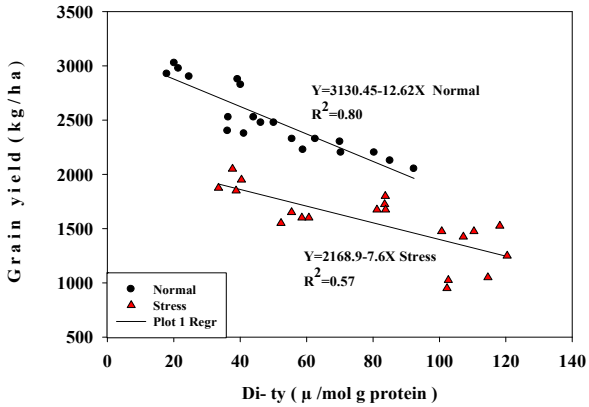

Fig. 2: Regresion curve grain yield and Di-Ty

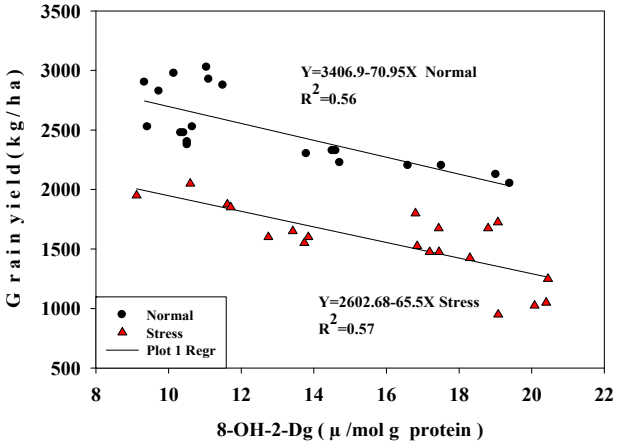

Fig. 3: Regresion Curve Grain yield and 8-OH-2-Dg

Table 1: Analysis variance for traits.

s.o.v	Df	Grain yield	Malondialdehyde	Dityrosine	8-Hydroxy-2-Deoxyguanosine
Replication	3	38432.2	133.92	20.95	0.62
Irrigation(I)	1	8906640**	22648.08**	8755.68**	101.66**
Error	2	3473.9	130.85	59.76	0.26
Second experimental treatment(T)	4	804273.43**	13818.29**	5773.96**	89.45**
I*T	4	26367.18**	399.03*	466.37**	15.06**
Error	24	4768.23	116.46	31.77	0.87
%Cv	-	3.4%	7.4%	8.7%	6.1%

ns,*and**;Non significant. Significant at the 5% and 1% levels probability respectively.

Table 2: Mean comparisons for measured traits.

Treatment	Grain yield (kg/ha)	MDA (µ/mol g protein)	Di-Ty (µ/mol g protein)	8-OH-2-DG (µ/mol g protein)
A1	2502.5^{a}	121.37^{b}	49.7^{b}	12.7^{b}
A2	1558.75^{b}	168.9^{a}	79.3^{a}	15.9^{a}
B1	1606.3^{e}	187.24^{a}	96.05^{a}	19.06^{a}
B2	1884.4^{d}	186.21^{a}	85.5^{b}	15.92^{b}
B3	2203.1^{b}	137.56^{b}	61.53^{c}	14.17^{c}
B4	2015.6^{c}	123.91^{b}	50.13^{d}	11.94^{d}
B5	2443.8^{a}	90.9^{c}	29.35^{e}	10.58^{e}
A1B1	2144^{e}	163.05^{b}	82.1^{b}	18.13^{d}
A1B2	2294^{d}	164.7^{b}	67.8^{c}	14.41^{c}
A1B3	2688^{b}	94.9^{cd}	40.02^{d}	10.33^{b}
A1B4	2431^{c}	106.2^{c}	43.5^{d}	10.45^{b}
A1B5	2956^{a}	77.8^{d}	21.1^{e}	10.4^{a}
A2B1	1069^{i}	209.3^{a}	110^{a}	20de
A2B2	1475^{h}	209.7^{a}	109.1^{a}	17.4^{e}
A2B3	1719^{g}	152.8^{b}	83.05^{b}	18.02^{cd}
A2B4	1600^{gh}	168.8^{b}	56.7^{c}	13.44^{c}
A2B5	1931^{f}	104^{c}	37.6^{d}	10.76^{a}

Similar letters in each column shows non- significant difference according to Duncan s multiple range test at 5% level.

4. References

[1] A. Guoth, I. Tari, A. Galle, J. Csiszar, L. Cseuz, L. Erdei, "Changes in photosynthetic performance and ABAlevels under osmotic stress in drought tolerant and sensitive wheat genotypes," *Acta Biologica Szegediensis*, 52: 91-92, 2008.

[2] R. G. Alscher, N. Erturk and L. S. Heath, "Role of superoxide dismutase (SODs) in controlling oxidative stress in plants," Exp.. Bot., 53:133-141, 2002.

[3] R. Amado, R. Aeschbach and H. Neukom. Dityrosine: in vitro production and characterization. Methods Enzy mol. 1984. 107: 377-388.

[4] B. R. Bird, S. S. O. Hung, M. Hadley and H.H. Draper, "Determination of malonaldehyde in biological materials by high-pressure liquid chromatography," Anal. Biochem. 1983.128: 240-244.

[5] M. B. Bogdanov, M. F. Beal, D. R. Meccabe, R. M. Griffin and W. R. Matson, “A carbon column based LCEC approach to routine 8-hydroxy-2-deoxyguanosine measurements in urine and other biological matrices,” Free. Rad. Biol. Med. 1999. 27:647-666.

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

[7] M. M. Chaves, J. P. Maroco and J.S. Pereira, “Understanding plant responses to drought—from genes to the whole plant,” Funct. Plant Biol. 2003. 30: 239–264.

[8] G. H. Badawi, Y. Yamauchi, E. Shimada, R. Sasaki, N. Kawano, K. Tanaka, K. Tanaka, (2004).Enhanced tolerance to salt stress and water deficit by over expressing superoxide dismutase in tobacco (Nicotianatabacum) chloroplasts, Plant Science. 166: 919-928.

[9] M. N. Alyemeny. The effect of drought on growth and dry matter allocation in seedling of VignaambacensisL, J. King Saud. Univ. (1988). 10: 41-51.

[10] Z. Moslemi, D. habibi, A. Asgharezadeh, M. R. Ardakani, A. Mohammadi, M. Mohammadi. Response of phytohormones and biochemical markers of maize to super absorbent polymer and plant growth promoting rhizobacteria under drought stress. American Eurasian J. Agric & Environ. 2011. Sci, 10 (5):787-796.

[11] H. Orhanl, N. P. E. Vermeulen, C. Tump, H. Zappey and J. H. N. Meerman. Simultaneous determination of tyrosine, phenylalanine and deoxyguanosineoxcidation products by liguid chromatography - tandem mass spectrometry as non-invasive biomarkers for oxidative damage. J. Chromatography. B. 2004. 799: 245-254.

[12] P. Valentovic, M. Luxova, L. Kolarovic, O.Gasparikova. Effect of osmotic stress on compatible solutes content, membrane stability and water relations in two maize cultivars, Plant Soil Environment. (2006). 52: 186-191.

[13] Q. X. Lai, Z. Y. Bao, Z. J. Zhu, Q. Q. Qian, B. Z. Mao, “Effects of osmotic stress on antioxidant enzymes activites in leaf discs of PSAG12-IPT modified gerbera,” J. Zhejiang UnivSci B.(2007). 8: 458-464.

[14] H. B. Shao, Z. S. Liang and M. A. Shao. Changes of some anti-oxidative enzymes under soil water deficits among 10 wheat genotypes at maturation stage. Colloids Surf. B: Bio interfaces .2005. 45: 7–13.

[15] Y. Pan, L. J. Wu, Z.L. Yu. Effect of salt and drought stress on antioxidant enzymes activities and SOD iso enzymes of liquor ice (Glycyrrhizauralensisfisch), Plant Growth Regul. (2006). 49: 157-165.

[16] J. X. Zhange, and M. B. Kirkham. Water relation of water-stressed, split-root C4(Sorghum bicolor, Poaceae) and C3 (Helianthus annuus; Asteraceae) plants. Am. J.Bot. 1995. 82: 1220-1229.