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Comparative Studies on Salt - Tolerant Wheat and Wheat - Grasses

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ABSTRACT

Wheat (*Triticum aestivum* L.) is one of the most important crop plants worldwide. It is the preferred cereal in most arid and semi-arid regions. Wheat genotypes exhibit different physiological responses to salt stress. Sakha 8 wheat variety as well as wheat-grasses *Sorghum virgatum* and *Lolium perenne* are salt-tolerant. *S. virgatum* is an annual desert grass, used extensively for its grains. Perennial ryegrass (*L. perenne*) is important in forage/ livestock systems. Salinization is a threat to global food security. The tolerance to high salinity as an abiotic stress is a quantitative trait. High salinity causes many problems in leaf and root growth. So we intended to compare the physiological and anatomical responses to salinity among the wheat variety sakha8 and the wheat-grasses *Sorghum* and *Lolium*.In the present study, salt stress caused a significant decrease in shoot, root growth parameters as well as seed germination percentage in sakha8, *Sorghum* and *Lolium* plants. In case of CMS, increasing in salinity stress showed increasing in cell membrane stability. From leaf and root anatomy of Sakha8, wheat plant treated with 0. 5% NaCl showed a decrease in leaf and root parameters were detected. Generally sakha8 wheat variety could afford salinity better than *Sorghum* and *Lolium* plants. Finally, further biochemical and molecular analysis are needed in this comparison to obtain wheat plants more salt tolerant.

Keywords: wheat, *Sorghum virgatum, Lolium perenne* and salinity

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important crop plants worldwide with annual production of about 736 million metric tons (FAO 2015). Wheat is the preferred cereal in most arid and semi-arid agricultural regions. Wheat genotypes exhibit different physiological responses or mechanisms to salt stress (Chen, Jiang *et al*. 2012). The rate of leaf electrolyte leakage that measures the amount of membranes leakage over a given time period due to membrane injury, can be considered as a useful screening protocol among wheat genotypes (Apostolova, Yordanova *et al*. 2008)

Wheat variety cv. Giza 168 was more salt tolerant while cv. Sohag variety was salt sensitive even at low salinity levels where wheat variety sakha 8 and cv. Giza 9 are salt-tolerant varieties (Shaddad *et al*. 2006). Some wheat-grasses tend to grow in the salt affected lands, as well as their growth in its normal habitats. There are several salt-tolerant annual wheat-grasses such as *Sorghum virgatum, Lolium perenne* , *Aeluropus lagopoides*, *A. littoralis* and Cutandia dichotoma.

S. virgatum is an annual desert grass, has some local names such as Hashish El-Faras or Garawa besides one synonyms; *Andropogon sorghum* (L.). In Africa, *Sorghum* is used extensively for its grains, fibres, biofuels, fodders and as a construction material. Perennial ryegrass (*L. perenne*) is a cool-season bunchgrass native to Europe, temperate Asia, and North Africa and widely distributed throughout the world. It is important in forage/ livestock systems (Mosimann 2002).

The continuous salinization of arable land is a threat to global food security (Mickelbart, Hasegawa *et al*. 2015). Salinized soils extend over all the continents leading to annual losses of arable land (Pessarakli and Szabolcs 1999).

Stresses, either abiotic or biotic, seriously influence agriculture and farming all over the world. The tolerance to high salinity as an abiotic stress is a quantitative trait. High salinity is one of the most widespread abiotic stress factors in agriculture; causing problems in leaf and root growth, the germination vigor and dry shoot weigh (Munns and Tester 2008). Depending on the level of the stress and the stage of plant development, high salinity may induce various physiological malfunctions (Fahad, Hussain *et al*. 2015) ; (Kranner and Seal 2013, Zagorchev, Seal *et al*. 2013). Water stress at anthesis stage had the most (Grewal 2010).

To achieve salt tolerance, three interconnected aspects of plant activities are important. Firstly, the damage resulting from salinity must be prevent or alleviate. Secondly, homeostatic conditions re-establish in the new stressful environment. Finally, the growth resumed albeit at a reduced rate (Borsani, Valpuesta *et al*. 2003); (Zhu, Bressan *et al*. 2005).

Plants have developed several survival mechanisms to reduce the damaging effects of high salinity: selection or exclusion of undesirable ions (sodium or chloride toxicity), the control of ion uptake by the roots and their transport into the leaves, the compartmentalization of ions at the cellular and whole-plant levels. Also, the adjustment of photosynthetic processes, alterations in membrane structure, the induction of antioxidant enzymes to reduce the level of oxidative stress and the reprogramming of plant hormone synthesis have encountered (Dang, Dalal *et al*. 2008); (Katerji, Mastrorilli *et al*. 2009). The objective of this work was to compare the physiological and anatomical responses to salinity among the wheat variety sakha8 and the wheat-grasses *Sorghum* and *Lolium*.

MATERIALS AND METHODS

1. Plants used:

Wheat, *Triticum aestivium* (Sakha 8) as well as two wheat grasses *Sorghum virgatum* and *Lolium perenne* are the three plants used in this study.

2. Seed germination:

Homogenous seeds of the three plants mentioned before were sterilized superficially by soaking in 0.3% sodium hypochloride for 3 minutes then washed 3 times by sterilized distilled water for 2 minutes. Finally, the sterilized seeds were divided into 4 equal groups. Each group has 10 seeds. The sterilized seeds were soaked in water overnight at room temperature. After that, treatments of seeds of the three plants (control, 0.125, 0.25 and 0.5% NaCl) applied on wet cotton at 28ºC for 2 days. Finally, the percent of germination was calculated.

3. Halotolerance test:

Healthy and homogenous seeds of the three plants sakha8, *S. virgatum* and *L. perenne* were sterilized as mentioned before. Sterilized seeds were germinated in aseptic conditions on Murashige and Skoog (MS) medium containing 3 different sodium chloride concentrations (0.125, 0.25 and 0.5%) besides the control one.

Germination had taken 2 weeks at room temperature in natural day/night light at November 2016.

4. Growth parameters:

The MS-cultivated plants under salinity stress as well as the control one were collected for measurement of growth parameters: shoot length, root length and number of roots per plant.

5. Cell membrane stability (CMS):

According to Sullivan (1972), CMS of the three plants was determined by selecting fully expanded young leaves from each treatment. Twenty leaf pieces (1cm diameter) were cut and submerged into distilled water. The leaf pieces were kept at 10^oC for 24h (T_1). After that, the leaf pieces were warmed at 25^oC (T_2) and the electrical conductivity (C_1 and T_1) of the control and treated contents were measured. The leaf discs were autoclaved for 15min and then the electrical conductivity was measured for control and treated samples after autoclaving (C_2 and T_2). Cellular injury was determined by using the equation:

$$\text{Cellular injury} = [1-(1-\frac{T1}{T2})/(1-\frac{C1}{C2})]\times 100$$

Where, C_1: **CMS before autoclaving** C_2: **CMS after autoclaving**

6. Leaf and root anatomy:

Anatomical features of root and leaf samples of sakha8 treated with 0.5% NaCl and control were examined using (Optika B-383PH, Italy) provided with a digital camera (Optika 4083 B9) at magnification power 100X and 400X. The cell size was measured by ImageJ software http://rsb.info.nih.gov/ij/. The data were analyzed by one-way analysis of variance (ANOVA). Means of treated and non-treated samples were compared by least significant difference (LSD) at the P≤ 0.05 (Snedecor and William 1989).

RESULTS

1. Effect of salinity on growth of sakha8, *Sorghum* and *Lolium*:

Generally, different NaCl concentrations (0.125, 0.25 and 0.5%) decreased the growth parameters representing in shoot, root length and number of roots of sakha8, *Sorghum* and *Lolium* plants (Table 1). Also the growth parameters of sakha8 were higher than the growth parameters of *Sorghum* and *Lolium* plants.

Table 1. Effect of different NaCl concentrations (control, 0.125, 0.25 and 0.5%) on growth parameters (sakha8, *Sorghum* and *Lolium*) plants

Cultivar	Treatment NaCl (%)	Growth parameters		
		Shoot length (cm)	Root length (cm)	Number of root
Sakha 8	Control	20.27	10.33	4.88
	0.125	18.00	8.50*	4.63
	0.250	10.95*	4.50*	4.20
	0.500	8.50*	4.20*	4.20
Sorghum	Control	15.57	9.00	1.90
	0.125	9.66*	5.66	1.60*
	0.250	8.50*	5.68	1.25*
	0.500	8.33*	5.82	1.20*
Lolium	Control	14.14	3.52	2.61
	0.125	13.66	3.33	2.55
	0.250	12.26*	2.52*	2.50
	0.500	8.64*	1.50*	1.71*

(*)=significant increase or decrease at P≤ 0.05 LSD

2. Effect of salinity on germination of sakha8, *Sorghum* and *Lolium*:

The obtained results in (Table 2) showed a decrease in seed germination percentage in three plants in response to treatment with NaCl (0.125, 0.25 and 0.5), where the most effective treatment is 0.5 % NaCl.

Table 2. Effect of different NaCl concentration (control, 0.125, 0.25 and 0.5 %) on seed germination for (Sakha 8, *Sorghum* and *Lolium*) plants

Cultivars	Treatment (%)	Percentage (%)
Sakha 8	Control	90
	0.125	81
	0.250	60*
	0.500	46*
Sorghum	Control	66
	0.125	63
	0.250	53
	0.500	44*
Lolium	Control	88
	0.125	79
	0.250	72
	0.500	50*

(*)= significant increase or decrease at P≤ 0.05 LSD

3. Effect of salinity on cell membrane stability of sakha8, *Sorghum* and *Lolium*:

The data of cell membrane stability of sakha8, *sorghum* and *lolium* (Table 3) showed an extrusive relationship between cell membrane stability and salinity, where increasing in salinity stress showed increasing in cell membrane stability.

Table 3. Effect of different NaCl concentration (control, 0.125, 0.25 and 0.5 %) on cell membrane stability for Sakha 8, *Sorghum* and *Lolium* plants

Cultivars	Treatment (%)	Before autoclaving	After autoclaving
Sakha 8	Control	102.4	146.2
	0.125 %	137.0*	170.3*
	0.250	145.0*	201.1*
	0.500	155.0*	219.3*
Sorghum	Control	61.90	113.2
	0.125 %	89.00*	153.6*
	0.250 %	105.0*	162.5*
	0.500 %	175.0*	179.0*
Lolium	Control	103.0	159.2
	0.125 %	119.0*	179.3*
	0.250 %	123.0*	183.2*
	0.500 %	130.4*	195.4*

(*)= significant increase or decrease at P≤ 0.05 LSD

Table 4. Effect of 0.5 % NaCl concentration and control on leaf and root anatomy of Sakha8 wheat plant

Leaf Anatomy		
Parameters (μm)	Control	0.5% NaCl
Leaf thickness	220.9	170.4
Mesophyll thickness	1447.9	775.1
Bundle size	4968.7	2616.5*
Mata-xylem size	415.2	355.1
Proto-xylem size	197.3	479.2*
Root Anatomy		
Root diameter	419.5	496.8
Stele size	24966.4	24746.1
Pith size	2430.3	3601.6
Cortex size	122.5	117.4
Meta-xylem size	165.9	238.9*

(*)= significant increase or decrease at 0.05 LSD

4. Effect of salinity on leaf and root anatomy of sakha8:

From leaf anatomy of Sakha8 wheat plant treated with 0. 5% NaCl, a decrease in leaf and mesophyll thickness besides a reduction in sizes of bundle and meta-xylem were recognized. While the area of proto-xylem cavity was highly increased with salt stress compared to control. On the other hand, root anatomy of Sakha8 wheat plant treated with 0.5% NaCl showed a slight increase in root diameter as well as an increase in pith and meta-xylem sizes evolved whereas a decrease in stele and cortex sizes were recorded when compared with control.

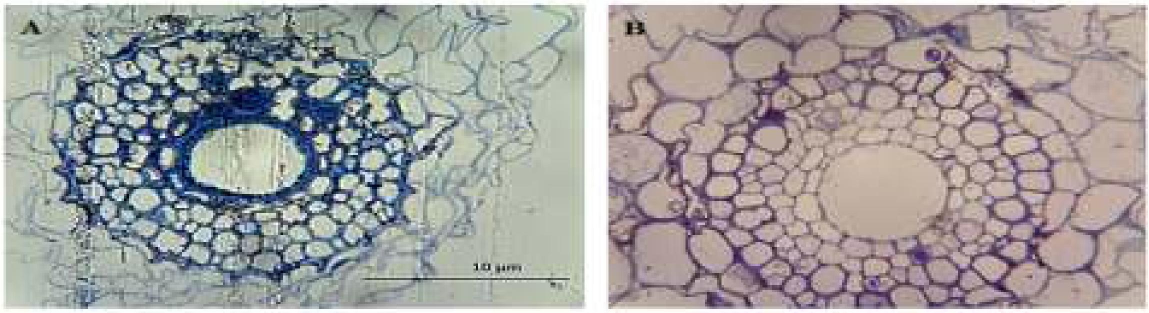

Plate 1. Leaf anatomy of Sakha8 wheat plant control (A) and treated plant with 0.5% NaCl concentration (B)

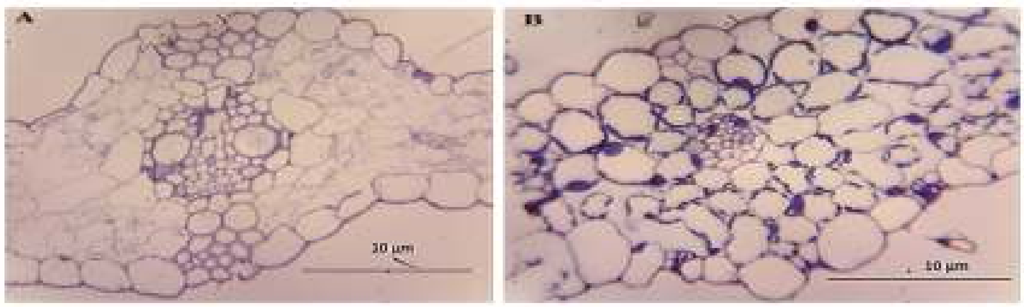

Plate 2. Root anatomy of Sakha 8 wheat plant control (A) and treated plant with 0.5% NaCl concentration (B)

DISCUSSION

Wheat (*Triticum aestivum* L.) is one of most important crop plants worldwide with annual production of about 736 million metric tons (FAO 2015). Strogonov (1962) was the first investigator who notes the growth of plant in media salinized with NaCl. It was evident from our results that NaCl treatment had a significant inhibitory effect on all the growth parameters of of sakha8, *Sorghum* and *Lolium* plants. However, some plants differed in their response to NaCl stress (Jan, Hadi *et al*. 2017). Plants are salt-sensitive at the seedling stage. It is well established that crop plants with better germination and seedling growth under salt stress will be more stress-tolerant at later stages and will produce better growth and productivity (Ahmadi and Ardekani 2006). Number of leave and root/plant decreased with increasing NaCl salt. Root and shoot length is the most significant traits for salinity tolerance because roots have direct contact with soil and absorb water and nutrients from soil. Therefore, root length provides important evidence of plant salinity tolerance (Ashraf, McNeilly *et al*. 1986).

Zerihun *et al*. (2000) also reported that under imposed osmotic stress shoot growth inhibited more than root growth. Also, the growth of leaf area is inhibited by salinity (Alberico and Cramer 1993). In present study, salt stress caused a significant decrease in shoot length of wheat plant with the increase of stress treatments. Probably the negative effect of salinity on plants provoked osmotic potential by salt in the culture medium, so root cells do not obtain required water from medium.

Some investigators thought that because of ion accumulation by changing membrane permeability, metabolism was negatively influenced (Grieve and Fujiyama 1987). In present work, increasing in salinity stress showed increasing in cell membrane stability in the three plants. Hayward and Long (1941) studied the effect of salinity on the anatomical structure. In leaf anatomy of Sakha8 wheat plant treated with 0. 5% NaCl, showed a decrease in leaf and mesophyll thickness besides a reduction in sizes of bundle and meta-xylem were recognized, while the area of proto-xylem cavity was highly increased with salt stress compared to control. Ginzburg (1964) made an intensive study of the interrelationships between root form, anatomy and the conditions of the habitat. He investigated numerous species collected in different habitats. From root anatomy of Sakha8 wheat plant treated with 0.5% NaCl showed a slight increase in root diameter as well as an increase in pith and meta-xylem sizes evolved whereas a decrease in stele and cortex sizes were recorded when compared with control. In conclusion, salt stress caused a significant decrease in shoot, root growth parameters as well as seed germination of sakha8, *Sorghum* and *Lolium* plants. Generally sakha8 wheat variety could afford salinity better than *Sorghum* and *Lolium* plants. Finally, further biochemical and molecular analysis are needed in this comparison to obtain wheat plants more salt tolerant.

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دراسات مقارنه على قمح مقاوم للملوحه منزرع واعشاب قمحيه

أشرف السيد ، محمد عباس ، ساميه هارون ، الشافعى المرسى و سمر الشرقاوى

قسم النبات- كليه العلوم- جامعه المنصوره

يعتبر القمح (*Triticum aestivum* L) احد أهم المحاصيل الزراعية في جميع أنحاء العالم. حيث ينتج الحبوب المفضلة في معظم المناطق القاحلة وشبه القاحلة. وتظهر الطرز الوراثية للقمح استجابات فسيولوجية مختلفة للإجهاد الملحي. توجد سلالات مختلفة من القمح تتحمل الملوحة مثل سخا (٨) وكذلك توجد أعشاب قمحية مختلفة تتحمل الملوحة مثل *Sorghum virgatum* و *Lolium perenne* . ويعتبر الـ *S. virgatum* عشب صحراوي حولي و يستخدم بكثافة لحبوبه.وكذلك تعتبر الأعشاب القمحية الدائمة مثل (*L. perenne*) مهمة في أنظمة العلف للماشية. ان الملوحة تهدد الأمن الغذائي العالمي حيث تسبب الملوحة العالية كضغوط غير حيوية تسمم الخلية. تؤدى الملوحة العالية الى العديد من المشاكل في نموالأوراق و الجذور. لذلك هدفت التجربة إلى مقارنة الاستجابات الفسيولوجية والتشريحية للملوحة بين صنف القمح sakha8 والأعشاب القمحية *Sorghum virgatum* و *Lolium perenne* .في هذه الدراسة ، تسبب الإجهاد الملحي في انخفاض ملحوظ في معلمات معدل نمو الجذر والساق وكذلك فى معدل إنبات بذور النباتات محل الدراسة *sakha8* و *Sorghum* و *Lolium*. و في تجربة الـ CMS كلما زادت كمية الملوحة زادت كمية CMS. من تشريح جذر نبات *sakha8* المعامل بـ ٠.٥٪ كلوريد الصوديوم انخفاضاً في حجم التراكيب التشريحية للوراقة والجذر. بشكل عام يمكن لصنف القمح sakha8 أن يتحمل الملوحة أفضل من نباتات *sakha8* و *Sorghum* و *Lolium*. وأخيرا ، هناك حاجة إلى مزيد من التحليل البيوكيميائي والجزيئي في هذه المقارنة للحصول على نباتات القمح التي تتحمل المزيد من الملوحة.