Effect of gibberellic acid foliar and kinetin on the antioxidant catalase anzymes and peroxidase in maize under drought stress

Shahram MEHRİ

Department of Agricultur Parsabad Branch, Islamic Azad University, Parsabad, Iran

Abstract. To study relation between water scarcity and gibberllic acid hormone and kinetin in three hybrids tested corn in two years as a split plot factorial based on randomized complete block design with 3 replications and antioxidant catalase enzymes and peroxidase leaves, the resulted measuremens are that drought stress is a change in the hormonal balance of corn so that amount of catalase and peroxidase enzymes compared to control were increased by foliar of hormones.however most of the characteristics attributed to the use of these hormones had different reactions. But seed function as the main character towards their use indicated a positive effect.

Keywords: peroxidase, drought, corn, catalase and hormones

1. INTRODUCTION

Environmental stresses are factors that limit agricultural production in all over the world. In addition, stresses are prevented an increase in the cultivation of plants and it is anticipated that a variety of environmental stresses such as high or low temperature, salinity, drought and so on are able to severely restrict plants production(individually or combined effect of them) in the coming decades. (Duncan,2000 and boyer,1982).

Great efforts to improve the tolerance of different stress characteristics and in particular drought, cold, heat, salinity and nutrients take place each year around the world and no doubt all of these efforts are associated with stress physiological in plants. Biotic and abiotic stresses cause changes in natural physiological processes of plant organs. In addition to the stresses imposed by the environment to the plant, some of them are caused by human activity (factors of stresses relate to human intervention) such that it can be toxic pollutants from pesticides, hazardous gases, photo oxidation and soil acidification, acid rains, overuse of fertilizers, heavy metals, ultraviolet radiation and etc. all of these stresses reduce biosynthetic capacity of the plant organs and change the normal activities and cause thermal damage, which leads to plant death (levitt, 1980). Sresses such as drought, salinity and cold causes oxidative stress in plants and production of reactive oxygen species (ROS) such as superoxide radicals, hydrogen peroxide and hydroxyl. Free radicals can reduce lipid peroxidation, protein denaturation and DNA mutation (Janmohammadie and et al., 2012).

Basically during ROS reactions, oxidation and recovery, and during the incomplete recovery of oxygen or water oxidation are formed by the electron transport chain in mitochondria or chloroplasts.

If ROS are not successful in stopping the oxidation reactions, finally cell death will occur. ROS specially OH largely can be devastating to lipids, proteins and nucleic acids. Lipid peroxidation and other essential components of the cell can be done through th production of free organic radicals.

Corresponding author. Email address: sh.mehri2000@gmail.com

During the course of evolution, organs of complex plant has developed the antioxidant protection system to deal with all of these effects.

Such as super oxide and hydroxide radicals accumulate under stress conditions, its necessary that this ROS species in fact reactive oxygen species or compounds can be controlled to maintain macro molecules healthy. This with balancing redox activity and recovery of cell has an important role in mark and growth regulation. Obviously, ROSes become possible by a group of enzymes and with assistance of metabolits(carol and dolan, 2006; calzadilla et al., 2014).

Generally, plant antioxidant systems are classified as follows:

A) Fat-soluble, antioxidant associated with the membrane, the Alpha-Tocopherol, Beta-Carotene that directly excrete the free radicals of lipid peroxidation.
B)
C) A water-soluble antioxidant glutathione and ascorbate that participate in $O2^-$ and $H2O2$.
D)
E) Catalase and peroxidase enzyme antioxidants and enzymes belong to ascorbate-glutathione cycle that participate in the detoxification of ROS, $O2^-$ and OH^-.
F)
Plant organs are able to overcome oxidative stress by activate one or all of these systems. However, in the case of fast, short-term stress, capacity of immune system is not responsive and hence it has significant damage and even lead to plant death.

-   The main places of activated oxygen production
-
Some cellular components are as the original places of production of activated oxygen and ROS is contained chloroplasts, mitochondria, endoplasmic reticulum, mykrvbadies, plasma membranes and walls(mittler, 2002).

2. THE MAIN DEFENSE MECHANISM AGAINST OXIDATIVE STRESS

Including original compositions act as the annihilator of activated oxygen and free radicals can be mentioned catalase and peroxidase.

In general, oxygen free radicals or activated oxygen indicate in the variety of environmental stresses that are involved in plants and animals in most of cell destruction circumstances.

Lipid peroxidation, inactivating protein and DNA mutations are obvious results of free radicals, but because these reactions occr rapidly and are often components of a complex chain reactions, we usually can only see the effects of it.

Active forms of oxygen are different and their role in the biosynthesis of complex organic molecules is very important and understanding their role in defense against all types of stress is essential.

Obviously, if the production of activate oxygen is more than plant capacity to detoxify it, corrosive hazardous reactions will occur and of symptoms of this conditions are lack of osmotic response, drought and necrosis and analysis of membranes and aggregation of proteins of cell surface are as signs of this type of damage.

Effect of gibberellic acid foliar and kinetin on the antioxidant catalase anzymes and peroxidase in maize under drought stress

Thus, the balance between production and removal of activated oxygen to maintain active growth and plant metabolism and tolerance to all environmental stresses is essential for all(mittler, 2002).

Materials and methods

This experiment came into force in field seasons of 1391 and 1392 in the research farm of natural resources and agricultural research center of Ardabil (Magi) in factorial split plot and based on randomized complete block trial design with 3 replications. Main plots consisted of 3 levels of irrigation: 1- complete irrigation based on water requirements of plant and customs of area 2- cut irrigation at vegetative stage (cut irritation after emergence to tassel emergence and continuing from appearance of tassel flower until the end of the reproductive period) 3- cut irrigation during grain filling stage (irrigation from planting until pollination ad seed formation and cutting off water to physiological maturity).

Sub plots consisted of a combination of two maize hybrids factors (S.C700, S.C647, S.C704) and hormonal treatments (no use of hormones, eight-leaf stage sprayed with 50 ppm kinetin) (Zare and et al., 2006; Hamidia and et al., 2014) eight- leaf stage sprayed with gibberllin detected at 50 ppm, and eight-leaf stage sprayed with gibberellin and kinetin detected 25 ppm of each) were conducted.

3. MEASUREMENT OF CATALASE (CAT)

To measure the activity of catalase (CAT) photochemical method (Cakmak and Horst. 1991) were used. 3 ml mixture of reaction containing 1.0 Mm bafrtys (8.6=pH), 10Mm H_2O_2 andenx=zyme extract were obtained and was read at a wavelength of 240 nm.

4. MEASUREMENT OF PEROXİDASE (POD)

4.1. Preparation Of Extraction Buffer

For this purpose, phosphate buffer mM (pH=7) was used. How to prepare it is based on following. Solution 1: 0.68 gr potassium dihydroen phosphate salt dissolved in a few ml of distilled water and then 0.0186 gr EDTA added to the solution and brought to the 100 ml volume.

Solution 2: 0.87 gr mono hydrogen phosphate potassium salt dissolved in a few ml distilled water and then 0.0186 gr EDTA added to the solution and brought to 100 ml volume with distilled water.

Both solutions are as storage solutions that 39 ml of solution 1 for every time mixed with 61 ml of solution 2 and then the pH regulated between 6.8- 7.we used of this solution as buffer of extracting peroxidase enzyme.

5. ENZYME EXTRACTION

0.5 gram from comminuted fiber leaves with liquid azoth was transferred into the 2 milliliter microtubules and 1 milliliter of extraction buffer is added to it, then it is centrifuged with circuit of 14000 (rpm) at the temperature of 4 C as long as 15 minutes. After finishing centrifuge, the

extraction above it, is been transferred to another microtubule and again been centrifuges with 14000 rpm as long as 10 min and then the extraction above it, after recording the volume, is transferred to the microtubule and is been conserved at the temperature of -70 in the freezer. Then the act of enzyme was computed by using formula of Birlambert codeand with the GayakulPeroxides black out modulus $\mu m^{-1} cm^{-1}$. Finally the act of enzyme computed at U/mg protein.

Analysis of combined variance according to the level of significance of square mean, interactions of the main factors (irrigation, hormones and hybrids), calculate and the F test and also resulted means are combined in the level of five percent by using danken test. Figures were drawn using software EXCEL2010 and statistical analysis has been done by using software MSTATC (Alizadeh and Tarinejad, 2012) and SAS.19.

6. RESULTS AND DISCUSSION

6.1. Catalase

Variance sameness of errors with Bartlett sameness experiment (congenering variance) has been done and did not show any tremendous difference about catalase level of leaf (table 1). Results of composite catalysis of variance denoted that effects of the year (Y), drought tension (I), irrigation in hormone (I×H), irrigation in variety (I×V), Hormone in variety (H×V), and irrigation in hormone in variety (I×H×V) at one percent possibility level and effects of year in irrigation (Y×I) and hormone (H) at five percent possibility level have had tremendous effects on catalase value (table 1) and this indicates that function of Gibberllic acid hormones and kinetin can have an effective role on the value of this enzyme at the germination and grain filling stage.

There was no significant difference between the levels of irrigation in comparison of the mean of interaction of irrigation in the hormone in variety (V × H × I). The highest rate of catalaseresulted at the time of applying tension in grain filling stage and by not using hormonal foliar (witness) and in hybrid 700 (figure 1). Although the above hybrids with other samples under the same conditions of tension and of using hormonalfoliar of Gibberellic acid, kinetin and composition of gibberellic acid with kinetin also at normal irrigation conditions, hybrid 647 using the hormone kinetin and 700 hybrid with hormonal combination of gibberellic acid and kinetinat the germination tension condition did not show any significant difference. Among Hybrids, 700 Hybrid had shown the highest rate of catalase changes. In terms of the levels of the hormone, the kinetin hormone showed the highest rate of catalase, though with not using the hormone (witness) had no significant difference (figure 1).

Effect of gibberellic acid foliar and kinetin on the antioxidant catalase anzymes and peroxidase
in maize under drought stress

Table 1. Results of analysis of variance for studied traits

S.O.V	df	Mean Square	(M.S)	
		Yield	Catalase	Peroxidases
Year	1	42.81**	0.013 n.s	169.05**
Yield(Replication)	4	1.1	0.0003	0.15
Irrigation	2	520.39**	0.099**	56.9*
Y×I	2	8.97*	0.003 n.s	2.85**
Error(a)	8	1.2	0.0004	0.235
Hormones	4	210.26**	0002*	0.065 n.s
Y×H	3	3.27**	0.0001 n.s	0.192 n.s
I×H	6	1.46 n.s	0.001**	0.164 n.s
Y×I×H	6	1.41*	0.0001 n.s	0.077 n.s
Variety	2	21.67*	0.0004 n.s	7.44 n.s
Y×V	2	0.36 n.s	0.0001 n.s	5.95**
I×V	4	1.75*	0.0002**	2.12**
H×V	6	0.8 n.s	0.001**	0.052 n.s
I×H×V	12	0.82 n.s	0.0004**	0.139 n.s
Y×I×V	4	0.06 n.s	0.00003 n.s	2.27**
Y×H×V	6	0.97 n.s	0.0001 n.s	0.16 n.s
Y×I×H×V	12	0.46 n.s	0.00004 n.s	0.114 n.s
Error(b)	132	0.68	0.0002	0.078
CV(/) ضریب تغییرات	-	12.32	12.88	15.94
Bartlett test	-	0.63	0.789	3.3

ns, * and ** mean significant and non-significant at the probability level of 5%, 1%, respectively.

Based on reportages of Jiang and Huang (2001) increase of the amount of catalase enzyme under the application of drought tension at the grain filling stage in 700 and 704 Hybrids can be due to the praline aggregation in the tension condition that may lead to the decrease of the oxidationreduction pace of praline to glutamate and finally decrease in praline consumption in protein synthesis and increase in protein metamorphosis.They also mentioned to catalase role in increase of endurance level towards mutants tension of corn in facing with the tension of post pollinating. Finally they conclude that an activity of anti oxidant enzymes depends on the plant specious resistance degree. After effective factors in resistance value towards drought tension, there is capacity increase of anti oxidant catalase enzyme. Thus it seems that reaction of catalase in plants, specious and even different numerics against of environmental tensions does not follow the similar alteration procedure.
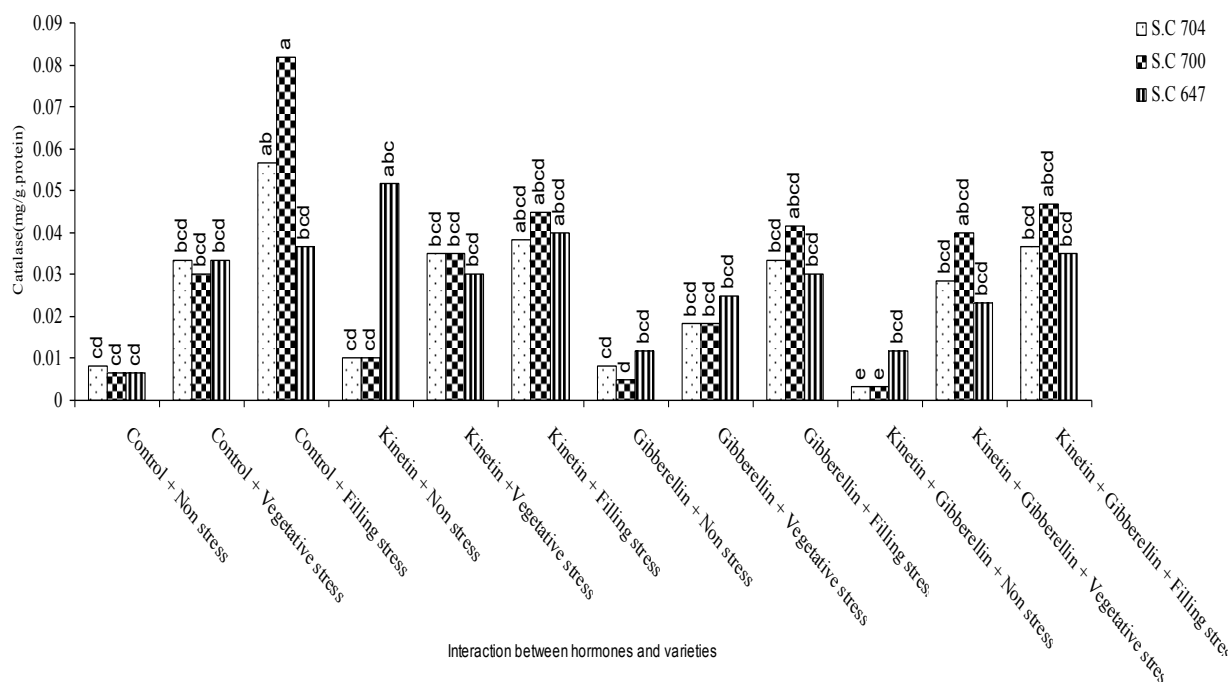
599

Figure 1. Interaction of irrigation in hormone at variety on amount of catalase

Huang and Guo (2005) in their study on the reactions of anti-oxidants system towards tensions declared that with increasing the value of catalase enzyme activities, the amount of corn plant tolerance increases against the conditions of water scarcity. Yang et al. (2004) also mentioned the role of catalase in increase of the amount of tolerance towards tension of maize. In addition, Feng et al. (2004)related the high levels of catalase in wheat cultivars with resistance to drought as well.

According to the results obtained in this experiment can be deduced that drought tension can cause the increase of catalase enzyme. The rate of increase is likely to be due to the tension that increases the amount of (H_2O_2) in cells. Because catalase is considered as one of the most specialized and original enzymes of sweepingthe H_2O_2. Since increasing the amount of catalase should be up to the amount that could turnthe H_2O_2 to the water and O_2in peroxisome, and through this way stultify the hazards of H_2O_2 (Taiz and Zeiger, 2005). Drought tension probably happens along with oxidative tension. So that the enzyme catalase is of basic compounds for toxic oxygen radicals in the detoxification of this tensions that this process also increases the amount of these enzymes. The enzyme catalase, which is known as the first spoiler of H2O2 in many plants and at least in corn has been emphasized by some researchers (Borzouie and Khazaei, 2001; Scandalios et al, 1980 and Prasad, 1995).

The reason of Catalase enzyme activity decrease, possibly is because of the effect of acidgibbrelik application + Kinetin through removing free radicals directly or by anti-oxidant enzymes that reduces the damage caused by the activities of this species. In this case, Jiang and Huang (2001) observed that the effect ofabsisic acid (ABA) onanti oxidative defense system and oxidative damage in leaves of corn plants was similar to their effect on levels of catalase amounts (CAT) and peroxides (POD).

-Peroxides

Bartlett test for the variance related to the 2-year experiment did not show any significant difference in amount of peroxides enzyme of leaf (table 1). Decomposition of compound

Effect of gibberellic acid foliar and kinetin on the antioxidant catalase anzymes and peroxidase in maize under drought stress

variance of data related to peroxides in a two-year experiment indicated that the effects of the year (Y), year in irrigation (I × Y), year in variety (V × Y),irrigation in variety(V × I) and year in irrigation in variety (V × H × I) at the level of the probability of one percent and the effects of drought tension (I) and variety (V) at the level of probability of five percent had a great impact on the amount of peroxides (table 1).

Comparison of mean of interactions of year in irrigation in variety (Y×I×V) indicated that between two years application of experiment there was a significant difference in the amount of peroxides. So that the most of the amount of peroxides in the second year had been regarded in all of the attendances. By application of levels of irrigation among Hybrids also there was observed significant different effects in amount of peroxides. So that the most of the amounts of peroxides in the conditions of applying drought tension at the grain filling stage and in 700 hybrid, and its least amount was been observed at the normal irrigation condition in 647 hybrids (figure 2). Thus applying tension causes the increase of peroxides in the leaves of corns.
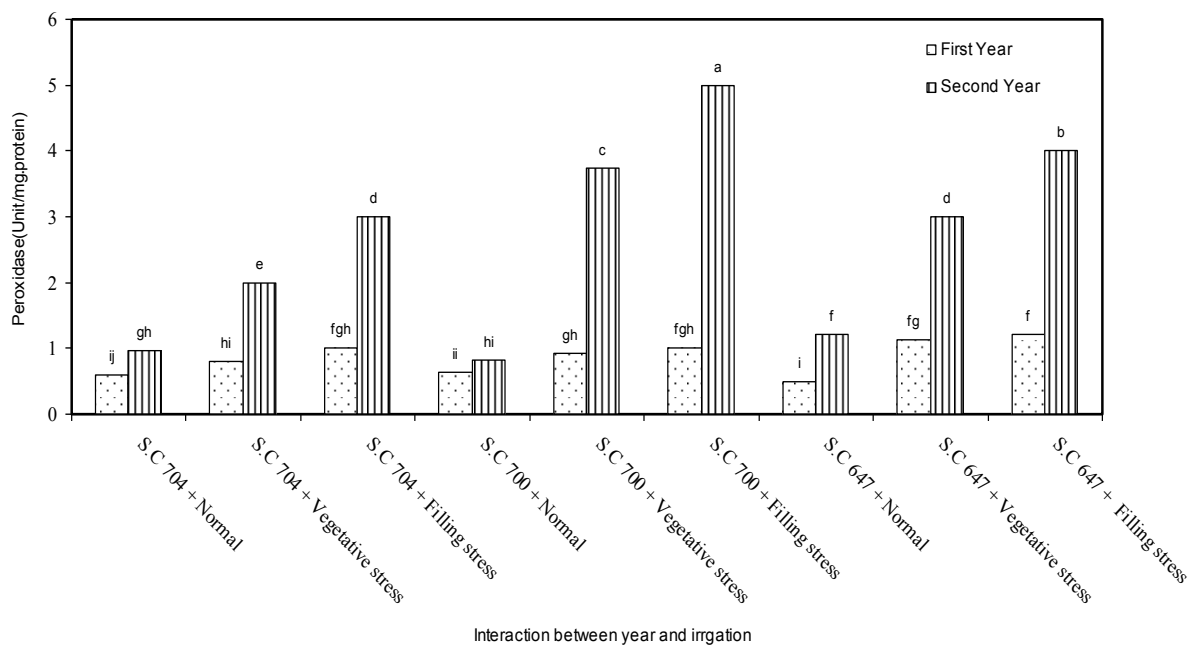

Figure 2. interaction of year in irrigation in Hormone on the amount of peroxides

Due to the fact that the amount of anti-oxidant enzymes such as peroxides increases by tension, it seems that water tension can lead to oxidative tension as well. Increase of the amount of peroxidesis one of the approaches that through it plant find the causes of compromise towards tension. It seems that hybrid 700 is been better practiced than other hybrids in this field. So, one way to determine the tension abiding hybrids may be due to the increase of the amount of antioxidant enzymes such as peroxides and catalaserespectively. The importance of peroxides in addition to their role in building lignin, subrisation, cell elongation, can also be effective in the biosynthesis regulation of cell wall and its flexibility. Some researchers have reported that this group of antioxidant enzymes affect the plant growth directly including the root system growth and development and generally peroxides act as detoxification enzymes of oxygen species (Yamasaki et al.; 1997 and Sakihama et al; 2002).

REFERENCES

[1] Borzouei, a and khazaee. 2001. The role of antioxidant enzymes in the increase of oxidative stress tolerance of plants. Olive magazine (the journal of agriculture specialist), number 179.

[2] Boyer, J.S. 1982. Plant productivity and environment. Science 218: 443–8

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

[4] Calzadilla, P.I., A. Gazquez, S.J. Maiale, O.S. Ruiz, and M.A. Bernardina. 2014. Polyamines as indicators and modulators in the abiotic stress in plants. CAB International. Plant Adaptation to Environmental Change (eds N.A. Anjum, S.S. Gill and R. Gill).

[5] Carol, R.J and, L. Dolan. 2006. The role of reactive oxygen species in cell growth: Lessons from root hairs. J. Exp. Bot. 57: 1829–1834.

[5] Davies, W.J, and Zhang, J. 1991. Root signals and the regulation of growth and development of plants in drying soil. Ann Rev Plant Biol. 42(1): 55-76.

[6] Duncan, R. R. 2000. Plant tolerance to acid soil constraints: Genetic resources, breeding methodology, plant improvement. In: Wilkinson R. E. (ed.). Plant-environment interactions 2nd edn. Marcel Dekker. New York. pp 1-38.

[7] Feng, Z., G. Jin-Kui, Y. Ying-Li, H. Wen-Liang, and Z. Li-in. 2004. Changes in the pattern of antioxidant exzymes in wheat exposed to water deficit and rewatering. Acta Physiologiae Plantarum vol. 26(3): 345-352.

[8] Foyer, C.H., H. Lopez-Delgado, J.F. Dat, and I.M. Scott. 1997. Hydrogen peroxide– and glutathione-associated mechanisms of acclimatory stress tolerance and signalling. Physiol Plant. 100:241–254.

[9] Hamdia, M., E.S, El-Samad, and M.A.K, Shaddad. 2014. The exogenous amelioration roles of growth regulators on crop plants grow under different osmotic potential. Journal of Stress Physiology & Biochemistry, 10: 203-213.

[10] Huang, M., and Z Guo,. 2005. Responses of antioxidative system to chilling stress in two rice cultivars differing in sensitivity. Biologia Plantarum 49 (1): 81-84, 2005.

[11] Janmohammadi, M., V. Enayati, and N. Sabaghnia. 2012. Impact of cold acclimation, de-acclimation and re-acclimation on carbohydrate content and antioxidant enzyme activities in spring and winter wheat. Icelandic Agricultural Sciences. 25: 3–11.

[12] Jiang, Y, and B. Huang. 2001. Drought and heat stress injury to two cool-season turfgrasses in relation to antioxidant metabolism and lipid peroxidation. Crop Sci. 41: 436-442 (2001).

Levitt, J. 1980. Responses of plant to environmental stresses. Vol. I. Academic Press, New York
Mittler R. 2002. Oxidative stress, antioxidants and stress tolerance trends. Plant Sci. 7: 405-410.

[13] Mohammad Khani, N, and R. Heidari. 2008. Effects of drought stress on soluble proteins in two maize varieties. Turk. J. Biol. 32 (2008). 23-30.

[14] Prasad T.K., M.D. Anderson, and C.R. Stewart. 1995. Localization and characterization of peroxidases in the mitochondria of chilling acclimated maize seedlings. Plant pHysiol. 108: 1597-1605.

[15] Sakihama, Y., M.F. Choen, S.C. Grace, and H. Yamasaki. 2002. Plant phenolic antioxidant and prooxidant activities: Phenolics-induced oxidative damage mediated by metals in plants. Toxicology 177: 67-80.

[16] Scandalios, J.G., W.F. Tong, and D.G. Roupakias. 1980. Cat 3, a third gene locus coding for a tissue-specific catalase in maize: Genetics, intracellular location, and some biochemical properties. Mol. Gen. Genet. 179: 33-41.

[17] Taiz L, and E. Zeiger. 2005. Plant physiology (4 th ed). Sinaver Associates, Inc. Publishers Sunderland, Massachusetts. Pp. 462-560.

[18] Yamasaki, H., Y. Sakihama, and N. Ikehara. 1997. Flavonoid-peroxidase reaction as a detoxification mechanism of plant cells against H2O2. Plant Physiol. 115: 1405-1412.

Effect of gibberellic acid foliar and kinetin on the antioxidant catalase anzymes and peroxidase in maize under drought stress

[19] Yang, J., X. loui, B. Kiu, J. Li, and D. He. 2004. Cytokinin concentration gradient in the winter wheat grown under reduced irrigation. J. Central European Agri. 10(3): 123–129.
[20] Zare, m., a. mehrabioladi. V, sh. SharafZadeh. Effects of gibberellic acid 2006 (GA3) and kinetin on seed germination and seedling growth stages kinetin wheat under salinity stress. Agricultural sciences research journal. Number 4. Page 855-865.