### **Effectiveness of Seed priming with Polyamines in Decreasing Drought Stress Adversities in Two Wheat Cultivars**

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#### Abstract

Two cultivars of bread Wheat (*Triticum aestivum*), Miser2 and Sakha 94 (Differ in their drought tolerance) were selected for drought and Polyamines (PAs) treatment. Seeds of both cultivars were presoaked in water or 100  $\mu$ M solutions of either putrescine (put) spermines (spm) separately or in mixture for 10 h. Thereafter, all seedlings (OR) germinated seeds were restricted to drought by withholding water beginning from the seventh day after sowing (DAS) for a total experimental period of 21days. The results revealed that drought caused significant decrease in growth parameters, pigments, total protein contents, total insoluble sugars, antioxidant enzymes activities and membrane stability index in both cultivars. The decrease was more pronounced in Sakha94.The level of proline, total soluble sugars, H<sub>2</sub>O<sub>2</sub> and lipid peroxidation increased under drought stress in Misr2 more than in Sakha 94 confirming thatMisr2 is more tolerant.

Presoaking seeds in putrescine solutions accumulated pigments, proline, soluble and insoluble sugars and total protein however; it decreased H<sub>2</sub>O<sub>2</sub> content and lipid peroxidation in Sakha 94, but soaking in spermine increased catalase activity while pretreatment with the mixture increased guaiacol peroxidase activity in Sakha94 only. The little effect of presoaking with polyamines was most detected in Misr2. In conclusion, presoaking with spm and/ or put play to some extent a protective role against drought stress through improving plant tolerance. This response differs according to the cultivar but more future research will be carried about PAs application as foliar spray.

*Keywords*: Wheat, Polyamines, Drought stress, Osmolytes, Enzymes activity, H<sub>2</sub>O<sub>2</sub>, Membrane stability index, lipid peroxidation.

#### Introduction

Wheat ranks first in global grain production and accounts for >20% of the total human food calories.Drought is a major abiotic stress that affects agricultural systems and food production

andalso induces several physiological, biochemical and molecular responses in several crop plants (Pennisi, 2008). Drought inhibits photosynthesis of plants\_causing damage the photosynthetic apparatus (Monakhova and Chernyadev 2002).Generally, the environmental stresses especially drought stress, give rise to

accumulation of soluble carbohydrates, proline and free amino acidsas a component of osmotic adjustment, either as a direct response to osmotic stress or a consequence of the growth reduction (Munns, 2002).Under drought stress, H<sub>2</sub>O<sub>2</sub> is generated as a result of electron leakage from he photosynthetic and respiratory electron transport chainsto oxygen or during photorespiration resulting from the oxygenase activity of Rubisco. Also, drought results in changes in antioxidant enzymes activity. High antioxidant activities could be interpreted as symptoms of oxidative stress or damage, the plant up regulates antioxidant enzymes because it produces more reactive oxygen species (ROS) (Nemat Alla and Hassan, 2007). Conversely, high antioxidant activity could be interpreted as higher tolerance to oxidative stress. So improving drought tolerance in plant cultivars is the most suitable solution to this problem. Polyamines (PAs) are small molecules, positively charged at physiological conditions (Takahashi and Kakehi, 2010). The positive charge of the amino groups is the most important feature in these molecules, because it allows electrostatic interactions with several macromolecules such as proteins, lipids and nucleic acids (Childs et al., 2003). At the cellular level, PAs affect cell division and differentiation, membrane stabilization, DNA replication, cell signaling. ion channel regulation, RNA transcription and protein translation (Handa and Mattoo, 2010). PAs have protective effects -during plant response to biotic and abiotic stresses, (Liu et al., 2011). The present study aims at evaluating the effectiveness of presoaking with polyamines (putrescine, spermine separately or in mixture) in mitigating adversities of drought stress in two wheat cultivars differ in their response to drought which of them plays the central role in increasing drought tolerance.

#### **Materials and Methods**

#### Plant Materials

#### Drought Tolerance Test

Seeds of Eight cultivars of wheat (Triticum aestivum) i.e., Gemiza 11, Sakha 94, Sakha 93, Gemiza 12, Gemiza 10, Giza 168, Shawendeel 1 and Miser2 cultivars were kindly obtained from Wheat Research Department, Agricultural Research Center (ARC), Giza, Egypt.In a preliminary experiment for evaluating drought tolerance, all cultivars were exposed to osmotic stress by treating them with 10 ml of PEG 6000 at concentration 20%. The germinated seeds were counted every 24 h allover 7 days to calculate seed germination (the cultivar which recorded the highest germination was considered as drought tolerant and the cultivar which recorded the lowest germination was considered as drought sensitive one). They were respectively, Miser 2and Sakha 94 which were selected for excessive treatment.

#### Experimental Design and Growth Conditions

Seeds of each cultivar were divided into four groups; the first group was left as a control and was soaked in tap water. Seeds of the other three groups were soaked either in 100 µM solution of putrescine, spermine or in a mixture of them (100 µM each). All groups were left for 10 h in the soaking solutions after that, seeds were spread on a filter paper overnight for air-drying their after, were sown in plastic pots. Thirty seeds of each cultivar were sown in each pot (13 cm in diameter) filled with compost + perlite mixtures at a ratio of 2:1, respectively. All pots were irrigated with tap water for a weak then irrigation was stopped and the seedlings were thinned to 10 seedlings per pot and the control only was irrigated twice a week throughout the experiment period of 21days after sowing (DAS) The pots were grouped based on treatment as follows: Control, Drought, Drought + putrescine, Drought + spermine and Drought + Mixture (spermine + putrescine). The experiment was replicated three times and left in the growth Chamber under controlled conditions (light intensity 20000 lux, and the temperature  $23 \pm 2$ °C). Samples were harvested randomly only once at the end of the experiment(21 DAS). At harvest, shoots were separated and used for growth parameters measurements. Leaves were collected, frozen immediately in liquid nitrogen and stored at -80° C for subsequent biochemical analyses. Shoot height (cm) and fresh and dry weights (mg per plant) were measured. Water contents were calculated on fresh weight basis.

#### Determination of pigment content

A known weight (about 0.2 g fresh weight) of leaves was homogenized in ice - cold 100% acetone, and extracted for 24 hr. Samples were centrifuged at 5000  $\times$ g for 15 min at 4°C. The supernatant was used for determination of chlorophylls a, b, and carotenoids content using spectrophotometer according to Lichtenthaler and Wellburn (1983).

## Determination of Total Soluble and Insoluble Sugars

About 0.05g of the oven dry samples were grounded in a mortar, then extracted with 5 ml of 80% ethanol overnight (Schortemeyer et al., 1997). The samples were centrifuged and the supernatant was dried in a water bath. The residues were resolved in 5 ml of distilled water and used for determination of total soluble carbohydrates (TSC). 100µl aliquots of the reconstituted samples were carefully mixed with anthrone reagent (8.6 mM anthrone in 80% H<sub>2</sub>SO<sub>4</sub>) heated for 10min then cooled in ice bath for 30 min. The absorbance was recorded at 623 nm (Schluter and Crawford 2001). The residues remained after extractions of soluble sugars were suspended in 1.6M perchloric acid in a water bath 70°C for 2h. Samples were centrifuged at 1000×g for 10 min and the nonsoluble carbohydrates were determined via the anthrone method as mentioned above.

#### Free Proline content

Free proline was determined according to the method of Bates et al. (1973). A known weight of dry plant material was homogenized in 3% sulfosalicylic acid and the residue was removed by centrifugation. One ml of the extract was reacted with 2 ml glacial acetic acid and 2 ml acid Ninhydrin (1.25 gm Ninhydrin warmed in 30 ml glacial acetic acid and 20ml 6M phosphoric acid until dissolved) for 1h at 100°C and the reaction was then terminated in an ice bath. The reaction mixture was extracted with 10 ml toluene. The chromophore-containing toluene was warmed to room temperature and its optical density was measured at 520 nm.

#### Membrane Stability Index (MSI)

The MSI was determined indirectly by measuring the electrical conductivity following the protocol of Kocheva et al. (2005). One g of leaf material was taken in 10mlof double distilled water in glass vials and kept at 10°C for 24h with shaking. The initial conductivity (C1) was recorded after bringing samples to 25° C by using conductivity meter. The samples were then autoclaved at 0.1 Mpa for 10min; cooled to 25°C and final conductivity (C2) was recorded. Leaf membrane stability index (MSI) and relative injury percentage (RI %) was determined according to Blum and Ebercon (1981) from the following equations:

 $MSI = [1 - (C1/C2)] \times 100.$ 

RI (%): 100-{[1-(CT1/CT2)]/[1-(C1/C2)] ×100} C and CT refer to electrical conductivity of control and treated samples respectively before (1) and after (2) autoclaving, respectively.

#### Hydrogen Peroxide Content

 $H_2O_2$  was measured according to Alexieva et al. (2001). Leaf Tissues (0.5 g) were homogenized with liquid nitrogen and suspended in 5ml of 0.1% (w/v) chilled trichloroacetic acid (TCA). The homogenate was centrifuged at 12,000 ×g for 15 min, and then 0.5 ml supernatant wasmixed with 0.5ml of 10mM potassium phosphate buffer (pH 7.0) and 1ml of 1M potassium iodide. The reaction was developed for 1h in darkness and the absorbance was measured at 390 nm.

#### Lipid peroxidation

Lipid peroxidation in leaves was assayed measuring the malondialdehyde (MDA) content according to Heath and Paker (1968). About 300 mg of the frozen material was ground in 2 ml of 0.1 %( w/v) TCA and centrifuged. An aliquot of 0.5 ml of the supernatant was reacted with 2 ml of 20% (w/v) TCA containing 0.5% thiobarbituric acid at 95°C for 30 min, and cooled in an ice bath. The resulting mixture was centrifuged at 12000 ×g rpm for 10 min and the absorbance of the supernatant was read at 532 nm. Measurements were corrected for unspecific turbidity by subtracting the absorbance at 600 nm. The concentration of MDA was calculated by using an extinction coefficient of 155 mM<sup>-1</sup> cm<sup>-1</sup>.

#### Protein content

A known weight (about 0.5g fresh weight) of leaves was homogenized in 2 ml of potassium phosphate buffer (0.05M, pH 7.4) containing 1 mM PMSF, 2mMdithiothreitol, 0.1mM EDTA and 20% polyvinyl polypyrrolione using a homogenizer for 1min. The samples were then centrifuged. The protein content was determined in the extract spectrophotometrically using Coomassie Brilliant Blue G-250 at 595 nm (Bradford, 1976).

#### Antioxidants Enzymes Activity

Frozen leaf tissue was ground in liquid nitrogen and homogenized in 50mM sodium phosphate buffer (pH 7.0) containing 2mM EDTA and 5mM mercaptoethanol βand 4% (w/v)polyvinylpyrrolidine-40. The homogenate was centrifuged at 8,000×g for 30min at 4°C. The supernatant was used for antioxidant enzyme catalase (CAT) and peroxidase (POX) assays. CAT activity was assayed by determining the rate of change in the absorbance at 240 nm in a reaction mixture (3ml) that consisted of 50mM potassium phosphate (pH 6.9), 11.6 mM H<sub>2</sub>O<sub>2</sub> and 10 mM dithiothretol at 25°C, and the decreased absorbance of  $H_2O_2$  ( $\epsilon = 39.4 \text{ mM}^{-1} \text{ cm}^{-1}$ ) at 240 nm was recorded 1 min later (Aebi, 1984). One unit of CAT activity is the amount of enzyme required for catalyzing the conversion of 1 mmol H<sub>2</sub>O<sub>2</sub> into water per min. POX activity was assayed in a reaction mixture containing 50 mM potassium phosphate (pH 6.4), 0.3mMguaiacol, at 470nm(Chance 0.14mM  $H_2O_2$ and Maehly.1955) by its ability to convert guaiacol to tetraguaiacol ( $\epsilon = 26.6 \text{ mM-1 cm -1}$ ).

#### Statistical analysis

For individual experiments, analyses of variance (ANOVA) were carried out for physiological traits using the Microsoft Excel software. Least significant difference (LSD) was calculated at 0.05.

#### **Results and Discussion**

#### Growth parameters

Shoots fresh weight, dry weight, height, and WC of both cultivars decreased significantly by drought (Fig.1 and 2). The greatest reductions were detected in Sakha 94. Meanwhile, seed priming with PAs specially Put caused significant increase in fresh weight and dry weight of drought stressed plants and improved to some extent the growth, these effects were most detected in Miser2 relative to Sahka94, nonetheless, shoot height in both cultivars was not affected by PAs treatment. Spm and mix caused significant increase in water content only in Sakha94 sensitive cultivar comparing with plants under drought conditions. The greater reductions in growth of Sahka94 than in Miser2 reveal that Miser2 could tolerate drought more than Sahka94. The decrease in fresh

weight under drought condition might suppress cell expansion and cell growth due to the low turgor pressure and reducing meristems (Khedr et al.2011a). These effects seemed higher in Sahka94 than Miser2, probably due to relative water content (RWC). In confirmation, RWC was more significantly reduced in Sakha, than in Miser2.RWC is very responsive to drought stress and has been shown to correlate well with drought tolerance. Therefore, the change in RWC in Miser2 synchronous with the slight decrease in fresh and dry weight relative to Sahka reveals that Miser2 could be considered as more tolerant to drought than Sahka. Pas regulate stomatal responses by reducing their aperture and inducing closure, and Put modulates ABA biosynthesis in response to abiotic stress (An et al., 2008; Alcazar et al., 2010). Thus, Pas are involved in the ABA mediated stress responses which affect the stomatal closure and increased water content.



**Fig** 1: Influence of priming of seeds of two wheat cultivars with 100  $\mu$ M polyamines (putrescine, spermine or their mixture) on the effect of drought on fresh weight and dry weight on the 21<sup>st</sup> day after sowing (DAS). Data are means of 3 replicates ± SE. Column with the same litters are not significant at 5 % level. Cv1= Misr 2 and Cv2 = Sakha 94.



**Fig. 2:** Influence of priming of seeds of two wheat cultivars with 100  $\mu$ M polyamines (putrescine, spermine or their mixture) on the effect of drought on shoot height and water content on the 21<sup>st</sup> day after sowing (DAS). Data are means of 3 replicates  $\pm$  SE. Column with the same litters are not significant at 5 % level. Cv1= Misr 2 and Cv2 = Sakha 94.

#### Changes in pigments content

Chla and Chl b contents were significantly decreased under drought conditions in both cultivars comparing with control. The greatest reduction occurred in drought sensitive cultivar. Seed priming with either Put or Spm separately caused significant increase in Chl a & b contents in Sakha 94 cultivar only comparing with plants under drought conditions. As general, the recovery was more or less comparable to the corresponding control levels. Put appeared to have the most effect upon increasing Chl a & b contents followed by Spm and the mix in Sakha 94 compared with the control (Fig.\_3). The great effects of water stress on Chl a & b appeared to be closely related to the metabolic regulation of Chl. This may be linked with the observation that under -0.34MPa water potential conditions Chl synthesis was severely inhibited with the result that the functioning of the apparatus became photosynthetic seriously impaired (Liuetal., 2007; Ashraf, 2010). Fig.3 also declares that the content of carotenoids was significantly decreased by water stress in both cultivars; the decrease was mostly restricted to the sensitive one. Seed presoaking with PAs increased the content of Car of only Sakha94to the level comparable to the corresponding control values. It has been reported that senescence is extremely affected by polyamines, which are commonly known as antisenescene agents and observed that polyamines retained chlorophyll and inhibited RNase and protease activity (Shallan et al., 2012; Zeid and Shedeed, 2006). Besford et al. (1993)attributed the positive effects of polyamines on chlorophyll and carotenoids levels to preservation of the thylakoid membranes at site of chlorophyll-protein complex.



**Fig. 3:** Influence of priming of seeds of two wheat cultivars with 100  $\mu$ M polyamines (putrescine, spermine or their mixture) on the effect of drought on chlorophyll a, chlorophyll b and carotenoids content on the 21<sup>st</sup> day after sowing (DAS). Data are means of 3 replicates  $\pm$  SE. Column with the same litters are not significant at 5 % level. Cv1= Misr 2 and Cv2 = Sakha 94.

#### Changes in osmoprotectants

Drought significantly increased soluble sugars content in both cultivars comparing with control

value; the increase was greatest in the tolerant one. Presoaking with PAs either separately or in combination caused more accumulation insoluble sugars in both cultivars comparing with respective well-irrigated plants but the accumulation was higher in sakha 94 than in Miser2 (Fig. 4). Conversely, drought decreased insoluble sugars accumulation in both cultivars but the decrease was greatest in tolerant one. Seed priming with PAs either separately or in combination caused slight accumulation in insoluble sugars contents comparing with respective non-irrigated plants in both, the change was most pronounced in the sensitive sahka94specially due to put pretreatment comparing with their values in plants under drought stress (Fig. 4). Soluble sugars are the main osmotic adjustment substances and could be considered as important indicators of drought tolerance. The results show that the soluble sugars contents of wheat seedlings increases under drought stress. This indicates that they may help regulate and maintain the activity of to physiological processes within the plant in a high water-stress environment by raising the osmotic potential of the cells (He et al., 2006). Our findings reveal that, under drought stress, soluble sugars tend to increase while starch concentration decreases. Photosynthesis is the mostimportant phenomenon inhibited under the stress resulting in reducing the whole producedstarch and soluble sugars within the seedlings (Demetriou et al., 2007). The modulation of Put or Spm in increasing the concentration of soluble sugars and decreasing the concentration of starch in seedlings under drought stress might be due to the constructive roles of Put and Spm in improving and maintaining the structure and function of photosynthetic system during stress. The solutes are low molecular weight, highly soluble compounds that are non toxic at high cellular concentration and protect cellular components from dehydration injury, thus are referred to as osmoprotectants and compatible solutes.

The impacts of water stress on proline were similar as in soluble sugars; its content increased under water stress in the shoots of both cultivars but its level was significantly accumulated in the tolerant one only. Pretreatment with PAs either separately or in combination caused high significant increase in proline content in both cultivars comparing with respective control values. The changes in proline accumulation due to PAs pretreatment, especially Put was most pronounced in sensitive cultivar Sahka94 comparing with their respective values in plants under stress or will irrigation conditions (Fig. 4). Proline is also known to play important roles in osmotic adjustment with its accumulation under water stress being observed in many species (Rengasamy, 2002). The results show that, along with a decrease in osmotic potential, the accumulation of free proline increased significantly in both cultivars. This increase would lower the osmotic potential in the cells which would help to maintain turgor and thus sustain the normal physiological and biochemical processes facing drought. In this concern, plants accumulate compatible osmolytes such as proline when they are subjected to stress (KaviKishor et al., 2005). In addition, proline probably detoxifies plants by scavenging ROS or prevents them from damaging cellular structures. There is strong evidence that proline as an amino acid play an adaptive role in mediating osmotic adjustment and protecting the sub cellular structures in stressed plants (Ashraf and Harris, 2004).



Fig. 4: Influence of priming of seeds of two wheat cultivars with  $100 \mu M$  polyamines (putrescine, spermine or their mixture) on the effect of drought on

soluble sugar, insoluble sugar and proline content on the  $21^{st}$  day after sowing (DAS). Data are means of 3 replicates  $\pm$  SE. Column with the same litters are not significant at 5 % level. Cv1= Misr 2 and Cv2 = Sakha 94.

#### Oxidative stress indices

Lipid peroxidation, measured as MDA, was significantly increased in plants under drought stress in the sensitive cultivar only. PAs pretreatment either separately or in combination maintained the level of lipid peroxidation more or less comparable to control value; however, significant decreases were detected in the sensitive cultivar. The Put and mix of PAs had the greatest effect in decreasing lipid peroxidation in the sensitive cultivar (Fig. 5). MDA is regarded as a marker for evaluation of lipid peroxidation or damage to plasmalemma and organelle membranes that increases with environmental stresses.

Fig.5 shows that H<sub>2</sub>O<sub>2</sub> contents werehighly accumulated in plants under drought stress in both cultivars, the increase was mostly pronounced in the sensitive one. Pretreatment with PAs either separately or in combination caused significant decrease in  $H_2O_2$  contents in the sensitive cultivar comparing with respective values in non-irrigated plants. However, Put and the mix caused decrease in H<sub>2</sub>O<sub>2</sub> accumulation in tolerant cultivar miser2 comparing to their values in plants under watered and stress conditions. Abiotic and biotic stresses enhance H<sub>2</sub>O<sub>2</sub> generation via enzymatic sources such as cell wall peroxidases (Nemat Alla and Hassan, 2007). Yiu et al. (2009) reported that exogenous Put reduces the oxidative damage in Allium fistulosum by increasing the antioxidant capacity. They found that exogenous application of Put resulted in reduced superoxide radical (O2--) and H<sub>2</sub>O<sub>2</sub> contents and thereby, less oxidative stress in plant cells.

#### Membrane Stability Index (MSI)

Membrane Stability Index significantly decreased in plants under drought stress in both cultivars comparing control value. put and the mix significantly increased MSI values in the tolerant and sensitive cultivars respectively comparing with respective value under stress condition otherwise, the change in MSI due to PAs was less detected (Fig. 5). Pretreatment of PAs was shown to stabilize plant cell membranes, protecting them from damage under drought stress. They maintain cells in a turgid status and reduced ion leakage from cells (Imai et al., 2004; Nayyar et al., 2005). In agreement PAs in the present results alleviate stress-induced growth inhibition possibly due to protection of membranes and minimization of oxidative damage. Polyamines being cationic in nature can associate with anionic components of membrane such as phospholipids thereby stabilizing the bilayer surface and retarding membrane deterioration. Protection of membranes from peroxidation by polyamines could involve both their ability to interact with phospholipids and their antioxidant activity (Roy et al., 2005).



**Fig. 5:** Influence of priming of seeds of two wheat cultivars with 100  $\mu$ M polyamines (putrescine, spermine or their mixture) on the effect of drought on lipid peroxidation, and H<sub>2</sub>O<sub>2</sub> contents and Membrane stability on the 21<sup>st</sup> day after sowing (DAS). Data are means of 3 replicates ± SE. Column with the same litters are not significant at 5 % level. Cv1= Misr 2 and Cv2 = Sakha 94.

#### Antioxidants Enzymes Activity

Catalase activity was inhibited in the sensitive cultivar but conversely it was stimulated in the tolerant one due to drought stress comparing control values (Fig 6). Pretreatment seeds with PAs either separately or in mix lead to recovery of catalase activity in the sensitive cultivar, the most effect were recorded with spm treatment while put was effective in only Miser2. These findings declare another strategy in Miser2 cultivar to tolerate drought through controlling ROS homeostasis, while the sensitive cultivar, Sahka94 lacked this strategy. On the other hand, significant inhibition of GPX activity by drought was restricted only to Sahka94(Fig 6). The CAT/POX system might act cooperatively to remove  $H_2O_2$  at a minimal expense of reducing power and at a maximal rate. Li and Wang (2002) suggested that CAT and POX participate in the regulating mechanism of liquorice cells withstanding a water stress environment. PAs pretreatment either separately or in combination enhanced GPX activity in the both cultivars comparing control values. The most pronounced enhancement was recorded due to the pretreatment with put and mix of PAs in the tolerant and sensitive cultivar respectively. PAs can regulate many enzyme activities by bonding with the enzyme protein or participation in the process of phosphorylation of the enzyme protein (Stark et al., 2011). The reduction in the accumulation of H<sub>2</sub>O<sub>2</sub> was also another result of the pretreatment of PAs, put or spm. Therefore, the increased activities of CAT and GPX activity against ROS seem to be one the mechanisms in which seed presoaking in put, spm separately or in mix found to alleviate to some extent, stress in wheat seedlings.

#### Changes in total soluble protein

Drought stress caused a reduction in protein content in seedlings of both cultivars; the decrease was higher in the sensitive than in the tolerant one. Pretreatment of put, spm or mix of them enhanced protein accumulation in the sensitive cultivar only the mix of PAs had the greatest effect (Fig. 6). Water stress significantly decreased total soluble proteins in plants (Nemat Alla and Hassan, 2012; Nemat Alla et al., 2011) may be due to intensified degradation of proteins as well as the overallinhibition in protein synthesis. Some evidences suggested that drought-sensitive species have higher proteolytic activity compared to resistant ones (khani and Heidari, 2008). Furthermore, production of free radicals such as H<sub>2</sub>O<sub>2</sub> under stress conjugates to proteins, and consequently destruction in their structures (Peltzer et al., 2002). Additionally, several proteins involved in Calvin cycle, glycolysis, and gluconeogenesis/glyoxylate cycle are commonly regulated by polyamines, resulting in the accumulation of starch and sucrose via the corresponding carbon metabolism (Shi et al., 2013a). PAs facilitate conformational changes, which have serious consequences for DNAs protein interaction in the cell (Heby and Persson, 1990). PAs stabilize other double helical structures such as stems and loops in mRNA, rRNA and tRNA conformation through binding tospecific sites, which may be the basis of polyamines stimulatory effects on DNA, RNA and protein (Heby and Persson, 1990).



Fig. 6: Influence of priming of seeds of two wheat cultivars with 100  $\mu$ M polyamines (putrescine, spermine or their mixture) on the effect of drought on catalase, and guiaicole peroxidase activity and protein content on the 21<sup>st</sup> day after sowing (DAS). Data are

means of 3 replicates  $\pm$  SE. Column with the same litters are not significant at 5 % level. Cv1= Misr 2 and Cv2 = Sakha 94.

#### Conclusion

Drought negatively affects wheat productivity worldwide therefore; an attempt was performed to improve the plant stress-tolerance to cope with this upcoming problem of food security through enhancing the protective role PAs. Water stress significantly inhibited wheat growth particularly sakha94. However, pre-soaking of mixture PAs overcame, to some extent, the impacts of drought upon fresh weight, dry weight, water content, protein content, membrane stability, H<sub>2</sub>O<sub>2</sub> content, lipid peroxidation and peroxidase activity. Moreover, putresine treatment followed by the mixture raised the contents of pigments and enhanced catalase activity. The response differ according to the PAs and the cultivar. More future study will be needed about PAs application as foliar spray.

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الملخص العربى

# عنوان البحث: فعالية نقع البذور مع الأمينات المتعددة في تقليل الإجهاد الناتج عن الجفاف في صنفين من القمح

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تم اختيار صنفين من قمح الخبز، مصر ٢ وسخا ٤٤ (مختلفين في تحمل الجفاف). تم نقع بذور كلا الصنفين في الماء أو ١٠٠ ميرولتر من محلول الأمينات (PAS) إما بوتريسين أو سبرمين بشكل منفصل أو الخليط منهما لمدة ١٠ ساعات. بعد ذلك، تركتالبذور للانبات لمدة أسبوع بعدها عرضت جميع الشتلات ما عدا الكنترول للجفاف من خلال حجب المياه بداية من اليوم السابغ بعد المنابغ و ١٠٠ ما عدا الكنترول للجفاف من خلال حجب المياه بداية من اليوم السابغ بعد المدة أسبوع بعدها عرضت جميع الشتلات ما عدا الكنترول للجفاف من خلال حجب المياه بداية من اليوم السابغ بعد المدة أسبوع بعدها عرضت جميع الشتلات ما عدا الكنترول للجفاف من خلال حجب المياه بداية من اليوم السابغ بعد البذر (DAS) لفترة التجرية الإجمالية و قدرها ٢ يوما. كشفت النتانج أن الجفاف سبب انخفاضا كبيرا في معايير النمو، والأصباغ، ومحتوي البروتين الكلى والسكريات الكلية غير القابلة للذوبان، وأنشطة الانزيمات المضادة للأكسدة وموشر الاستقرار في الغشاء البلزمي في كلا الصنفين لكن الانخفاض كان أكثر وضوحا في . والمولين، السكريات الكلية أن الجفاف من خلال حجب المياه بداية من النمو، والأصباغ، ومحتوي البروتين الكلى والسكريات الكلية غير القابلة للذوبان، وأنشطة الانزيمات المضادة للأكسدة وموشر النمو، والأصباغ، ومحتوي البروتين الكلى والسكريات الكلية غير القابلة وضوحا في . المنظرة الانزيمات المضادة للأكسدة وموشر النمو، والأصباغ، ومعتوي البرولين، السكريات الكلية الاستقرار في الغشاء البلازمي في كلا الصنفين لكن الانخفاض كان أكثر وضوحا في . والمشطة الانزيمات المضادة وموسلاء عد من عد المنوين، المكريات الكلية المائين المالية الذوبان، وأنشطة البلازمي في كلا الصنفين لكن الانخفاض كان أكثر وضوحا في . Misr2 أنه المكريات الكلية القابلة الذوبان، وأنشطة البلازمي في كلا الصنفين لكن الانخفاض كان أكثر وضوحا في . المنوين، المكرمان عليه في علماء ملاما المالية الذوبان، وأنشطة الذوبان، والمولين، المكرمان عليه من الموليان الكثر وضوحا في . Misr2 ألمان عليه في الغاب الكثر معا كام القابلة الذوبان، وأنه ما كله ما ي

نقع البذور في محلول بوتريسين أدى الى تراكم الأصباغ ، البرولين والسكريات الذائبة وغير الذائبة والبروتين الكلي ولكن انخفض محتوى H2O2 وبيروكسيد الدهون في سخا ٩٤، أما النقع في السبرمين أدى الى زيادة نشاط الكاتلاز بينما المعالجة مع الخليط زادت النشاط فى انزيم جواياكول البيروكسيديز في Sakha94 فقط. كان تأثير النقع مع الأمينات أقل وضزحا في ٢ Misr

في الختام، النقع مع الأمينات المستخدمة منفردة أو على صورة خليط لعب دورا وقانيا إلى حد ما ضد إجهاد الجفاف من خلال تحسين تحمل النبات لكن هذا التأثير يختلف وفقا لصنف النبات وهذا يتطلب المزيد من البحوث في المستقبل و سوف يتم عنطريق تطبيق االرش الورقي.