# THE EFFECT OF SALINITY ON LIBYAN SOFT WHEAT TRITICUM AESTIVUM L AT GERMINATION STAGE

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## **Abstract**

Salinity is the major abiotic stress that reduces plant growth and productivity worldwide. The objectives of this study were to quantify the effects of salinity on seedling characters of soft wheat Triticum aestivum L genotypes and to define if responses varied among wheat genotypes. For that, 12 genotypes of wheat were evaluated for the salinity tolerance in artificially induced with NaCl at their germination and early seedling stage. Seeds were subjected to four levels of salinity (0, 50, 100 and 150 mM NaCl) and raised for eight days under optimum conditions to calculate the final germination percentage, speed of germination, mean daily germination, shoot and root length, and seedling fresh and dry weight. The response of genotypes were divers among the studied traits. At higher concentration of NaCl (100 and 150mM), speed of germination and mean daily germination were delayed and final germination percentage was decreased. At higher salinity level, significant decrease in shoots and roots length, seedling dry weight and seedling vigor. Additionally, the study showed considerable variation in salinity tolerance among wheat genotypes for studied traits. The study concluded that there is a genetic variability among genotypes and that genotypes varied in their response to salinity stress and that genotypes Sabha, Salambo, Makkawi and Bushi were the most tolerant genotypes.

**Keywords:** Triticum aestivum L, germination, salinity tolerance, seedling vigor.

# Introduction

Agricultural productivity is cruelly affected by soil salinity. Soil and water salinity are caused by the presence of excessive amounts of salts. At least 20 percent of the world's irrigated land is salt affected and/or irrigated with saline waters (Qadir et al., 2008, Shrivastava and Kumar, 2015). In addition, additional of about two million of cropping land are affected by salinity every year (Rengasamy, 2006; Tuteja, 2007, Jamil et al., 2011, Munns and Gilliham, 2015). Because of salinization increases of agriculture land, it is projected that about 50% loss of cropping acreage by the middle of the 21st century (Wang et al, 2007, Patel et al., 2011, Shahbaz and Ashraf, 2013). In arid and semi-arid regions, high temperatures during the summer season causes severe evaporation losses, hence leaving behind large amounts of salts. In Libya, specifically in the North close to the coast of the Mediterranean Sea, the problem of salinity in agriculture becomes more and more severe because of too much irrigation water is

applied for crop production. In addition, bad drainage systems and hot weather increase evaporation which tends to create salinity. Soil salinity causes great losses to agriculture by lowering the yields of various crops.

Wheat is moderately tolerant to salinity stress (Shannon, 1997). However, salt stress effects plant as it reduces water potential, cause ions imbalance, disturbances in ion homeostasis, and toxicity (Maas, and Grattan. 1999; Munns, 2002; Sairam, 2002). This results in changing the water status, which leads to initial growth reduction and limitation of plant productivity due to reduced cell growth and leaf area (Acevedo et al., 2002). Under saline condition, relative water content, leaf water potential, water uptake, and water use efficiency decreased (Nishida et al., 2009). Plant salinity tolerance is the inherent ability of the plant species to tolerate the effects of high salts without a significant adverse effect on the plant (Munns et al., 2006). Some plants have developed different adaptation mechanisms, which include the accumulation of osmo-protectants, exclusion of sodium and chloride, tissue tolerance to accumulated sodium and chloride (Rathinasabapathy, 2000; Zhang et al., 2001; Munns and Tester, 2008; Ashraf et al., 2010).

Crops have different sensitivity to salinity at different growth stages. Many studies showed that plants are tolerant during germination stage and become more sensitive to salinity during the seedling stage (Kingsbury et al., 1986; Maas et al., 1999, Giuffrida e al., 2016). Seed germination is a factor which contributes to yield of the plants. Among the abiotic factors, salinity is considered an important problem for wheat germination, because it influences the rate of water up take by seeds which is essential for plant growth and development. Many studies point out that crops are more sensitive to salinity during the seedling stage than during germination, and this depends on the crop ((Maas et al., 1999). A study by Maas and his team on corn and wheat reported that the dry matter yields of these plants are reduced as the concentrations of salt increased (Maas et al. 1983). Studies showed that there is a linear decrease in yield components as the level of salinity increased. However, these findings recommended that seedling emergence and early seedling growth stages are most sensitive to salinity (Zeng and Shannon, 2000). Salinity levels in soil and/or irrigation water have variable effects on different plant species. Plant species and genotypes within species show differential responses to salinity stress (Djanaguiraman and Prasad, 2013; Namvar et al., 2018). To our knowledge, screening Libyan soft wheat germplasms to salinity stress and understanding the genetic variability for seedling character was not studied in detail. Therefore, the objective of this study is to evaluate Libyan soft wheat genotypes for salinity tolerance at the germination stages and to determine seedling growth traits associated with salinity tolerance.

#### **Materials and Methods**

This study was conducted in controlled environment facilities at the Plant Science Department, University of Az Zawia, Libya. Experiments were conducted in fall of 2018 to

determine the impact of salinity stress on germination and seedling characteristic of soft wheat genotypes.

## **Plant Material**

12 genotypes of Libyan soft wheat were used in this study and these genotypes were obtained from Libyan National GenBank in Tajoura. These genotypes include some very old local genotypes that include: (Fritcea, Makkawi, Bushi, Solala 22); old improved genotypes include: (Sidi El-Masry, El-Mukhtar, Bohot 208, Germa, Sabha); New improved genotypes include: (Abu Al-Joud, Abu Al-Khair); and imported genotype include (Salambo).

## **Experimental Details and Growth Conditions**

The experiments were conducted during 2018 at Plant Science Department, University of Zawia, Libya. Four different concentrations of saline solution prepared with tap water (0, 50, 100 and 150 mM NaCl with electric conductivity [EC] value of < 0.7, 4.5, 8.3 and 15.5 dSm<sup>-1</sup>) were used for salinity treatments and tap water used as control solution (0 mM NaCl). Healthy seeds of each genotype were surface sterilized with sodium hypochlorite solution (5 %) for three minutes, washed with distilled water, air dried and used for the experiment. A set of 20 seeds were placed in a petri dish with Whatman no. 1 filter paper disc; was moisturized 5 mL of the different saline solutions (0, 50, 100 and 150 mM NaCl). The filter paper was moisturized on daily basis till the end of experiment and filter papers were changed once in every two days to prevent salt accumulation due to evaporation. The seeds moisturized with tape water instead of NaCl solution saved as absolute control treatment for the experiment. A total of four replications used for control and NaCl treatments. All the petri dishes were placed in the dark throughout the germination period (total 8 days) at room temperature 24±2°C. Seeds were considered germinated (Feekes 0.9) when both shoot, and root extended more than 2 mm from the seed (Islam et al., 2012). The following traits were recorded during germination period and 8 days after sowing.

#### **Germination Traits**

Germination per cent (G %) was expressed according to Nasri et al., 2011. The following formula was used to calculate G%:

$$G\% = (NSG \div TNSS) \times 100$$

Where NSG is the number of seeds germinated at the end of the experiment (8 day after sowing). TNSS is the total number of seeds sown.

Germination index (GI %) in each NaCl treatment was calculated according to the equation given by Karim et al. (1992). The following formula was used to calculate GI %:

$$GI\% = (\% GNaCl \div \% GC) \times 100$$

Where % GNaCl is per cent germination at different NaCl treatments. % GC is per cent germination in control treatment.

The germination rate (GR) was calculated according to the equation given by Rubio-Casal, (2003). The count of germinated seeds was recorded at 24 hours interval from sowing till the end of experiment (8 day after sowing) and used to calculate GR. The following formula was used to calculate GR:

$$GR = (n1t1) + (n2t2) + \cdots + (nxtx) \div TNGS$$

Where  $n_I$  is the number of seeds germinated on the first day of germination,  $t_I$  is the number of days taken for first germination and TNGS is the total number of seeds germinated.

Mean daily germination (MDG) was calculated as per Gairola et al. (2011). The following formula was used to calculate MDG:

$$MDG = TNGS \div TNDG$$

Where *TNGS* is total number of germinated seeds and *TNDG* is total number of days taken for final germination.

## **Early Seedling Traits**

Morphological traits *viz.*, shoot and root length and dry weight were subsequently measured from 5 uniform seedlings from each replication at early seedling stage (Feekes 1). Selected seedlings were dissected and shoot, and root length were recorded. The length from the seed to the tip of the root and leaf blade was calculated to measure the root length and shoot length, respectively. The fresh weight of shoot and root was recorded using a weighing balance, and then dried in an oven maintained at 40 °C till it attains stable weight. After that, shoot and root dry weights were recorded. Using the morphological traits, the salinity tolerance index (STI) and Seedling vigor index (SVI) were calculated.

The following formula was used to calculate STI (Tsegay et al., 2014):

$$STI = (SdwNaCl \div SdwC) \times 100$$

Where *SdwNaCl* is dry weight of seedling from NaCl treatment. *SdwC* is dry weight of seedling from control treatment.

The following formula was used to calculate SVI (Abdoli1 et al. 2013):

$$SVI = (SL \times G\%)100$$

Where SL is seedling length and G% is germination per cent.

# **Statistical Analysis**

The experimental design was a randomized complete block design (RCBD) with four replications. Analysis of variance (ANOVA) performed on genotypes using generalized linear model (GLM) procedure in SAS 9.4 (SAS Institute Inc., Cary, NC, USA) for seed germination and related traits. Separation of means was carried out using the least significant differences (LSD; P < 0.05). The means were compared using Ducan's multiple range test.

## **Results**

Significant differences were found among the genotypes for all the traits measured. Analysis of variance results showed that salinity had significant effect (P < 0.05) on germination %, germination index, mean daily germination, speed of germination and seedling vigor index. Whereas salinity and genotype interactions were significant (P < 0.05) for almost all germination traits except for on germination %, germination index Table (1). Regarding seedling traits, the result showed that salinity treatments, genotype and their interaction had significant effect (P < 0.05) on all studied seedling traits excluding seedling fresh weight Tables (1) and (2).

#### **Germination Parameters**

Most of germination traits of wheat genotypes was significantly affected by the salinity stress (p<0.05) Tables (1) and (2). Germination per cent was reduced from 100 mM NaCl salt concentration onwards for almost all the genotypes. Generally increasing level of salinity stress caused decrease in germination per cent over control. Still, in some genotypes, the germination per cent was not affected at 50 mM NaCl but decreased by 18 % at 150 mM NaCl over the control (0 mM NaCl; Fig. 1a). Salinity stress significantly decreased mean daily germination (MDG) in all genotypes. However, in some genotypes, moderate salinity decreased mean daily germination by lesser extent and severe stress decreased to a greater extent.

Table (1): Probability Values of Effects of Salinity (S), Genotype (G) And Salinity X Genotype Interaction on Germination and Early Seedling Traits at Germination Stage (Feekes 0.9)

	Variables			
Traits	Salinity (S)	Genotype (G)	SxG	
Germination per cent (%)	<.0001	0.0416	0.8613	
Germination index (GI; %)	<.0001	0.0291	0.7540	
Mean daily germination (MDG)	<.0001	0.0003	0.0447	
Speed of germination (SG)	<.0001	<.0001	<.0001	
Shoot length (cm)	<.0001	<.0001	0.0392	

Root length (cm)	<.0001	00148	0.0256
Seedling length (cm)	<.0001	<.0001	0.0051
Seedling fresh weight (g)	<.0001	0.0018	0.0905
Seedling dry weight (g)	<.0001	0.0001	0.0164
Salt tolerance index (STI)	<.0001	<.0001	0.0036
Seedling vigor index (SVI)	<.0001	<.0001	0.0578

Table (2) Effect of Salinity Stress on Studied Traits Under Different Salinity Levels in Soft Wheat at Germination Stage (Feekes 0.9).

Traits	NaCl levels (mM)			
	0	50	100	150
Germination percent (%)	99a*	97ª	91 <sup>b</sup>	83°
Germination index (%)	100 <sup>a</sup>	98 <sup>b</sup>	85°	85°
Mean daily germination	6.3ª	5.7 <sup>b</sup>	4.3°	2.8 <sup>d</sup>
Germination Speed	14ª	13 <sup>b</sup>	8°	6 <sup>d</sup>
Shoot length (cm)	8 <sup>a</sup>	7 <sup>b</sup>	6°	4 <sup>d</sup>
Root length (cm)	9ª	8 <sup>b</sup>	5°	3 <sup>d</sup>
Seedling length (cm)	17ª	15 <sup>b</sup>	10°	7 <sup>d</sup>
Seedling fresh weight (g)	1.5ª	1.4 <sup>b</sup>	1.3°	1.2 <sup>d</sup>
Seedling dry weight (g)	0.59 <sup>a</sup>	0.48 <sup>b</sup>	0.32°	0.16 <sup>d</sup>
Salt tolerance index	100a	88 <sup>b</sup>	62°	32 <sup>d</sup>
Seedling vigor index	16 <sup>a</sup>	15 <sup>b</sup>	10°	6 <sup>d</sup>

<sup>\*</sup> Individual value is the mean of 12 genotypes under different salinity levels. Values followed by different letters are significantly different according to Duncan's multiple range test (P < 0.05).

The results of salinity stress were almost prominent from 150mM salt concentration for most wheat genotypes resulting into reduction in mean daily germination (MDG). In some genotypes the per cent reduction over the control reached 65% in Sidi Elmassry genotype Figure (1b).

Speed of germination of wheat genotypes were significantly affected because of salinity stress from 50 mM NaCl concentration onwards.

There was substantial reduction in the speed of germination for almost all the genotypes. however, the reduction was higher for few wheat genotypes in case of 100 mM and 150 mM

NaCl concentration. At 150 mM NaCl concentration, the percent reduction over the control in Abu Al-Joud genotype was 65%, where as in more tolerant genotypes the per cent reduction over the control was 46% Figure (1c). Also, the results displayed small reduction in germination index (GI) at moderate and high concentrations with the highest decrease at 150 mM NaCl. Figure (2a). Results regarding salt tolerance (ST) of different soft wheat genotypes showed that genotypes MAKKAWI, SALAMBO and Sabha were tolerant to salinity stress at germination stage, whereas genotypes ABU AL-KHAIR, GERMA and SOLALA 22 were sensitive to salinity stress Figure (2b). High level of salinity (100 to 150 mM NaCl increasingly decreased seedling vigor index. Low salinity level (50 mM NaCl) showed small reduction on seedling vigor index. Figure (2c) shows that the highest seedling vigor index was observed in control, while salinity at 150 mM NaCl decreased significantly seedling vigor index by (70%). Speed of germination of wheat genotypes were significantly affected because of salinity stress from 50 mM NaCl concentration onwards.

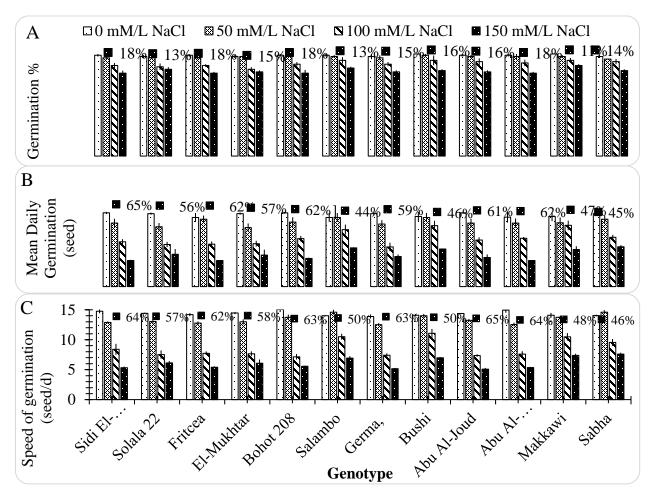


Figure (1): The Effects of Different Salinity Levels (0, 50, 100, 150 Mm Nacl) on (A) Germination Percentage, (B) Mean Daily Germination and (C) Germination Speed of Twelve Soft Wheat Genotypes. Percent Decline of each Trait Due to High Level of Salinity (150 Mm Nacl) as Compared to Control is Indicated. Vertical Lines on Top of Bars Indicate Standard Error of Means (n = 4).

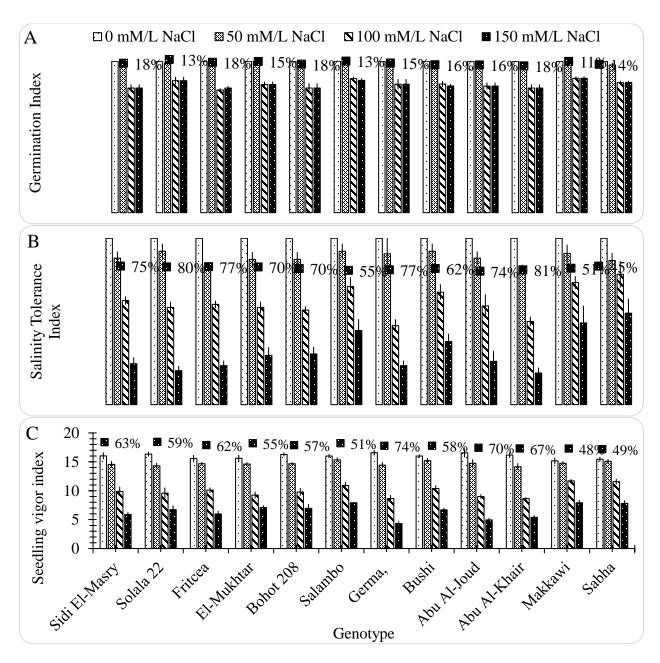


Figure (2): The Effects of Different Salinity Levels (0, 50, 100, 150 Mm Nacl) On (A) Germination Index, (B) Salinity Tolerance Index and (C) Seedling Vigor Index of Twelve Soft Wheat Genotypes. Percent Decline of Each Trait Due to High Level of Salinity (150 Mm Nacl) as Compared to Control is Indicated. Vertical Lines on Top of Bars Indicate Standard Error of Means (n = 4).

# **Seedling Parameters**

Most of seedling parameters decreased with increasing salinity level Tables (1) and (2). Under non-saline conditions genotypes showed no substantial differences in terms of shoot length. However, under both levels 100mM and 150 M of NaCl condition there were significant

differences in response of genotypes to salinity levels Table (2). In addition, salinity caused a significant (p<0.05) reduction on root length and seedling length at the higher NaCl concentration (100, 150 mM NaCl) .Increase in the salinity from 0 to 50mM NaCl had small effect on root and seedling length , though more increase from 100mM NaCl onwards significantly reduced the root length and seedlings length Figure (3a) and (3b).

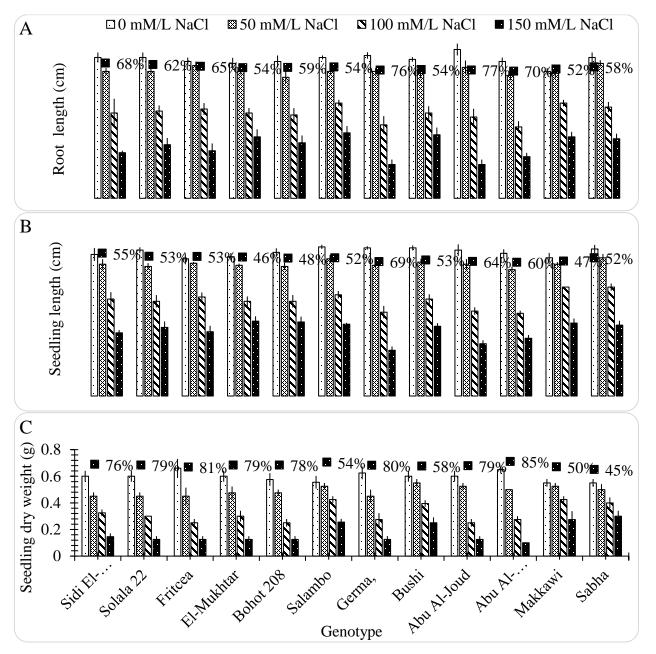


Figure (3): The Effects of Different Salinity Levels (0, 50, 100, 150 Mm Nacl) on (A) Root Length (Cm), (B) Seedling Length and (C) Seedling Dry Weight of Twelve Soft Wheat Genotypes. Percent Decline of Each Trait Due to High Level of Salinity (150 Mm Nacl) As Compared to Control Is Indicated. Vertical Lines on Top of Bars Indicate Standard Error of Means (n = 4).

However, root length was more affected by salinity stress than shoot length. The result indicated that some genotypes were more tolerance to salinity in root growth as compared with other genotypes. For example, genotypes GERMA, ABU AL-JOUD and ABU AL-KHAIR showed a percent reduction of above 70 %, whereas genotypes MAKKAW, SALAMBO and SABHA showed a percent reduction of 52%,54%, 58% respectively Figure (3a). The seedling dry weight displayed a significant reduction in response to the increasing levels of salinity (p<0.05). under effect of 150 mM NaCl, the seedling dry weight in some genotypes decreased by 85% compared to those of the control Figure (3c) and Table (2).

The genotypes were ranked based on the seedling vigor index, such that those with the smallest and largest percent reduction over the control were ranked respectively as the most and least tolerant genotypes at 150 mM NaCl. Based on that, genotypes were separated into two categories. (1) tolerant to salinity at germination stage (MAKKAWI, SABHA, BUSHI AND SALAMBO), (2) susceptible to salinity at germination stage (SIDI EL-MASRY, SOLALA 22, FRITCEA, EL-MUKHTAR, BOHOT- 208, ABU AL-JOUD and ABU AL-KHAIR).

## **Discussion**

The environmental condition in the North of Libya is characterized by soil and water salinity resulting from shortage of rain which influences negative growth and productivity of crop plants. To avoid stress environments, plants have evolved complex mechanisms to counter NaCl toxicity and low water potential in growth condition caused by salinity (Munns and Tester, 2008). Salinity affects early seedling growth of plants by changing water relations due to salt gathering in intercellular spaces (Zhang et al., 2006). From the results of this study it can be concluded that seeds of different wheat genotypes were susceptible to high concentrations of salt solutions at germination stage which was agreed with the works of (Ungar et al., 1996; Gul et al., 1999). This study indicated that during the germination phase the seeds were sensitive to salinity stress. This finding agreed with many studies on the effect of salinity stress on seed germination under saline condition (Ashraf et al., 2010; Faroog et al., 2011). The reduction of seed germination may be due to (1) damage of viability at higher salinity level (2) delaying germination of seeds at salinities that cause some stress to but not percent germination (3) due to salinity induced high oxidative stress as stated in other studies (Gulzar et al., 2001; Amor et al., 2005). This study found that the effect of salinity stress was finally inhibitory at 150 mM NaCl concentrations for almost all wheat genotypes except SALAMBO, MAKKAWI, SABHA and BSHI. In this study the higher concentration of salinity has a more distinct effect on root length as compared with shoot length maybe since roots are directly exposed to salinity solution. Similar tendency was detected by other studies ((Jamil and Rha, 2004). The reduction in root and seedling growth may be due to the toxic effects of the increased level of NaCl concentration in addition to decrease in water uptake by the plant roots.

## **Conclusions**

In conclusion, the results of this study conclude that some wheat genotypes such as SALAMBO SABHA and MAKKAWI appeared to be moderately salinity tolerant at germination stage than other genotypes of wheat involved in this study. Whether such results can be achieved under natural condition and at later growth stages needs the significance of field assessment through screen field trials. Besides, comparative physiological and biochemical foundation of such tolerance amongst genotypes similarly needed.

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