



## The response of spring rapeseed genotypes (*Brassica napus* L.) under salinity stress

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### Abstract

Abiotic stresses such as salinity, are factors that severely affects agricultural production. To evaluate the effect of salt stress on some morphological and physiological traits related to salt tolerance, 22 spring type genotypes of rapeseed (*Brassica napus* L.) in the vegetative growth stage an experiment was conducted as a split plot form based on Randomized Complete Blocks Design using levels of salinity: zero (control), 150 and 300mM sodium chloride with three replications were done in the research greenhouse of Tabriz university. In present study, the Na<sup>+</sup> contents of leaves and roots and electrolyte leakage were significantly increased. Also the K<sup>+</sup> contents of leaves and roots, K<sup>+</sup>/Na<sup>+</sup> ratios in shoot and root, shoot height and root length were measured significantly in response to salinity stress. Significant differences between genotypes in all traits except Na<sup>+</sup> contents of root were observed. Cluster analysis led to the separation of genotypes in to four groups. Results showed identified Safi-7, Safi-3, Hyola308, SAN-17 and SAN-2 in most traits has higher means and ranked as tolerant genotypes. The genotypes Sarigol, Safi-5, Option500, SAN-3, SAN-13, Zafar, Goliath, SAN-8 and Heros in most traits has lower means and ranked as sensitive genotypes to salinity stress. So can be used this genotypes to understanding the mechanisms involved in salt tolerance and salt tolerance genes involved in this process.

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## Introduction

Salinity is the major abiotic stress limiting plant growth and productivity (Allakhverdiev *et al.*, 2000). Over 6% of the world's land is affected by high salinity (Munns and Tester, 2008). Salinity will reduce plant growth by osmotic stress, ion toxicity and nutritional disturbances. Oilseed crops after the cereals is a important food source (Ashraf and McNeilly, 2004). Canola (*Brassica napus* L.) has a high adaptability under the salinity stresses and is a semi-tolerant group (Ashraf and McNeilly, 2004). Salt stress disturbed many physiological and biochemical changes in different crops and reduced the plant growth and yield (Athar *et al.*, 2009). The most common adverse effect of salinity on the crop of Brassica is the reduction in plant height, size and yield (Zamani *et al.*, 2010). Farhoudi *et al.* (2010) reported that salt stress decreased canola cultivar root length, shoot length and seedling dry weight. High levels of  $K^+$  in young expanding tissue is associated with salt tolerance in many plant species (Bandehhagh *et al.*, 2008; Khan *et al.*, 2009; Mer *et al.*, 2000). The research by Ghaedr ahmati *et al.* (2013) has shown that in response to Salinity stress decreased  $K^+$  concentration and  $K^+/Na^+$ ; however  $Na^+$  concentration was increased in genotypes. Electrolytic leakage increases under salinity stress (Dolatabadi and Toorchi, 2017) and tolerant genotypes usually indicate lower electrolytic leakage (Sairam, *et al.*, 2002). Bandehhagh *et al.* (2008) reported that the effect of salinity on canola genotypes, With increasing NaCl,  $Na^+$  accumulated while  $K^+$  decreased. The salinity stress tolerance of cultivars are mostly liable to osmotic adjustment by removing sodium and absorption of potassium (Dolatabadi *et al.*, 2012). The objective of this study was to investigate the response of rapeseed genotypes to salinity in terms of physiological and biochemical traits and grouping genotypes.

## Materials and methods

### *Plant material and experimental treatments*

In this research 22 spring type genotypes of rapeseed (SAN-2, SAN-3, SAN-6, SAN-8, SAN-13, SAN-17, Safi-3, Safi-5, Safi-6, Safi-7, Zabol-0, Zafar, RGS003,

S8-901-123, Hyola401, Amica, Olga, Goliath, Option500, Heros, Hyola308 and Sarigol) constituted the plant material for this experiment. Seeds were sterilized and germinated in petri dishes and 7 days later these genotypes were arranged in a split plot based on randomized complete block design with three replications was conducted in hydroponics culture system under greenhouse conditions. Salinity treatments of zero (control), 150 ( $s_1$ ) and 300 ( $s_2$ )mM of NaCl were imposed to the plants in which they were irrigated four times daily with a modified Hogland nutrient solution (Bandehhagh *et al.*, 2008). One week after putting the plantlets salinity stress was imposed gradually by adding 50mM of NaCl per day.

### *Measurements*

Measuring different characteristics were done four weeks after imposing salinity stress. Electrolyte leakage, plant height, root length, sodium and potassium content of leaf and root, and  $K^+/Na^+$  ratio in leaf and root were measured/calculated. Electrolyte leakage was calculated by  $EL=L_1/L_2$  (Nayyar, 2003) where  $L_1$  is electric conduction of leaf after putting in the deionized water in 25°C and  $L_2$  is the electric conduction of the autoclaved samples. Shoot and root dry weights were determined after drying the samples in oven at 75°C for 48 hours. 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*

These measured data were analyzed as a split plot experiment based on randomized complete block design with three replications, and mean comparison of genotypes were done by Duncan's Multiple Range Test. The genotypes were then grouped by Ward and Squared Euclidean distance. To identify the cutting point in the tree dendrograms, discriminant analysis was performed using MSTATC and SPSS 22 software.

**Result and discussion**

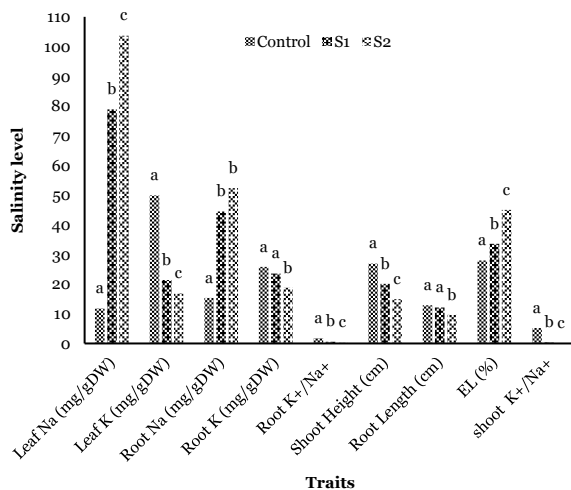
Analysis of variance showed significant difference between salinity treatments in all the traits (Table 1). The response of genotypes was different to salinity treatments. Leaf sodium content, leaf K<sup>+</sup>/Na<sup>+</sup> ratio in leaves, root length, electrolyte leakage and shoot height differences were significant between genotypes at 1% probability level which shows high variability among the genotypes for these traits. K<sup>+</sup>/Na<sup>+</sup> ratio in

root, leaf and root potassium content a significant difference at 5% probability level was observed among genotypes. There was no significant difference between genotypes in root sodium and potassium content. The traits of electrolyte leakage, sodium content of leaf and sodium content of root increased by the increment of the stress, also leaf K<sup>+</sup>, root K<sup>+</sup>, leaf K<sup>+</sup>/Na<sup>+</sup>, root K<sup>+</sup>/Na<sup>+</sup>, shoot height, root length were decreased in response to salinity stress (Fig. 1).

**Table 2.** Variance analysis of rapeseed genotypes in different levels of salinity.

Source of variance	Degrees of freedom	Mean of Square				
		Leaf Na <sup>+</sup>	Leaf K <sup>+</sup>	Leaf K <sup>+</sup> /Na <sup>+</sup>	Root Na <sup>+</sup>	Root K <sup>+</sup>
Replication	2	13.48 <sup>**</sup>	1.31 <sup>ns</sup>	25.92 <sup>ns</sup>	6.59 <sup>ns</sup>	724.63 <sup>*</sup>
Salinity (S)	2	836.23 <sup>**</sup>	166.59 <sup>**</sup>	532.86 <sup>**</sup>	203.56 <sup>**</sup>	852.34 <sup>*</sup>
Error (a)	4	2.61	0.40	19.95	8.67	55.64
Genotype (G)	21	3.55 <sup>**</sup>	0.63 <sup>*</sup>	2.31 <sup>**</sup>	1.54 <sup>ns</sup>	97.62 <sup>*</sup>
(G × S)	42	2.37 <sup>ns</sup>	0.31 <sup>ns</sup>	2.17 <sup>**</sup>	2.05 <sup>ns</sup>	58.68 <sup>ns</sup>
Error (b)	126	1.69	0.36	0.78	1.55	50.74
			Mean of Square			
Source of variance	Degrees of freedom	Root K <sup>+</sup> /Na <sup>+</sup>	Shoot Height	Root Length	EL	
Replication	2	0.25 <sup>ns</sup>	278.19 <sup>ns</sup>	76.68 <sup>*</sup>	19.451 <sup>*</sup>	
Salinity (S)	2	8.07 <sup>**</sup>	2377.81 <sup>**</sup>	189.09 <sup>**</sup>	30.950 <sup>**</sup>	
Error (a)	4	0.03	100.04	6.18	1.491	
Genotype (G)	21	0.05 <sup>*</sup>	35.41 <sup>**</sup>	12.21 <sup>**</sup>	1.412 <sup>**</sup>	
(G × S)	42	0.03 <sup>ns</sup>	6.55 <sup>ns</sup>	5.90 <sup>ns</sup>	0.628	
Error (b)	126	0.03	9.84	5.23	0.485	

\*\*and\* respectively indicate significant difference at 1% and 5% probability level; ns, is not significant.



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

Results of means comparison of rapeseed genotypes under salinity stress (Table 2) was showed The sodium content of Leaf in genotypes SAN-2, SAN-17 and Safi-7 the lowest and the genotypes RGS003, Hyola401 and Amica were highest. Control of Na<sup>+</sup>

accumulation by the exclusion mechanism, or tolerance of high internal levels of Na<sup>+</sup> (tissue tolerance), are important factors for the mechanism of salt tolerance in different plant species (Munns *et al.*, 2006, Munns and Tester, 2008). SAN-17, Safi-3 and Safi-7 were more potassium content in their leaf compared to other genotypes also SAN-8, Zabol-o and Amica were lower potassium than other genotypes. The presence of Na<sup>+</sup> in the environment abundantly prevents the absorption of K<sup>+</sup> (Bandehhagh *et al.*, 2008). At the potassium content of root trait greatest amount related to SAN-2, Safi-7 and Sarigol and least amount was related to SAN-8, Safi-6 and Option500. SAN-2, Safi-3 and Safi-7 Genotypes were higher ratio of potassium to sodium compared to other genotype, also SAN-3, Option500 and Safi-6 had the least ratio. Plant salt tolerance is their ability to maintain optimal K<sup>+</sup>/Na<sup>+</sup> ratio in the cytosol (Shabala and Pottosin, 2014). Plant height Genotypes SAN-2, Safi-3 and Safi-7 had maximum height and

genotypes Hyola401, Goliath and Option500 had the least height. In root length, SAN-2, SAN-6 and Safi-7 genotypes is maximum and Goliath, Option500 and Hyola308 was least length. Bandehhagh *et al* (2013) showed that a significant differences in studied canola cultivars in root system. Also highest electrolyte leakage on the Olga, Heros and Amica genotypes and the lowest In Safi-7, Safi-3 and Hyola308 genotypes were observed. In

response to the increment of metabolites and electrolytes due to high content of sodium chloride, electrolyte leakage increased under the salt stress (Nayyar, 2003). Accumulation of sodium chloride and cumulative entering of Cl<sup>+</sup> and Na<sup>+</sup> in absence of K<sup>+</sup> cause electrolytes leakage (Iqbal *et al.*, 2008). Previous studies have shown that high K<sup>+</sup>/Na<sup>+</sup> ratio shows a Direct relationship with salt tolerance (Kusvuran *et al.*, 2007).

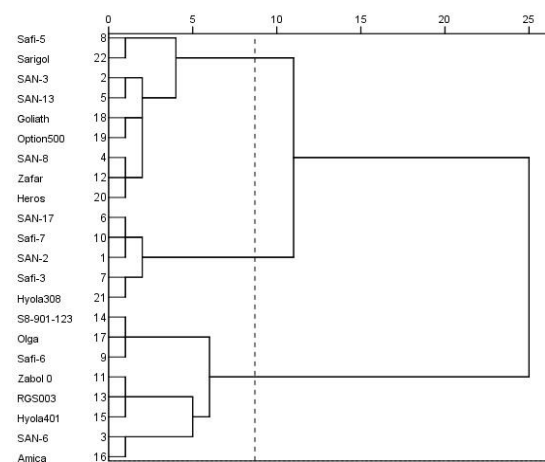
**Table 2.** Means comparison of rapeseed genotypes under salinity stress.

Genotypes	Leaf Na <sup>+</sup> (mg/gDW)	Leaf K <sup>+</sup> (mg/gDW)	Root K <sup>+</sup> (mg/gDW)	Root K <sup>+</sup> /Na <sup>+</sup>	Leaf K <sup>+</sup> /Na <sup>+</sup>	Shoot Height (cm)	Root Length (cm)	EL (%)
SAN-2	44.97 a	31.77 abc	27.03 ab	1.19 a	2.96 a	24.40 a	12.89 ab	36.21 a_d
SAN-3	57.29 a_d	30.89 a_e	20.95 a_d	0.59 cd	1.75 c_f	20.73 b_f	12.08 abc	30.55 ab
SAN-6	69.06 cd	31.76 a_d	25.79 abc	0.92 bcd	1.57 c_f	21.67 a_d	13.76 a	41.51 cd
SAN-8	52.58 a_d	24.63 cde	18.96 bcd	0.79 a_d	1.68 c_f	20.32 b_f	12.02 abc	38.09 bcd
SAN-13	62.39 a_d	29.16 a_e	22.98 a_d	0.72 bcd	1.99 a_e	21.98 a_d	11.24 a_e	34.81 abc
SAN-17	45.36 a	33.93 a	22.40 a_d	0.77 a_d	2.37 a_d	21.85 a_d	11.59 a_d	30.42 ab
Safi-3	46.63 ab	32.48 ab	23.20 a_d	1.19 a	2.22 a_d	23.25 ab	12.10 abc	28.39 a
Safi-5	45.94 ab	28.89 a_e	26.22 abc	1.01 a_d	2.21 a_d	21.67 a_d	11.02 b_e	34.42 abc
Safi-6	63.29 a_d	29.58 a_e	15.15 d	0.62 bcd	1.70 c_f	21.36 a_e	11.32 a_d	34.74 a_d
Safi-7	42.13 a	32.29 ab	26.81 abc	1.08 ab	2.23 a_d	23.72 ab	13.20 ab	28.29 a
Zabol-o	67.58 bcd	24.70 e	21.85 a_d	1.00 a_d	2.21 a_d	20.81 b_f	12.35 ab	32.32 abc
Zafar	51.05 a_d	29.03 a_e	25.92 abc	0.93 a_d	2.35 a_d	19.55 c_g	11.38 a_d	40.17 bcd
RGS003	69.57 cd	28.02 a_e	25.49 abc	1.03 a_d	1.63 c_f	19.54 c_g	11.63 a_d	30.61 ab
S8-901-123	56.69 a_d	31.62 abc	19.27 bcd	1.07 a_d	1.56 c_f	22.31 abc	11.58 a_d	41.23 cd
Hyola401	71.30 d	30.08 a_e	22.91 a_d	0.93 a_d	1.46 def	17.42 fg	11.05 b_e	30.87 ab
Amica	69.85 cd	25.08 de	19.81 bcd	0.74 bcd	1.71 c_f	19.06 c_g	11.59 a_d	45.68 d
Olga	61.87 a_d	25.95 b_e	22.22 a_d	1.00 a_d	0.98 f	19.43 c_g	11.64 a_d	42.52 cd
Goliath	53.11 a_d	30.15 a_e	21.96 a_d	1.05 abc	1.77 b_f	18.11 efg	9.53 cde	35.58 a_d
Option500	52.94 a_d	29.34 a_e	18.75 cd	0.71 bcd	1.44 def	16.67 g	8.82 e	31.58 abc
Heros	57.65 a_d	28.34 a_e	22.95 a_d	0.87 bcd	1.07 ef	20.42 b_f	11.25 a_e	41.85 cd
Hyola308	48.02 abc	26.63 a_e	20.11 bcd	0.78 a_d	2.74 ab	18.52 d_g	9.26 de	30.03 ab
Sarigol	46.48 ab	30.73 a_d	28.50 a	0.79 a_d	2.48 abc	20.42 b_f	11.79 a_d	40.31 bcd

In each column, means that at least one common letter are statistically not significant differences in 5 %

Cluster analysis was carried out using WARD method according to the Euclidean distance was performed (Fig. 3). Discriminant function analysis identified three groups of genotypes (Table 3). The means of each cluster and the deviation percentage from the mean for each of the attributes in Table (4) is inserted. The first group included 9 genotypes (Sarigol, Safi-5, Option500, SAN-3, SAN-13, Zafar, Goliath, SAN-8 and Heros) with higher mean in root sodium, and potassium, and electrolyte leakage. The Second group included 5 genotypes (SAN-17, Safi-7, Hyola308, SAN-2 and Safi-3) has higher mean in leaf and root potassium, Leaf and root potassium to sodium ratio, root length, shoot height and root length. The third group included 8 genotypes (S8-901-123, Olga, Safi-6, Amica, RGS003, Hyola401, Zabol-o and SAN-6) with higher mean in leaf and

root sodium, Leaf and root potassium to sodium ratio, root length, electrolyte leakage and shoot height.



**Fig. 1.** Cluster analysis of rapeseed genotypes based on the studied traits.

The first group had low average in most of the traits so according to the evaluated traits and character of these traits that had a high and low average, group one can called susceptible genotypes to salinity. Based on the investigated characteristics under salinity stress, the second group can be considered as the superior cluster in terms of tolerance to salinity, also the third group includes semi-tolerant genotypes under salinity stress. Van Beuningen and Busch (1997), used cluster analysis for the genetic variation among 270 North American wheat

cultivars in three regions of the United States, Canada, and Mexico, and they were able to produce 20 large groups, each containing 4 or more cultivars and 6 small groups.

**Table 3.** Discriminant function analysis to identify the cutting point.

Wilk's lambda	Probability	Number of groups
0.151	0.002	2
0.052	0.001	3
0.408	0.072	4

**Table 4.** Means and deviation percentage from total mean for difference traits under salinity stress.

Groups	Genotyps	Leaf Na <sup>+</sup> (mg/gDW)	Leaf K <sup>+</sup> (mg/gDW)	Root Na <sup>+</sup> (mg/gDW)	Root K <sup>+</sup> (mg/gDW)	Shoot K <sup>+</sup> /Na <sup>+</sup>	Root K <sup>+</sup> /Na <sup>+</sup>	Shoot Height (cm)	Root Length (cm)	EL (%)	
1	Sarigol	53.27	29.02	42.50	23.02	1.86	0.82	19.98	11.01	36.38	Average
	Safi-5										
	Option500										
	Goliath										
	SAN-3										
2	SAN-13	-5.16	-1.02	12.80	1.45	-3.12	-8.68	-0.40	-4.26	2.59	deviation
	SAN-8										
	Zafar										
	Heros										
	Safi-7										
3	SAN-2	45.42	31.42	33.71	23.91	2.51	1.01	22.34	11.81	30.67	Average
	SAN-17										
	Safi-3										
	Hyola308										
	Amica										
3	Olga	-19.14	7.16	-10.53	5.37	30.73	12.47	8.45	2.70	-13.51	deviation
	zabol-0										
	S8-901-123										
	SAN-6										
	Hyola401										
3	RGS003	66.15	28.35	34.73	21.56	1.61	0.91	20.20	11.86	37.43	Average
	Safi-6										
3	Safi-6	17.76	-3.31	0.92	-4.98	-1.14	1.34	-1.94	3.13	5.56	deviation
	Safi-6										

**Conclusion**

Tendency to use similar parents and the lack of knowledge and use of new species in breeding programs leads to a reduction in genetic variation. However, furthest cultivars with more polymorphism show more genetically different variation, more variation, higher heterostasis, or transmission rare traits will follow. This experiment indicated significant genetic variability among genotypes, which includes genotypes of groups 1 and 2 so can be used this genotypes in breeding programs and understanding the mechanisms involved in salt tolerance and salt tolerance genes involved in this process.

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