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 Antioxidant Enzyme Activity of Wheat under Drought Stress

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

1- Department of Agronomy and Plant Breeding, Roudehen Branch, Islamic Azad University, Roudehen, Iran.

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

3- Department of Agronomy and Plant Breeding, Science and Research Branch, Islamic Azad University, Tehran, Iran

Abstract. Reducing of drought damage in plants by using of biofertilizers as plant growth promoting bacteria, silicic acid and amino acids and improving physiological parameters and thus raising the level of plant yield in arid and semi arid areas are emergency management for drought control agriculture and also in wheat agronomy. 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.Analysis of variance showed that the antioxidant enzymes in the 1 percent level is significant, so that the highest levels of superoxide dismutase, catalase and glutathione peroxidase related to cut irrigation after flowering. effect second experimental treatment that the antioxidant enzymes in the 1 percent level is significant and highest levels of antioxidant enzymes 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 superoxide dismutase, catalase and glutathione peroxidase belonged to normal irrigation and seed inoculated with bacteria and sprayed silicic acid with amino acids (235, 86.3 and 54.7 U/g protein) ,respectively.

Keywords: wheat, drought stress, antioxidant enzyme, plant growth promoting bacteria, silicic acid, amino acid.

1. Introduction

Environmental stresses, such as drought stress and high temperature, influence almost all aspects of plants physiology and biochemistry, and considerably reduce yield [15]. Water is very important for growth and development of plants [17]. Mechanisms of active oxygen species detoxification exist in all the plants and include activation of enzymatic defense system [12]. Moreover, activities of antioxidant enzymes and the amount of elevated antioxidants under drought stress are very changeable among plant species [19] and even between the two cultivars of identical plant species [2]. A large amount of the damage to plants exposed to drought stress is owing to oxidative damage at the cellular level [8, 4].

[1] Mahdidavoodifard @gmail.com.
Tell.:+989122369651,+982144413185.

Drought stress induces cellular accumulation of ROS which can damage membrane lipids, proteins and nucleic acids [1, 2, 9, 11, 14]. A correlation between the antioxidant capacity and NaCl tolerance has been demonstrated in some plant species [6, 7, 10].Several studies have pointed out that drought-tolerant species increased their antioxidant enzyme activities and antioxidant contents in response to drought treatment, whereas drought-sensitive species failed to do so [5, 18].This research has been done to investigation the effect of moisture stress on the enzyme 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.

After drought stress treatment, three leaves of each plant were removed. The samples were washed and then frozen in liquid N2 and then stored at -80°Cpending biochemical analysis. Leaf sample was homogenized in a mortar and pestle with 3 mL ice-cold extraction buffer (25 mM sodium phosphate, pH 7.8).The homogenate was centrifuged at 18000 g for 30 min at 48°C and then supernatant was filtered through paper. The supernatant fraction was used as a crude extract for the assay of enzyme activity. All operations were carried out at 48°C.

2.2. Enzyme Extractions and Assays

Catalase activity was estimated by the method of [3]. There action mixture contained 100 crude enzyme extract, 500µL 10 mM H2O2 and 1400 µL 25 mM sodium phosphate buffer. The decrease in the absorbance at240 nm was recorded for 1 min by spectrophotometer, model Cintra 6 GBC (GBC Scientific Equipment, Dandenong, Victoria, Australia). CAT activity of the extract was expressed as CAT units per milligram of PROT. Superoxide dismutase activity was determined with the reaction mixture contained 100 µL 1 µ Mriboflavin, 100 µL 12 mM L-methionine, 100 µL0.1 mM EDTA (pH 7.8), 100 µL 50 mM Na2 CO3 (pH10.2) and 100 µL 75 µM Nitroblue Tetrazolium (NBT)in 2300 µL 25 mM sodium phosphate buffer (pH 6.8),200 µL crude enzyme extract in a final volume of 3 mL.SOD activity was assayed by measuring the ability of the enzyme extract to inhibit the photochemical reduction of NBT glass test tubes containing the mixture were illuminated with a fluorescent lamp (120 W); identical tubes that were not illuminated served as blanks. After illumination for 15 min, the absorbance was measured at 560 nm. One unit of SOD was defined as the amount of enzyme activity that was able to inhibit by 50% the photo reduction of NBT to blue formazan. The SOD activity of the extract was expressed as SOD units per milligram of PROT. Activity of GPX (EC 1.11.1.9) was determined as described by [16]. The leaf sample was homogenized in 0.4 m Tris–HCl buffer (pH 7.0), and the reaction mix true contained 0.2 ml of tissue homogenate, 0.2 ml of 0.4 M Tris–HCl buffer (pH 7.0), 0.1 ml of 10 mM sodium azide, 0.2 ml of glutathione and 0.1 ml of 0.2 mm hydrogen peroxide. The contents were incubated at 37 °C for 10 min. The reaction was stopped by the addition of 0.4 ml of 10% trichloroacetic acid (TCA), and centrifuged. The supernatant was assayed for

glutathione content by using Ellman's reagent. One unit of enzyme activity is the amount of glutathione consumed per minute at 37 °C.

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 $p<0.05$ [20].

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. Antioxidant Enzymes

3.2.1. Superoxide dismutase

Result of analysis of variance (Table1) showed that there was significant differences between irrigation levels at %1 level of probability. In normal condition lowest activity of SOD observed (315.5 U / g protein).Results also showed significant differences for second experimental treatments .Application of PGPR + Silicic acid +Amino acid significantly (%1) decreased SOD 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 [13]. Correlation between grain yield and SOD (Fig 1) was negative and significant (R^2=0.60).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. Catalase

Result of analysis of variance (Table1) showed that there was significant differences between irrigation levels at %1 level of probability. In normal condition lowest activity of CAT observed (137.6 U / g protein).Results also showed significant differences for second experimental treatments .Application of PGPR + Silicic acid +Amino acid significantly (%1) decreased CAT 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 [13]. Correlation between grain yield and CAT (Fig 2) was negative and significant (R^2=0.64).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. Glutathione Peroxidase

Result of analysis of variance (Table1) showed that there was significant differences between irrigation levels at %1 level of probability. In normal condition lowest activity of GPX observed (78 U / g protein).Results also showed significant differences for second experimental treatments .Application of PGPR + Silicic acid +Amino acid significantly (%1) decreased GPX 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 [13]. Correlation between grain yield and GPX (Fig 3) was negative and significant (R^2=0.53).This shows plants for cope with environmental stress increase this antioxidant enzyme activity, but this action cost decrease of yield in the plant.

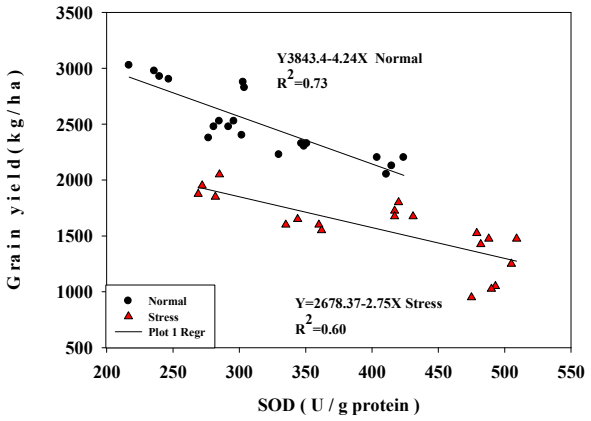

Fig.1.Regrrsion curve grain yield and SOD

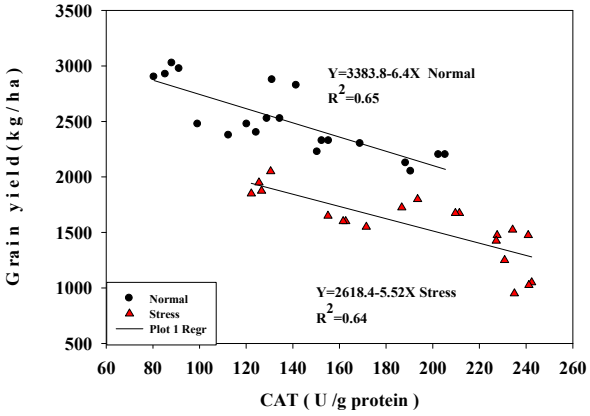

Fig.2.Regresion curve grain yield and CAT

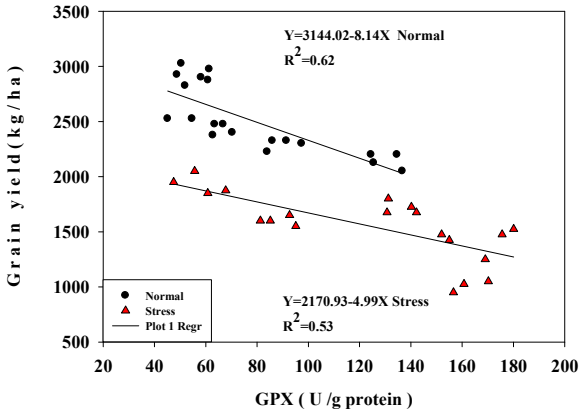

Fig.3.Regresion curve grain yield and GPX

Table1. Analysis variance for traits.

s.o.v	Df	Grain yield	Superoxide dismutas	Catalase	Glutathione Peroxidase
Replication	3	38432.2	170.7	79.53	22.77
Irrigation(I)	1	8906640**	81360.4**	29376.4**	19105.6**
Error	2	3473.9	136.2	32.09	56.12
Second experimental treatment(T)	4	804273.43**	48414.4**	15492.51**	10898.81**
I*T	4	26367.18**	3731.02**	513.73**	2375.98**
Error	24	4768.23	103.92	59.75	60.34
%Cv	-	3.4%	2.8%	4.6%	7.7%

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)	SOD (U / g protein)	CAT (U / g protein)	GPX (U / g protein)
A1	2502.5^{a}	315.5^{b}	137.6^{b}	78^{b}
A2	1558.75^{b}	405.7^{a}	191.8^{a}	122.4^{a}
B1	1606.3^{e}	452.13^{a}	217.04^{a}	147.23^{a}

B2	1884.4[d]	416.88[b]	194.65[b]	127.7[b]
B3	2203.1[b]	359.13[c]	167.19[c]	94.63[c]
B4	2015.6[c]	319.13[d]	138.43[d]	77.22[d]
B5	2443.8[a]	256[e]	106.28[e]	56.33[e]
A1B1	2144[e]	413.5[b]	192.7[b]	130.3[b]
A1B2	2294[d]	344.25[c]	156.8[c]	89.7[c]
A1B3	2688[b]	297[d]	134.05[d]	53.2[d]
A1B4	2431[c]	288[d]	114.1[e]	65.8[d]
A1B5	2956[a]	235[e]	86.3[f]	54.7[d]
A2B1	1069[i]	490.7[a]	237.3[a]	164.1[a]
A2B2	1475[h]	489.5[a]	232.4[a]	165.6[a]
A2B3	1719[g]	421.25[b]	200.3[b]	136.07[b]
A2B4	1600[gh]	350.25[c]	162.7[c]	88.5[c]
A2B5	1931[f]	277[d]	126.2[de]	57.9[d]

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

4. References

[1] R.G. Alscher, J.L. Donahue and C.L. Cramer. Reactive oxygen species and antioxidants: Relationships in green cells. Physiol. Plant,100: 224-233. DOI: 10.1034/j.1399-3054.1997.1000203.x.

[2] A. Blum. Crop responses to drought and the interpretation of adaptation. Plant Growth Regul .1996. 20: 135–148.

[3] I .Cakmak and W.J. Horst. Effect of aluminum on lipid peroxidation, superoxide dismutase, catalase and peroxidase activities in root tip of soybean (Glysin max). J. Plant. Physiol.1991. 83: 463–468.

[4] M.Farooq,A. Wahid, N. Kobayashi, D. Fujita and S.M.A. Basra. Plant drought stress: effects, mechanisms and management. Agron. Sustain. 2009. Dev., 299: 185–212.

[5] C.H. Foyer, M. Lelandais and K.J. Kunert. Photooxidative stress in plants. Physiol. Plant. 1994. 92: 696-717.

[6] F.Ghanati, A. Morita and H. Yokota. Induction of suberin and increase of liginin content by exess boron in Tabacco cell. Soil Sci. Plant Nutr. 2002. 48: 357-364.

[7] A .Hegedus, S. Erdei and G. Horvath,. Comparative studies of H_2O_2 detoxifying enzymes in green and greening barley seedlings under cadmium stress. Plant Sci.2001. 160: 1085-1093. DOI:10.1016/S0168-9452(01)00330-2.

[8] J. Hernandez, F.J. Corpas, M. Gomez, L.A. Del Rio and F. Sevilla. Salt-induced oxidative stress mediated by activated oxygen species in pea leaf mitochondria. Plant Physiol.1993. 89: 103–110.

[9] M.S. Johnson and R.T.Leah. Effect of superabsorbent polyacrylamide on efficacy of water use by crop seedlings. J. Sci. Food Agric. 1990. 52: 431-434. DOI: 10.1002/jsfa.2740520316.

[10] G. Kar, A. Kumar and M. Martha.Water use efficiency and crop coefficients of dry season oilseed crops. Agric. Water Manage .2007. 87: 73-82. DOI: 10.1016/J.AGWAT.2006.06.002.

[11] S. Lovelli , M. Perniola, A. Ferrara and T. Di Tommaso. Yield response factor to water (Ky) and water use efficiency of Carthamus tinctorius L. and Solanum melongena L. Agric. Water Manage. 2007. 92: 73-80.

[12] D.A. Meloni , M.A. Oliva, C.A. Martinez and J.Cambraia. Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. Environ. Exp. Bot. 2003. 49: 69–76.

[13] 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.

[14] R. Munns. Comparative physiology of salt and water stress. Plant Cell Environ. 2002. 25: 239-250.

[15] M.G. Pitman and A. Lauchli,. Global impact of salinity and agricultural ecosystems. In: Lauchli, A. and V. Luttge (eds.), Salinity: Environment-Plants-Molecules. Kluwer, Netherlands. 2002.

[16] J.T. Rotruck, A.L. Pope, H.E. Ganther, A.B. Swanson, D.G. Hafeman, W.G. Hockstra. Selenium: biochemical roles as a component of glutathione peroxidase. Science.1973. 179: 588–590.

[17] H.B. Shao, Y. Chu, C.A. Jaleel and C.X. Zhao. Water deficit stress induced anatomical changes in higher plants. J. Plant Biol. Pathol.2008. 331: 215–225.

[18] R.G. Steel and J.H. Torrie. Principals and Procedures of Statistics: A Biometric Approach. MCGraw-Hill, New York, USA. 1980. ISBN: 2587584587, pp: 421.

[19] A. Zaman, and P.K. Das.Effect of irrigation and nitrogen on yield and quality of safflower. Indian J. Agron.1991. 36: 177–179.

[20] 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.