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RESEARCH PAPER

# Effect of salinity stress on some physiological traits of spring rapeseed genotypes at seedling stage

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**Key words:** *Brassica napus* L., growth parameters, physiological traits, $K^+/Na^+$, salt stress.

## Abstract

To study the effects of salinity on rapeseed (*Brassica napus* L.) and clear the role of $K^+$ and $Na^+$ in salt stress tolerance, an experiment was carried out in three salinity levels (0, 150 and 300 mM) and 16 genotypes with three replications under hydroponic culture system in greenhouse of university of Tabriz. Growth and physiological traits were measured 28 days after imposing NaCl stress at the end of seedling stage (42 days old).Salinity stress was affected all of the traits significantly. All of the traits were decreased by salt stress except electrolyte leakage and $Na^+$ content of shoot and root. Significant differences were observed among genotypes in all of the studied traits, which indicate valuable genetic variability between the genotypes. Safi7 and Option500 genotypes with the highest and lowest performance in growth and physiological traits were identified as tolerant and susceptible genotypes, respectively. Safi7 genotype has the lowest $Na^+$ content of shoot and highest shoot $K^+/Na^+$ ratio while genotype Option500 has the highest $Na^+$ content of shoot and the lowest shoot $K^+/Na^+$ ratio. Due to the amount of $K^+$ in shoots and roots weren't significantly changed in genotypes; it seems rapeseed genotypes cope with salt stress by removing undesirable $Na^+$ ions, not through adsorption of $K^+$ ions.

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## Introduction

Soil salinity is a global problem that via the effect on plant growth and limit the production of agricultural lands, has made agricultural production difficult (Joseph *et al.*, 2010). Salt as one of the most important a biotic stresses in arid and semi-arid areas, reduces the average crop yield about 50% (Kandil *et al.*, 2012). After cereals and beans the oil seeds have third place in the human needs of food and among the oil seed crops, *Brassica* species holds third position (Shirazi *et al.*, 2011). Although rapeseeds classified as semi-tolerant plants (Miyamoto *et al.*, 2012), but water and soil salinity are the most important factor that limiting production of rapeseeds (Purty *et al.*, 2008), and breeding and deployment of tolerated genotypes to salinity, especially in the seedling stage (Ashraf and McNeilly, 2004) can be a suitable solution in order to maintain the production levels in the face of salt stress (Ashraf and Akram, 2009).

Since the salt stress cause's dehydration and drying of the tissues, the ionic toxicity and food imbalances or a combination of them, salt tolerance has a complex mechanism (Zhu, 2001). Rapeseed cultivars and genotypes which have good seedling performance under salt stress, in other developmental stages was also more tolerant and keep their performance high (Athar *et al.*, 2009) so this doubled the importance of studying the salinity in the seedling stage. Salt stress at seedling stage decreased traits such as shoot and root dry weight and fresh weight, root length, shoot height and leaves number (Miyamoto *et al.*, 2012).

Root growth compared to growth of shoot less affected by salinity, but it causes morphological changes in some species (Bandehagh *et al.*, 2013). Salinity also affects water relations. Osmotic adjustment in plants is one of the mechanisms involved in tolerance to salinity. Osmotic adjustment is a decrement in osmotic potential by increasing soluble or decreasing the water level of plant in response to decreasing of the soil water potential (Chinnusamy *et al.*, 2006). In response to salt stress, osmotic adjustment by accumulation of a series of organic regulators (e.g., proline) and minerals (such as $Na^+$ and $K^+$) occurs in cells that caused absorb water into them (Shirazi *et al.*, 2011) and cellsturgor will be maintained (Nayyar, 2003). It should be noted that absorption of sodium and potassium ions is a less costly osmotic adjustment way (Morant-Manceau *et al.*, 2004). Proline increasing has been reported as one of the salinity injuries in rapeseed genotypes (Bandeh-hagh *et al.*, 2008), which is explained the high proline content in susceptible rapeseed genotypes (Dolatabadi *et al.*, 2012).

In general, two mechanisms were known as salt tolerance in higher plants: By the first mechanism, the plant in response to salinity, excrete toxic ions such as sodium and chlorine from their leaves (De Lacerda *et al.*, 2005). In the second mechanism, ions are absorbed by the cells accumulate in the vacuoles (Parida and Das, 2005). High concentration of sodium ions disturbs the food balance and also creates ionic toxicity and disorder osmotic adjustments (Munns and Tester, 2008).

High concentrations of salt cations such as $Na^+$ (key elements of salt stress) can disrupt or even prevent the biochemical processes in plant tissues and synthesis of required proteins were changed quantitatively and qualitatively under such circumstances, which in turn disrupt the growth process (Gul *et al.*, 2014). By studying the effect of salinity on *Brassica* species, it is known that the amount of sodium and potassium ions under saline conditions are increased and reduced receptively in shoots and roots (Asghari *et al.*, 2011; Shirazi *et al.*, 2011; Toorchi *et al.*, 2011). In the other hand a significant positive correlation between the performance and the ratio of $K^+/Na^+$ have been reported (Bandeh-hagh *et al.*, 2008) whereas negative correlation between performance and increasing the amount of sodium ions have seen in rapeseed genotypes under salinity stress (Shirazi *et al.*, 2011). It seems that the $K^+/Na^+$ ratio in shoot and root is a good selection criterion for identification of salt tolerant varieties in breeding programs. Hence, this investigation was planned to study the effect of salinity on the growth of rapeseed seedlings to clear the role of $K^+/Na^+$ ratio to salt tolerance under varying levels of NaCl stress.

## Materials and methods

### *Plant material and experimental treatments*

To study the effects of salinity on rapeseed genotypes a split plot experiment based on randomized complete block design with salinity as main factor and genotypes as sub factor in three replications was conducted in hydroponics culture system under greenhouse conditions. Seeds of 16 genotypes of spring rapeseed (SAN-2, SAN-6, SAN-13, SAN-17, Safi-3, Safi-5, RGS003, S8-901-123, Hyola401, A mica, Olga, Goliath, Option 500, Hero's, Hyola308 and Sarigol) were sterilized and germinated in Petri dishes and seven days later these plantlets were transferred to hydroponic culture system in which they were irrigated four times daily with a modified Hoagland nutrient solution. One week after putting the plantlets in the hydroponic system, salinity stress (0, 150, 300 mM) was started by adding 50 mM of NaCl in some continuous days.

### *Measurements*

Measuring different traits were done 28 days after imposing salinity stress (42 days old) at the end of seedling stage just before flowering stage began. Shoot and root dry weight, number of leaves, plant height, root length, electrolyte leakage (EL) and relative water content (RWC), sodium and potassium content of shoot and root and $K^+/Na^+$ ratio in shoot and root were measured/calculated. To calculate RWC the third fully expanded youngest leaf was taken and five leaf discs (1.0 cm diameter) of each leaf were sampled and immediately weighted as fresh weight (FW). Then, they were immersed in distilled water in Petri dishes for 24 h at 4°C in darkness to obtain turgid weight (TW). The discs were dried in an oven at 75°C for 48 h and the dry weight (DW) determined. Finally RWC was calculated as $RWC\ (\%) = (FW-DW)/(TW-DW) \times 100$ (Morant-Manceau *et al.*, 2004). EL was measured by using five discs of third young leaf for each treatment. Discs were washed with deionized water to remove surface adhered electrolytes. Leaf discs were placed in closed vials containing 10 ml of deionized water and were shaken for 24 h. Then electrical conductivity of the solutions was determined ($L_1$). Samples were autoclaved at 100°C for 30 min and then the electrical conductivity of each sample was defined ($L_2$) after cooling the samples to the laboratory temperature. EL was calculated using the formula $L_1/L_2 \times 100$ (Nayyar, 2003). Shoot and root dry weights were determined after drying the samples in oven at 75°C for 48 h. The amounts of sodium and potassium ions were measured in dried leaf and root samples. Then dried samples were powdered and weighted. Samples were dissolved in 8ml nitric acid (7.2 N) by heating on hot plate. Extract of the samples were filtered and by adding distilled water bring the final volume to 50 ml. Finally K and Na content were assayed by flame photometer.

### *Statistical analysis*

Analysis of variance of traits was performed in a split plot with a randomized complete block design with three replications and mean comparisons were done by using Duncan's multiple range test. MSTATC, IBM SPSS Statistics 20 and Excel computer programs were used for statistical analysis.

## Result and discussion

Analysis of variance based on split plot in randomized complete block design for 16 rapeseed genotypes and salinity levels has been shown in Tables 1 and 2. In all traits a significant difference were observed between salinity levels. Significant difference between salinity levels in measured traits indicates that these traits which respond to salinity could be the possible mechanisms of salt tolerance in rapeseed genotypes. Shoot fresh and dry weight and plant height differences were significant between genotypes at 1% probability level which shows high variability among the genotypes for these traits. Root fresh and dry weight, root length, leaf number, electrolyte leakage, relative water content, leaf sodium content and $K^+/Na^+$ ratio in leaves a significant difference at 5% probability level was observed among genotypes. There was no significant difference between genotypes in root sodium and potassium content, eaves potassium content and root volume. For all traits, genotypes salinity levels interaction was not significant at 5% probability level. The shoot and root fresh and dry weight, leaf number, RWC, shoot potassium content $K^+/Na^+$ ratio in shoot and root were decreased in response to salinity stress, While electrolytic leakage, shoot and root sodium content, were increased under salinity (Fig. 1-3).

**Table 1.** Analysis of variance for some studied traits in rapeseed genotypes under salinity treatments.

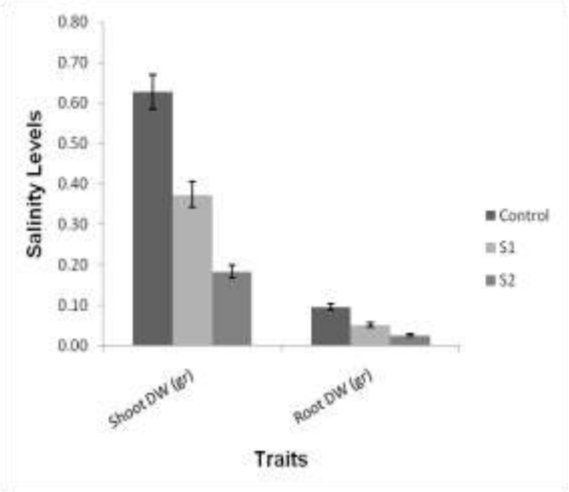
| | | Mean of Square | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Source of Variance | Degrees of Freedom | Shoot Dry Weight | Root Dry Weight | Shoot Height | Root Length | Leaves Number | EL | RWC |
| Replication | 2 | $1.857^{*}$ | $4.282^{**}$ | $186.083^{ns}$ | $18.920^{ns}$ | $6.498^{ns}$ | $5152.413^{**}$ | $484.183^{**}$ |
| Salinity (S) | 2 | $4.025^{*}$ | $5.025^{**}$ | $1402.069^{**}$ | $70.501^{*}$ | $16.755^{*}$ | $5802.957^{**}$ | $1322.125^{**}$ |
| Error (a) | 4 | 0.236 | 0.188 | 44.868 | 7.059 | 1.336 | 268.856 | 12.711 |
| Genotype (G) | 15 | $0.144^{**}$ | $0.137^{*}$ | $23.760^{**}$ | $5.252^{*}$ | $0.576^{*}$ | $176.948^{*}$ | $28.732^{*}$ |
| S×G | 30 | $0.043^{ns}$ | $0.068^{ns}$ | $4.830^{ns}$ | $2.450^{ns}$ | $0.195^{ns}$ | $78.174^{ns}$ | $8.887^{ns}$ |
| Error (b) | 90 | 0.058 | 0.070 | 6.938 | 2.908 | 0.284 | 81.760 | 14.611 |
| CV% | | 44.40 | 18.51 | 12.75 | 15.06 | 10.97 | 24.03 | 5.38 |

*Significant at the 5% probability level; ** significant at the 1% probability level; ns: Not significant.

**Table 2.** Analysis of variance for some other studied traits in rapeseed genotypes under salinity treatments.

| | | Mean of Square | | | | | |
|---|---|---|---|---|---|---|---|
| Source of Variance | Degrees of Freedom | Leaf $Na^+$ | Leaf $K^+$ | Leaf $K^+/Na^+$ | Root $Na^+$ | Root $K^+$ | Root $K^+/Na^+$ |
| Replication | 2 | $121.362^{*}$ | $1.809^{ns}$ | $9.741^{ns}$ | $1729.717^{ns}$ | $865.139^{**}$ | $0.332^{ns}$ |
| Salinity (S) | 2 | $655.812^{**}$ | $115.859^{**}$ | $58.193^{**}$ | $21492.094^{**}$ | $249.949^{*}$ | $4.526^{**}$ |
| Error (a) | 4 | 7.056 | 0.820 | 1.518 | 705.316 | 25.126 | 0.050 |
| Genotype (G) | 15 | $4.431^{*}$ | $0.360^{ns}$ | $0.402^{*}$ | $564.882^{ns}$ | $68.309^{ns}$ | $0.019^{ns}$ |
| S×G | 30 | $2.012^{ns}$ | $0.416^{ns}$ | $0.235^{ns}$ | $564.980^{ns}$ | $112.410^{*}$ | $0.026^{ns}$ |
| Error (b) | 90 | 1.986 | 0.435 | 0.211 | 416.324 | 60.557 | 0.023 |
| CV% | | 18.59 | 12.49 | 26.97 | 51.02 | 32.45 | 23.57 |

*Significant at the 5% probability level; ** significant at the 1% probability level; ns: Not significant.



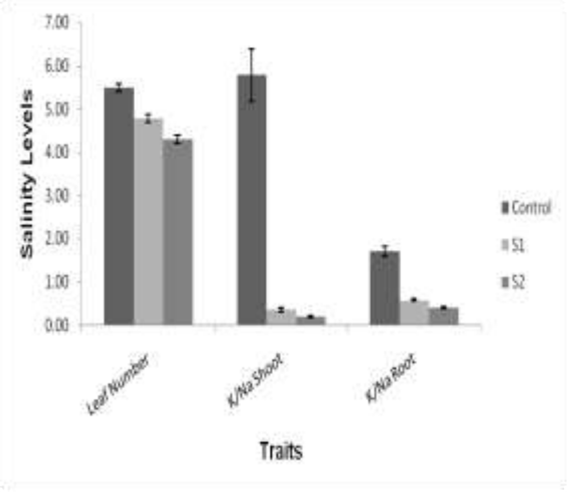


**Fig. 1.** Mean of some traits according to salinity levels.

**Fig. 2.** Mean of some other traits according to salinity levels.

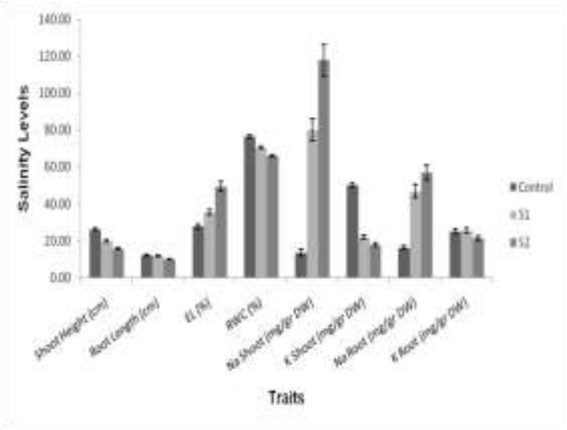


**Fig. 3.** Mean of some other traits according to salinity levels.

Electrolyte leakage was increased under salinity due to the increment of metabolites and electrolytes leakage in response to concentration of ions, which associated with increment of $Cl^-$ and $Na^+$ entering and the exclusion of $K^+$. Accordingly, more tolerant cultivars have lower electrolyte leakage (Sairam *et al.*, 2002; Bennabi *et al.*, 2013). Shoot and root dry weight, leaves number and shoot height significantly decreasing under salt stress were reported previously (Mer *et al.*, 2000; Ashraf and McNeilly, 2004; Bandeh-hagh *et al.*, 2008).

Under salt stress, leaf area expansion and the height decrease much faster than other morphological characteristics and resistant cultivars were tended to retain much more of these variables (Heidari *et al.*, 2011; Hajiaghaei Kamrani *et al.*, 2013). Increment of sodium in shoot and root and $Na^+/K^+$ ratio in the shoots and roots has been reported under salt stress (Ashraf and McNeilly, 2004; Parida and Das, 2005). The same reports showed a reduction in shoot and root potassium concentration due to antagonistic relationship between $Na^+$ and $k^+$ under salt stress. The presence of $Na^+$ in the environment prevents the absorption of $K^+$. Susceptible and tolerant cultivars were identified as high concentrations of sodium and potassium ions in their tissues, respectively (Haq *et al.*, 2014). Therefore high $K^+/Na^+$ ratio in shoot and root identified tolerant genotypes. Previous reports suggesting that cultivars with higher shoot and root dry weight are tolerant varieties (Ashraf, 2004; Bandeh-hagh *et al.*, 2008; Gupta and Huang, 2014). Also genotypes with lower reduction rate of plant height, root length, leaves number under salt stress conditions were considered as tolerant varieties. Leaf relative water content decreased under salt stress and genotypes with less decreasing are more tolerant to salt stress (Parida and Das, 2005).

Safi 7 for most traits such as shoot and root dry weight, plant height, electrolyte leakage, leaf sodium and $K^+/Na^+$ has the best performance and has a suitable conditions in number of leaves per plant and RWC traits (Fig. 4-6). Option 500 showed the lowest performance for all traits, especially in growth parameters such as shoot and root dry weight; plant height and root length were obvious (Fig. 4-6). This genotype in biochemical traits such as sodium content of shoot and shoot $K^+/Na^+$ ratio has the lowest performance, while has a good relative water content. This indicates the fact that despite the availability of enough water, the plant is still unable to maintain its performance under salt stress. One of the main reasons could be ionic toxicity which is known as the main factor of reducing the plants performance under salt stress. The high sodium content of shoot and low potassium to sodium ratio in the leaves of Option 500 confirm $Na^+$ toxicity. In previous studies the importance of osmotic adjustment by excretion of sodium ions and replace it by potassium is mentioned in rapeseeds (Ashraf and McNeilly, 2004; Toorchi *et al.*, 2010).

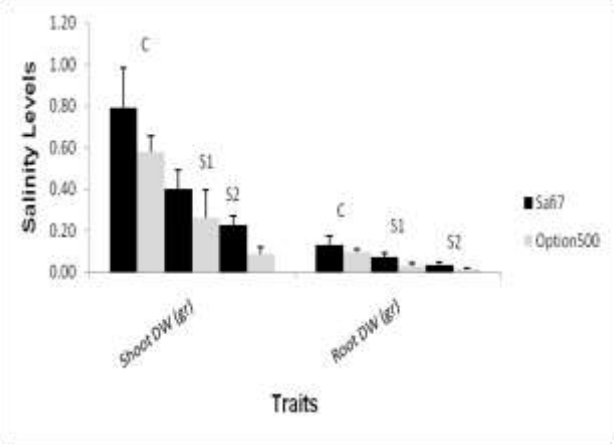


**Fig. 4.** Comparing of Safi7 and Option500 in

different salinity levels (C=zero, S1=150 and S2=300 mM of NaCl treatment).

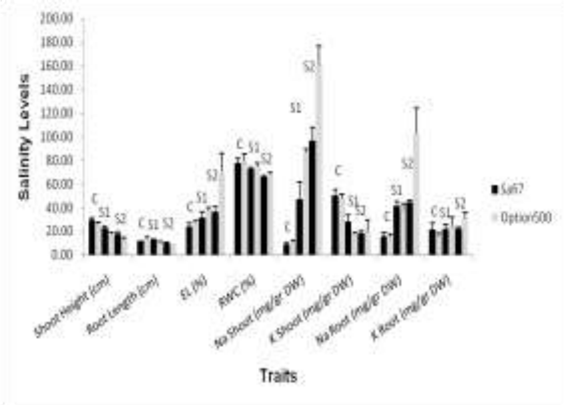


**Fig. 5.** Comparing of Safi7 and Option500 in different salinity levels (C=zero, S1=150 and S2=300 mM of NaCl treatment) by some traits.

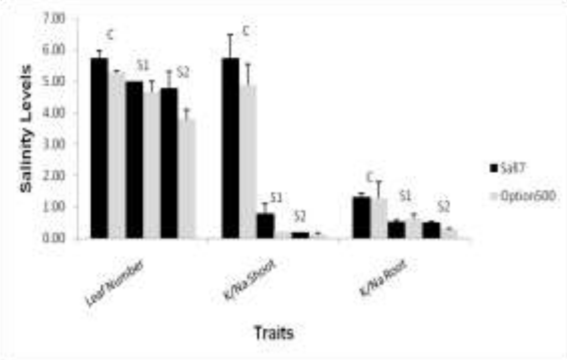


**Fig. 6.** Comparing of Safi7 and Option500 in different salinity levels (C=zero, S1=150 and S2=300 mM of NaCl treatment) by some other traits.

## Conclusion

In general, by studying the tolerant and sensitive genotypes of rapeseed to understand physiological mechanisms of tolerance to salinity, it was revealed that tolerant genotypes tend to repel $Na^+$ to prevent the devastating effects of it. The high concentration of sodium ions in plant tissues not only disrupt the biochemical processes even can be prevented them from implementing. Also the syntheses of required proteins under such conditions were changed quantitatively and in the same way qualitatively, which caused a disturbance in the growth process of plants. Our results suggest that tolerant genotypes are more successful in excretion of sodium ions. And the excretion of $Na^+$ from leaves which resulting high $K^+/Na^+$ ratio nor $K^+$ absorption was one of the several strategies that were evolved in salt tolerant rapeseed genotypes to maintain growth under high NaCl concentrations.

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