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EFFECT OF POLYAMINES PRETREATMENTS ON THE ACTIVITY OF ANTIOXIDANT ENZYMES IN SALT STRESSED WHEAT PLANTS

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ABSTRACT

A pot experiment was conducted to study the effect of polyamines pretreatments, putrescine (2, 4, 6, 8 &10 mM) or spermine (2,4, 6,8 & 10 mM),on the activity of catalas, peroxidase, and polyphenol oxidase in salt stressed wheat plants. NaCl (0.0, 6000, 8000,10000 ppm) led to a gradual increase in the activity of catalase, peroxidase and polyphenol oxidase in wheat plants. On the other hand, activity of antioxidant enzymes was decreased by the application of different polyamines treatments, especially with higher levels, in plants grown under salt stress (6000, 8000 and 10000 ppm NaCl)

INTRODUCTION

High salt concentrations disturb the ion homeostasis resulting ion toxicity, osmotic stress and production of reactive oxygen species (ROS) (Ashraf, 1994; Munns, 2002; Mittler, 2002). In addition, the production of ROS is enhanced in plants in response to different environmental stresses such as salinity, drought and temperature extremes etc. Mittova *et al.,* 2002.

Enhanced production of oxygen free radical is responsible for peroxidation of membrane lipids and the degree of peroxidative damage of cells was controlled by the potency of antioxidantive peroxidase activity in response to salinity Sreenivasulu *et al.,* 1999. Polyamines are now regarded as a new class of growth substances and are also well known for their anti-senescence and anti-stress effects due to their acid neutralizing and anti-oxidant properties, as well as to their membrane and cell wall stabilizing abilities Velikova et al., 2000.

Polyamines have been reported to be involved in the plant response to salt and osmotic stress by playing a role in the ROS mediated damage caused by high salt and osmotic conditions (Borsani *et al*., 2001; Zhu 2002).

MATERIALS AND METHODS

Experiment was carried out during the growth period, November (2005) to April (2006) to study the effect of salinity application on some antioxidant enzymes of wheat plants and to investigate the effectiveness of putrescine and spermine treatments on the activity of the same enzymes

The used grains of wheat plant (*Triticum aestivum* L.) sakha 93 were obtained from the Department of Wheat Research, Agriculture Research Center, Giza, Egypt.

Four levels of salinity at the rate of zero, 6000, 8000 and 10000 ppm

NaCl were used for irrigation of the plants of each treatment. Mature grains of wheat (cv. sakha 93) were soaked for 8 h at 20 + 1°C in distilled water (control) or in putrescine at the concentrations, 2, 4, 6, 8 and 10 mM and or in spermine concentrations at 2, 4, 6, 8 and 10 mM.

Method of planting:-

Grains of wheat cultivar sakha 93 were sown in pots (39 cm diameter) at depth of 4-5 cm from soil surface. The pots were irrigated with tap water until the complete germination (7 days) then the plants were thinned to identical 8 plants/ pot. Wards after, the plants were irrigated every 15 days either with tap water (in the control treatment) or with one of the different concentrations of saline water to keep the soil at the level of 70% of the field capacity. Saline soil was washed with tap water from time to time to decrease salt accumulation in the soil. The plants were harvested at 150 –day –old.

Estimation of antioxidant enzymes

Antioxidant enzymes were determined in two stages, tillering and anthesis (45 and 75- day- old wheat plants).

-Enzyme extraction and assay

The fresh leaf samples, weighting about 200mg, were homogenized with 10ml of phosphate buffer pH 6.8 (0.1M). Five ml of homogenate was centrifuged at 2°C for 15 min at 17.OOO g in a refrigerated centrifuge. The clear supernatant was taken as the enzyme source.

Catalase assay:

The activity of catalase as well as peroxidase was assayed after the method of Chance and Maehly (1955) with the following modifications. Five ml of the assay mixture for the catalase activity comprised 300 µmoles of phosphate buffer, pH 6.8, 100 µmoles of H_2O_2, and 1ml of the twice diluted enzyme extracted. After incubation at 25°C for I min, the reaction was stopped by adding 10ml of 2% (v/v) H_2SO_4 and the residual H_2O_2 was titrated against 0.01 N $KMnO_4$ until a faint purple color persisted for at least 15 sec.

A control was run at the same time in which the enzyme activity was stopped at "zero" time as that amount of enzyme which breaks down 1 µmol of H_2O_2/min under the assay conditions described.

-Peroxidase assay

Five ml of the assay mixture for the peroxidase activity comprised: 125 µmol of phosphate buffer, pH 6.8, 50 µmoles of pyrogallol, 50 µmoles of H_2O_2, and 1ml of the 20 times-diluted enzyme extract. This was incubated for 5min at 25°C after which the reaction was stopped by adding 0.5 ml of 5% (v/v) H_2SO_4. The amount of purpurogallin formed was determined by taking the absorbance at 420nm.

-Polyphenol oxidase assay:

Five ml assay mixture for polyphenoloxidase activity consisted of the same assay mixture as that of peroxidase without H_2O_2. The absorbancy of the purpurogallin formed was taken at 420 nm. Peroxidase and polyphenoloxidase activities were expressed in absorbancy units.

RESULTS AND DISCUSSION

Antioxidant enzymes

1- Catalase

Results in Table 1 show the effect of different salinity levels as well as polyamines application on the activity of catalase at tillering and anthesis stages, 45 and 75-day-old plant.. Catalase activity was increased gradually with increasing NaCl concentrations. This increment was true at different sampling dates, tillering and heading stages, especially at heading stages where the increases were more pronounced as compared to control plants.

Concerning polyamines application, putrescine and spermine, catalase activity was decreased at both growth stages in shoots of wheat plants under all polyamines treatments as compared to their control plants. Concerning the effect of interaction between salinity and polyamines, it can be noticed that polyamines decreased the catalase activity in plants grown under salinity. This was true at both tillering and heading stages.

Table 1: Effect of Polyamines and Salinity on Activity of Catalase (Enzymatic Activity/g Fresh Weight /1Hour) at Different Growth Stages in Shoots of Wheat Plant

plant stage (day)	Polyamines (mM) / NaCl(ppm)	polyamines (mM)										
		Control	Putrescine (mM)					Spermine (mM)				
			2	4	6	8	10	2	4	6	8	10
Tillering (45)	zero	320	270	284	257	194	252	145	153	203	230	194
	6000	338	230	302	225	221	270	324	221	225	234	230
	8000	355	243	266	275	239	297	234	248	302	194	234
	10000	387	194	266	279	234	315	243	243	279	257	239
Anthesis (75)	zero	684	500	549	609	606	658	652	659	669	616	696
	6000	797	554	589	652	746	786	706	787	739	652	737
	8000	950	787	702	769	702	787	787	900	877	706	810
	10000	1048	882	1025	900	499	697	832	949	454	666	909

2- Peroxidase

Data recorded in Table 2 show that peroxidase activity in shoots of wheat plant was increased gradually with increasing salinity levels comparing to the control (unsalinized one). Results presented in Table 2 also show the effect of different salinity levels as well as polyamines treatment on the activity of peroxidase at tillering and heading stages of wheat plants. In control plants (treated with polyamine) peroxidase activity had a little increase under different levels of putrescine or spermine treatments. Peroxidase activity had a pronounced decrease with polyamine application in salinized plants. This result was true in the two samples, 45 and 75-day-old plants.

3- Polyphenol oxidase

Data presented in Table 3 show the effect of NaCl concentrations on the activity of polyphenol oxidase in wheat plants (45 and 75-day-old plant). Polyphenol oxidase activity at both growth stages was increased gradually by increasing salinity levels.

2+3

Additional data in Table 3 show the effect of polyamines application on the activity of polyphenol oxidase. Diamine (putrescine) and/or polyamine (spermine) application lead to an increase in polyphenol oxidase activity in wheat tissues at both tillering and heading stages.

Data also, in Table 3 show the effect of polyamines treatment on salt stressed wheat plants. Polyphenol oxidase activity was decreased with polyamine treatments. This results were true in the two taken samples, (45 and 75-day-old plants).

It is observed generally from the previous results that, the enzymatic activities of catalase, peroxidase and polyphenol oxidase in shoots of wheat plants were increased gradually and associated with the increase in sodium chloride concentration.

High salt concentrations disturb the ion homeostasis resulting ion toxicity, osmotic stress and production of reactive oxygen species (ROS) (Ashraf, 1994; Munns, 2002; Mittler, 2002). Excess of ROS trigger phytotoxic reactions such as lipid peroxidation, proteins degradation and DNA mutation (Alshcher *et al.,* 1997, McCord, 2000). To overcome salt-mediated oxidative stress plants detoxify ROS by up-regulating antioxidative enzymes, like superoxide dismutase (SOD), Ascorbate peroxidase (APX) and catalase (CAT).

It is now widely accepted that reactive oxygen species (ROS) are responsible for various stress-induced damage to macromolecules and ultimately to cellular structure (Fridovich, 1986; Imlay and Linn, 1988). Consequently, the role of antioxidant enzymes like superoxide dismutase, ascorbate peroxidase, glutathione reductase and catalase, and metabolites like ascorbic acid, glutathione, α-tocopherol, flavonoids, carotenoids responsible for the quenching of ROS becomes very important (Bowler *et al.,* 1992; Menconi *et al.,* 1995).

Azooz *et al.* (2009) found that the activity of antioxidant enzymes, catalase, peroxidase, ascorbate peroxidase and superoxide dismutase in the salt tolerant maize cultivars increased markedly during salinity stress, while they were mostly decreased by salinity stress in the salt sensitive cultivar.

Salt stress was found to modify polyamine distribution between seedling organs indicating that polyamine responses to salt stress were functional in whole plants Ghoulam and Fares, 2001. Polyamines have been reported to be involved in the plant response to salt and osmotic stress by playing a role in the ROS mediated damage caused by high salt and osmotic conditions (Borsani *et al.,* 2001; Zhu 2002).

In this respect, Ghoulam and Fares, 2001 demonstrated that polyamines reduced salt induced oxidative damage by increasing the activities of antioxidant enzymes and decreasing lipid peroxidation. Putrescine is more effective in increasing the activities of ascorbate peroxidase, glutathione reductase and suproxide dismutase in Virginia Pine, compared to both spermidine and spermine.

Enhanced production of oxygen free radical is responsible for peroxidation of membrane lipids and the degree of peroxidative damage of cells was controlled by the potency of antioxidantive peroxidase activity in

response to salinity. In this respect, Sreenivasulu *et al.* (1999) reported that diamine putrescine under control conditions increased polyphenol oxidase and catalase activities but decreased peroxidase. In saline conditions, putrescine increased peroxidase and catalase activities, while it decreased polyphenol oxidase activity.

Moreover, Huang *et al.* (1990) noted that polyamines prevented the loss of chlorophyll and protein hydrolysis. Also, applying 0.5 mM spermidine stabilized superoxide dismutase and peroxidase activities and prevented the appearance of a new peroxidase isoenzyme band after incubation for 48 h.

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تأثير المعاملة بعديدات الأمين على نشاط بعض الإنزيمات المضادة للأكسدة فى نبات القمح المجهد ملحياً

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أجريت تجربة أصص لدراسة تأثير المعاملة بعديدات الأمين مثل (البتروسين والإسبرمين) على نشاط بعض إنزيمات المضادة للأكسدة لنبات القمح المجهد ملحياً. أوضحت النتائج المتحصل عليها وجود زيادة كبيرة في نشاط الإنزيمات المضادة للأكسدة وهي الكتاليز ، البيروكسيديز ، البولي فينول أوكسيديز وكانت الزيادة مرتبطة بزيادة تركيز كلوريد الصوديوم في التربة . أدت معاملات عديدات الأمين المختلفة إلي حدوث نقص ملحوظ في نشاط إنزيم الكتاليز بالمقارنة بالنباتات المعاملة بالملح وغير معاملة بعديدات الأمين . بالنسبة لنشاط إنزيمي البيروكسيديز والبولي فينول أوكسيديز ، كان هناك زيادة في النباتات الغير مجهدة ملحياً والمعاملة بعديدات الأمين بينما أدي استخدام معاملات البتروسين والاسبرمين إلي وجود نقص ملحوظ فى نشاطها وذلك فى وجود الإجهاد الملحى بالمقارنة بالنباتات المجهدة وغير المعاملة.

قام بتحكيم البحث

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Table 2 : Effect of Polyamines and Salinity on Activity of Peroxidase (Enzymatic Activity/g Fresh Weight /1 Hour) at Different Growth Stages in Shoots of Wheat Plant

Plant Stage (day)	Polyamines (mM) / NaCl (ppm)	Control	Putrescine (mM)					Spermine (mM)				
			2	4	6	8	10	2	4	6	8	10
Tillering (45)	zero	7.38	11.07	8.46	11.07	9.18	8.4	9.63	9.9	8.03	9.81	8.1
	6000	10.17	9.27	7.56	9.25	9.81	7.65	10.15	10.17	8.91	10.17	9.27
	8000	11.88	9.9	9	11.42	10.35	10.08	11.04	10.97	10.17	11.07	10.26
	10000	13.77	10.35	10.62	12.33	11.34	10.17	12.49	12.06	9.54	10.8	10.53
Anthesis (75)	zero	19.53	21.51	20.09	19.68	20.3	20.7	29.88	38.25	28.98	31.95	20.35
	6000	24.25	17.55	20.7	15.3	22.59	23.29	24.24	24.19	19.8	24.13	16.84
	8000	34.65	21.15	21.42	81.18	27.18	18.81	29.61	28.08	34.65	25.29	27.9
	10000	41.04	34.56	32.4	20.16	18	30.15	28.17	40.75	34.2	13.5	37.8

Table 3 : Effect of Polyamines and Salinity on Activity of Polyphenol Oxidase (enzymatic activity/g fresh weight /1hour) at Different Growth Stages in Shoots of Wheat Plant

Plant Stage (day)	Polyamines (mM) / NaCl (ppm)	Control	Putrescine (mM)					Spermine (mM)				
			2	4	6	8	10	2	4	6	8	10
Tillering (45)	zero	1.26	2.99	2.44	2.99	2.08	2.54	2.08	2.9	2.18	2.08	2.99
	6000	1.53	1.08	1.71	1.17	1.17	0.9	1.08	0.99	0.99	1.08	1.17
	8000	2.07	1.35	2.07	1.35	1.17	1.17	1.26	1.17	1.17	1.35	1.44
	10000	3.15	1.8	2.25	1.44	1.44	1.35	1.44	0.9	1.17	1.26	1.71
Anthesis (75)	zero	6.75	9.45	13.5	17.2	15.4	14.94	24.93	37.53	19.53	19.44	25.56
	6000	15.12	6.57	12.96	5.4	9.18	13.31	12.55	12.42	14.32	9.8	9.81
	8000	16.74	9.27	12.96	7.02	14.85	7.74	9.08	12.5	13.17	13.86	12.52
	10000	19.89	15.66	17.72	18.09	12.34	14.94	17.55	13.84	13.05	18.36	12.68