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# **Tolerance of Wheat Doubled Haploids to Elevated Levels of NaCl at Germination and Seedling Stages**

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# *Authors' contributions*

*This work was carried out in collaboration between all authors. Correspondence author AMMAN designed the study, wrote the protocol, and wrote the first draft of the manuscript. Authors MMMA and SRSS managed analyses of the study and the literature searches. Author OMAEA performed the experimental processes. All authors read and approved the final manuscript.*

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# **ABSTRACT**

Genetic variability in salt tolerance of bread wheat could be induced via crossing diverse parents and producing doubled haploid (DH) lines from such crosses. One hundred seventeen DH lines resulted via anther culture technique from the cross between Sakha-8 (salt tolerant old variety) and Line-25 (a high yielding promising line) along with the two parents and two check cultivars (Sakha-93 and Sids-1), making a total number of 121 genotypes, were evaluated at different salinity levels under controlled greenhouse conditions at germination (in petri dishes) and seedling (in pots) stages. The aims were to identify the most tolerant DH lines to elevated levels of salinity and to determine the traits of strong association with salinity tolerance. A factorial experiment based on randomized complete blocks design in three replications was used. Full strength Hoagland solution was added to four saline solutions, namely zero (distilled water + Hoagland solution), 3000, 6000 and 9000 ppm NaCl. Increasing concentration of NaCl caused a gradual and significant decrease for most studied traits. On the contrary, increasing salt level caused a significant increase in mean germination time (MGT) (delay) at 6000 and 9000 ppm, shoot dry weight (SDW) at 3000 ppm and root:shoot ratio (RSR) at 9000 ppm NaCl level. At the highest salinity level (9000 ppm NaCl), the 121 genotypes under study were classified based on their salinity tolerance index (STI) into four

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categories, i.e., tolerant (T), moderately tolerant (MT), sensitive (S) and very sensitive (VS), with a number of 39, 36, 38 and 8 genotypes, respectively; 28 DH lines showed much higher salinity tolerance than the highest salt tolerant check cultivar in this study (Sakha-93). The relationship between NaCl levels and means of studied traits is of quadratic (curvilinear) nature for all categories ofgenotypes, expect for root dry weight (RDW) of all categories, root fresh weight (RFW) of the two groups S and VS and shoot length (SL) of the group VS, where the relationship was near linear regression. The quadratic regression was in the direction of decrease with increasing of NaCl levels for all studied traits, except for MGT of all wheat categories and RSR of S and VS groups of genotypes, which showed a curvilinear increase. Salinity tolerance trait index (STTI) of T group of genotypes increased by increasing NaCl levels, while STTI of MT, S and VS groups decreased for most studied traits. In general, increasing salinity level increased correlation coefficients among absolute means and each of STTI's and STI for all studied traits. Traits showing strong correlations with STI, high heritability estimates, high expected selection gain and wide phenotypic and genotypic variability were shoot fresh weight (SFW), SDW, RFW and shoot length (SL) at 9000 ppm and RDW and SL at 3000 and 6000 ppm; they were recommended as selection criteria for salinity tolerance.

*Keywords: Triticum aestivum; salinity tolerance index; quadratic response; germination; seedling traits; doubled haploids; selection criteria.*

# **1. INTRODUCTION**

In Egypt, about 25% of total cultivated lands are salt affected; these lands are located in north, east and west Delta and some other areas in Wadi Al Natron, Al-Tal Al-Kebeir, Al-Wahat and Al-Fayoum regions [1]. Negative effects of salinity on agriculture are a concern because it affects growth and development and yield of crop plants. Typically, decrease in growth of plant occurs linearly after attending threshold value of salinity. Salinity decreases root growth as well as shoot growth, but this reduction is lower in roots compared to tops growth [2].

Bread wheat (*Triticum aestivum* L.) is considered a moderately salt tolerant crop; its productivity deceases by 25% at 9.5 dSm<sup>-1</sup> and 50% at 13  $dSm^{-1}$  soil salinity [3,4]. Raising the tolerance of wheat genotypes to salinity is considered an important target for developing wheat cultivars of improved adaptation for growing under salinity conditions. Improvement of wheat genotypes that tolerate salinity is facing a number of problems, viz, reduction in genetic variability available to the plant breeder, non availability of effective evaluation methods for salinity tolerance character and the low selection efficiency by using the conventional agronomic characters, since salinity tolerance is a complicated quantitative character that depends on several physiological and biochemical characters, which are yet completely undiscovered.

Genetic diversity in wheat has been reduced mainly due to narrow genetic base of the wheat germplasm [5]. Therefore, wheat breeders are always looking for new methods to enrich breeding material of better tolerance to salinity stress. The anther pollen culture technique can help in developing doubled haploids (DH) in a very short period of time from wheat crosses that show new genetic variation amenable for efficient selection for salinity tolerant genotypes [6].

Several studies suggested that selection for salinity tolerance by evaluating physiological traits is more successful in controlled conditions than the yield in salt affected fields [7,8]. Germination is a crucial stage for plant establishment [9]. Numerous plants are sensitive to ion stress during germination [10] or seedling growth [11,12]. Saboora [13] evaluated nine wheat cultivars at germination and early seedling growth under six salt treatments and reported that salt treatment had significant negative consequence on germination percentage, rate of germination, total dry weight and dry weight of shoot and root. Wheat plants are sensitive to salt stress at all growth stages, but early germination and seedling stages are more sensitive [14,15]. Early germination and seedling stage also reflects responses of plant growth to salinity [15,16]. Therefore, evaluation of wheat genotypes for salinity tolerance is essential at both stages [17,18].

The objectives of the present investigation are (i) studying the genetic variation in salinity tolerance at germination and seedling stages among 121 wheat genotypes (117 DH lines, 2 parents and 2 check cultivars), (ii) identifying the differential response of tolerant, moderately tolerant, sensitive and very sensitive categories of wheat genotypes at germination and seedling stages under elevated concentrations of NaCl and (iii) determining the germination and seedling traits of strong association with salinity tolerance and of high heritability to be recommended as selection criteria for such tolerance.

#### **2. MATERIALS AND METHODS**

#### **2.1 Plant Materials**

Seeds of 121 bread wheat genotypes, including 117 doubled haploid lines, their two parents (Sakha-8 and Line-25) of the cross from which they were produced *via* anther culture technique and the 2 check cultivars Sids-1 and Sakha-93 (Table 1) were obtained from Wheat Research Department, Field Crops Research Institute (FCRI), Agricultural Research Center, (ARC), Egypt.

The reason of using this large number of lines in this study is to exploit the maximum variation resulted from the crossing between a salt tolerant cultivar (Sakha-8) and a high yielding promising line (Line-25) in an attempt to find new line(s) that assemble more tolerance to salinity and higher yielding ability as compared to their parents and the check cultivars, i.e., new sources of salinity tolerance.

## **2.2 Sowing Method**

Ten seeds of each of the 121 genotypes were surface-sterilized and sown in 10 cm petri dishes under environmentally controlled green house (temperature was 22-23ºC and photoperiod was 16-14 h darkness) in summer of 2013 (off season) for evaluation under four salinity treatments, viz. zero or control (only distilled water), 3000, 6000 and 9000 ppm NaCl. Seeds were sown on filter papers and were irrigated with saline solution of different concentrations. A factorial experiment based on a randomized complete blocks design was used with three replications. Full strength Hoagland solution [19] was used in each treatment by adding it to the saline solution used for irrigation twice a week as follows:

Treatment 1: (control or no stress)[Distilled water + Hoagland solution].

Treatment 2: [Saline solution (3000 ppm NaCl) + Hoagland solution].

Treatment 3: [Saline solution (6000 ppm NaCl) + Hoagland solution].

Treatment 4: [Saline solution (9000 ppm NaCl) + Hoagland solution].

NaCl solutions were made by dissolving analytical grade NaCl in distilled water. Seeds were germinated in three petri dishes and data of germination percentage, time and rate were recorded every day [20] until a constant count was achieved.

Three seedlings from each replicate (petri dish) were transplanted to 10 cm plastic pots (containing washed sand) in environmentally controlled green house (same used for germination) and used for data collection on seedling traits. Harvesting was done 40 days after seed sowing date. Data were recorded for following traits:

#### **2.3 Germination Traits**

## **2.3.1 Germination percentage (GP)**

A seed was considered germinated when radical length exceeded 2 mm. Germination percentage (GP) for every genotype was calculated after every 24 hours for 12 days by using the following formula:  $GP = 100(No.$  of seeds germinated / Total No. of seeds).

#### **2.3.2 Germination rate index (GRI)**

Daily count of germinating seeds was made till it reached a constant count. The number of seeds germinated was divided by days from first germination. The GRI reflects the percentage of germination on each day of the germination period. Higher GRI values indicate higher and faster germination GR was calculated according to the formula described by Kader [21] as follows:

$$
GRI = X_i / Y_i + X_{ii} / Y_{ii} + X_{iii} / Y_{iii} + \dots + X_n / Y_n
$$

Where:  $X =$  number of seeds germinated for the day.

> $Y =$  number of days from the sowing date.

i, ii, iii  $\ldots \ldots$  n = No. of days.

#### **2.3.3 Mean germination time (MGT)**

The lower MGT, the faster a population of seeds has germinated [21]. The formula used is as follows:

$$
MGT (d) = \sum (N_i T_i) / \sum N_i
$$

Where,

 $N_i$  = number of seeds germinated for the day.

i,  $T_i$  = number of days from the sowing date.

#### **2.4 Seedling Traits**

#### **2.4.1 Shoot length (SL)**

Shoot length was measured with the help of scale and average of 3 seedlings was used for statistical analysis.

#### **2.4.2 Root length (RL)**

Root length was measured with the help of scale and average used for statistical analysis.

#### **2.4.3 Shoot fresh weight (SFW)**

Fresh weight of shoot was taken with the help of electric balance and average was used for statistical analysis.

#### **2.4.4 Shoot dry weight (SDW)**

Shoots were dried in the oven at 70ºC till it reached constant weight and dry weight for each was weighed.

#### **2.4.5 Root fresh weight (RFW)**

Root fresh weight was taken with the help of electric balance and average was used for statistical analysis.

## **2.4.6 Root dry weight (RDW)**

Roots were dried in the oven at 80ºC till constant weight and dry weight of roots for each seedling was weighed.

## **2.4.7 Root/shoot ratio (RSR)**

Root/shoot ratio (RSR) was calculated as follows:

## RSR = RDW / SDW

The previously mentioned traits were used to calculate the following parameters:

#### **2.5 Salinity Tolerance Trait Index (STTI)**

Salinity tolerance trait index (STTI) modified from dry matter or grain yield efficiency index suggested by Fageria [2] to classify genotypes for tolerance to salinity. The formula used is as follows:

STTI = 
$$
(Y_1/AY_1) \times (Y_2/AY_2)
$$

Where,

 $Y_1$  = trait mean at low salinity level.

 $AY_1$  = average trait of genotypes at low salinity level

 $Y_2$  = trait mean at high salinity level.

 $AY<sub>2</sub>$  = average trait of genotypes at high salinity level.

## **2.6 Salinity Tolerance Index (STI)**

Salinity tolerance index (STI)was estimated as average of STTI's according to Munir et al. [18] as follows:

 $STI = (STT_1I + STT_2I + \dots + STT_nI)/n$ 

Where,

 $STTI<sub>1</sub>$ ,  $STTI<sub>2</sub>$  ……  $STTI<sub>n</sub>=Trait$  No.1, Trait No.2 ……. Trait No.n n = number of measured traits When STI is  $> 1$ , it indicates that genotype is tolerant (T) to salinity. If STI is  $> 0.5$  to 1, it indicates that genotype is moderately tolerant (MT) to salinity.

#### **Table 1. Pedigree, salinity tolerance of the bread wheat genotypes used in the present investigation**



*Source: Wheat Res. Dept., FCRI, ARC, Giza, Egypt*

If STI is  $> 0$  to  $< 0.5$ , it indicates that genotype is sensitive (S) to salinity.

If STI is zero, it indicates that genotype is very sensitive (VS) to salinity.

This is our own classification based on Fageria [2].

## **2.7 Statistical Analysis**

After verifying the normality of data, they were subjected to analysis of variance (ANOVA) using MSTAT C21 and Assistat 7.7 software's, and comparisons of means were made using the least significant difference (LSD) test at P < 0.05 and 0.01 levels of confidence; coefficients of simple and rank correlations between attributes were also calculated according to Steel et al. [22].

For the purpose of determining genetic parameters, each salinity treatment was analyzed separately as a randomized complete blocks design (RCBD). Expected mean squares were estimated from ANOVA table of RCBD according to Hallauer et al. [23] and used to estimate the genotypic  $(\sigma_g^2)$ , phenotypic  $(\sigma_p^2)$ and error  $(\sigma_e^2)$ ) variances. Broad-sense heritability  $(h^2)$  and expected genetic advance (GA%) from direct selection for all studied traits under each salinity treatment were calculated according to Singh and Chaudhary [24]. The GA was calculated as follows: GA% = 100 Kh<sup>2</sup><sub>b</sub> $\sigma_{\rm p}$  /X where  $X =$  general mean of the appropriate salinity treatment,  $\sigma_{p}$  = square root of the phenotypic variance estimate and K= selection differential (K= 1.76 for 10% selection intensity in this study).

## **3. RESULTS AND DISCUSSION**

## **3.1 Analysis of variance**

Analysis of variance of the studied germination and seedling traits of 121 wheat genotypes (117 DH's, 2 parents and 2 checks) under 4 NaCl levels (Table 2) showed that mean squares due to salinity levels (S) were highly significant for all studied traits. This indicates that salinity stress had a significant effect on all studied wheat germination and seedling traits. Results also exhibited that mean squares due to wheat genotypes (G) were highly significant for all studied traits, except for mean germination time (MGT) trait, where mean squares due to genotypes were only significant. This suggests that wheat genotypes used in this study were

significantly different for all studied germination and seedling growth traits. Moreover, mean squares due to genotypes x salinity levels, (G x S) were highly significant for 9 out of 10 studied traits and only significant for MGT trait, indicating that wheat genotypes in this study behaved differently under different salinity levels. This conclusion is supported by Munir et al. [18], who reported that 187 wheat landraces / cultivars responded differently to the different NaCl stress levels. Mean squares due to salinity levels were much greater in magnitude than those due to wheat genotypes for all studied germination and seedling growth traits, indicating that salinity effects were more pronounced than genotypic effects for such traits; same as reported by Munir et al. [18].

## **3.2 Effect of Salt Stress**

Increasing NaCl salinity levels adversely affected germination and seedling growth attributes of the 121 wheat genotypes (Figs. 1 and 2). Significant  $\geq 0.05$  and / or  $\geq 0.01$ ) gradual reductions were observed due to increasing NaCl salinity level from 0 to 3000, 6000 and 9000 ppm, for all studied traits, except for GP, GRI and RL traits at 3000 ppm NaCl level, where reductions were non-significant. On the contrary, salt stress at 6000 and 9000 ppm caused a significant  $( \geq 0.01)$ increase (delay) in mean germination time (MGT) by 188.40 and 234.62% (3.91 and 4.87 days), respectively. A similar conclusion was reported by Rahman et al. [25].

Maximum reductions due to salinity stress were observed at 9000 ppm NaCl solution concentration in all studied germination and seedling growth stages, except for RSR trait, where maximum reduction was observed at 3000 ppm NaCl level. Reductions at 6000 ppm NaCl level ranged from 39.21% for root length to 86.87% for GRI. Root/shoot ratio trait that showed a significant increase of 31.63% and root length that showed the lowest decrease of 39.21% at 9000 as compared to control (no salt stress) could be considered the most tolerant traits to salinity in the present study. Husain et al. [26] reported that seedling root length of wheat showed least suppression in root elongation at higher salt concentrations.

Munir et al. [18] also concluded that seedling root characters are the most tolerant characters to salinity stress.





*\* and \*\* indicate significant at 0.05 and 0.01 probability levels, respectively*

It is evident from our results that shoot growth was more inhibited by salinity stress than root growth; the reason for which root: shoot ratio was increased by increasing salinity. Similar results were reported by Zerihun et al. [27] and Munir et al. [18]. According to them shoot growth was more reduced as compared to root growth under imposed stress. Probable reason of better root growth may be that the salt stress causes physiological drought; due to this reason roots proliferated more under stress conditions to take up more water [18].

Impaired seed germination is the major factor limiting the establishment of plant under salinity [28]. The inhibitory effect of salinity on germination attributes of different crops has been reported earlier [29,30,31]. The decline in germination under salinity has been attributed to combined effect of osmotic pressure [32] and toxicity of salts [13] or due to the effect of added chlorine ion [33] that gave rise to osmotic stress. Rahman et al. [25] reported that salinity significantly delayed the germination mainly due to altered water relations caused by high salt accumulation in intracellular spaces [28,34].

The inability of seeds to germinate under salinity conditions may be due to embryo damage by Na<sup>+</sup>/Cl<sup>-</sup> ions [35] or inhibition of seed water uptake [13,26,36] or exosmosis [25].

Soil salinity affects early seedling growth of plants by altering water relations due to salt accumulation in intracellular spaces [34], injurious effects of toxic ions [13], osmotic stress [33] and reduced water use efficiency [37]. Moreover, salinity stress can generate a wave of reactive oxygen species such as super oxide,

hydrogen peroxide; hydroxyl radical resulting in oxidative damage to cell ultra structures [13].

In the present study, it is worthy to note that salinity treatment with 3000 ppm NaCl had a stimulating effect on shoot dry weight and had no significant undesirable effect on GP, GRI, MGT and RL traits as compared to control treatment (distilled water), for most studied wheat genotypes.

## **3.3 Effect of Genotype**

Means of 121 wheat genotypes showed a wide range (difference between the minimum and maximum) for studied traits under control as well as salinity stress treatments; the ten highest and ten lowest genotypes under all salinity levels for selected seedling growth traits are presented in Table 3.

It was observed that with increasing salinity levels, the range decreased (narrowed) for RL, SFW, DFW, SDW and RDW traits and increased (widened) for SL trait . Under 9000ppm NaCl salinity conditions, eight out of 121 genotypes (L19, L28, L49, L54, L63, L69, L101 and L114) did not germinate (6.6%) and 37 genotypes (30.6%) could not develop into seedlings (non survived).

For SL, RL, SFW, RFW, SDW and RDW, the genotypes L86, L109, L75, L11, L72 and L5, respectively had the highest values under 9000 ppm salinity conditions. The genotypes L86, L70, L74 and L5 under 6000 ppm, L9, L16, L11, L90, L2 and L74 under 3000 ppm and L76, L80, L27, L82. L11 and L86 under 0 ppm NaCl salinity conditions had the highest absolute means for



**Fig. 1. Effect of different NaCl concentrations on studied germination and seedling growth traits. Means with different letters differ significantly at 0.05 probability level. The bar above each column denotes the standard error of three replications. LSD for interaction is (1) 31.2, (2) 0.61, (3) 3.36, (4) 3.09, (5) 1.55, (6) 117.2, (7) 46.6, (8) 34.8, (9) 29.37 and (10) 0.133**



**Fig. 2. Seedlings of a tolerant line (No. 11) and sensitive line (No. 10) under different salinity levels at 40-days age**

SL, RL, SFW, RFW, SDW and RDW, respectively. The lowest means were shown by genotypes L41, L6, L59 and L47 for SL, L68, L18, L77 and L42 for RL, L7, L22, L76 and L66 for SFW, L68, L36, L45 and L5 for RFW, L39, L26, L26 and L34 for SDW and L81, L104, L73 and L68 for RDW at 0, 3000, 6000 and 9000 ppm, respectively (Table 3).

In general, under salt stress, the DH lines no. 11, 14, 12, 75, 21, 86, 80, 113, 2, 3, 4, 72, 48, 5, 74 were amongst the highest genotypes in one or more seedling traits under 6000 and 9000 ppm NaCl conditions. The highest germination percentage (66.7%) at 9000 ppm NaCl level was shown by genotypes L48, L72, L94, L96, L110, L4, L11, L12, L13, L14, L16, L24, L25, L29, L41, L45, L53, L55, L56, L60, L74, L79 and L87. respectively. The lowest means were shown by<br>genotypes L41, L6, L59 and L47 for SL, L68,<br>1018, L77 and L42 for RL, L7, L22, L76 and L66<br>for SFW, L68, L36, L45 and L5 for RFW, L39,<br>L26, L26 and L34 for SDW and L81, L104, L7

#### **3.4 Salinity Tolerance**

Summarizing estimates of salinity tolerant trait indices (STTI's) indicated that under severe salinity stress (9000 ppm NaCl) the germination characters (GP, GRI and GMT) exhibited the lowest mean, maximum and minimum STTI estimates, indicating that germination stage is more sensitive to severe salinity stress than seedling growth stage. This conclusion was previously reported by Munir et al. [18].

On the contrary, under 9000ppm NaCl conditions, RDW trait followed by SL trait showed the highest mean and maximum STTI and RSR traits showed the highest maximum STTI, indicating that these traits were the most tolerant ones to the most severe salinity stress in this study. riously reported by Munir et al. [18<br>the contrary, under 9000<br>ditions, RDW trait followed by SL<br>highest mean and maximum ST

Salinity tolerance index (STI) estimates calculated across germination traits showed that L36, L42, L43, L48, L53, L56, L64, L67, L76, 20 wheat genotypes (L5, L12, L16, L24, L35,<br>L36, L42, L43, L48, L53, L56, L64, L67, L76,<br>L78, L79, L94, L104, L110 and Sd-1) were tolerant under the three salinity stress treatments(3000, 6000and 9000 ppm NaCl). Nine more genotypes (L4, L14, L21, L25, L41, L57, L70, L73 and L108) showed tolerance to salinity at both 6000 and 9000 ppm levels and one more genotype (L96) showed tolerance under 9000 ppm NaCl salinity stress conditions. under the three salinity stress (3000, 6000and 9000 ppm NaCl).<br>
9 genotypes (L4, L14, L21, L25, L41, L73 and L108) showed tolerance to<br>
both 6000 and 9000 ppm levels and<br>
9 genotype (L96) showed tolerance<br>
1 ppm NaCl salin

the (STI) 20 wheat genotypes (L5, L12, L16, L24, L35, Regarding salinity tolerance index for vegetative stage (seedling growth traits), 29 genotypes showed tolerance at the three levels of salinity stress (3000, 6000 and 9000 ppm L2, L3, L4, L5, L7, L9, L11, L12, L14, L21,L24, L25, L34, L44, L48, L57, L62, L67, L72, L75, L80, L85, L86, L94, L103, L111, L113, L117 and Sk-93. Two more genotypes (L65 and L106) were tolerant at both 6000 and 9000 ppm NaCl levels. Based on STI's calculated across both germination and vegetative traits, the 121 wheat genotypes were grouped into four categories at 9000 ppm NaCl level, namely tolerant (39 genotypes), moderately tolerant (36 genotypes), sensitive (38 genotypes) and very sensitive (8 genotypes) (Table 4). Number of tolerant, moderately tolerant and sensitive genotypes under 6000 ppm NaCl conditions was 53, 66 and 2, respectively. However, under 3000 ppm NaCl salinity conditions, there were only two categories, namely tolerant (83 genotypes) and moderately tolerant (38 genotypes), indicating that wheat genotypes in the present study do not include a sensitive genotype at this low salinity stress. In general, wheat is a moderately salt tolerant crop [32]. When salinity increased to about 10 dSm<sup>-1</sup>, wheat performance decreases marginally compared to other crops like rice [38]. about 10 dSm<sup>-1</sup>, wheat performance decreases<br>marginally compared to other crops like rice [38].<br>Remarkable reductions in germination and L3, L4, L5, L7, L9, L11, L12, L14, L21, L24,<br>i, L34, L44, L48, L57, L62, L67, L72, L75,<br>l, L85, L86, L94, L103, L111, L113, L117 and<br>93. Two more genotypes (L65 and L106)<br>re tolerant at both 6000 and 9000 ppm NaCl<br>els. Bas (Table 4). Number of tolerant,<br>tolerant and sensitive genotypes<br>Dppm NaCl conditions was 53, 66 and<br>vely. However, under 3000 ppm NaCl<br>onditions, there were only two<br>namely tolerant (83 genotypes) and<br>tolerant (38 genotype

seedling growth traits observed in the present study often started from the moderate salinity stress (6000 ppm NaCl) upwards. It is worthy to note that only the check cultivar Sakha-93 was salt stress tolerant at 9000 ppm NaCl level; Sids-1 was tolerant only at 6000 ppm NaCl level, but was moderately tolerant at 9000 ppm NaCl level and the promising Line 25 and Sakha-8 cultivar (parents of the cross from which DH lines used in this study were produced) were moderately tolerant at 6000 ppm NaCl level, but were sensitive at 9000 ppm NaCl level (Table 4). Under the most severe salt stress in the present study (9000 ppm NaCl), 28 DH lines showed much better salinity tolerance (based on both germination and vegetative stages) than the salt stress tolerant cultivar Sakha-93; the 12 most tolerant of them were L11, L14, L3, L75, L12, L4, L21, L86, L48, L72, L94 and L2 in a descending order.

The relative ranking for some genotypes also varied between the different treatments, which may be due to large genetic variation of the DH lines and the complex nature of salt tolerance mechanisms. However, the salt tolerance response of some genotypes was stable over the different NaCl concentrations. These genotypes showed the potential to provide good plant stand establishment and biomass production at early seedling stage, which are essential to get high production under high salt stress.

The most 12 tolerant wheat genotypes under all studied salinity stress conditions were L11, L14, L3, L62, L21, L86, L4, L2, L12, L113 and L5, On the contrary, the 12 most sensitive genotypes to salinity at 6000 and 9000 ppm NaCl levels were L66, L61, L98, Line-25, L89, L43, L36, L68, Sakha-8, L18, L20 and L116 (Table 4).





To describe the differences between salinity tolerant (T) and sensitive (S) genotypes, data of studied characters were averaged for the two groups of genotypes differing in their tolerance at 9000 ppm NaCl stress level (the 12 most tolerant and the 12 most sensitive genotypes) and presented in (Table 5).

Under 9000 ppm NaCl conditions, significant higher values were exhibited in T than in S by about 323.1% for SFW followed by 306.4% for RDW, 262.9% (SDW), 206.8% (RFW), 102.9% (SL), 79.9% (RL), 68.6% (GP), 66.7% (RSR) and 33.3% (GRI). In contrast, a significant lower value (desirable) exhibited in T than in S by about 27.8% for MGT at 9000 ppm NaCl level, indicating that T genotypes were earlier than S genotypes in completing germination under severe salt stress conditions. The same trend of superiority of T over S was observed under 6000, 3000 and 0 ppm NaCl levels, but in much less magnitude (Table 5), especially for SFW, RDW, SRW and RFW.

Salt-tolerant *Triticum* spp. had lower rate of Na<sup>+</sup> accumulation than the salt sensitive ones [26,39] reported that wheat genotypes AARI-2011 and Sehar-2006 appeared more susceptible to salinity levels  $(6.8, 13.2 \text{ and } 19.0 \text{ dSm}^{-1})$ regarding germination attributes as compared to Millat-2011. They reported that the better performance of Millat-2011 under salinity suggests its superiority and greater ability to cope with salinity levels. Almodares et al*.* [33] stated that some plants are sensitive to salinity at early seedling growth stage because the mechanism of the tolerance to salinity is not yet fully developed. Differential suppression of wheat genotypes under salinity might originate from variable metabolic deficiencies under stress induced carbon deficit and activity of enzymes of anti-oxidative defense as these have been positively correlated with stress tolerance [40]. Moreover, difference in cell membrane stability and macro molecule stability under salinity might also be the possible cause of differential response.

#### **3.5 Differential Response of T, MT, S and VS Wheat Genotypes**

Mean performance and salinity tolerance trait index (STTI) of studied germination and seedling traits were averaged across the four groups of wheat genotypes, i.e., tolerant (T), moderately tolerant (MT), sensitive (S) and very sensitive (VS) under 9000 ppm NaCl concentration. Data were analyzed to evaluate traits means and STTI

responses of T, MT, S and VS genotypes across varying levels of salt stress. For each group of genotypes quadratic regression function was performed for salt stress effects on mean performance (Fig. 3) and on STTI (Fig. 4). The regression functions were also performed to distinguish which for each group of genotypes.

It is obvious from Fig. 3 that the relationship between NaCl levels and means of studied germination and seedling traits is of quadratic (curvilinear) nature for all categories of genotypes, expect for RDW trait of all categories, RFW of the two groups S and VS and SL of the group VS, where the relationship was near linear regression. The quadratic regression function observed in this study was in the direction of decrease with increasing of NaCl levels from 0 to 3000, 6000 and 9000 ppm for all studied traits, expect for MGT of all wheat categories and RSR of S and VS groups of genotypes which showed a curvilinear increase.

The optimum NaCl concentration varied among the 4 wheat categories and among the studied germination and seedling traits. The optimum NaCl levels to get the most favorable means for all wheat categories was 1500 ppm for GP, GRI, MGT and SFW traits. It was 9000 ppm NaCl for tolerant (T) and moderately tolerant (MT) groups of genotypes for RSR trait and 6800 ppm NaCl level for RL trait of T genotypes group, indicating that the most important trait for salt tolerance is RSR followed by RL.

For the tolerant group of genotypes, the optimum salt concentration was also ca 1750 ppm NaCl level for SDW and ca 1400 ppm for SL. For the MT group of genotypes, it was also ca 5250 ppm for RL, ca. 2250 ppm for SDW and ca 800 ppm for SL trait. The non-stress salt concentration (0 ppm NaCl) was the optimum for RDW of all groups of genotypes, RFW of S and VS groups and SL of VS group. Moreover, The NaCl concentration 6500 ppm was the optimum for S and VS groups of genotypes for RSR trait as compared to 9000 ppm level which was the optimum salt concentration for same trait of T and MT groups of genotypes.

It is worth noting that at the severe salt stress (9000 ppm NaCl), the salt tolerant group of genotypes achieved the best (most favorable) means for all studied germination and seedling traits followed by the moderately tolerant group and the sensitive, but the very sensitive group of genotypes came in last rank (Fig. 3). Under 6000

ppm, NaCl concentration, all characters of T group, SL, SFW, SDW and RFW of MT group and RL, RDW and RSR of S and VS groups occupied the 1<sup>st</sup> position. Under 3000 ppm NaCl level, the T group for SL, SFW and RDW and MT group for GR, RL, RDW and RSR showed the best means. Moreover, under no stress (distilled water) the T group of genotypes achieved the best means for SL, RL, SFW, SDW and RDW traits.





<b>Traits</b>	0 ppm				3000 ppm				6000 ppm				9000 ppm			
				<b>Superiority %</b>				<b>Superiority %</b>		c		<b>Superiority %</b>			Superiority %	
GP	93.	93.1	0.C	NS	95.8	91.	4.5	ΝS	73.6	83.3	$-11.$	ΝS	37.5	22.2	68.6	$***$
<b>GRI</b>	2.5	2.6	$-1.6$	<b>NS</b>	2.7	2.5	5.6			.0	10.0	$***$	0.4	0.3	61.5	$***$
<b>MGT</b>	2.0	2.1	-2.9	<b>NS</b>	2.2	2.2	$-1.8$	ΝS	5.6	6.3	$-10.3$	<b>NS</b>	4.3	5.5	$-22.0$	ΝS
<b>SL</b>	32.0	29.7	7.7	$\star$	29.4	25.6	14.6	**	19.1	14.8	29.5	**	15.2	7.5	102.9	**
RL	19.4	16.8	15.9	$* *$	18.5	17.1	8.2	**	19.6	18.6	5.2	ΝS	14.0	7.8	79.9	$***$
<b>SFW</b>	988	783	26.3	$* *$	864	754	14.6	**	392	313	25.0	**	277	66	323.1	$***$
<b>RFW</b>	555	477	16.4	$* *$	462	427	8.3	$***$	307	231	32.7	$***$	152	50	206.8	$***$
<b>SDW</b>	472	283	66.6	$***$	474	418	13.4	$***$	287	219	31.1	$***$	100	28	262.9	$***$
<b>RDW</b>	267	135	97.4	$***$	229	130	76.7	$***$	168	97	72.9	$***$	92	23	306.4	$***$
<b>RSR</b>	0.6	0.5	20.8	$***$	0.5	0.3	48.5	**	0.6	0.5	20.0	$***$	1.4	0.8	66.7	$***$

**Table 5. Superiority (%) of tolerant (T) over sensitive (S) DH lines based on a salinity tolerance index (STI) of vegetative traits under 9000 ppm evaluated under elevated NaCl levels (0, 3000, 6000 and 9000 ppm)**

*NS, \* and \*\* indicate non significance and significance at 0.05 and 0.01 probability levels, respectively*

#### *Al-Naggar et al.; IJPSS, 5(5): 249-267, 2015; Article no.IJPSS.2015.079*



Fig. 3 Relationships between elevated NaCl level and means of studied germination and seedling traits of the four categories of wheat genotypes, i.e., tolerant (blue), moderately tolerant (red), sensitive (green), very sensitive (orange).

0.40

 $0.20$ 

 $0.00$ 

Control

 $-0.1215x^2 + 0.819$ 

Salinity levels (ppm)

6000

9000

3000

261



Fig. 4 Relationships between elevated NaCl level and salinity tolerant trait index (STTI) value of studied germination and seedling traits of the four categories of wheat genotypes, i.e., tolerant (blue), moderately tolerant (red), sensitive (green), very sensitive (orange).



Fig. 4 clearly shows that tolerant genotypes had a quadratic response of increase in STTI's for GP, GRI, SFW, RFW, SDW and quadratic regression of decrease for STTI's of GMT and SL traits, Moderately tolerant group of genotypes showed a quadratic regression of increase in STTI's for RSR, near linear regression of increase in STTI for GP, RL and RDW and a quadratic response of decrease in STTI for the remaining traits. Sensitive and very sensitive groups of genotypes show a quadratic response of decrease in STTI for all studied germination and seedling traits. Salinity tolerance of T group of genotypes increased by increasing NaCl levels for all studied germination and seedling traits, except for GMT and SL trait. For MT group of genotypes, salinity tolerance increased by elevating NaCl levels for only RSR trait. For the rest of traits of MT group and all traits of S and VS groups of genotypes, salinity tolerance decreased by increasing NaCl levels.

Correlations between salinity tolerance index (STI) and studied germination and seedling traits of 121 wheat genotypes (Table 6) were generally stronger at 9000 ppm than 3000 and 6000 ppm levels. Under 9000 ppm NaCl salinity conditions, SFW, SDW, RFW, SL, RL and RDW were strongly correlated with salinity tolerance index (r = 0.901, 0.858, 0.852, 0.850, 0.818 and 0.769, respectively). This suggests that these characters could be recommended for wheat breeders as selection criteria for improving salinity tolerance at 9000 ppm NaCl stress level, especially if heritability of these traits are high in magnitude.

Under 6000 ppm NaCl stress conditions, STI had a high positive and significant correlation with GRI (0.858), GP (0.817), SL (0.613) and RDW (0.605). These characters were common to 6000 ppm salinity level and could also be considered as selection criteria for salinity tolerance. At 3000 ppm NaCl level, STI showed high positive and significant correlation coefficients with RDW (0.719), GP (0.677), GRI (0.671) and SL (0.646).

The present study concluded that the germination traits GP and GRI and the seedling vegetative traits shoot and root fresh and dry weight and seedling length are good selection criteria for salinity tolerance of wheat. Moud and Maghsoudi [32] concluded that germination and emergence rates could be used as selection criteria for wheat salt stress tolerance at early growth stages. Munir et al. [18] reported that both root and shoot weights possessed positive

correlation with all seedling traits studied, indicating that root and shoot dry weights might be good selection criteria to evaluate genotypes under salinity stress. Maas [4] and Ashraf et al. [41] also suggested that root and shoot dry weight can be used as selection criteria under salinity stress. Meneguzzo et al. [42] suggested that dry weight can be a good indicator of salinity tolerance in controlled conditions.

## **3.6 Variability, Heritability and Selection Gain**

Phenotypic (PCV) and genotypic (GCV) coefficients of variation for germination and seedling growth characters of the 121 wheat genotypes at different salt solution concentrations are presented in (Table 7). Maximum variability at the phenotypic and genotypic levels was recorded by root: shoot ratio (RSR) trait under all salinity concentrations, followed by RDW at 0, 3000 and 6000 ppm NaCl for PCV and GCV and GRI (for PCV) and SFW (for GCV) at 9000 ppm NaCl level, and RFW, SDW and SFW for both PCV and GCV at 0, 3000 and 6000 ppm NaCl levels, respectively and SFW (for PCV) and SDW (for GCV) at 9000 ppm NaCl. Such wide variability at both phenotypic and genotypic levels for RSR, RDW, SDW, SFW and RFW is a pre-requisite for practicing successful development of salt tolerant varieties of wheat.

Roots are important as a plant trait because they have direct contact with soil and are used to absorb water and mineral nutrients. Thus, roots give significant indication of plant salinity tolerance under stress conditions [18]. Vigorous shoots having more leaves carry out photosynthesis efficiently and add to root growth [43]. Consequently genotypes having more dry weight might be good option under salinity stress.

Heritability percentages in the broad-sense (Table 8) were at maximum for root and shoot dry and fresh weight traits (SDW, RDW, SFW, RFW and RSR) under all NaCl solution concentrations, ranging from 98.0% for RSR under control to 100.0% for the same trait under 6000 ppm NaCl stress conditions. On the contrary, the moderate to high estimates of heritability were recorded for germination traits (GP, GRI and MGT), ranging from (44.26%) for GRI at 9000 ppm NaCl level to (91.42%) for GP at 6000 ppm level. The lowest estimates of heritability of germination traits were observed at 9000 ppm NaCl level, except for GP at 6000 ppm level (91.42%). Heritability estimates for SL and RL traits were very high (99.15 and 99.25% at 9000 ppm NaCl level and 94.65 and 94.69% at 6000 ppm level) and moderate (81.58 and 77.31%) at control treatment, respectively.

In general, heritability values under severe salinity were found to be lower as compared to control (no stress) for all germination traits (GP, GRI and MGT), higher for SL and RL trait and similar for the rest of studied seedling traits. However, Munir et al. [18] suggested that high salinity level had more inhibiting effects on heritability of seven studied seedling traits.

The highest expected genetic advance (GA) from selection (based on 10% selection intensity) was generally observed if selection was done under severe salinity stress (9000 ppm NaCl) for root: shoot ratio (RSR) followed by SFW, SDW, RFW and RDW. RSR showed also the highest expected GA under 3000 and 6000 ppm NaCl followed by RDW under both treatments, SFW under 6000 ppm and SDW under 3000 ppm. The RDW under control treatment (no salt stress) showed the highest GA percentage followed by RFW, SDW and SFW.

**Table 6. Rank correlation among absolute means and each of STTI's and STI's for studied traits of 121 wheat genotypes under different NaCl concentrations**

Trait	<b>STTI's</b>						STI					
	3000		6000		9000		3000		6000		9000	
Germination percentage	0.753	**	0.956	$***$	0.987	$***$	0.677	$***$	0.817	$***$	0.630	$***$
Germination rate index	0.724	**	0.957	$***$	0.991	$***$	0.671	$***$	0.858	$***$	0.594	$***$
Mean germination time	0.781	**	0.934	$***$	0.993	**	0.054	ΝS	0.304	$***$	0.666	$***$
Shoot length	0.108	<b>NS</b>	0.948	$***$	0.992	$***$	0.646	$***$	0.613	$***$	0.850	$***$
Root length	0.924	**	0.702	$***$	0.963	$***$	0.530	$***$	0.260	$***$	0.818	$***$
Shoot fresh weight	0.792	**	0.894	$***$	0.977	**	0.556	$***$	0.464	**	0.901	$***$
Root fresh weight	0.593	**	0.533	$***$	0.952	$***$	0.235	*	0.473	$***$	0.852	$***$
Shoot dry weight	0.517	**	0.602	$***$	0.902	$***$	0.459	$***$	0.559	$***$	0.858	$***$
Root dry weight	0.566	**	0.872	$***$	0.936	$***$	0.719	$***$	0.605	$***$	0.769	$***$
Root shoot ratio	0.895	**	0.881	$***$	0.986	$***$	0.452	$***$	0.232	*	0.401	$***$

*NS, \* and \*\* indicate non significance and significance at 0.05 and 0.01 probability levels, respectively*

**Table 7. Phenotypic (PCV) and genotypic (GCV) coefficient of variation for germination and seedling traits of 121 wheat genotypes under different NaCl concentrations**

<b>Traits</b>		<b>PCV (%)</b> ppm NaCl		<b>GCV (%)</b> ppm NaCl					
	0	3000	6000	9000	0	3000	6000	9000	
Germination percentage	10.16	8.80	24.63	71.45	8.45	7.51	20.58	47.95	
Germination rate index	10.26	9.51	35.19	89.81	8.01	8.05	29.77	59.75	
Mean germination time	9.26	12.11	24.14	74.52	7.61	10.25	19.11	52.59	
Shoot length	9.31	12.96	23.99	57.98	8.41	12.61	23.59	57.74	
Root Length	15.92	19.41	16.39	58.39	13.99	18.89	15.25	58.17	
Shoot fresh weight	21.39	14.57	38.95	88.99	21.35	14.57	38.92	84.55	
Root fresh weight	30.82	17.49	20.72	76.17	30.81	17.48	20.69	76.07	
Shoot dry weight	27.45	19.66	22.03	76.12	27.42	19.63	22.01	76.08	
Root dry weight	64.71	42.09	43.65	74.49	64.58	41.74	43.63	74.42	
Root/shoot ratio	195.74	42.96	50.95	94.76	61.29	42.62	50.95	94.59	



## **Table 8. Broad-sense heritability (h<sup>2</sup> b%) and expected genetic advance from selection (GA %) for germination and seedling traits of 121 wheat genotypes under different NaCl levels**

# **4. CONCLUSION**

In conclusion, traits showing strong correlations with STI, high heritability estimates, high expected selection gain in this study are SFW, SDW, RFW and SL at 9000 ppm and RDW and SL at 3000 and 6000 ppm. These traits showed also wide variability at the phenotypic and genotypic levels and therefore are recommended as selection criteria for seedling salinity tolerance under elevated levels of controlled salinity conditions.

# **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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