# Assessment of Salt Stress Effect on Wheat (*Triticum aestivum* L.) Cultivars at Seedling Stage

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## Summary

Salinity is one of the major abiotic environmental stresses affecting plant crops. The present study was conducted at the regional lab of the National Seed and Plant Control and Certification Center (CNCC) of Sétif, Algeria. The purpose of this study was to assess the behavior of twenty bread wheat (*Triticum aestivum* L.) varieties under different salt stress concentrations (0, 50, 100 and 150 mM NaCl) at seedling stage under hydroponic conditions. Accordingly, the results indicated that NaCl induced significant decreases in roots length and number, coleoptile length, root and shoot fresh weights; and each variety reacted differently as indicated by the 'genotype x salinity' effect. Moderate (100 mM) and high (150 mM) salt stress were the most discriminating traits between sensitive and tolerant cultivars. Based on salt sensitivity index (SSI), the evaluated genotypes were grouped into three clusters. SSI identified Mezghana ($V_1$), Almirante ($V_8$), Sensas ($V_{18}$), Florence Aurore ($V_{19}$) and Pinzon ($V_{20}$) as the most tolerant cultivars. These genotypes could be used in local wheat breeding programs for the improvement of salt tolerance.

## Key words

*Triticum aestivum*, NaCl, tolerance, germination, growth, SSI

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## Introduction

Cereals are an important part of the food resources of humans and animals. In Algeria, cereals are the main crops and are grown on an annual area of 3.3 million hectares (INRAA, 2016). Among cereals, bread wheat (*Triticum aestivum* L.) occupies a prominent place in the diet of Algerian population. The areas reserved to this species are located on the interior plains and highlands, in which this crop is exposed to different types of biotic and abiotic constraints (Fellahi et al., 2018). Among these constraints, salinity is one of the major abiotic stresses that has negative effect on wheat growth. According to Zörb et al. (2019), moderate soil salinity (80 – 100 mM ie 8 dS $m^{-1}$ – 10 dS $m^{-1}$) results in an average 28% wheat yield loss. Large parts of Algerian agricultural lands are threatened by salinity, especially those in arid zones, where irrigated cereals are annually cultivated (Belkhodja and Bidai, 2004). However, the exploitation of these lands has become inevitable to meet the needs of a constantly growing population since domestic production remains unable to overcome the local demand. This situation forces the country to import large quantities of wheat grain with highly estimated invoices (Fellahi et al., 2018). Salt stress is an excess of ions, particularly, but not exclusively, to $Na^+$ and $Cl^-$ ions (Zörb et al., 2019). By growing wheat plants in different salt solutions with or without $Na^+$ or $Cl^-$, Kingsbury and Epstein (1986) attributed the salt toxicity to $Na^+$ rather than $Cl^-$. The accumulation of these ions to toxic levels reduced the availability of water for the plants (Zhu, 2002). The damage caused by long-term salt stress exposure is mainly due to the ionic imbalance and toxicity due to $Na^+$ rather than the effect of salt on the water potential (Munns, 2002). Apart from $Na^+$ and $Cl^-$ ions, $Mg^{2+}$, $SO_4^{2-}$ and $HCO_3$ also contribute to salt toxicity. The amount of salts in the soil that plants can withstand without much damage varies with families, species (Flowers and Yeo, 1995; Levigneron et al., 1995), varieties (Niu et al., 2010) and even the stage of plant development (Flowers and Yeo, 1995). Among cereals, bread wheat is a moderately salt tolerant crop and can be grown in salinities up to 150 mM NaCl as long as rainfall and/or irrigation can rescue the crop at critical stages (Shabala and Munns, 2017). Durum wheat (*Triticum turgidum* ssp. *durum*) is less salt tolerant than bread wheat (Munns et al., 2006), rice (*Oriza sativa* L.) is much more salt sensitive (Shabala and Munns, 2017), and barley (*Hordeum vulgare* L.) is by far the most tolerant (Zörb et al., 2019). Selection of wheat cultivars for salinity tolerance can be done directly for a limited number of lines. However, when it is carried out on a large number of genotypes, the most suitable approach resides on the use of agronomic characteristics easily measurable and highly heritable (Adjel et al., 2013). According to Quarrie and Mahmood (1993), the plant vigor and yield stability are the main traits that can improve salt tolerance. Indeed, despite its limitations, plant vigor based-selection is an effective agronomic approach to select high yielding individuals under salt stress (Conway, 1997). Early stage selection is only possible following experiments conducted under controlled conditions, since larger-scale trials require more seed and fixed plant material (Hollington, 1998). As a result, germination tests and monitoring of seedling growth are an important step in the process of assessing salt stress tolerance. This work aims to identify the relative importance of agronomic parameters associated with salt tolerance in bread wheat (*Triticum aestivum* L.) genotypes from different origins, to screen the different wheat genotypes for their salt tolerance at seedling stage, and to evaluate the effectiveness of salt sensitivity index for screening and identification of salt tolerance wheat genotypes.

## Materials and Methods

### Growth conditions

The study was conducted at the National Seeds and Plants Control and Certification Center (CNCC), regional laboratory of Sétif, during March-April 2018. It was focused on 20 bread wheat (*Triticum aestivum* L.) registered varieties from different origins. Seeds were provided by the CNCC center. Table 1 shows the name, pedigree, and origin of each variety tested.

In order to determine the harmful effects of NaCl on wheat seedlings, a germination test was initially carried out, in the absence of salinity, under controlled conditions. Seeds of each genotype were surface sterilized with 5% sodium hypochlorite solution for 5 min and then rinsed with sterile distilled water thrice (Askari et al., 2017). Seeds were then germinated in 10 cm Petri dishes containing three layers of towel paper, which was moistened with 10 to 15 ml of distillated water. The experimental unit consisted of a Petri dish, carrying 100 seeds per variety with similar size. The dishes were then placed in a culture chamber of an automatic seed germinator (Ing. Climas type) at 22 °C, a 16/8 hours photoperiod, and 85.0% relative humidity average. Four dishes were used per variety, where three dishes served for germination test and the fourth one was intended to assess the effect of NaCl on seedlings growth.

For germination test, daily counts of germinated seeds were made during the seven days period. Seeds were considered germinated when their radicle was at least 2 mm out (Adjel et al., 2013). The final germination percentage (G, %) was determined, after 7 days of sowing, as the ratio of the number of germinated seeds (GS) to the total number of seeds incubated: $G\ (\%) = 100\ (GS/TS)$ (Shiferaw and Baker, 1996). The mean daily germination (MDG, seeds day-1) was obtained by dividing the cumulative germination percentage by the number of days since sowing (Scott et al., 1984).

For seedlings growth, germinated seeds (abnormal seeds were discarded) from the fourth Petri dish were transferred after 48 hours into test tubes containing 25 ml of distilled water (control) or NaCl solutions at three concentration levels: 50, 100 and 150 mM (i.e 3, 5.844 and 9 g $l^{-1}$). Ten seeds were used for each treatment. Salinized solutions were prepared by dissolving NaCl in distilled water at the required concentrations. Cotton was used as seed carrier in the test tubes, where one germinated seed per tube was considered. Salt levels were maintained daily by dripping out and applying fresh salt solution. A two-factorial experiment, arranged in a completely randomized design, with ten replications, was used. After 10 days of planting, seedlings at Zadoks scale 12 (Zadoks et al., 1974) were collected from the test tubes, their shoots and roots were carefully separated, and fresh weights (SW, mg and RW, mg) immediately recorded. Seminal roots number (RN, No.) and the maximum length of the seminal roots (RL, cm) were determined for each treatment. The length of the coleoptile (CL) was also measured using a graduated ruler.

**Table 1.** Name, pedigree and origin of the twenty wheat genotypes tested

| N° | Name | Pedigree | Origin |
|---|---|---|---|
| $V_1$ | Mezghana | Orchestrexs.306 | Serasem-France |
| $V_2$ | Anza | $LR/N_{10}B$ x $ANE_3$ | Cimmyt-Mexico |
| $V_3$ | Arz | $Mayo_{54}e/LR_{64}//H_{490}/3/LR_{64}//TPP/Yaktana_{54}$ | Cimmyt-Mexico |
| $V_4$ | Djanet | Acsad529/4/C182.24/C168.3/3/Cno*2/7C//CC/Tob | Acsad-Syria |
| $V_5$ | El Wifak | $K_{134}$/4/Tob/Bman/Bb/3/Cal/5/Bucc | Cimmyt-Mexico |
| $V_6$ | Mahon Demias | Land race | Balearic Islands |
| $V_7$ | Anapo | Eg 52 x Bel 118 | PRO.SE.ME-Italy |
| $V_8$ | Almirante | $H_{77215}$C/Recital | Serasem-France |
| $V_9$ | Djemila | 529//Prl4s4/Vee"S" | Acsad-Syria |
| $V_{10}$ | Hidhab | $HD_{1220}$/3*Kal/Nac | Cimmyt-Mexico |
| $V_{11}$ | Boumerzoug | CMSS93B00255S-48Y-010M-010Y-10M-7Y-0M- 4KBY-0KBY-0M | Cimmyt-Mexico |
| $V_{12}$ | Rmada | Vee's/Bow's//Alondra's/Pavon's | Acsad-Syria |
| $V_{13}$ | Hodna | Hodna | Acsad-Syria |
| $V_{14}$ | Bonpain | Prinqual x Cornette | Florimond Desprez-France |
| $V_{15}$ | Buffalo | 521/45 363/Cimmyt 12 | Serasem-France |
| $V_{16}$ | Tidis | Erena CM91575-28Y-0M-0Y-2M-0Y | Cimmyt-Mexico |
| $V_{17}$ | Salama | Salama | Florimond Desprez-France |
| $V_{18}$ | Sensas | So 179 x 32203 | Serasem-France |
| $V_{19}$ | Florence Aurore | Florence x $Aurore_{588}$ | Local Landrace |
| $V_{20}$ | Pinzon | Pinzon | Spain |

Acsad: Arab Center for the Studies of Arid Zones and Dry Lands, Cimmyt: International Maize and Wheat Improvement Center.

Salt sensitivity index (SSI) was calculated for each trait, as any decrease or increase relatively to the mean values of the control treatment, as follow:

$$SSI = \frac{1 - \frac{Y_S}{\bar{Y}_C}}{SI}$$

where:

$$SI = \frac{1 - \bar{Y}_S}{\bar{Y}_C}$$

$Y_S$ and $Y_C$ are the means of genotypes evaluated under saline (150 mM) and non-saline (0 mM) conditions, and $\bar{Y}_S$ and $\bar{Y}_C$ represent the mean value of all genotypes evaluated under saline and non-saline conditions, respectively (Fisher and Maurer, 1978).

### Data Analysis

The data concerning the variables measured were statistically analyzed using the Balanced analysis of variance (ANOVA) to test 'genotype', 'salinity' and 'genotype x salinity' effects. The model for the two-way ANOVA with interaction is:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_{ij} + \varepsilon_{ijk},$$

where $Y_{ijk}$ is the observation of the $i^{th}$ genotype, evaluated in the $j^{th}$ concentration level of salinity in the $k^{th}$ replicate; $\mu$ is the overall mean of the experiment; $\alpha_i$ is the effect of the ith genotype; $\beta_j$ is the effect of the concentration level of salinity; $\gamma_{ij}$ is the effect of the interaction between levels $i$ and $j$ of factors genotype and salinity; and $\varepsilon_{ijk}$ is the error. Whenever the ANOVA *F-test* was significant, the significance of the difference between treatment means was determined using Fisher's least significant difference test at the 5% probability level ($LSD_{0.05}$) according to Snedecor and Cochran (1980):

$$LSD_{0.05} = t_{0.05}\sqrt{2MSE/r}$$

where: $t_{0.05}$ is the tabulated value of the *t*-test at 5% probability level for *(g-1)(r-1)* residual degrees of freedom; *MSE* is the residual variance and *r* refers to the number of replications. Reduction of the traits measured in response to salinity level was assessed, in percentage, as compared to the control non-stressed. Genotypes with decreases less than 20% were considered as tolerant. When decreases were comprised between 21-40%, genotypes were qualified as semi-tolerant. Genotypes with decreases above 40% were considered as sensitive. In order to determine different bread wheat genotypes and their relationships, cluster analysis was applied (Jolliffe et al., 1989). The cluster analysis based on Euclidean distance was performed on the basis of the salt sensitivity index (SSI) by using Ward's method in 150 mM level of salinity. The statistical analyses were performed using CropStat 7.2.3 (2009) and Past software packages (Hammer et al., 2001), and a Microsoft Excel© spreadsheet.

## Results and Discussion

The results of the analysis of the variance showed a significant 'genotype', 'salinity' and 'genotype x salinity' significant effects ($p < 0.0001$) for all the traits measured (Table 2), suggesting the presence of a large genetic variability among the cultivars tested. A significant salt stress effect indicated that the NaCl concentration levels resulted in different reactions to the seedling growth parameters. The 'genotype x salinity' interaction effect revealed that the response of wheat cultivars varied among salt treatments. As indicated by the significant salinity main effect, NaCl accounted most of the total variation for all the measured traits including RL (67%), RN (85%), CL (70%), RW (61%) and SW (80%). The 'genotype' effect explained 30%, 9%, 26%, 30% and 16%; while the 'genotype x salinity' interaction accounted 3% 5%, 4%, 7% and 2% of the total variation observed, respectively for the same traits in the same order. The assessment of the relative importance of these sources of variations (S, G and G x S sum squares) is justified to take advantage of the salinity effect (Table 2). Hilal et al. (1998) as well as Askari et al. (2017) reported that contribution of the NaCl effect was proportionally greater than the main effect of the genotype and 'genotype x salinity' interaction.

In the absence of salinity, the germination percentage ranged from 78.4 to 97.6% (Fig. 1). This range may be attributed to the quality of seed used. According to the International Seed Testing Association rules (ISTA, 2019), seed with a germination percentage less than 85% is of quality. Seed quality is not only affected by the time between harvesting and sowing, but also by harvesting and storage conditions, including changes in temperature and relative humidity (Elis et al., 1990). Seed-associated diseases can also result in poor quality. Among the tested varieties, only Boumerzoug ($V_{11}$) had a germination rate lower than the 85% threshold. The other genotypes had on average germination estimates varying from 86.19% for Djanet ($V_4$) to 97.57% for Mezghana ($V_1$). In rice and barley, Alam et al. (2005) as well as Adjel et al. (2013) reported low seed germination in the absence of salt and related this to poor seed quality. The daily germination mean was recorded for Mezghana ($V_1$), Arz ($V_3$), El Wifak ($V_5$), Anapo ($V_7$), Almirante ($V_8$), Sensas ($V_{18}$), Florence Aurore ($V_{19}$) and Painzon ($V_{20}$) genotypes, with an average estimate greater than 14 seeds day$^{-1}$. The lowest rates were observed in Djanet ($V_4$) and Boumerzoug ($V_{11}$) varieties with average estimates lower than 12.5 seeds day$^{-1}$ (Fig. 1). These results exhibited a high relationship between the germination rate and daily germination mean, the more germination per day, the higher germination percentage (Fig. 1). These results are in agreement with the earlier reported findings of Aflaki et al. (2017).

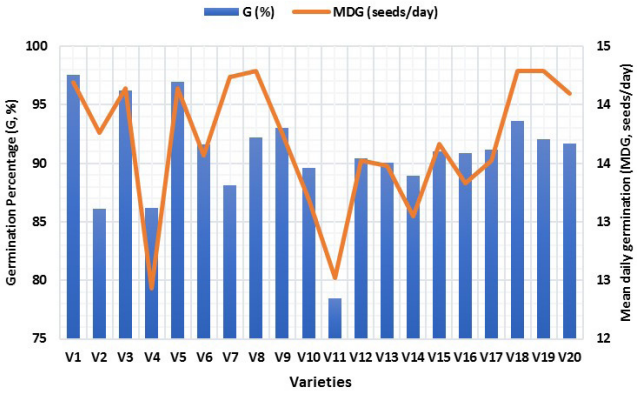


**Figure 1.** Germination percentage and mean daily germination seed averages of the tested varieties in the absence of salinity

For growth parameters, the average extreme minimum and maximum values recorded vary according to the trait measured, the variety tested, and the level of stress employed as indicated by the results of the analysis of variance (Table 2). Averaged over salt concentration levels, means of the measured variables (Table 3) indicated that El Wifak ($V_5$) exhibited the shortest roots (4.82 cm) and coleoptiles (1.43 cm) lengths, the lowest roots number (2.51 roots seed$^{-1}$), roots (13.25 mg) and shoot (29.98 mg) fresh weights. On the other hand, Florence Aurore ($V_{19}$) was distinguished by the highest number of roots (3.53 roots seed$^{-1}$) and the longest coleoptile (4.20 cm). Painzon ($V_{20}$) produced the highest roots (51.80 mg) and shoot (107.20 mg) fresh weights. The longest roots (13.42 cm) were recorded by Mezghana ($V_1$) cultivar.

Averaged over genotypes, NaCl decreased the roots length from 10.71 to 6.95 cm, the number of roots from 3.31 to 2.27 roots per germinated seed, the coleoptile length from 2.73 to 1.87 cm, shoot fresh weight from 78.81 to 34.75 mg and root fresh weight from 33.24 to 17.32 mg, as salinity increased from none to 150 mM NaCl treatment (Table 4). These results are in harmony with those obtained by Benderradji et al. (2011), Ben Naceur et al. (2001), Kadri et al. (2009), and Jlassi et al. (2014), who reported that growth parameters decreased significantly as salinity stress level increased. These researchers also mentioned that discrimination between tolerant and sensitive genotypes was effectively observed for the measured traits at high rather than at low NaCl concentration levels. Munns and Tester (2008) mentioned that the presence of salt ions around the roots had an immediate impact on the cell growth and all associated metabolisms. Zaman-Allah et al. (2009) added that species that maintain a relatively good root growth under high salt stress were more tolerant.

**Table 2.** Mean squares of the analysis of variance of measured traits in the twenty genotypes of wheat tested

| SV | df | G% | RL | RN | CL | RW | SW |
|---|---|---|---|---|---|---|---|
| Genotypes (G) | 19 | 53.80*** | 271.46*** | 5.026*** | 14.929*** | 4427.5*** | 16545*** |
| Salinity (S) | 3 | /// | 604.21*** | 47.352*** | 40.610*** | 8915.7*** | 81125*** |
| G x S | 57 | /// | 22.65*** | 2.759*** | 2.074*** | 1021.3*** | 2363*** |
| Error | 40 | 10.4 | 6.74 | 0.803 | 0.555 | 191.3 | 1315 |

SV = source of variation, df = degrees of freedom, G% = percent of seed germination, RL = root length, RN = roots number, CL= coleoptile length, RW = root fresh weight, SW = shoot fresh weight, *** = significant effect at 0.1% probability level.

**Table 3.** Mean values of measured traits in the twenty genotypes of wheat tested

| Genotype | RL (cm) | RN (No) | LC (cm) | RW (mg) | SW (mg) |
|---|---|---|---|---|---|
| $V_1$ | 13.42$^{a}$ | 3.05$^{bcd}$ | 2.75$^{c}$ | 73.95$^{b}$ | 34.28$^{b}$ |
| $V_2$ | 9.97$^{c}$ | 3.08$^{bc}$ | 2.70$^{c}$ | 63.20$^{b}$ | 34.03$^{b}$ |
| $V_3$ | 6.45$^{gh}$ | 2.25$^{gh}$ | 1.82$^{h}$ | 45.80$^{defg}$ | 18.73$^{defg}$ |
| $V_4$ | 5.60$^{hi}$ | 2.68$^{def}$ | 1.89$^{gh}$ | 39.50$^{fg}$ | 17.08$^{fg}$ |
| $V_5$ | 4.82$^{i}$ | 2.15$^{h}$ | 1.43$^{i}$ | 29.98$^{g}$ | 13.25$^{g}$ |
| $V_6$ | 6.92$^{fg}$ | 2.68$^{def}$ | 2.46$^{cde}$ | 49.03$^{defg}$ | 18.85$^{defg}$ |
| $V_7$ | 9.86$^{c}$ | 3.20$^{ab}$ | 2.11$^{fgh}$ | 61.43$^{c}$ | 25.33$^{c}$ |
| $V_8$ | 9.39$^{cd}$ | 3.25$^{ab}$ | 2.43$^{cdef}$ | 66.68$^{cd}$ | 24.13$^{cd}$ |
| $V_9$ | 9.17$^{cd}$ | 2.88$^{bcde}$ | 2.10$^{fgh}$ | 52.48$^{b}$ | 31.43$^{b}$ |
| $V_{10}$ | 8.40$^{de}$ | 2.58$^{efg}$ | 1.81$^{h}$ | 40.25$^{cdef}$ | 19.98$^{cdef}$ |
| $V_{11}$ | 7.52$^{efg}$ | 2.58$^{efg}$ | 2.49$^{cde}$ | 45.85$^{defg}$ | 19.03$^{defg}$ |
| $V_{12}$ | 9.87$^{c}$ | 2.73$^{cdef}$ | 2.17$^{efg}$ | 48.15$^{cde}$ | 23.43$^{cde}$ |
| $V_{13}$ | 6.82$^{fg}$ | 2.48$^{fgh}$ | 1.87$^{gh}$ | 47.33$^{fg}$ | 16.93$^{fg}$ |
| $V_{14}$ | 7.11$^{fg}$ | 2.63$^{efg}$ | 2.03$^{gh}$ | 51.35$^{efg}$ | 17.75$^{efg}$ |
| $V_{15}$ | 7.86$^{ef}$ | 2.90$^{bcde}$ | 2.13$^{fgh}$ | 43.23$^{cdef}$ | 21.03$^{cdef}$ |
| $V_{16}$ | 12.81$^{a}$ | 3.10$^{bc}$ | 2.36$^{def}$ | 62.15$^{g}$ | 13.53$^{g}$ |
| $V_{17}$ | 11.30$^{b}$ | 3.08$^{bc}$ | 2.53$^{cd}$ | 61.25$^{cdef}$ | 20.95$^{cdef}$ |
| $V_{18}$ | 11.22$^{b}$ | 3.08$^{bc}$ | 3.11$^{b}$ | 85.09$^{cde}$ | 23.65$^{cde}$ |
| $V_{19}$ | 12.91$^{a}$ | 3.53$^{a}$ | 4.20$^{a}$ | 102.70$^{a}$ | 48.50$^{a}$ |
| $V_{20}$ | 12.95$^{a}$ | 3.20$^{ab}$ | 3.12$^{b}$ | 107.20$^{a}$ | 51.80$^{a}$ |
| Mean | 9.22 | 2.85 | 2.37 | 58.83 | 24.68 |
| $LSD_{0.05}$ | 1.14 | 0.39 | 0.33 | 15.92 | 6.07 |

RL = root length, RN = roots number, CL= coleoptile length, RW = root fresh weight, SW = shoot fresh weight, Means followed by the same letter are not significantly different at 5% probability level by the Fisher's LSD test.

**Table 4.** Salinity main effects of measured traits in the twenty genotypes of wheat tested

| Salinity (mM) | RL (cm) | RN (No) | LC (cm) | RW (mg) | SW (mg) |
|---|---|---|---|---|---|
| 0 | 10.71$^{a}$ | 3.31$^{a}$ | 2.73$^{a}$ | 33.24$^{a}$ | 78.81$^{a}$ |
| 50 | 10.44$^{a}$ | 3.20$^{a}$ | 2.72$^{a}$ | 25.75$^{b}$ | 71.59$^{b}$ |
| 100 | 8.77$^{b}$ | 2.65$^{b}$ | 2.26$^{b}$ | 22.43$^{c}$ | 50.17$^{c}$ |
| 150 | 6.95$^{c}$ | 2.27$^{c}$ | 1.78$^{c}$ | 17.32$^{d}$ | 34.75$^{d}$ |
| Mean | 9.22 | 2.85 | 2.37 | 24.68 | 58.83 |
| $LSD_{0.05}$ | 0.510 | 0.176 | 0.146 | 2.715 | 7.120 |
| *F-test* | 24.78* | 36.57* | 81.56* | 16.49$^{ns}$ | 63.50* |

RL = root length, RN = roots number, CL= coleoptile length, RW = root fresh weight, SW = shoot fresh weight, Means followed by the same letter are not significantly different at 5% probability level by the Fisher's LSD test, ns and * = non-significant and significant effect at 5% probability level, *F-test* was used to test the regression linearity of the measured traits over the NaCl stress.

Relative to the control, the results of the present study indicated that low salt stress intensity (50 mM) had mild effect on the measured variables, suggesting tolerance to this salt stress level. Thus, this concentration level had a very limited ability to discriminate between the genotypes tested. However, under severe salt stress intensity (150 mM), the RL, RN, CL, RW, and SW estimates declined, as compared to the control non treated, by 35.10, 31.47, 47.91, 34.66, and 55.91%, respectively (Fig. 2).

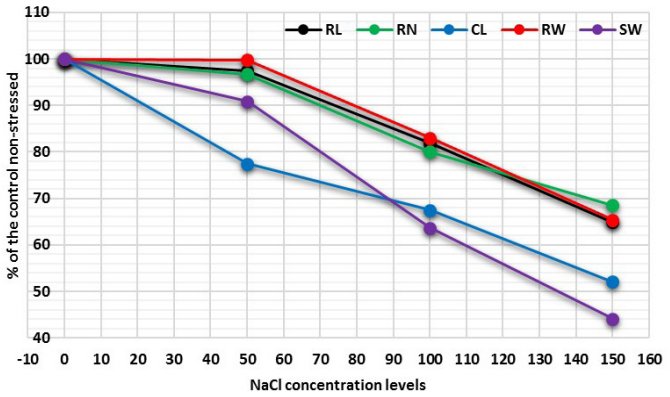


**Figure 2.** Decreases of the traits means in % to the control non-stressed

The decrease in root and shoot development may be attributed to toxic effects of the higher level of NaCl concentration as well as unbalanced nutrient uptake by the seedlings. These results are consistent with those of Zaman-Allah et al. (2009) who also showed that growth significantly decreased when salinity exceeded 4 g $l^{-1}$. Averaged over genotypes, except for RW that exhibited a quadratic decline ($p > 0.05$), RL, RN, CL, and SW decreased linearly ($p < 0.05$) (Table 4, Fig. 2), fitting the following regression equations:

$RL = -12.091\ [NaCl] + 116.29,\ (R^2 = 0.9253)$

$RN = -11.104\ [NaCl] + 114.07,\ (R^2 = 0.9481)$

$CL = -15.372\ [NaCl] + 112.68,\ (R^2 = 0.9761)$

$RW = -4.359\ [NaCl]^2 + 9.714\ [NaCl] + 95.43,\ (R^2 = 0.9847)$

$SW = -19.49\ [NaCl] + 123.37,\ (R^2 = 0.9695)$

The regression models explained 92.53%, 94.81%, 97.61%, 98.47% and 96.95% of the total variation, respectively, for RL, RN, CL, RW and SW as indicated by the coefficients of determination. This was pointed out in the study by Adjel et al. (2004), who clearly showed in their study on sixteen durum wheat cultivars that the regression equations could be used to estimate more conveniently and accurately the NaCl concentrations that reduce 50% the average genotype capacity for the measured variables. This advantage will be more obvious when a large number of genotypes have to be evaluated in salt tolerance breeding. In our current study, these salt concentrations were 224.1, 239.8, 228.0, 254.8 and 138.2 mM for RL, RN, CL, RW, and SW, respectively.

As compared to the control, reduction of the traits measured varied among genotypes in response to salinity level (Table 5). Reductions, in absolute values, below 20% (green symbol) for such a genotype indicated its tolerance in the considered NaCl level of stress. Similarly, absolute decrease estimates over 40% (red symbol) suggested that the genotype in question was sensitive to the NaCl concentration considered. Genotypes with reductions in absolute values comprised between 20% and 40% (yellow symbol) are semi tolerant with respect of the NaCl level of stress.

For root length, $V_4$, $V_{12}$, $V_{13}$ and $V_{15}$ were sensitive/semi tolerant over the whole range of salt treatments tested, showing a root length reduction varying from -20 to -31% under 50 mM NaCl and -32 to -69% under 150 mM NaCl. Except these four varieties, the others were tolerant under 50 mM NaCl stress. However, only $V_6$ and $V_8$ were tolerant under both 100 and 150 mM NaCl treatments. Genotypes $V_2$, $V_7$, $V_{11}$, $V_{14}$ and $V_{19}$ were tolerant under 100 mM, but sensitive or semi tolerant under 150 mM NaCl treatment (Table 5). The roots number showed approximately a similar pattern of responses. Cultivars $V_5$, $V_{13}$ and $V_{15}$ were sensitive/semi tolerant to salt stress while the remaining varieties showed tolerance at 50 mM NaCl, the relative decline of the roots number varied from -21 to -24%. Varieties $V_1$, $V_8$, $V_{10}$, $V_{12}$, $V_{16}$, $V_{17}$, $V_{18}$, $V_{19}$ and $V_{20}$ were also tolerant under 100 and 150 mM NaCl concentrations. $V_6$, $V_9$, $V_{11}$ showed their tolerance to salinity at both 50 and 100 mM NaCl stress, while the remaining genotypes were sensitive/semi tolerant under 100 mM and sensitive under 150 mM NaCl concentration levels (Table 5).

The coleoptile length measured at 50 mM NaCl was almost similar to that of the control treatment for all the tested entries, excluding $V_9$, suggesting tolerance to this salt stress level. The genotypes responded differently at 100 and 150 mM NaCl treatments (Table 5). Wheat genotypes including $V_3$, $V_4$, $V_5$, $V_7$, $V_9$, $V_{12}$, $V_{13}$, $V_{14}$ and $V_{15}$ were sensitive under 100 and 150 mM NaCl, while $V_2$, $V_6$ and $V_{11}$, and to a lesser extent $V_{16}$ and $V_{17}$ exhibited sensitivity under 150 mM NaCl stress.

Cultivars $V_3$, $V_4$, $V_6$, $V_8$, $V_{14}$, $V_{16}$, $V_{17}$, $V_{19}$ and $V_{20}$ were tolerant to the salt treatments tested at 50 mM NaCl stress, since their root fresh weights decreased by less than 20% from the values measured in the control non-stressed (Table 5). However, except for $V_{14}$, which was tolerant under 100 mM NaCl stress, all the plant material tested was sensitive/semi tolerant under 100 and 150 mM NaCl, showing a root fresh weights reduction varying from -48 to -84% and -26 to -89% under, respectively. Salt tolerant wheat genotypes were $V_1$, $V_2$, $V_6$, $V_7$, $V_8$, $V_9$, $V_{11}$, $V_{16}$, $V_{17}$, $V_{18}$ and $V_{19}$, reducing their shoot fresh weights to less than 20% at the 50 mM NaCl. In addition, $V_8$ and $V_{19}$ were tolerant under 100 mM, but the remaining genotypes were all semi tolerant or sensitive at 100 and 150 mM NaCl treatments, showing sizeable reduction in their shoot fresh weights over 20% and 40%, respectively (Table 5).

The overall results indicated that the behavior of wheat genotypes responses varied within each trait and between traits. This showed the variation of the tolerance among genotypes according to the various traits used as selection criteria. A given cultivar is tolerant or sensitive depending on the trait used for its classification, indicating the complex inheritance of salinity tolerance. In their study, Benderradji et al. (2016) reported that NaCl at 50, 100 and 200 mM caused significant decreases for all the morpho-physiological traits studied on durum and bread wheat cultivars, in contrast to biochemical traits that exhibited increased estimates. These authors concluded that the response to the salt stress varied depending on the genotype and the species, durum wheat seemed to be more affected by the NaCl abiotic constraints than bread wheat species.

**Table 5.** Decreases of measured traits in % of non-stressed treatment (genotypes with green, yellow and red symbols were considered as tolerant, semi tolerant and sensitive to the considered level of NaCl stress, respectively)

| Traits | RL | | | RN | | | CL | | | RW | | | SW | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NaCl (nM) | 50 | 100 | 150 | 50 | 100 | 150 | 50 | 100 | 150 | 50 | 100 | 150 | 50 | 100 | 150 |
| $V_1$ | -8 | -20 | -30 | -6 | -9 | -15 | -1 | -5 | -8 | -35 | -55 | -60 | -13 | -24 | -44 |
| $V_2$ | -4 | -20 | -48 | -20 | -50 | -63 | -9 | -11 | -56 | -43 | -61 | -84 | -2 | -32 | -75 |
| $V_3$ | -10 | -66 | -71 | -16 | -70 | -70 | -3 | -83 | -84 | 0 | -84 | -89 | -24 | -91 | -93 |
| $V_4$ | -24 | -32 | -69 | -3 | -31 | -69 | -8 | -49 | -65 | -10 | -57 | -68 | -20 | -65 | -76 |
| $V_5$ | -2 | -41 | -64 | -21 | -45 | -73 | -14 | -71 | -66 | -34 | -62 | -80 | -20 | -73 | -77 |
| $V_6$ | -6 | -13 | -15 | -7 | -13 | -23 | -12 | -13 | -57 | -9 | -21 | -30 | -12 | -31 | -68 |
| $V_7$ | -14 | -19 | -25 | -15 | -37 | -37 | -3 | -25 | -27 | -30 | -35 | -42 | -7 | -30 | -39 |
| $V_8$ | -8 | -12 | -15 | -3 | -17 | -19 | -8 | -10 | -10 | -17 | -21 | -26 | 0 | -14 | -25 |
| $V_9$ | -18 | -30 | -32 | 0 | -3 | -26 | -22 | -21 | -30 | -23 | -35 | -57 | -9 | -38 | -40 |
| $V_{10}$ | -4 | -37 | -42 | -7 | -11 | -14 | -7 | -15 | -19 | -28 | -53 | -54 | -25 | -36 | -38 |
| $V_{11}$ | -5 | -11 | -50 | -13 | -17 | -27 | -7 | -19 | -44 | -58 | -63 | -76 | -15 | -40 | -71 |
| $V_{12}$ | -20 | -26 | -32 | -10 | -10 | -17 | -15 | -22 | -30 | -37 | -52 | -55 | -28 | -38 | -54 |
| $V_{13}$ | -21 | -26 | -67 | -24 | -24 | -52 | -17 | -25 | -78 | -48 | -79 | -84 | -38 | -44 | -81 |
| $V_{14}$ | -2 | -4 | -47 | -11 | -29 | -60 | -4 | -29 | -65 | -11 | -12 | -73 | -28 | -48 | -80 |
| $V_{15}$ | -31 | -37 | -60 | -21 | -40 | -62 | -1 | -23 | -59 | -66 | -72 | -74 | -37 | -46 | -68 |
| $V_{16}$ | -15 | -31 | -44 | -3 | -3 | -18 | -3 | -4 | -28 | -17 | -27 | -38 | -9 | -21 | -51 |
| $V_{17}$ | -14 | -37 | -48 | 0 | -12 | -15 | -6 | -10 | -21 | -10 | -26 | -42 | -12 | -28 | -48 |
| $V_{18}$ | 0 | -25 | -34 | -3 | -3 | -9 | -3 | -11 | -11 | -23 | -63 | -72 | -6 | -33 | -43 |
| $V_{19}$ | -12 | -12 | -27 | -13 | -15 | -20 | 0 | -2 | -12 | -12 | -30 | -43 | 0 | -13 | -35 |
| $V_{20}$ | -6 | -21 | -27 | -3 | -6 | -15 | -4 | -6 | -8 | -20 | -51 | -52 | -53 | -59 | -66 |

RL = root length, RN = roots number, CL= coleoptile length, RW = root fresh weight, SW = shoot fresh weight

According to our results, a genotype with a highly appropriate response to a certain salinity level for a such trait cannot necessarily be considered a tolerant genotype. In order to have a clear picture of the response pattern of the tested genotypes, the salt sensitivity index (SSI) was calculated for each trait. SSI estimates ranged from 0.20 in $V_{20}$ to 2.19 in $V_3$ for the coleoptile length (data not shown). The lower values of SSI indicated lower differences in biomass accumulated across stressed and non-stressed conditions and hence more stability and indicated genotypes performing well under stress with sufficient plasticity to respond to the potential environment. To summarize the results of SSI obtained, the genotype responses were sought through hierarchical clustering technique. The advantages of using this approach in the evaluation of salt tolerance are that it allows: (i) a simultaneous analysis of multiple parameters to increase the accuracy of the genotype ranking; (ii) the ranking of genotypes even when plants are evaluated at different salt levels and salt tolerance varies with salinity levels (i.e. high 'genotype x salinity' interaction); and (iii) a more convenient and accurate estimation of salt tolerance among genotypes by simply adding the numbers in cluster group ranking at different salt levels (Zeng et al., 2002). The cluster analysis grouped the twenty genotypes tested into three clusters (Fig. 3).

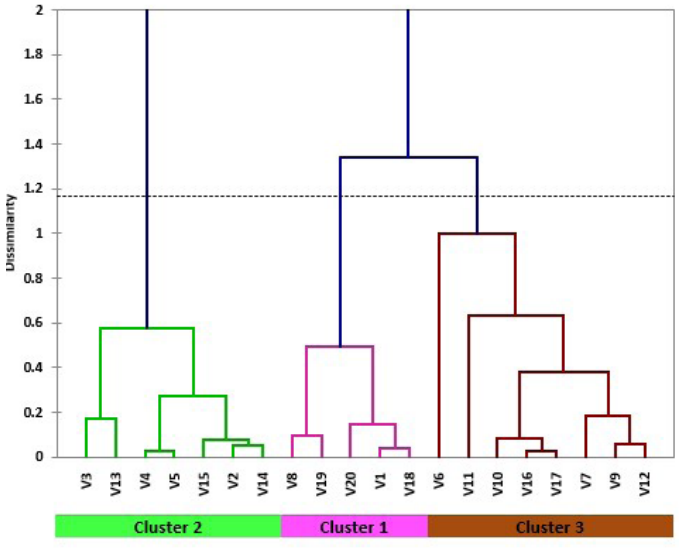


**Figure 3.** Dendrogram of classified genotypes using hierarchical cluster analysis

Cultivars $V_1$, $V_8$, $V_{18}$, $V_{19}$ and $V_{20}$ clustered together (cluster 1), $V_2$, $V_3$, $V_4$, $V_5$, $V_{13}$, $V_{14}$ and $V_{15}$ formed the second cluster (cluster 2), while the third cluster (cluster 3) contained the remaining varieties (Fig. 3).

Genotypes of the first cluster had low SSI values, they are considered as the most tolerant and desirable for salty growth conditions. In the sensitive group (cluster 2), the genotypes had high SSI values, demonstrating their susceptibility to salt and aptness only under normal conditions. Genotypes of the third group (cluster 3) had intermediate SSI estimates, they were qualified as semi tolerant. The average of SSI per cluster indicated that genotypes belonging to clusters 2 and 3 significantly decreased their roots length (62.38% and 26.37%), roots number (54.32% and 15.82%), coleoptile length (69.24% and 40.05%), root (71.15% and 40.29%) and shoot (74.70% and 45.54%) fresh weights under 150 mM salt treatment compared to the tolerant genotypes of cluster 1. The results obtained through cluster analysis indicated that SSI-based selection may provide more suitable for improving salt tolerance of wheat at early growth stage. Askari et al. (2017) used multiple statistical procedures to assess the effect of salinity stress in barley and showed that mean productivity and geometric mean productivity indices could be a useful indicator of desirable genotypes in high level of salinity at early growth stage. Generally, in the present screening experiment, wide genotypic differences were observed for all the studied parameters indicating that evaluation for salt tolerance among genotypes can be done at early stage of plant growth.

## Conclusion

In this study salinity affected all the measured traits which decreased relatively to the values of the control treatment at early stage. The results indicated too that the sensitivity/tolerance of the tested genotypes varied among the measured traits. Moderate and high salt treatments were more discriminating between salt tolerant and salt sensitive genotypes than the low salt treatment. Due to a significant 'genotype x salinity' interaction effect, selection of genotypes with best performance for a level of salinity based on their mean value in other levels seems to be less efficient. Nevertheless, some genotypes maintained a certain level of tolerance under moderate and high salinity stress. Mezghana ($V_1$), Almirante ($V_8$), Sensas ($V_{18}$), Florence Aurore ($V_{19}$) and Pinzon ($V_{20}$) behaved as tolerant bread wheat cultivars as confirmed by the SSI index and cluster analysis. These findings are very useful for the planning of further wheat breeding programs for wheat salt tolerance.

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