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MORPHOLOGICAL AND BIOCHEMICAL EVALUATION OF SOME EGYPTIAN WHEAT GENOTYPES UNDER SALINITY STRESS CONDITIONS

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ABSTRACT

Three Egyptian wheat genotypes namely; Landrace (LR1), landrace (LR2) and Sakha 93 (S93) cultivar were evaluated under salinity stress using different concentrations of NaCl (0, 50, 100 and 150 mM) at seedling stage. The results revealed that germination percentage, growth parameters and photosynthetic pigments (chlorophyll A and chlorophyll B) were decreased by increasing NaCl concentrations in all wheat genotypes. Moreover, proline accumulation and enzymatic activity of catalase (CAT) and peroxidase (POX) enzymes increased by increasing salt concentrations. However, S93 achieved the highest value of germination percentage, growth parameters, photosynthetic pigments, proline accumulation and enzyme activity of CAT and POX at the highest salt concentration, followed by LR1, while LR2 had the lowest values. On the other hand, the SDS-PAGE showed that S93 had the highest number of protein bands followed by LR1compared with LR2, which had the lowest number of bands under salinity stress. The changes in the number of bands and molecular weight of protein profile can be used as a marker to compare between wheat genotypes. Finally, it could be concluded that S93 cultivar may be more salt tolerance followed by LR1, while LR2 was the most sensitive to salinity stress.

Key words: Enzyme activity, Pigments, Proline, Salinity stress, SDS-PAGE,Wheat.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the staple food of most of the world's population. It is the first grain crop in developing countries. In Egypt, wheat is a main source of bread, providing 80% of the protein needed for human consumption. Egypt's production for wheat represents 40% of it's annual needs while it's imports about 60% from foreign countries. So, it was necessary to increase the cultivated area with an increase in productively of wheat to meet domestic consumption and one of the obstacles that facing to increase wheat crop was salinity stress **(Taregh *et al.* 2011**). Salinity stress is a major problem that caused a decline in productivity of wheat plants in the world (**Irshad *et al.*2002**). Seeds germination are the most sensitive stage in plant life and it is the most affected by salinity.**Al-Taisan (2010)** indicated that seeds of plants achieved the maximum growth in distilled water but it was more sensitive at high salinity levels during the germination stage. Increasing of salinity level by NaCl is harmful to plant growth and results in remarkable decreases in the shoot, root length, dry weight and total pigments content **(Khattab 2007).** Total chlorophyll and carotenoids are an important parameters that have a role in the process of photosynthesis and protection of plants from large-scale radiation and have an important role in the activity of antioxidants **(Redzic *et al.* 2008).** Salt stress breaks chlorophyll and is due to increased toxic effect of sodium chloride (**Yang *et al.* 2011**). Also, proline is an important compound that helps protect cells by proteins fixation and cellular membranes. Proline accumulates play important role in salinity tolerant in plants and reducing salt sensitive plants **(Wang and Han 2009).** Salinity stress gives rise to the formation of reactive oxygen species (ROS). Overmuch formation of ROS leads to severe damage to plants such as oxidation of plant pigments and destruction of proteins and nucleic acids **(Yordanov *et al.* 2000).** Plants use enzymes such as peroxidase (POX), catalase (CAT) and superoxide dismutase (SOD) that act as antioxidants to scavenging of ROS **(Harinasut *et al.* 2000)**. Previous studies were showed that salt tolerant plants increase their antioxidant content because of salinity stress while decreasing in salt sensitive plants **(Ashraf and Harris 2004**). **Ehab *et al.* (2011); El-hamamsy and Behairy (2015)** indicated that protein electrophoresis (SDS-PAGE) method, can be used to show the change of proteins during growth of

plants under salinity conditions. Led to the appearance and disappearance of specific protein bands, also molecular weight (MW) and relative front (RF) of the bands can be used as a marker to distinguish among genotypes under study. The main target of this study was carried out to evaluate three Egyptian wheat genotypes under salinity stress by different concentrations of NaCl at seedling stage, to examined the morphological and biochemical characteristics changes as references.

MATERIALS AND METHODS

Three Egyptian wheat genotypes included tow Landraces; A. # 14201 (LR1) from Northern West Coast, A. #14194 (LR2) from New valley and Sakha 93 cultivar (S93) (Released in 1999 for salt affected soils of North Nile Delta). Which were obtained from Field Crops Research Institute and National Gene Bank (NGB), Agricultural Research Center (ARC), Egypt. Seeds were sterilized by immersion in 1% sodium hypochlorite (NaOCl) solution for five min, then for 1 min with 70% ethanol and then washed with streaming water then distilled water to remove any residue.

The effect of different levels of salinity on seed germination were done using four concentrations of NaCl (0, 50, 100, 150 mM) in 15 cm diameter dishes (three replicates). 25 seeds were randomly selected per dish containing on Whatman filter paper. The Whatman filter paper in Petri dish was moistened by 5 ml of salt concentrations. Place the Petri dishes in the incubator at 20 ° C for 10 days. After the germination period for 10 days, the percentage of germination, shoot length, root length, seedling fresh weight and seedling dry weight were measured according to **(Krishnasamy and Seshu, 1990 as well as ISTA 1999).**

Chlorophyll a, chlorophyll b and total carotenoids were determined using spectrophotometrically (Jenway 6305 spectrophotometer, UK) according to **(Metzner *et al*. 1965)**. Pigments were calculated by following equations: Chlorophyll a (µg / ml) = 10.3 E 663 - 0.918 E 644, Chlorophyll b (µg / ml) = 19.7 E 644 - 3.87 E 663, Carotenoids (µg / ml)= 4.2 E 425 - (0.0264 chlorophyll a + 0.426 and then converted into mg chlorophyll/g fresh plant weight.

Proline content was determined according to **Bates *et al*. (1973).** The content of proline was calculated based on a standard curve and was expressed as μg/g F.W.

Preparation of enzymes extract from vegetative samples was according to **Esfandiari *et al*. (2007).** Peroxidase activity was determined according to the method by **Addy and Goodman (1972).** Catalase activity was assayed according to the procedure of **Chandlee and Schandalios (1984).**

Protein extracts of vegetative samples of three Egyptian wheat genotypes were separated by Sodium Dodecyl Sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to **Laemmli (1970).** The protein gel was scanned and analysis was carried out by image analysis software.

Statistical analysis was performed using randomized complete block design (three replicates) and the differences between means were calculated by L.S.D test according to **Steel and Torrie (1980).**

RESULTS AND DISCUSSION

Effect of salinity stress on germination and growth parameters:

Seed germination percentage and growth parameters affected by salt stress of different wheat genotypes are shown in **Figures (1, 2, 3, 4 and 5).** Results indicated that seed germination percentage and different growth parameters were significantly decreased with increasing NaCl concentrations. Cultivar S93 achieved the highest values of germination percentage (85%) and growth parameters such as shoot length (7.4 cm), root length (6.8 cm), seedling fresh weight (0.74 g) and seedling dry weight (0.09 g) at the highest salt concentration (150 mM), followed by LR1, which is considered moderate tolerance in germination percentage and different growth parameters. On the other hand, LR2 was sensitive to salt stress and had the lowest values of germination percentage (65%), shoot length (4.5 cm), root length (3.5 cm), seedling fresh weight (0.42 g) and seedling dry weight (0.038 g) at 150 mM NaCl. The increasing of salt concentration reduces the osmotic potential, which leads to the prevention or delayed absorption of water uptake to mobilize seed nutrients during seed germination and the components of salt or ions may be toxic to the embryo, all this leads to decrease seed germination **(Mujeeb *et al*. 2008)**. Also, under a high concentration of NaCl, the

rate of photosynthesis decreases, the transportation of appropriate nutrients reduced and the discontinuation of enlargement and cell division, leading to a decrease in shoot length, root length, reduction of plant growth and accumulation of dry weight. Similar results were found by **(Sharma 2003; Datta *et al*. 2009; Alom *et al*. 2016; Hasan *et al*. 2017).**

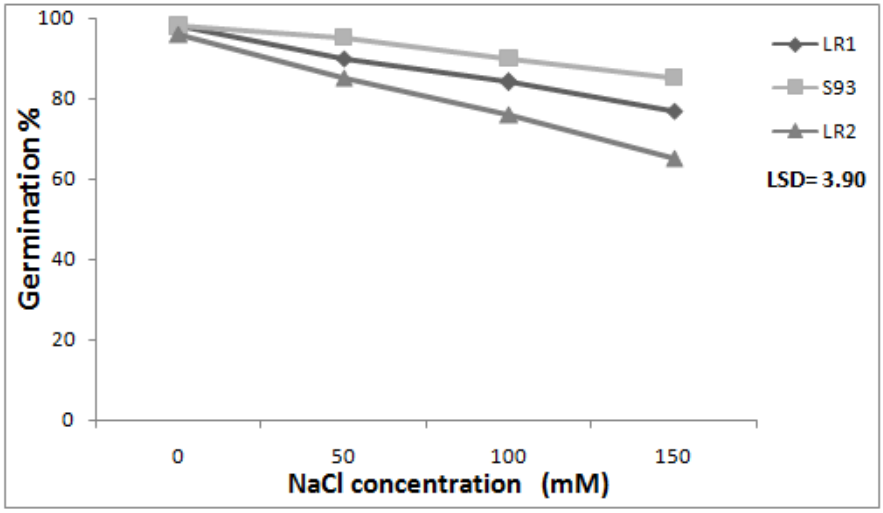

Fig. (1): Effect of salinity stress on germination in three wheat genotypes

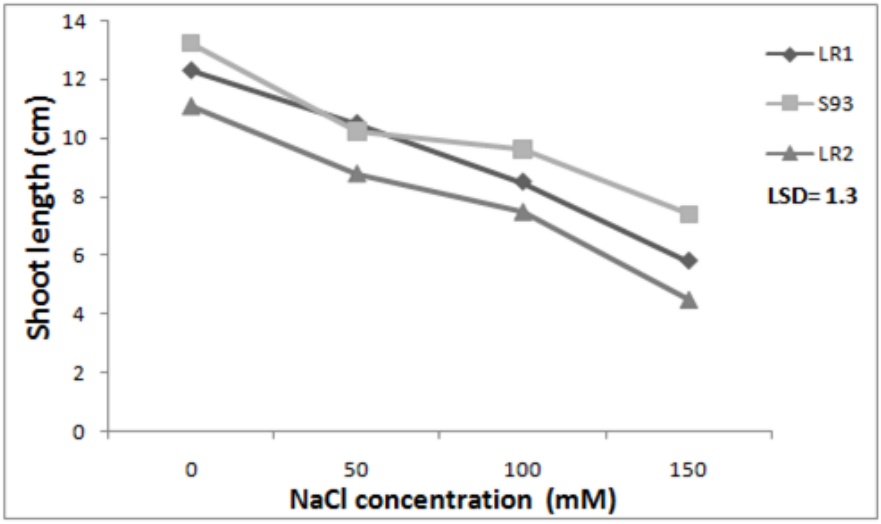

Fig. (2): Effect of salinity stress on shoot length in three wheat genotypes

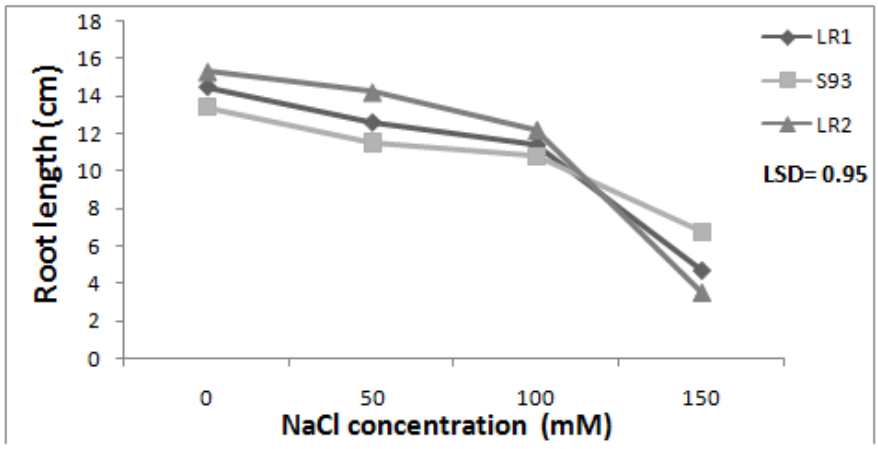

Fig. (3): Effect of salinity stress on root length in three wheat genotypes

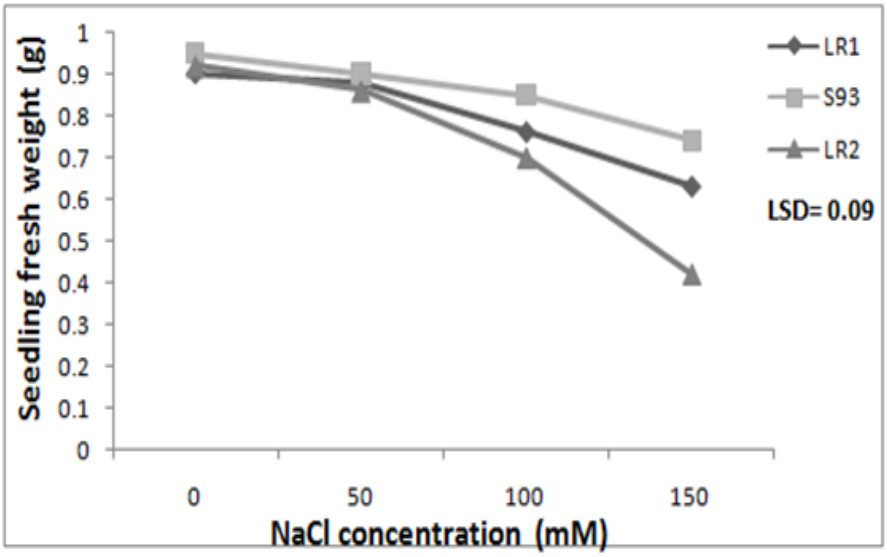

Fig. (4): Effect of salinity stress on seedling fresh weight in three wheat genotypes

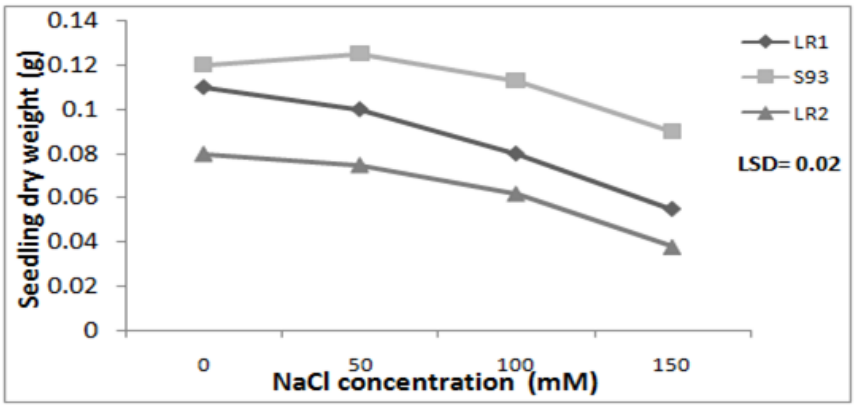

Fig. (5): Effect of salinity stress on seedling dry weight in three wheat genotypes

Effect of salinity stress on plant pigments

Figures (6 and 7) showed that Chl. A and Chl. B were significantly decreased with increasing NaCl concentrations. LR1 had maximum contents of Chl. A and Chl. B 1.35 and 0.650 mg/g F.w., respectively under normal condition, while LR2 had minimum contents of Chl. A and Chl. B 0.44 and 0.202 mg/g F.w., respectively at NaCl conc.150 mM. S93 was more resistant to salinity by containing the highest amounts of Chl. A and Chl. B 0.82 and 0.340 mg/g F.w., respectively at the highest level of salt stress (150 mM). On the other hand, carotenoids content under salt stress had non-significant decrease in the three wheat genotypes (Fig. 8). Degradation of plant pigments under salt stress refers to the high activity of proteolytic or increased activity of chlorophyllase enzyme **(Iyengar and Reddy 1996; Munne *et al*. 1999)**. Salt stress caused the destruction of photosynthetic pigments, as well as deterioration of thylakoid membranes **(Kannan and Kulandaivelu 2011)**. These results are consistent with the results of **Farahat *et al*. (2013); Behairy and El-khamissi (2017)** and **Jiang *et al*. (2017)** reported that accumulation of sodium (Na^+) effects on the synthesis of chlorophyll and photosynthesis process by affecting the photosystem-II in plants.

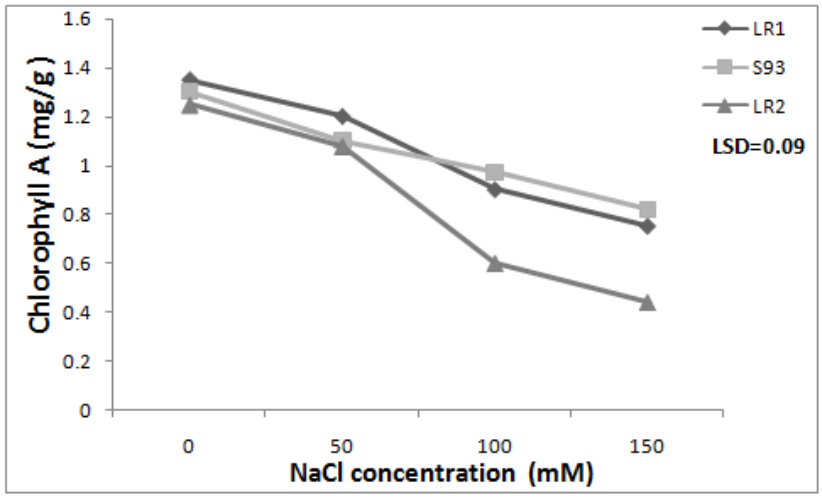

Fig. (6): Effect of salinity stress on chlorophyll A in three wheat genotypes

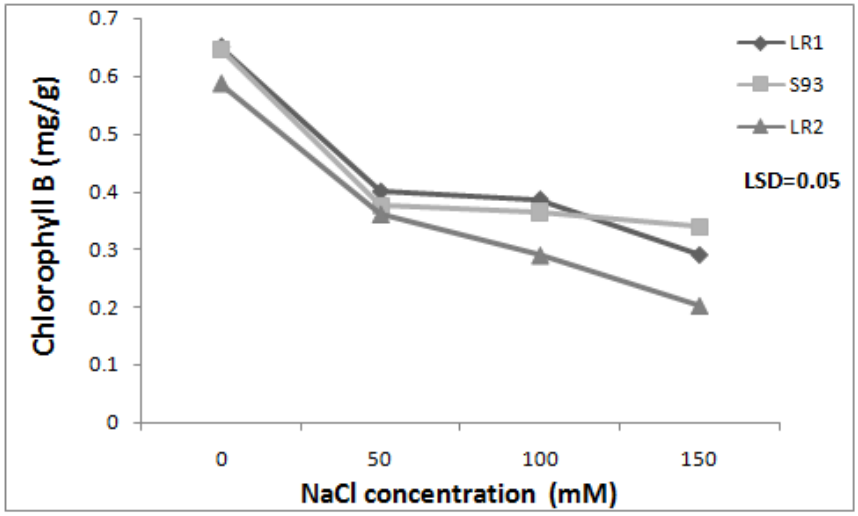

Fig. (7): Effect of salinity stress on chlorophyll B in three wheat genotypes

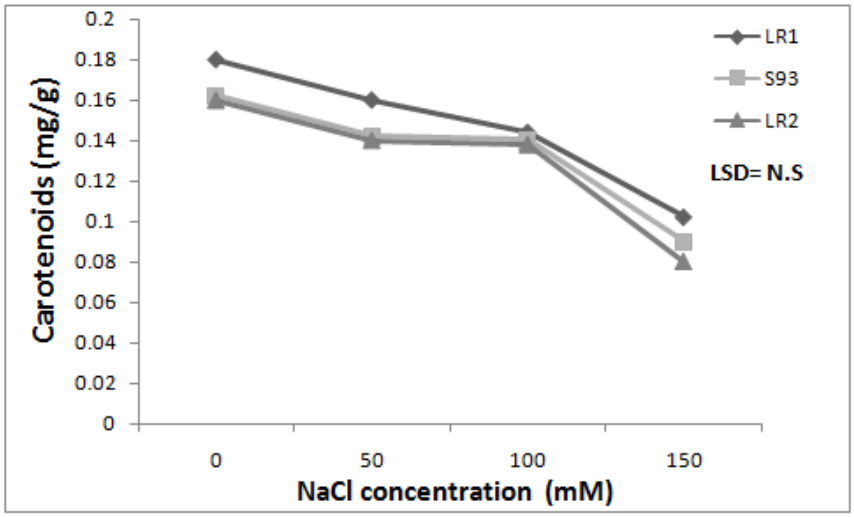

Fig. (8): Effect of salinity stress on carotenoids in three wheat genotypes

Effect of salinity stress on proline accumulation ;

Figure (9) indicated that proline content accumulated with increasing NaCl concentration in all wheat genotypes. The lowest value of proline (6.3 µg /g F.w) was found in LR1 under normal condition, while the highest value (14 µg /g) was observed in S93 at 150 mM. Also, Proline had a significant increase from 7 µg /g under normal condition to 13 mg/g at 150 mM of NaCl in LR2. **Figure (9)** showed that S93 was the highest value in proline accumulation, followed by LR1 then LR2 at the high concentration of salt stress. Proline is one of the important keys in reducing the effect of salt stress on wheat genotypes by adjusting the osmosis. Proline has a role in promoting salinity tolerance by protecting cellular membranes. Proline accumulation may be related to plant tolerance for salt stress **(Pirasteh *et al*. 2014).** Previous reports had suggested that proline accumulation is a reaction to salt stress and has nothing to do with salt tolerance. The accumulation of proline may be due to the increased stimulation of the enzymes in the biosynthesis of proline (**Claussen 2005).** The similar results have been reported by **Kumar *et al*. (2017).**

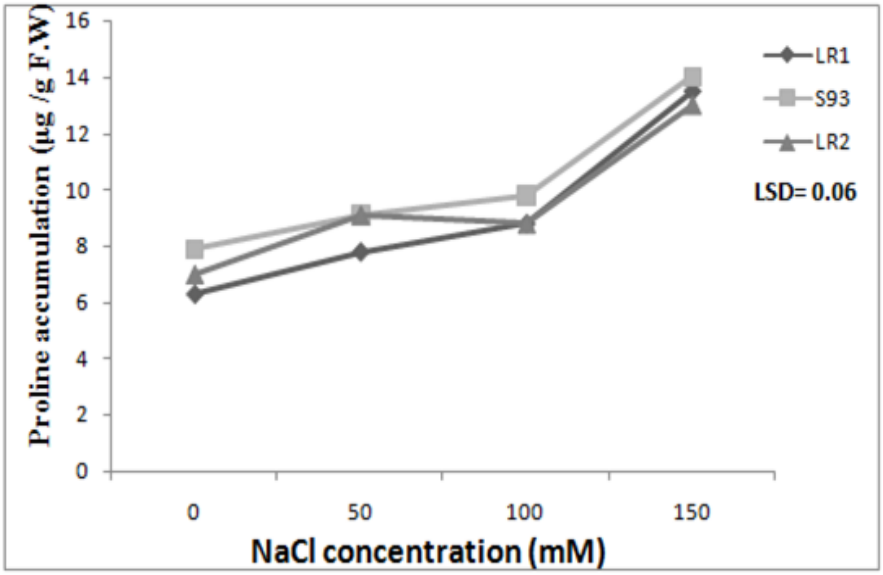

Fig. (9): Effect of salinity stress on proline accumulation in three wheat genotypes

Effect of salinity stress on enzyme activity :

Results of **figures (10 and 11)** indicated that, a significant gradual increase in enzymatic activity of catalase (CAT) and peroxidase (POX) enzymes with increasing NaCl concentrations. CAT activity nonsignificant increased at 50 mM in LR1 and S93 while significantly increased in LR2 at 50 mM as compared to normal condition. The highest value of CAT activity 17.04 U/mg F.W was found in S93, followed by (15.84 U/mg F.W) in LR1 at 150 Mm NaCl. While the lowest value for CAT activity 10.6 U/mg F.W as observed in LR2 under normal condition. POX activity was increased in LR1, S93 and LR2 from 20.19, 23.16 and 16.89 U/mg F.W at normal condition to 40.49, 42.68 and 35.11 U/mg F.W at 150 mM NaCl, respectively. S93 had the highest value of POX activity at 150 mM followed by LR1. While LR2 had the lowest value of POX activity under normal condition or any salt stress level. Increasing in salts concentration led to oxidative damage and increased enzymatic antioxidants can be a defensive strategy for the plant **(Shalata *et al.* 2001; Mittova *et al.* 2004).** Our study, CAT and POX activities was higher in salt tolerant genotypes (S93) than LR1 and LR2. These results are in agreement with findings reported by **Darko *et al.* (2017).**

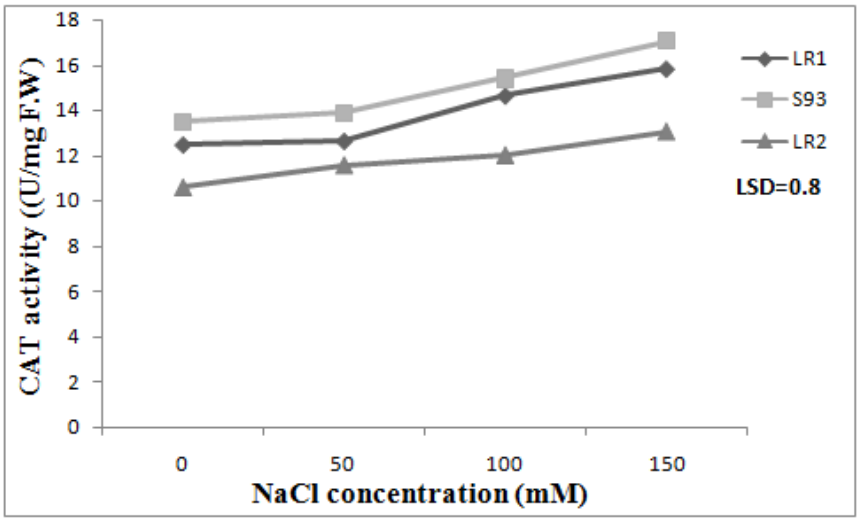

Fig. (10): Effect of salinity stress on CAT activity in three wheat genotypes

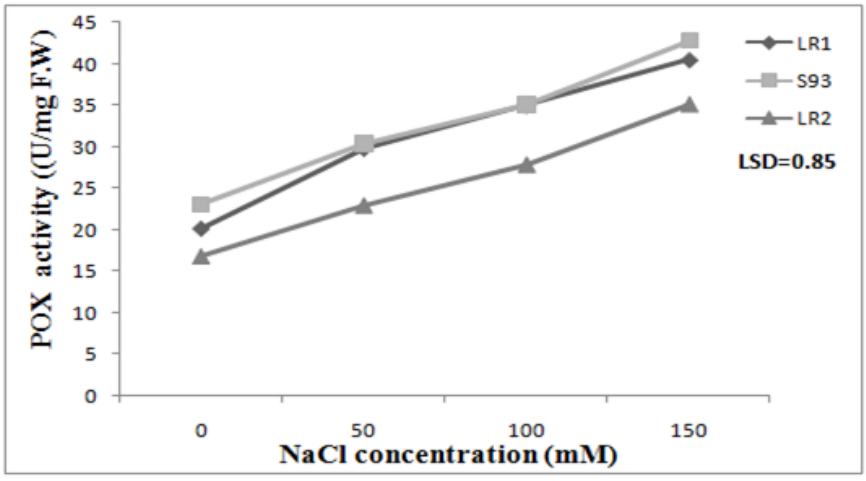

Fig. (11): Effect of salinity stress on POX activity in three wheat genotypes

Protein electrophoretic :

Protein electrophoretic for three Egyptian wheat genotypes, were exposed to four levels of salinity stress 0, 50, 100 and 150 mM. The results revealed that there large variance in electrophoretic protein pattern. The total number of bands were 25 bands, were distributed along of gel between 11 to 96 KDa at Rf 0.91 to 0.11, respectively. **Table (1)** showed that the number of monomorphic bands were 7

bands while the polymorphic bands were 18 bands. Also, the results indicated that the S93 cultivar has the biggest number of bands followed by LR1 while LR2 has the lowest number of bands. S93 had a specific band, which distinguished among genotypes, for instance, molecular weight with 21 KDa at Rf 0.70 with 100 mM of salinity stress also, S93 had specific bands with molecular weights 29, 19 and 15 kDa at Rf 0.6, 0.75 and 0.78 respectively, at 150 mM of NaCl concentration while disappeared in all of the other treatment genotypes. Molecular weight with 11 KDa at Rf 0.91 was presented as a marker or specific band in S93 and LR1 at 150 mM concentration of salt stress while was vanished in the rest of other cultivar treatments. These results agreed with **Yildiz and Terzi (2008)** suggested that the increase of the amount of proteins may lead to an increase in the tolerance mechanisms towards NaCl salinity of wheat species. Also, they revealed that changes in the number of bands and molecular weight of protein profile can be used as a marker to compare between wheat varieties. Results agree with **Moradpour *et al*. (2014)** which they found that the presence of a specific band, which may be used as a protein marker between wheat cultivar. Molecular weight with 82 KDa at Rf 0.29 was presented as a specific band in LR1 and LR2 at 150 and 50 mM of salinity concentration respectively, while was disappeared in S93 genotypes. Molecular weights 80 and 53 KDa at Rf 0.30 and 0.43 respectively were appeared in all cultivars under control condition while these bands disappeared at salt stress conditions. Also, **Table (1)** showed that protein profile had about common protein bands which were presented in the three genotypes under the four salinity stress treatments, for instance, molecular weights with 96, 94, 58, 56, 33, 26 and 13 at Rf 0.11, 0.13, 0.40, 0.41, 0.59, 0.63 and 0.81 respectively, were found in all of control and treatments. Our study explained that these changes that showed in all genotypes are one of the plant reactions of gene expression. Since the reaction process in wheat genotypes are different, we can be concluded that these differences are probably related to their genetic and variability of each genotypes toward salinity.

Table (1): Effect of salinity stress on protein banding pattern extracted from the shoots of the three Egyptian wheat genotypes using SDS-PAGE technique.

Genotypes / Molecular Weight			S93				LR1				LR2			
No. of bands	MW	RF	0m M	50m M	100m M	150m M	0m M	50m M	100m M	150m M	0m M	50m M	100m M	150m M
1	96	0.11	+	+	+	+	+	+	+	+	+	+	+	+
2	94	0.13	+	+	+	+	+	+	+	+	+	+	+	+
3	82	0.29	-	-	-	-	-	-	-	+	-	+	-	-
4	80	0.30	+	-	-	-	+	-	-	-	+	-	-	-
5	76	0.32	+	+	+	+	+	-	+	-	+	-	-	-
6	73	0.33	+	+	+	+	-	+	-	+	-	+	+	-
7	70	0.34	-	-	-	-	-	-	-	-	+	-	-	-
8	67	0.36	+	-	-	-	+	+	+	+	-	-	-	+
9	66	0.36	-	-	+	-	-	-	-	-	-	-	-	-
10	64	0.37	-	+	-	-	-	+	-	-	-	-	-	-
11	58	0.40	+	+	+	+	+	+	+	+	+	+	+	+
12	56	0.41	+	+	+	+	+	+	+	+	+	+	+	+
13	53	0.43	+	-	-	-	+	-	-	-	+	-	-	-
14	45	0.48	+	+	-	-	+	+	-	-	+	+	+	+
15	42	0.50	-	-	-	-	-	-	+	+	-	-	-	-
16	39	0.51	+	+	+	+	+	-	-	-	-	-	-	-
17	33	0.59	+	+	+	+	+	+	+	+	+	+	+	+
18	29	0.60	-	-	-	+	-	-	-	-	-	-	-	-
19	26	0.63	+	+	+	+	+	+	+	+	+	+	+	+
20	23	0.67	+	+	-	-	-	-	-	-	+	-	-	-
21	21	0.70	-	-	+	-	-	-	-	-	-	-	-	-
22	19	0.75	-	-	-	+	-	-	-	-	-	-	-	-
23	15	0.78	-	-	-	+	-	-	-	-	-	-	-	-
24	13	0.81	+	+	+	+	+	+	+	+	+	+	+	+
25	11	0.91	-	-	-	+	-	-	-	+	-	-	-	-
Total number of bands			15	13	12	14	13	11	10	12	12	10	9	9

Conclusion:

It could be concluded that, Sakha 93 cultivar was identified as the more salt tolerant genotypes, followed by LR1 which was moderately salt tolerant, while LR2 was the most sensitive to salt stress at seedling stage. It is therefore recommended to conduct further studies and experiments on Egyptian wheat genotypes so that they can be introduced into breeding and genetic improvement programs for the production of high-yielding and distinct production varieties that are tolerance to adverse environmental conditions.

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التقييم المورفولوجى والبيوكيمائى لبعض التراكيب الوراثية للقمح المصري تحت ظروف الاجهاد الملحي

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تم تقييم ثلاثة تراكيب وراثية من القمح المصرى(LR1، LR2 ، صنف سخا 93) تحت تاثير الاجهاد الملحى باستخدام تركيزات مختلفة من كلوريد الصوديوم (0، 50، 100، 150 مللى مول) في مرحلة الانبات. أظهرت النتائج ان نسبة الانبات ومقاييس النمو المختلفة وصبغات البناء الضوئى (كلوروفيل أ، كلوروفيل ب) انخفضت مع زيادة تركيزات الملوحة باستخدام كلوريد الصوديوم فى جميع التراكيب الوراثية للقمح. علاوة على ذلك، تراكم تركيز حمض البرولين مع زيادة النشاط الانزيمى لانزيمات الكتاليز والبروكسيديز بزيادة تركيزات الملوحة .أيضا حقق صنف سخا 93 اعلى القيم فى نسبة الانبات ومقاييس النمو المختلفة والصبغات النباتية وتراكم البرولين والنشاط الانزيمى للكتاليز والبيروكسيديز عند اعلى تركيز للملوحة (150 مللي مول) ويتبعها التركيب الوراثى LR1 بينما حصل LR2 على اقل القيم لكل ما سبق .من ناحية اخرى، أظهرت نتائج التفريد الكهربى باستخدام طريقة SDS-PAGEان صنف سخا 93 احتوى على أكبر عدد من الحزم البروتينية ويليه LR1 بينما احتوى LR2 على اقل عدد من الحزم البروتينية وذلك تحت الاجهاد الملحى، وبالتالي يمكن استخدام التغييرات فى عدد الحزم البروتينية مع الاوزان الجزيئية المختلفة كأداة للمقارنة بين جميع التراكيب الوراثية المختلفة للقمح. وأخيراً يمكن ان نستنتج ان صنف سخا 93كان أكثر تحملاً للإجهاد الملحى يتبعه LR1 حيث كان معتدل التحمل بينما كانت LR2 الأكثر حساسية للإجهاد الملحي .لذا نوصى بإجراء المزيد من الدراسات والاختبارات على العديد من التراكيب الوراثية والاصناف البلدية للقمح بحيث يمكن إدخالها في برامج التربية والتحسين الوراثي لإنتاج أصناف ذات صفات ذات قدرة إنتاجية عالية ومتميزة ومتحملة للظروف البيئية المعاكسة.