

## **COMPARATIVE PHYSIOLOGICAL STUDIES ON SOME WHEAT (*Triticum aestivum*, L.) CULTIVARS UNDER SALINE CONDITION DURING GERMINATION AND SEEDLING STAGE**

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### **ABSTRACT**

The effect of NaCl-salinity (0, 8, 12  $\text{dsm}^{-1}$ ) on germination, as well as seedlings growth and their components from water status, chlorophyll, proline, reducing sugars,  $\text{Na}^+$ ,  $\text{K}^+$ , indole acetic acid (I.A.A) and I.A.A. oxidase activity, in three wheat cultivars (cvs), denoted Sakha 94, Masr 1 and seds 12 were investigated. Data indicated that, although 100% of all three cultivars caryopsis was germinated during 48h under all salinity levels; growth behavior of the seedlings showed different responses during the subsequent periods. Also, cv. Sakha 94 appeared more tolerant to high saline level, subsequent by Masr 1 and seds 12. Sakha 94 recorded the maximum values in most vegetative parameters and photosynthetic pigments. Relative water content and osmotic potential were decreased to very low levels accompanied with higher accumulation of  $\text{K}^+$ ,  $\text{K}^+/\text{Na}^+$  ratio, proline and reducing sugars in the leaves of the same cv. In the sensitive cv (Seds 12), I.A.A.oxidase activity was increased by 1.5-5 folds in leaves and roots which a led to decreases in auxine levels (plant growth activator) in the seedlings contrary to more tolerant cv. Sakha 94. It could be recommended that cv. Sakha 94 was more suitable to cultivate under saline condition and the physiological indicators studied may be used as screening markers of early selection for salt tolerant in wheat cultivars.

### **INTRODUCTION**

Salinity has become a critical problem worldwide due to its dramatic effects on plant physiology and performance (Ghassemi *et al.*, 1995). Seed germination is usually the most critical period of seedling establishment, determining subsequent plant growth and production (Bhattacharjee, 2008). Germination and development of seedling are reduced under salt stress with varying responses for species and cultivars (Hampson and Simpson, 1990). Salinity may also affect the germination of seeds by creating an external high osmotic pressure that prevents water uptake or due to toxic effects of  $\text{Na}^+$  and  $\text{Cl}^-$  ions on the enzymes activity during seed germinating (Khajeh-Hosseini *et al.*, 2003).

Wheat (*Triticum aestivum* L.) is the most widely grown crop in the world. World annual production being over 685.6 m ton while reached to 8.5 m ton in Egypt in 2009 (FAO-STAT, 2009). Wheat is considered a moderately salt-tolerant plant (EC: 4-8  $\text{dsm}^{-1}$  without reduction of yield), as well as drought-tolerant crop compared with other cereals and, with barley, it is the preferred cereal in most arid and semi-arid agricultural regions (Mass and Hoffman, 1977). The effect of salinity stress on wheat growth and production has been extensively studied by several authors (Colmer *et al.*, 1995; Derra *et al.*, 1973; Goudarzi and Pakniyat, 2009). Therefore it will be important to quantify the impact of such saline conditions on the germination and seedling establishment of wheat in an effort to improve their yield. Stressed plants

diminish osmotic potential by accumulating free amino acids as proline, ions and dissolvable substances (Salama *et al.*, 1994). Moreover, Schachtman and Munns (1992) reported that sodium exclusion was a general characteristic of salt tolerance in wheat lines; whereas, salt tolerant display much higher shoot sodium level than sensitive lines. Screening large numbers of wheat genotypes for salinity tolerance in the field is difficult, due to spatial heterogeneity of soil chemical and physical properties, and to seasonal fluctuations

in rainfall (Srivastava and Jana, 1984). Therefore Screening techniques that can be carried out under controlled environments have often been used.

This short-term experiment was conducted to screen the more salt-tolerant wheat cultivar to cultivate in the new reclaimed soil and determined useful physiological and biochemical indicators of tolerance to salinity in wheat.

## **MATERIALS AND METHODS**

This study was carried out at the Department of Agric. Botany, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt. The grains of *Triticum aestivum*, L. cvs Masr 1, seds 12, Sakha 94 provided by the Egyptian crops Research Center, Ministry of Agriculture, Egypt. After surface sterilization with sodium hypochlorite 1% (3min.), the grains were cultivated in 10cm Petri plates on Whatman filter papers with 10 ml of tested solutions. Grains were exposed to 2 saline solutions: 8 dsm<sup>-1</sup> ( $\approx 80\text{mmol NaCl}$ ), 12 dsm<sup>-1</sup> ( $\approx 120\text{mmol NaCl}$ ) and control (dionized water). The experimental design was 2 factorial, arranged in a completely randomized design with 3 replications and 30 seeds per replicate. Caryopsis were allowed to germinate under controlled condition using growth chamber at  $25\pm 1^\circ\text{C}$  in the dark for 12 days during the two growing seasons of 2010 and 2011.

### **Measurements and observations**

#### **Seed germination, and seedling growth:**

Caryopsis was considered to have germinated when the emerging radical elongated to 1mm (ISTA, 1976). Root and shoot length (cm), fresh and dry weight of 10 seedlings 12 days-old for each replicate were determined using gravimetric method. Seedling vigor index (SVI) was calculated using formula of Abdul-baki and Anderson, (1973):  $\text{SVI} = (\text{seedling length (cm)} \times \text{germination percentage})$ . Salt tolerance index was calculated as reported by Cano *et al.*, (1998) using the following relation:  $\text{STI} = \text{Fresh weight in NaCl-saline solution} / \text{Fresh weight in control} \times 100$ .

#### **Water content, relative water content and osmotic potential ( $\Psi_s$ ):**

Water content of the seedlings was calculated after determines dry weight by fixing plant material at  $90^\circ\text{C}$  and drying at  $70^\circ\text{C}$  up to constant weight (Bohm, 1979). The relative water content was determined according to Henson *et al.*, (1981) using the following formula:  $\text{RWC} = 100 \times (\text{fresh mass} - \text{dry mass}) / (\text{turgid mass for 24 h} - \text{dry mass})$ . Osmotic potential ( $\Psi_s$ ) was determined with constant weight method using serial sucrose solutions according to Moore (1974).

**Determination of reducing sugars and I.A.A:**

Ethanol extract (96% ETOH) of leaves and roots were prepared according to Abdel-Rahman *et al.*, (1975), Reducing sugars were determined with alkaline copper and arsenomolybdate reagents spectrophotometrically at 540nm according to Moore (1974). I.A.A was determined spectrophotometrically using Beckman DK-2 at 525 nm with Ehrlich reagent, I.A.A was used as a standard (Fliosson, 1969).

**Photosynthetic pigments and chlorophyll stability index:**

0.5 g leaves was ground with 10ml acetone 85% and filtered. Optical density was measured at 662.644 and 440.5 nm using a Beckman DK-2 Spectrophotometer. Concentration of Chl a, Chl b and carotenoids as mg/g FW were calculated according to Fadl and Sari Eldeen (1978). The chlorophyll stability index (CSI) was determined according to Sairam *et al.*, (1997) and calculated as follows:  $CSI = (\text{Total Chl under stress} / \text{Total Chl under control}) \times 100$ ; Genotypic variation coefficient (GVC) was recorded according to Baz *et al.*, (1984) as: reducing sugars/chlorophyll a+b x carotenoids

**Determination of free proline, Na<sup>+</sup> and K<sup>+</sup>:**

Free proline was assayed in fresh plant material according to Bates *et al.*, (1973). L-proline was used as a standard. The content of K<sup>+</sup> and Na<sup>+</sup> was determined using a flame photometer; model III Carl Zeiss Jena, Germany as described by Jackson, (1973).

**Preparation of enzyme extracts of leaves and root:**

0.5 g fresh leaves or roots was homogenized by using a mortar and pestle with 0.1M phosphate buffer (pH 6.5) at 4 °C and stirred for 20 min. The suspension obtained was filtered through one layer of muslin cloth and then centrifuged at 18,000×g for 15 min, 4 °C. The supernatant was used to determine activity of enzymes (Urbanek *et al.*, 1991) as follows:

**I.A.A oxidase (I.A.A.O.) (EC 1.13.11.17) activity:**

1 ml enzyme extract, 2 ml 1 mM aqueous MnCl<sub>2</sub>, 1 ml 1 mM aqueous 2,4-dichlorophenol, 2 ml 1 mM aqueous IAA and 5 ml phosphate buffer (pH 6) were mixed in a 30°C water bath for 30 min. Then 2 ml of this mixture and 4 ml of reaction solution (1.0 ml 0.5M FeCl<sub>3</sub> + 50 ml 35% perchloric acid) were mixed in the dark in a 30°C water bath for 30 min. IAAO absorbance values were recorded at 530 nm. One unit of I.A.A.O. activity was expressed in terms of µg of IAA degraded/mg protein/min. Protein content of the extracts was determined according to Bradford, (1976) using bovine albumin serum (BSA) as a standard.

**Statistical analyses:**

All data were statistically analyzed as randomized complete blocks design (Steel *et al.*, 1997); using the MSTAT-C statistical package (M-STAT, 1990) and the mean values of the two growing seasons were separated by LSD test,  $P \leq 0.05$ .

## **RESULTS AND DISCUSSION**

### **Germination, Seedling biomass and performance:**

Salinity tolerance among three wheat cultivars (cvs) was estimated using different physiological and biochemical parameters during germination and seedling stages. Although 100% (data non-tabled) of the three examined wheat caryopsis were germinated during 48h under all NaCl-salinity levels (8 and 12  $\text{dsm}^{-1}$ ) and control, seedlings showed different growth rate in subsequent periods. Similar results were reported by Khayatnezhad *et al.*, (2010) who found that germination and water uptake were decreased in five wheat cultivars under high concentration of NaCl -salinity. Moreover, Malcolm *et al.*, (2003) reported that, many species, such as wheat and barley, have the ability to germinate at very high salinity (over 300 mM NaCl), but the emerged radical cannot grow further at this level of salinity. Some authors affirm that low salt concentrations can act as primers improving germination and initial growth of many species by preventing membrane damage during imbibitions (Sivritepe *et al.*, 1999).

Table (1) and figure (1) showed that FW, DW, seedling vigor index (SVI), shoot and root length in seedlings of cvs Masr 1 and Sakha 94 were significantly stimulated under 8  $\text{dsm}^{-1}$  NaCl compared with control and the other wheat cv. seds12, therefore wheat considered moderately salt tolerant genus. Accumulation of either FW was continued only in Sakha 94 under 12  $\text{dsm}^{-1}$  NaCl by approximately 43 mg or for shoot length by 2.4 cm in compared to their control. Maximum significant shoot/root ratio (2.2) and Salt tolerance index (STI) (130.7) were recorded in Sakha 94 seedlings under high level of salinity (12  $\text{dsm}^{-1}$ ) compared with control and other cvs under investigation. Root length was more affected than shoots length in wheat seedlings under different NaCl levels, due to direct exposure to saline solution. Moreover, increasing of salt concentrations in the root medium led to inhibition of seedling biomass in cv. Seds 12, therefore this cv. describe as a sensitive wheat cultivar to salinity. Table (1) also demonstrated that, Sakha 94 was more salt tolerant wheat cv. than Seds 12 and Masr 1 due to their high ability to maintain plant water content at high level. In this respect, Zahid *et al.*, (2002) found that with the gradual increase of salinity dry weight of shoot and root, potassium, calcium contents both in shoot and root and leaf area decreased, whereas sodium, chloride and chlorophyll contents increased in all wheat cultivars except chlorophyll contents decreased at 100  $\text{mol m}^{-3}$  salinity in cv. Bao-119. Also, the reduction of dry weight due to increased salinity may be a result of a combination of osmotic and specific ion effects of  $\text{Cl}^{-}$  and  $\text{Na}^{+}$  on enzymes activity (Munns, 2002).

### **Water content, relative water content (RWC) and osmotic potential ( $\Psi_s$ ):**

Leaves water content is one of the important parameters describing water status in plant under stress. High significant levels of water content (81.5 and 79.2 %) were obtained with Sakha 94 seedlings under 8 and 12  $\text{dsm}^{-1}$  NaCl, respectively compared with control and other cvs. (Table 1). The same table showed that, RWC was gradually decreased in both Saka 94 and Seds 12 cvs. with increasing of NaCl concentration in contrary to cv. Masr 1.

Table (1): seedling biomass and water status of three Seedlings wheat cvs. grown under different concentrations of NaCl-salinity (Combined analysis of the growing seasons 2010 and 2011).

Parameters		Seedling biomass		Seedling performance					Water status		
Cultivars	NaCl (ds/m)	weight(g)		Length(cm)		Shoot/root ratio	SVI	STI	Water content %	RWC	$\Psi_s$ (Mpa)
		FW	DW	shoot	root						
Masr 1	0.0	0.159b	0.046bc	8.6d	6.3g	1.37b	1490g	100d	70.1e	91.1d	-1.26e
	8	0.186a	0.053a	10.5b	12.4a	0.84de	2290a	117 b	71.5d	92.5c	-1.43d
	12	0.148cd	0.046bc	6.5f	7.5f	0.87d	1400h	93.1e	68.9f	96.2a	-1.8c
Seds 12	0.0	0.138d	0.038d	8.7d	11.3b	0.77ef	2000b	100d	72.4d	95.3b	-1.33de
	8	0.116f	0.037de	7.6e	8.2e	0.93d	1580f	84.1f	68.1g	70.6g	-1.7c
	12	0.127e	0.044c	4.5g	6.5g	0.69f	1100i	92 e	65.4h	71.5f	-1.8c
Sakha 94	0	0.140d	0.034e	9.5c	9.7c	0.98c	1920d	100d	75.7c	58.5i	-1.2e
	8	0.152bc	0.028f	11.2a	8.7d	1.3b	1990c	108.5c	81.5a	75.4e	-2.3b
	12	0.183a	0.038d	11.9a	5.4h	2.2a	1730e	130.7a	79.2b	68.4h	-2.6a
L.S.D.0.05%		0.008	0.003	0.51	0.31	0.109	22.5	1.5	0.46	0.5	1.5

Seedling vigour index (SVI), Salt tolerance index (STI)

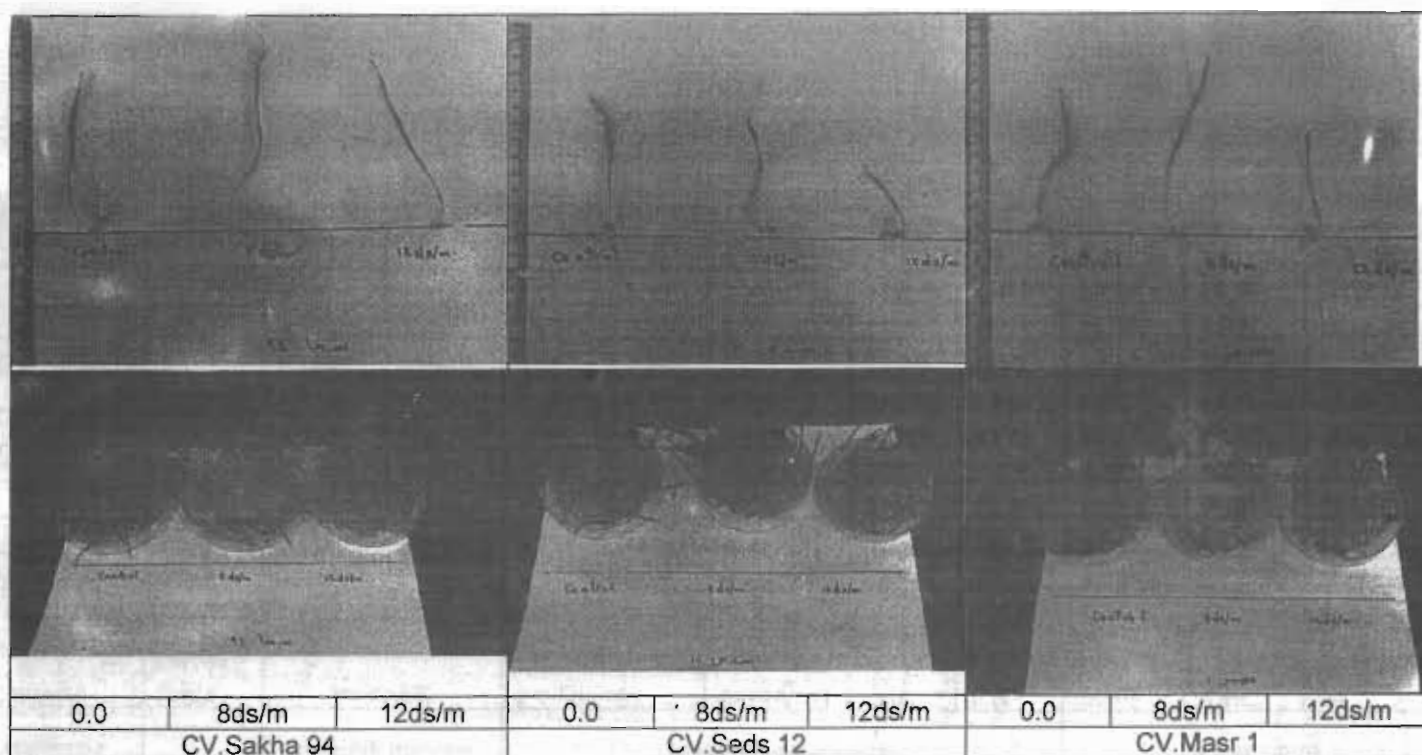


Figure (1): 12-days Seedlings of three wheat cultivars under different NaCl-salinity levels.

The more tolerant cv. (Sakha 94) was recorded the minimum significant value (68.4) in this concern compared with other wheat cvs. under study.

Dissolved solutes in the rooting zone generate a low (more negative) osmotic potential that lowers the soil water potential. The general water balance of plants is thus affected because leaves need to develop even lower water potential to maintain a "downhill" gradient of water potential between the soil and the leaves (Taiz and Zeiger, 2002). Decreasing of osmotic potential of leaves was observed in all three wheat cvs. accompanied with high salinity concentration around root medium. High potential of seedlings cv. Sakha 94 to lower their leaves osmotic potential to -2.6 MPa under high levels of salinity ( $12 \text{ dsm}^{-1}$ ) may be caused by more intensive uptake of inorganic ions and synthesis of high different compatible osmolytes as proline (Table 1). These results were agreed with Flower and Ludlow, (1986) who reported that RWC is considered an alternative measure of plant water status, reflecting the metabolic activity in tissues. Neumann, (1995) found that salinity can rapidly inhibit root growth and hence capacity of water uptake and essential mineral nutrition from soil. Also, high intracellular concentrations of both  $\text{Na}^+$  and  $\text{Cl}_2$  can inhibit the metabolism of dividing and expanding cells.

#### **Osmolytes concentration:**

Table (2) shows that  $\text{Na}^+$  concentration in wheat seedlings was increased insignificantly in the leaves of all three cvs with an increase salinity level. Schubert and L  uchli, (1986) affirmed that, no correlation between  $\text{Na}^+$  exclusion and salt resistance of maize in a short-term experiment (17 d). Similarly, James *et al.*, (2002) showed that,  $\text{Na}^+$  became potentially toxic only when concentration exceeded  $1.25 \text{ mmol g}^{-1} \text{ DW}$  in durum wheat. Berkowitz, (1998) added that high soil solution sodium impairs cell metabolism and photosynthesis by imposing an osmotic stress on cell water relations and by toxicity of sodium in the cytosol.

The same table showed that,  $\text{K}^+$  concentration was increased by 1-2.5 times in all three cvs. wheat with salt increment. Seedlings of cv. Sakha 94 was significantly recorded the maximum values of  $\text{K}^+$  content ( $216.7\text{-}213.3 \mu \text{ equiv.}$ ) and  $\text{K}^+/\text{Na}^+$  ( $5.5\text{-}5.6$ ) under 8 and  $12 \text{ dsm}^{-1}$ , respectively compared with other examined wheat cvs. According to Chhipa and Lal, (1995) potential of seedlings to maintain high ratio  $\text{K}^+/\text{Na}^+$  in their tissue rather than  $\text{Na}^+$  alone is important parameter to estimate wheat salt tolerance. In the same line, Bavei *et al.*, (2011) found that, clear decline in  $\text{K}^+$  and  $\text{Ca}^{2+}$  concentrations and increase in  $\text{Na}^+$  and proline contents were observed in the root and leaf tissues at each NaCl concentration in sensitive sorghum varieties during the NaCl treatment.

On the other hand, osmoprotectants as reducing sugars and proline concentrations were also increased in both leaves and roots of all three wheat cvs under study with high levels of NaCl (Table 2). Seedlings of cv. Sakha 94 was more efficient in biosynthesis of reducing sugars ( $1.8$  and  $0.73 \text{ mg/g FW}$ ) and ( $56.7 - 20.3 \mu \text{mol/g FW}$ ) for proline concentration in leaves and roots respectively, under  $12 \text{ dsm}^{-1}$  NaCl compared with control and other cvs. Increasing of reducing sugars concentration in the leaves may occur due to decrease of translocation of photoassimilates from leaves (source) to

(roots) sinks while root has high amount of reducing sugars at seedling stage could result by continues supplement of degraded carbohydrates from grains during germination (Taiz and Zeiger, 2002). Data in the present investigation showed also that, proline was accumulated by 2-6 times in leaves or roots of wheat seedlings grown under salinity stress. Similar results were found by Aloni and Rosenshtein, (1984) who reported that, proline play an important role as osmoregulator under drought and salinity conditions, proteins stabilizer, prevention of heat denaturation of enzymes and conservation of nitrogen and energy for a post-stress period. Goudarzi and Pakniyat, (2009) found that proline, protein contents and peroxidase activity were increased in most tolerant cultivars of wheat grown under 6.8 and 13.8  $\text{dsm}^{-1}$  NaCl. Also, salinity induced an accumulation of proline in the wheat seedlings to a level twice that in the control with 2% NaCl and three times higher with 4% NaCl (Shakirova *et al.*, 2003). These results were non-coordinated with Colmer *et al.*, (1995) who did not find any significant role of proline in salt tolerance in a wheat x *Lophopyrum elongatum* amphiploid.

**Table (2): Effect of NaCl-salinity on osmolytes accumulation in 12-days seedlings of wheat cvs (Combined analysis of the two growing seasons 2010 and 2011)**

Parameters	NaCl (ds/m)	Reducing sugars mg/g FW		Proline $\mu\text{mol/g}$ FW		Leaves ions $\mu\text{equiv./g}$ FW		
		leaves	Root	leaves	Root	$\text{Na}^+$	$\text{K}^+$	$\text{K}^+/\text{Na}^+$
Masr 1	0.0	1.43ab	0.47cd	11.7c	3.7de	38.5a	85.2d	2.2d
	8	1.47ab	0.5bcd	30.1b	6.7cde	37.3a	155.2b	4.2a
	12	1.47ab	0.63abc	30.3b	10.7 cd	39.6a	153.6b	3.9b
Seds 12	0.0	1.5ab	0.37d	11.5c	3.2e	33.5a	91.7d	2.8c
	8	1.5ab	0.73a	18.3c	12.2bc	40.3a	126.7c	3.1c
	12	1.2b	0.73a	18.3c	12.3bc	41.1a	125.3c	3.1c
Sakha 94	0	1.2b	0.47cd	11.8c	3.1e	31.7a	88.4d	2.8c
	8	1.6ab	0.7ab	50.2a	17.7ab	39.7a	216.7a	5.5a
	12	1.8a	0.73a	56.7a	20.3a	38.2a	213.3a	5.6a
L.S.D.0.05%		0.35	0.19	8.5	6.1	ns	14.3	0.31

**Content of chlorophyll, carotenoids, chlorophyll stability index (CSI) and genotypic variation coefficient (GVI):**

Data presented in Table (3) showed that, concentrations of Chl.a, Chl.a+b, Chl.a/b and chlorophyll stability index (CSI) were decreased gradually in all wheat cvs. with increasing of NaCl content. Seedlings of cv.Sakha 94 showed the lowest significant reduction in chl.a especially under 12  $\text{dsm}^{-1}$  concentration. This reduction in chl.a was determined by approximately 37%, 137 and 146% in leaves of Sakha 94, Masr 1 and Seds 12, respectively compared with their control. Furthermore, seedling leaves of cv. Sakha 94 recorded 2-folded content of Chl.a+b (2.8 mg /gFW) and CSI (72.9) under 12  $\text{dsm}^{-1}$  NaCl compared with other wheat cvs. under investigation. However, maintain of stationary in Chl.a / b ratio was noticed in Sakha 94 under all NaCl levels, while this ratio was reduced in the other

wheat cvs. The same table also showed that concentration of Chl.b was stimulated under low NaCl level (8  $\text{dsm}^{-1}$ ), and then decreased under high salinity one (12  $\text{dsm}^{-1}$ ) in all three investigated cvs. According to Taiz and Zeiger, (2002), the most visual symptoms of salinity were yellowing of leaf tips and margin due to degradation of chlorophyll and biosynthesis of carotenoids.

**Table (3): Effect of NaCl salinity on chlorophyll and carotenoids concentration in leaves of three wheat cvs Seedlings after 12 days from germination (Combined analysis of the two growing seasons 2010 and 2011)**

Cultivars	NaCl (ds/m)	mg/g FW					CSI	GVI
		Chl.a	Chl.b	Chl.a+b	Chl.a/b	carotenoids		
Masr 1	0.0	2.03ab	1.4c	3.37cd	1.4a	0.3e	100b	1.41a
	8	1.6bc	1.8a	3.3cd	0.77b	0.4de	32.6g	1.07b
	12	0.66d	0.87e	1.6f	0.5c	1.6a	45.5e	0.56de
Seds 12	0.0	2.03ab	1.4c	3.5c	1.5a	0.7c	100b	0.64d
	8	1.3c	1.8a	3.1d	0.77b	1.3b	87.5c	0.42e
	12	0.57d	0.77e	1.4f	0.53bc	1.3b	40f	0.73cd
Sakha 94	0	2.07a	1.6b	3.8b	1.4a	0.4de	100b	0.73cd
	8	1.97ab	1.7ab	4.03a	1.3a	0.4de	107.1a	0.86c
	12	1.7abc	1.2d	2.8e	1.4a	0.53d	72.9d	1.2b
L.S.D.0.05%		0.41	0.16	0.25	0.23	0.15	4.3	0.19

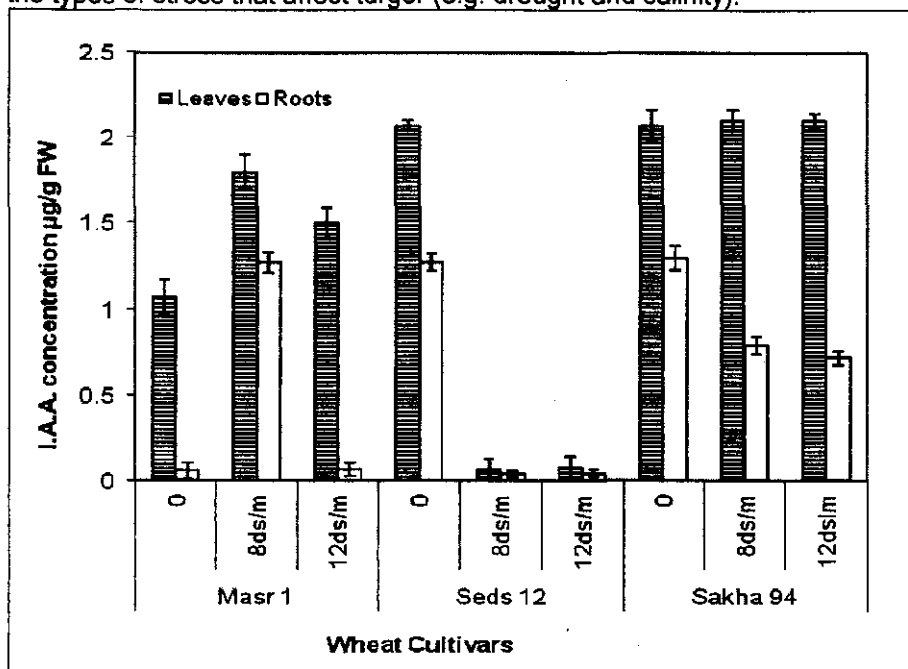
(CSI) chlorophyll stability index, (GVI) genotypic variation coefficient

Results in the present investigation, demonstrated that concentration of carotenoids was increased with different rates according to wheat cv. Seedlings of cv. Masr 1 was more efficient in carotenoids biosynthesis (1.6 mg/g FW) compared with other cvs. Genotypic variation coefficient (GVI) demonstrated the relation of photosynthesis products (reducing sugars) and the photosynthetic pigments (Chl.a+b and carotenoids). The maximum GVI was recorded in seedlings of cv.Sakha 94 (1.2) compared with 0.56 and 0.73 in seedlings of cvs. Masr 1 and seds 12, respectively. These findings agreed with Sabater and Rodriguez, (1978), who found that, Chlorophyll a and b content decreased in response to water stress. The decrease may be due to the formation of proteolytic enzymes such chlorophyllase, which is responsible for chlorophyll degradation. Also, Zahid *et al.*, (2002), found that Photosynthesis was increased in all wheat cultivars at 100  $\text{mol/m}^3$  salinity then decreased at 200  $\text{mol/m}^3$  salinity.

#### **Indole acetic acid (I.A.A.) and the activity of indole acetic acid oxidase(IAAO):**

Data represented in Figure (2) showed that concentration of indole acetic acid (I.A.A.) was significantly increased in leaves (2.1 and 1.8  $\mu\text{g/g}$  FW) in both Sakha 94 and Masr 1 cvs. under low NaCl level (8  $\text{dsm}^{-1}$ ) compared with the more sensitive cv. Seds 12 (0.07  $\mu\text{g/g}$  FW). Furthermore, Seedlings of both Sakha 94 and Masr 1 maintained the concentration of I.A.A. at high level in their leaves to 2.1 and 1.5  $\mu\text{g/g}$  FW under 12  $\text{dsm}^{-1}$  NaCl. According to Mansfield and Mc-Ainsh, (1995), I.A.A. eliminating osmotic inhibition; enhancing water uptake, stomatal opening and water

movement in roots and is thought to act with abscisic acid in responding to the types of stress that affect turgor (e.g. drought and salinity).



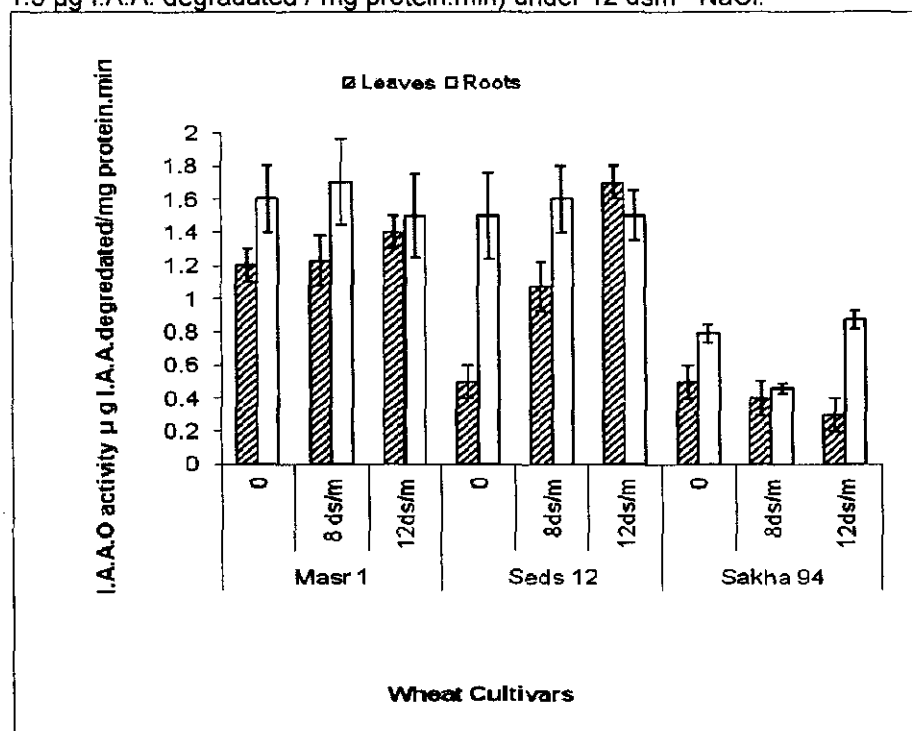
**Figure (2): Effect of different levels of NaCl on I.A.A. concentrations in leaves and roots of three wheat cvs. after 12 days from germination. (means $\pm$ SE, n = 4).  $P \leq 0.05$  as determined by Duncan's test (Combined analysis of the two growing seasons 2010 and 2011) .**

Polar transportation of I.A.A. from leaves to roots was cleared in seedling of all three wheat cvs. under control and low NaCl concentration (8  $\text{dsm}^{-1}$ ). Our results were similar to that of Wolters and Jurgens, (2009) who found that during development of root system, the establishment of an auxin gradient with its maximum at the tip is essential for proper lateral root primordia development. Also, Wang *et al.*, (2009) demonstrate that auxin transport activities are required for remodeling lateral root formation and elongation and for adaptive root system development under salt stress. Derra, (1973) added that, auxins like indole butyric acid and indole acetic acid promote germination and seedling growth of wheat better than kinetin by eliminating osmotic effect of salinity.

Maximum significant values of I.A.A. concentrations were recorded in roots of cv. Masr 1 (1.27  $\mu\text{g/g FW}$ ) under 8  $\text{dsm}^{-1}$  NaCl compared with other wheat cvs. Moreover, roots of cv. Sakha 94 showed the minimum degradation levels of I.A.A. (0.79  $\mu\text{g/g FW}$ ) under 12  $\text{dsm}^{-1}$  NaCl which contributed with the most root length (Table 1). This finding agreed with Munns, (2002) who reported that the osmotic stress of the salt outside the

roots reduces the rate of formation of new leaves and the rate of tiller production. This response is probably under the control of hormonal signals from roots. Furthermore, incubation of wheat seedlings cv. Saratovskaya 29 on the medium containing 2% NaCl resulted in a transitory accumulation of ABA, a progressive decline in cytokinins and a decrease in the level of IAA (Shakirova *et al.*, 2003). In contrary, Naqvi, (1994) showed that, salinity stress reduces the recovery of the free or diffusible IAA from maize (*Zea mays*) coleoptile tips due to enhanced their conjugation or oxidation. The regulation of IAA concentration in the plant tissues may be due to its oxidation caused by indole acetic acid oxidase to non effective compound as reported by Taiz and Zeiger, (2002).

Regarding the effects of salinity on the activity of indole acetic acid oxidase (I.A.A.O.) Figure (3), shows that activity of I.A.A.O. was higher in both leaves and roots in all three wheat cvs. with increasing of NaCl concentration especially under 12 dsm<sup>-1</sup> NaCl. The maximum values of I.A.A.O. activity was recorded at the more salt sensitive cv. Seds 12 (1.7 and 1.5 µg I.A.A. degraded / mg protein.min) under 12 dsm<sup>-1</sup> NaCl.



**Figure (3): Effect of different levels of NaCl on I.A.A.O activity in leaves and roots of three wheat cvs. after 12 days from germination. (means±SE, n = 4). P<0.05 as determined by Duncan's test (Combined analysis of the two growing seasons 2010 and 2011).**

The activity of I.A.A.O. was higher by 1.5- 5 times in roots and leaves of cv. Seds 12 under  $12 \text{ dsm}^{-1}$  NaCl compared with the most salinity tolerant cv. Sakha 94, which recorded the minimum significant values of I.A.A.O. activity ( $0.3$  and  $0.87 \mu\text{g I.A.A. degraded / mg protein.min}$ ) in leaves and roots, respectively under the same level of NaCl. These results were agreed with Shukla and Bajjal, (1977) who found that, exposure for longer time periods to Na-salt conditions caused a sharp enhancement in the IAA-oxidase activity in different wheat cultivars. Meneguzzo *et al.*, (1999) added that, the activity of antioxidant enzymes were increased in wheat shoot under salinity conditions. Also, Tuna *et al.*, (2008) found that,  $10 \text{ dsm}^{-1}$  NaCl-salinity reduced the total dry matter, chlorophyll content, relative water content (RWC), but increased proline accumulation, superoxide dismutase, peroxidase and polyphenol oxidase enzyme activities and electrolyte leakage in maize cv. DK 647 F1. Unlike these results of Naqvi, (1994) did not find any change for endogenous enzyme activity in vitro in the coleoptiles (leaf structures) of salinity-stressed maize seedlings.

**Conclusion:**

Germination % of wheat grains cultivars was not influenced by 8 or  $12 \text{ dsm}^{-1}$  NaCl salinity but reduction in seedlings biomass was observed. Seedlings of cv. Sakha 94 appeared more tolerant to high saline concentration, subsequent by Masr 1 and seds 12. Sakha 94 recorded the maximum values in most vegetative parameters and photosynthetic pigments. Relative water content and osmotic potential were decreased to very low levels accompanied with higher accumulations of  $\text{K}^+$ ,  $\text{K}^+/\text{Na}^+$  ratio, proline and reducing sugars in the leaves of the most tolerant cultivar. Activity of I.A.A. oxidase increased by 1.5-5 folds in leaves and roots of the sensitive wheat cv. (Seds 12) to NaCl salinity accompanied with decreasing of auxine levels (plant growth activator) in seedlings contrary to more tolerant one (cv. Sakha 94). It can be recommended that cv. Sakha 94 was more suitable to cultivate in saline soil. Physiological indicators could be used in short term experiment of early selection for salt tolerant in wheat cultivars.

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### **دراسات فسيولوجية مقارنة على ثلاثة اصناف من القمح تحت ظروف ملوحة كلوريد الصوديوم في مرحلتى الانبات وتكوين البادرة**

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**قسم النبات - كلية الزراعة - جامعة قناة السويس - ٤١٥٢٢ - الاسماعيلية - مصر**

تم دراسة تأثير ملوحة كلوريد الصوديوم (صفر - ٨ - ١٢ ديسى سيمنز/متر) على الانبات وتكوين البادرة وحالة الماء وكمية الكلوروفيل والبرولين والسكريات المختزلة والصوديوم والبوتاسيوم واندول حامض الخليك ونشاط انزيم اكسدة اندول حامض الخليك فى ثلاثة اصناف قمح هى سخا ٩٤ ومصر ١ و سنس ١٢. اوضحت النتائج انه رغم ان نسبة انبات حبوب الاصناف الثلاثة وصلت ١٠٠% خلال ٤٨ ساعة تحت المستويات المختلفة من الملوحة، الا ان معدل نمو البادرات كان متباينا بين الاصناف الثلاثة. اظهر الصنف سخا ٩٤ قدرة عالية على تحمل الملوحة، يليه الصنف مصر ١ ، يليه الصنف سنس ١٢. فقد سجل الصنف سخا ٩٤ اعلى القيم المعنوية لكلا من الصفات الخضرية وصيغات البناء الضوئى بينما اظهر اقل القيم فى المحتوى النسبى للماء والجهد الاسموزى والذى ارتبط بتراكم كمات عالية من البوتاسيوم ونسبة البوتاسيوم/صوديوم والبرولين والسكريات المختزلة مقارنة بالاصناف الاخرى. من ناحية اخرى، فقد لوحظ ارتفاع نشاط انزيم اكسدة اندول حامض الخليك بمقدار من ١.٥ - ٥ مرات أضعاف ما قدر فى الصنف سنس ١٢ الحساس ، وقد يرجع إنخفاض تركيز الاوكسين فى البادرات الى ارتفاع نشاط الانزيم المؤكسد مما كان له اثر واضح على النمو بصفة عامة. ويمكن التوصية بزراعة الصنف سخا ٩٤ فى الاراضى المتأثرة بالملوحة كما يمكن الاعتماد على الصفات الفسيولوجية فى انتخاب التراكيب الوراثية للقمح المقاوم للملوحة.

### **قام بتحكيم البحث**

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