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EFFECT OF SPERMINE AND AMINO FERTILIZER ON GROWTH OF WHEAT PLANT UNDER WATER STRESS

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Abstract

This research was spilt-spilt plot experiment was conducted in hoop house to study the effect overlap between of water stress and exogenous of amino fertilizer and spermine on some biochemical attributes of wheat *Triticum aestivum* L. variety buhooth (22) this experiment was carried out in according to Randomized Complete Block Design (RCBD) the experience included three periods of water stress (3, 7 and 14) day and gave the symbols (D1, D2 and D3) respectively, and four concentrations of amino fertilizer (0, 2, 4 and 8) ml. L^{-1} and two concentrations of spermine (0 and 1) mM , the fresh leaves was harvested to study some characteristics, including enzymatic antioxidants of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) and nutrients (N, P and K) the results showed that when increasing water stress it led to a significant decrease in nutrient concentrations (N, P and K) by (15.94%, 53.12% and 11.24%) respectively, While the effectiveness of enzymes(SOD, POD and CAT) increased significantly with increased water stress (D1 to D3) by (42.75 %, 91.39 % and 69.87%) respectively, The study also showed that foliar spray of amino fertilizer and spermine had a positive effect on a significant effect in reducing the activity of enzymatic antioxidant, While the values of nutrients (N, P and K) increased significantly under water stress.

Key world: Spermine, Amino fertilizers, water stress, Enzymatic antioxidants

Introduction

Wheat is Monocotyledone from the family plant of the grassroots family. Wheat produces compound grains, and in the form of spikes, global trade in wheat is greater than all other crops combined. making it the second most prolific grain after corn, an important source of carbohydrates. (Shewry *et al.,* 2015) which is considered one of the important foods in the world and has many uses as food or treatment. It is also used in herbal medicine and Wheat embryos and apostasy are used in animal feed. (water stress) is one of the most important factors of abiotic stress that affects the life of all organisms. Dehydration occurs when the soil moisture level in the air is low while the temperature is also high (Hansen *et al.*, 2015). Also, water stress in the plant results in oxidative stress, which is the high production of toxic free radicals from the Nitrogen and Oxygen group. Effective RNS and ROS, inhibiting the process of carbon fixation from the air and the speed of decomposition of live tissue and cells, leading to aging and death if no external factors are added that address and limit the effect of stress (Gupta *et al*., 2016). The leaf additives of amino fertilizer depend on the extent of the need of the plant during the different growth stages, especially the critical stages of growth such as pollination, fertilization, nodes or exposure to any conditions of environmental stress or patients. The added amino fertilizer as a food supplement for the plant help absorb the basic nutrients That you naturally need to produce more amino acids (Pollegioni and Servi, 2012). Polyamine is an organic compound consisting of two or more amine groups (-NH2) Colorless, hygroscopic, soluble in water. (Karsten *et al*., 2005) Aliphatic, the most abundant types of polyamine in plants are potresin PUT, spermidine SPD and spermine SPM. Polyamines additionally direct different cell procedures, for example, signal molecules that show resistance of abiotic stress is generally influenced by the job of polyamines in signaling procedures as opposed to their gathering Most abiotic stress reactions share normal components in their pathways. The role of Polyamine in reducing water stress by in protecting the cellular membrane from oxidation peroxidation, activating the antioxidant immune system and reducing ROS by suppressing it. The purpose of implementing the

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experiment is to evaluate the role of both amino fertilizers and polyamines in treating the harmful effects of water stress.

Materials and Methods

The experiment was designed according to the split split plot design of Randomize Complete Block Design (RCBD) as global experience and three replicates. As The experiment included the following. Holding water (3, 7 and 14) days, which gave the symbols (D1, D2 and D3), respectively, which represented the main factor, while the secondary factor is represented by amino fertilizer in concentrations (0, 2, 4 and 8) ml. L^{-1} and sub- secondary spermine in (0 and 1)mM The spermine was sprayed in the morning and according to the concentration prepared after 20 days of germination and repeated praying every four weeks. After two days, the leaf spray was carried out with concentrations of amino fertilizer and repeated spraying as well every four weeks for all the vegetative parts of the wheat plant Control treatments were sprayed with plain water, After 80 days of planting, vegetable samples were taken from the shoot and kept in the refrigerator to study the biochemical characteristics of wheat.

Extraction of enzyme

One g of the vegetable part was weighed and mashed well in a ceramic mortar after adding 10 ml of potassium phosphate K_2HPO_4 buffer (0.1 m), then filtered with a piece of gauze and the filtrate was subjected to centrifugation by centrifuges to 4° at a speed of 4000 r/ min For a period of one hour, and the resulting leachate was placed for each sample in test tubes and kept in the refrigerator in cooling conditions, it was used to estimate of enzymatic antioxidants within the experiment . Pitotti, *et al.,* (1995).

Superoxide dismutase (SOD) : was evaluated by NBT and riboflavin. According to the method (Beyer and Fridowich, (1987). Prepare mix Working mixture (21.60 mL) Prepared by mixing the buffer potassium phosphate solution (18.35 mL) with the amino acid solution L-methionine 14 mM. (1.50 mL) with Triton X-100 solution (0.75 mL) with Nitro blue tetrazolium solution (1 mL) and the total volume of solution becomes (21.60 mL(1.5 ml of the work mixture was added in test tubes and 500 microliters of distilled water were added by a graduated pipette. 40 microliters of perch were added to each tube and 40 micro liters of Riboflavin solution (47.7 micromol) were mixed well with shake Each tube is hand held and then I read the absorbance with a Spectrophotometer at a wavelength of 560 nm. Then the tubes were exposed to lighting for ten minutes, then I read the absorbance directly below the wavelength of 560nm.

Estimating of peroxidase enzyme (POD) : was estimated according to the described method Muftugil (1985)1 ml of Guaiacoal solution was mixed with 1 ml of hydrogen peroxide solution and read the absorbance at 420nm wavelength in the Spectrophotometer apparatus. The enzyme activity was estimated by adding 2 ml of the reaction mixture in the spectroscopy cell, then 0.1 ml of the sample was added, and the absorbance was calculated at Wavelength of 420 nm.

Estimating of the catalase enzyme (CAT): was estimated according to the method. (Aebi.1974).0.1 ml of the filtrate of the sample was mixed with 1.9 ml phosphate buffer solution and 1 ml of hydrogen peroxide solution was mixed well then read the sample with a Spectrophotometer U.V device at wavelength 470. Nm reading was followed up every 30 seconds for 3 minutes.

Protein estimation: The method developed by Bradford (1976)

Concentration of (Nitrogen, Phosphorous and Potassium)

After drying the samples well with an electric oven it is ground by an electric grinder, then taking 0.2g for each treatment and digesting according to the method Gresser and Parsons (1979) which includes 0.2g of dry weight for each treatment, then grinding it and placing it in the glass digestion tubes capacity of 100 ml followed the method of digestion by adding 5ml of concentrated sulfuric acid to the beaker and adding 2ml of concentrated perchloric acid, then the tubes were placed in a sandy bath with a thermal source until the color of solution became clear and cooled. Then complete the volume to100ml with distilled water and the above elements were estimated according to the methods for each an item Nitrogen according to a method Champman and Pratt, (1961) and the percentage of Phosphorus was measured by a device to measure the spectrophotometer at the wavelength 883nm according to a method Matt, (1970), the proportion of Potassium was measured by film photometer, according to a method Page*, et al*., (1982).

The Statistical Analysis System (2012) -SAS statistical program was used in data analysis significant differences were compared to the least significant difference (LSD) at $p \leq 0.05$.

Results

Table 1 indicated an increase in the activity of SOD when the wheat plant was exposed to holding water from 3 to 14 days, when the SOD activity increased from (30.36

to 43.34) units. Mg. Protein^{-1} and decreased (from 54.21 to 24.65) units. Mg. Protein^{-1} under the effect of spraying with amino fertilizer at a concentration of 8 ml. L^{-1} a decrease by 54. 52%, as well as the activity of the enzyme decreased (from 50.60 to 22.19) units. Mg. Protein^{-1} when spraying of the spermine at a concentration (1) mM a decrease by 56.14% As for the Triple overlap between the spraying of the spermine at a concentration (1) mM and the amino fertilizer (8) ml.L^{-1} and (D3) stress decreased Efficacy of SOD from 98.76 to 15.29 units. Mg. Protein^{-1}, with a decrease by 84.51%. Table 2 results showed that when the wheat plant was exposed to water stress from D1 to D3, it resulted in an increase in the effectiveness of the enzyme (POD) as it increased from 3.02 to 5.78 units. Mg. Protein^{-1} while when spraying the plant with amino fertilizer (4) mL. L^{-1} decreased efficacy of (POD) from 5.27 to 4.06 units. Mg. Protein^{-1}, with a decrease rat 22.96%. Also, spraying the plant with spermine a concentration (1) mM resulted in decreased efficacy of POD enzymes which amounted to 3.69 units. Mg. Protein^{-1} compared control 5.33 units. Mg. Protein^{-1}. a decrease by 30.67% As for the Triple overlap between the spraying of the spermine at a concentration (1) mM and the amino fertilizer (8) ml.L^{-1} and when treating (D3) stress decreased of (POD) from 7.35 to 3.87 units. Mg. Protein^{-1}, under stress D3 with a decrease rat 47.34%.

Table 3 showed an increase in the activity of the CAT enzyme from 4.68 to 7.95 units. Mg. Protein^{-1} when the plant was subjected to a stress treatment from D1 to D3, but when spraying the plant with amino fertilizer (8) ml.L^{-1} The effectiveness of the CAT enzyme decreased from 7.67 to 4.70 units. Mg. Protein^{-1} With a decrease rat 38.72% As for the Triple overlap between the spermine at a concentration (1) mM and the amino fertilizer (8) ml.L^{-1} and when treating D3 stress cause the decrease in the effectiveness of (CAT) from 11.70 to 4.86 Unit. Mg. Protein^{-1} a decrease rat 58.46%. When increasing water stress intensity nitrogen concentration decreased from 1.38% at D1 to 1.16% stressD3, and the percentage of decrease 15.94% while nitrogen concentration increased when spraying amino fertilizer from 1.11% control treatment to 1.41% at amino fertilizer with concentration of (8) ml.L^{-1} by an increase 27.2% and also results showed spraying spermine at concentration of 1mM resulted in an increase in the Nitrogen concentration from 1.18% to 1.38% comparison with the control treatment with an increase by 16.94% table 4, the results of the triple overlap between the three factors showed that there were significant differences for spraying spermine and amino fertilizer as the highest nitrogen concentration reached 1.79% when spraying spermine at a concentration of 1mM and an amino fertilizer at concentration of 8 ml.L^{-1} compared to the control treatment that was 1.20% and with an increase rat 49.16% at D1 and the lowest value reached in this capacity is 1.07% when not spraying factors and under the treatment of stress D3.

The result of table 5 confirmed significant differences when increasing water stress intensity P concentration decreased from 0.64% at treatment D1 to 0.30% at stress D3 and the percentage of decrease 53.12% while P concentration increased when spraying amino fertilizer concentration increased from 0.30% control treatment to 0.47% at amino fertilizer with concentration of 4ml.liter

Table 1: Effect of Triple overlap between Spermine and Amino fertilizers and Water Stress on (SOD) units. Mg. Protein^{-1}.

Mean sper mine	Water stress			Amino fertilizer ml.L^{-1}	Sper mine (mM)
	D3	D2	D1		
50.60	98.76	67.72	63.23	0	0
	54.29	46.28	40.76	2	
	45.34	44.37	38.93	4	
	42.36	40.86	24.43	8	
22.19	41.95	29.02	24.59	0	1
	26.02	23.50	20.92	2	
	22.75	19.19	18.16	4	
	15.29	13.08	11.94	8	
	43,34	35.50	30.36	Mean water stress	
8	4	2	0	Mean Amino fertilizer	
24.65	31.45	35.29	54.21		
Spermine	Amino fertilizer		Sterss	LSD	
10.52	21.97		11.15		

Table 2: Effect of Triple overlap between Spermine and Amino fertilizers and Water Stress in (POD) units. Mg. Protein^{-1}.

Mean sper mine	Water stress			Amino fertilizer ml.L^{-1}	Sper mine (mM)
	D3	D2	D1		
5.33	7.35	6.57	4.72	0	0
	6.25	5.54	3.43	2	
	6.21	5.14	3.01	4	
	7.93	4.92	2.99	8	
3.69	5.46	4.78	2.81	0	1
	5.23	3.87	2.59	2	
	3.97	3.62	2.44	4	
	3.87	3.56	2.21	8	
	5.78	4.74	3.02	Mean water stress	
8	4	2	0	Mean amino fertilizer	
4.24	4.06	4.48	5.27		
Spermine	Amino fertilizer		Sterss	LSD	
0.65	0.95		1.35		

[1] by an increase rat 56.66% and also results showed spraying spermine at concentration of 1mM resulted in an increase in the concentration Phosphorus increased from 0.40% to 0.43% comparison with the control treatment with an increase rat 7.5% the results of the triple overlap between the three factors showed that there were significant differences for spraying spermine and amino fertilizer as the highest P concentration reached 0.92% when spraying spermine at a concentration of 1 mM and an amino fertilizer at concentration of 8 ml. liter^{-1} at treatment (D1) compared to the control treatment that was 0.27% at the same stress and with an increase rat 24.07% and the lowest value reached in this capacity is 0.32% when not spraying factors and under the treatment of stress D3.

The result of table 6 indicated significant differences when increasing water holding K concentration decreased from 2.49% under D1 to 2.21% under stress D3 percentage of decrease 11.24% while Potassium concentration increased when spraying amino fertilizer from 2.20% control treatment to 2.48% at amino fertilizer with concentration of 8 ml.liter^{-1} by an increase rat 12.72% and also results explained spraying spermine at Potassium concentration of 1 mM resulted in an increase in the concentration from 2.34% to 2.39% comparison with the

Table 3: Effect of Triple overlap between Spermine and Amino fertilizers and Water Stress on (CAT) units. Mg. Protein^{-1}.

Mean sper mine	Water stress			Amino fertilizer ml.L^{-1}	Sper mine (mM)
	D3	D2	D1		
8.02	11.70	10.36	8.34	0	0
	10.94	9.94	6.85	2	
	9.25	7.21	4.35	4	
	7.32	5.69	4.83	8	
4.62	6.80	5.40	3.47	0	1
	6.74	5.04	3.50	2	
	6.53	3.94	3.75	4	
	4.86	3.18	2.38	8	
	7.95	6.34	4.68	Mean water stress	
8	4	2	0	Mean amino fertilizer	
4.70	5.83	7.08	7.67		
Spermine	Amino fertilizer		Sterss	LSD	
1.21	2.25		1.95		

Table 4: Effect of Triple overlap between Spermine and amino fertilizer and water stress on Nitrogen concentration in leaves plant (%).

Mean sper mine	Water stress			Amino fertilizer ml.L^{-1}	Sper mine (mM)
	D3	D2	D1		
1.18	1.07	1.09	1.20	0	0
	1.15	1.16	1.21	2	
	1.16	1.20	1.22	4	
	1.17	1.21	1.28	8	
1.38	1.03	1.07	1.22	0	1
	1.25	1.31	1.47	2	
	1.36	1.47	1.66	4	
	1.40	1.67	1.79	8	
	1.16	1.27	1.38	Mean water stress	
8	4	2	0	Mean amino fertilizer	
1.41	1.35	1.20	1.11		
Spermine	Amino fertilizer		Stress	LSD	
0.05	0.07		0.06		

Table 5: Effect of Triple overlap between Spermine and amino fertilizer and water stress on Phosphours concentration in leaves plant (%).

Mean sper mine	Water stress			Amino fertilizer ml.L^{-1}	Sper mine (mM)
	D3	D2	D1		
0.40	0.32	0.28	0.27	0	0
	0.30	0.26	0.77	2	
	0.30	0.30	0.78	4	
	0.32	0.31	0.66	8	
0.43	0.31	0.32	0.35	0	1
	0.27	0.27	0.36	2	
	0.28	0.34	1.02	4	
	0.34	0.35	0.92	8	
	0.30	0.34	0.64	Mean water stress	
8	4	2	0	Mean amino fertilizer	
0.47	0.55	0.37	0.30		
Spermine	Amino fertilizer		Stress	LSD	
0.01	0.15		0.13		

Table 6: Effect of Triple overlap between Spermine and amino fertilizer and water stress on Potassium concentration in leaves plant (%).

Mean sper mine	Water stress			Amino fertilizer ml.L^{-1}	Sper mine (mM)
	D3	D2	D1		
2.34	2.04	2.46	2.35	0	0
	2.25	2.91	2.37	2	
	2.10	2.17	2.26	4	
	2.39	2.42	2.40	8	
2.39	1.93	2.23	2.25	0	1
	2.23	2.27	2.41	2	
	2.23	2.35	2.89	4	
	2.60	2.33	3.02	8	
	2.21	2.36	2.49	Mean water stress	
8	4	2	0	Mean amino fertilizer	
2.48	2.46	2.40	2.20		
Spermine	Amino fertilizer		Stress	LSD	
0.04	0.10		0.09		

control treatment by 2.13% the results of the triple overlap between the three factors explained that there were significant differences for spraying spermine and amino fertilizer as the highest Potassium concentration reached 3.02% when spraying 1 mM spermine and an 8 ml.liter^{-1} amino fertilizer with treatment (D1) compared to the control treatment that was 2.35% at the same stress and with an increase rat 28.51%.

Discussion

The increase in the duration of water stress causes an increase in the effectiveness of enzymatic antioxidants, which includes peroxidase, Catalase and *Superoxide* dismutase enzymes, with a treatment at D3 tables 1, 2, 3 respectively. The increased activity of enzymes is produced as an anti-oxidant reaction, as the water stress Stimulates the cell to produce free radicals $^1O_2$, O^-_2, OH^- (Kusvuran, 2010) Where it accumulates in chloroplast, peroxisomes and mitochondria and moves from the activation stage to the stage of interactions between free radicals, which causes the plant to stimulate the activation of the enzymatic system lead to the removal of the toxic effect of these radicals (Rabiei *et al.*, 2015) such as hydroperoxide that results from the metabolism of lipids (Shabala, 2012), may be attributed to the high effectiveness of the antioxidant enzyme superoxide dioxide when the wheat plant is exposed to water stress resulting in cell stimulation. Production Free radicals H_2O_2 and $^1O_2$ The radical attack the cell components, causing damage to nucleic acids, proteins and cell membranes. This leads to stimulation of the plant to resist and scavenge the effect of the ROS by the SOD enzyme and protect the cells from the negative effects of the active types of oxygen. These results are consistent with Their study Yin, *et al*., (2012) in their study of wheat. As higher OH production results in increased enzyme activity (POD) believed to be vital evidence for plant antioxidant counseling. Shanker and venkate, (2011 b); Rao, *et al*., (2006) and these results are consistent with what he mentioned (Saeedfar and Negahban, 2015). POD, CAT and SOD enzymes have the catalytic ability to convert the H_2O_2 and O^-_2 radical to H_2O and O_2 and rid the cell of oxidative stress Manivannan *et al*., (2007 a). The results of table 3 showed that the effectiveness of the CAT increased by increasing the stress times and gave the highest value when treating D3 stress and this effect on the total protein content in the leaves was explained by Luhova, *et al.,* (2003). This provides the plant with an opportunity to develop and grow as a means of resistance to the conditions of water stress The water stress at D3 led to a decrease in the macro elements (NPK). It is believed that the reason for this decrease is a result of a decrease in photosynthesis and is caused by a decrease in the total concentration of chlorophyll and an imbalance in the distribution of dry matter, which leads to reduced absorption of nutrients (Hussein, 2010) and believes the accumulation of free radicals It affects the effectiveness of Nitrogen-fixing bacterial activity (Aroca, 2012) which causes a decrease in Nitrogen concentration table 4 and an increase in phosphorous concentration was observed when treating D1 compared to a stress treatment of D3 table 5 high phosphorous concentration in the plant is believed when switching from the growth stage Vegetative to the stage of proliferative to escape the influence of the facades hydrothermal (Kooyers, 2015) and these results were consistent with mentioned Ardakani *et al*., (2014) increase in potassium concentration was also observed when treating D1 compared to the treatment of D3 stress table 6 and its reason that the intensity of water stress reduces the concentration of potassium which in turn leads Slowing the growth of the plant and less expansion of the cells, Amino acids stimulate plant growth in addition to increased leaf content of Nitrogen, phosphorous and potassium as a result of the presence of these elements in the synthesis of amino fertilizers and their reflection on the growth of Plant (AL- Said, 2008) The reason for the increase in the above characteristics is, the use of an amino fertilizer solution may stimulate vital activities, especially the processes of cell expansion in the plant and their division, as well as its role in increasing the activity of enzymes that analyze organic compounds and which liberate the elements, which leads to an increase in their readiness, which results in increased plant growth rates. The amino fertilizers has led to a high concentration of Nitrogen, phosphorous and potassium tables (4, 5, 6) in the vegetative part. The reason for this rise is that the amino fertilizer is a nitrogenous source, absorbed by the plant's lungs, The concentration of Nitrogen and phosphorus increased when adding the Spermine Table (5, 4). The reason is due to the role of spermine in activating nucleic acids and sulfur phosphate in cell membranes, which leads to high nitrogen concentration and growth rate (AL-Khafaji, 2014), as well as its presence as ammonium derivatives And it contains two groups of the active amine and thus represents a source of Nitrogen (Karsten, *et al.,* 2005), While increasing the potassium concentration when adding spermine, the reason is that the polyamine including spermine, is rates for some of the ion channels, including propionic acid receptor, which prevents the flow of ions to the direction Reversed inside the potassium channels and thus are preserved To cellular energy, the potassium ion gradient across the cell membrane (Takano, *et al.,* 2012).

Conclusion

It could be concluded that exogenous of amino fertilizers and spermine can alleviate water stress havoc on wheat plants.

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