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Rapeseed (*Brassica napus* L.) genotypes response to NaCl salinity

Nima Dolatabadi, Mahmoud Toorchi*

Department of Plant Breeding and Biotechnology, University of Tabriz, Tabriz, Iran

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Abstract

In order to evaluate the response of rapeseed genotypes to NaCl salinity, a hydroponic experiment at seedling stage with three replications was performed in greenhouse. Twelve spring rapeseed genotypes were treated by sodium chloride on three levels (0 (control), 175 and 350 mm) to assessed salinity effects on rapeseed. Traits were measured 28 days after imposing salt stress. Variance analysis showed that all traits except for leaf water potential were significantly affected by salinity. Also, differences between genotypes in most traits were significant. Hyola308 as the most tolerant and SW5001 as the most susceptible genotypes were identified. Cluster analysis of genotypes based on WARD method divided the genotypes to three groups and two genotypes Hyola308 and SW5001 were grouped separately. These results indicated that these traits which associated to salinity stress can be used in future researches as salt tolerance indicators. Due to the significant differences between the genotypes in most traits, this diversity can be used in breeding programs to find the location of genes which involved in salinity tolerance and also for researching the mechanisms of salt tolerance.

*Corresponding Author: Mahmoud Toorchi ✉ mtoorchi@tabrizu.ac.ir

Introduction

Oilseeds are in the third place of the human food needs, and rapeseed due to the high oil yield and quality of oil thorough the large amounts of polyunsaturated fatty acids is preferable compared to other oilseeds (Shirazi *et al.*, 2011). Salinity as one of the most common environmental stresses in arid and semi-arid areas reduced crop yield severely (Joseph *et al.*, 2010). For optimal use of the saline soils, planting the resistant crops and cultivars to salinity is one of the essential strategies. Because the saline soil improvement needs more time and it is not economical, therefore, finding tolerant genotypes are very important (Purty *et al.*, 2008). Before starting and performing any breeding program to identify varieties and genotypes resistant to salinity, recognition of characteristics which related to salt tolerance in plants and their genotypes will be important (Ashraf, 2004).

Salt stress researches indicated, salt tolerance is not a simple and monogenic trait (Zhu, 2001), so in breeding programs for resistance to salt, various secondary traits that was associated with stress were examined to obtain more precise and comprehensive results (Zhang *et al.*, 2014). Due to the features of hydroponic method, it was observed that problems resulting from the effect of salinity on farm and natural conditions do not exist in this method. As the indices of salt tolerance, dry weight of shoot and root, leaf area and plant height can be mentioned (Bandehagh *et al.*, 2008; Shirazi *et al.*, 2011). Studies have shown that salinity stress not only reduces plant height, also can cause leaf area, total dry weight and harvest index reductions (Heidari *et al.*, 2011). Morphological indexes of leaf area and plant height responded quickly than the other trait and reduced, because of the dry matter accumulation by plants, resulted by the amount of the net photosynthesis and the photosynthetic area of plants (Ashraf and McNeilly, 2004). Studies about the effects of salinity on agricultural crops characteristics including rapeseed indicated negative effects of salinity on fresh and dry weight and root system (Bandehagh *et al.*, 2013).

Various studies suggested that an indirect relationship was existed between salinity and water and osmotic potentials (Kaymakanova and Stoeva, 2008; Sobhanian *et al.*, 2011). *Brassica napus* and *Brassica campestris* were two species which have been classified as semi-tolerant species (Miyamoto *et al.*, 2012). But yet in salinity levels above the threshold, more reducing crop yield than other crops has shown (Purty *et al.*, 2008). Also tolerance in the early stages of rapeseed growth was not associated with the later stages (Ashraf and McNeilly, 2004). So the cultivars with salt tolerance in each developmental stage of the plant should be detected separately (Athar *et al.*, 2009). This study was designed and performed to evaluate the effect of salinity on physiological and agronomic characteristics of spring rapeseed cultivars in seedling stage.

Materials and methods

Plant material and salt treatments

In this research 12 spring rapeseed cultivars (Olga, Wild Cat, Sarigol, Heros, Cracker, Option500, Comet, Hyola308, Amica, Eagle, SW500l and RGS003) affected by salinity of NaCl at three levels (0 (control), 175 mm and 350 mm) was performed in hydroponic culture. A week after the transferring of plantlets, salinity stress was performed slowly over 3-5 days depending on the salinity levels.

Measurements

Traits were measured 4 weeks after the stress performing at the end of the seedling stage and just before the beginning of flowering stage. These traits included dry weight of shoot and root, leaf area, number of leaves, shoot height, root length, leaf osmotic potential and water potential (Dolatabadi *et al.*, 2012), leaf relative water content (RWC) (Morant-Manceau *et al.*, 2004), electrolyte leakage and chlorophyll content (Sabagh *et al.*, 2015).

Statistical analysis

The experiment was performed in Split plot with randomized complete block design with three replications and mean comparisons were done by Duncan's multiple range test. MSTATC, IBM SPSS Statistics 20 and Excel computer programs were used for statistical analysis.

Result and discussion

Significant differences were shown between levels of salinity for all traits except for the leaf water potential (Tables 1, 2). Among the varieties there were a significant difference (1%) in shoot dry weight, root dry weight, leaf area, number of leaves, shoot height, root length, RWC, and chlorophyll content. Salinity decreased shoot and root dry weight, leaf area, shoot height, number of leaves, leaf relative water content and leaf osmotic potential (Fig. 1). But the traits of electrolyte leakage and chlorophyll content increased by the increment of the stress (Fig. 1). Root length showed an increment by 175 mM salt stress while by increasing the salinity stress to 350 mM, decreased. Adverse effect of salinity on growth and weight of root has also been reported previously (Parida and Das, 2005; Dolatabadi *et al.*, 2016). Electrolyte leakage increased under the salt stress, in response to the increment of metabolites and electrolytes due to high content of sodium chloride (Nayyar, 2003).

The accumulation of NaCl was caused by the increment of Na⁺ and Cl⁻ entering and exclusion of K⁺ (Rezaei *et al.*, 2010).

Leaf area and plant height reduced much faster than other morphological characteristics under the salt stress and tolerant varieties have the tendency to maintain in the high levels of these traits (Dolatabadi *et al.*, 2016). In this experiment the osmotic potential under salt stress significantly decreased. Leaf water potential and osmotic potential decreased by increment of salinity due to decreasing of leaves water content which is known as the secondary effects of salt stress (Chimenti *et al.*, 2002; Moghaieb *et al.*, 2004). Despite the increment of chlorophyll content in this research, there are many reports that have shown the decrement of this trait in different plants under the salt stress (Yang *et al.*, 2009; Chakraborty *et al.*, 2012). This could be caused by increasing the density of chlorophyll in leaf area due to leaf area decline under salt stress.

Table 1. Analysis of variance for some studied traits in rapeseed genotypes under salt stress.

| Source of variance | Degrees of Freedom | Mean Square | | | | |
|--------------------|--------------------|---------------------|---------------------|---------------------|----------------------|----------------------|
| | | Shoot dry weight | Root dry weight | Leaf area | Number of leaves | Shoot Height |
| Replication | 2 | 0.007 ^{ns} | 0.001 ^{ns} | 0.33 ^{ns} | 2.346 ^{ns} | 68.691 ^{ns} |
| Salinity (S) | 2 | 1.102 ^{**} | 0.161 ^{**} | 4.219 ^{**} | 19.976 ^{**} | 733.33 ^{**} |
| Error (a) | 4 | 0.057 | 0.008 | 0.146 | 0.650 | 12.881 |
| Genotype (G) | 11 | 0.052 ^{**} | 0.005 ^{**} | 0.099 ^{**} | 0.947 ^{**} | 14.596 ^{**} |
| S × G | 22 | 0.009 ^{ns} | 0.001 ^{ns} | 0.012 ^{ns} | 0.120 ^{ns} | 0.980 ^{ns} |
| Error (b) | 66 | 0.007 | 0.001 | 0.014 | 0.179 | 1/392 |
| CV% | | 27.19 | 16.09 | 7.09 | 10.06 | 6.78 |

*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 salt stress.

| Source of variance | Degrees of Freedom | Mean Square | | | | | |
|--------------------|--------------------|---------------------|---------------------|---------------------|-----------------------|-----------------------|----------------------|
| | | Root Length | RWC | Osmotic potential | Leaf water potential | Electrolytic leakage | Chlorophyll content |
| Replication | 2 | 2.709 ^{ns} | 1.258 ^{ns} | 0.003 ^{ns} | 0.007 [*] | 314.11 ^{ns} | 42.381 ^{ns} |
| Salinity (S) | 2 | 46.199 [*] | 4.978 ^{**} | 2.811 ^{**} | 0.001 ^{ns} | 10653.30 [*] | 347.391 [*] |
| Error (a) | 4 | 3.424 | 0.189 | 0.042 | 0.001 | 708.15 | 26.801 |
| Genotype (G) | 11 | 4.332 ^{**} | 0.119 ^{**} | 0.013 ^{ns} | 0.00036 ^{ns} | 154.70 ^{ns} | 15.450 [*] |
| S × G | 22 | 1.667 ^{ns} | 0.054 ^{ns} | 0.009 ^{ns} | 0.00027 ^{ns} | 127.61 ^{ns} | 5.178 ^{ns} |
| Error (b) | 66 | 1.365 | 0.042 | 0.012 | 0.00035 | 113.64 | 6.431 |
| CV% | | 11.69 | 11.66 | 14.39 | 25.64 | 19.40 | 5.69 |

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

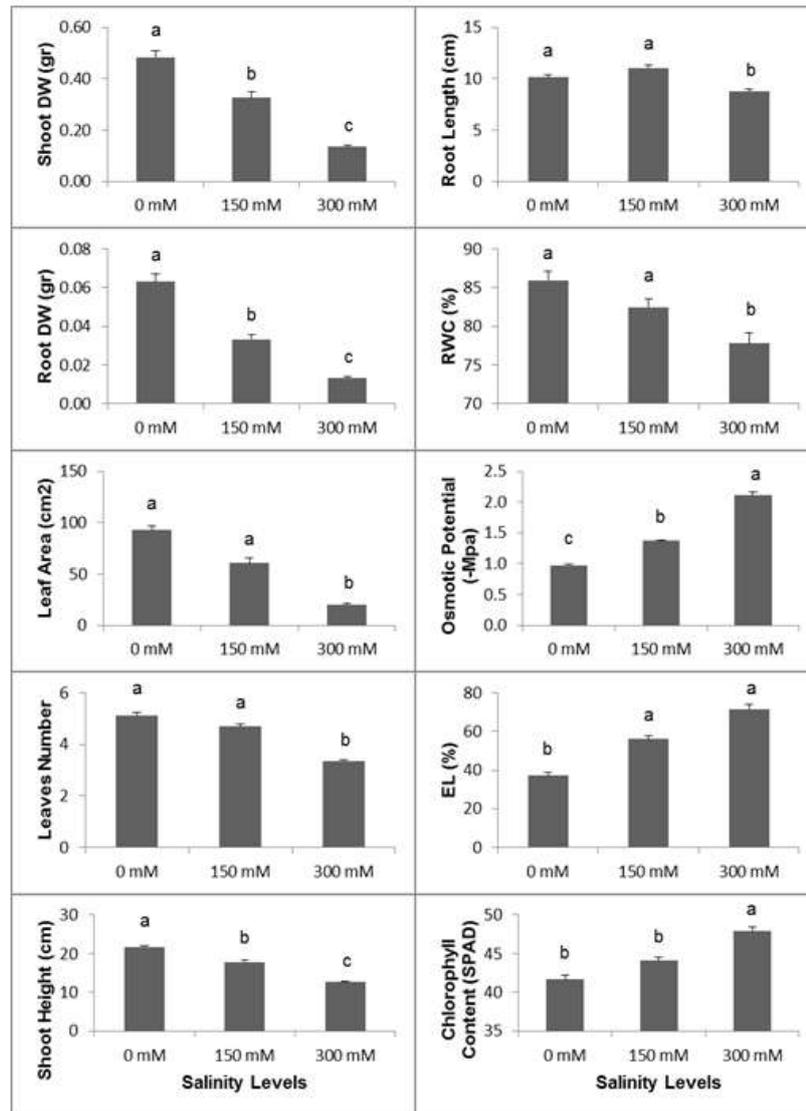


Fig. 1. Effects of salt stress (0, 175 and 350 mM NaCl) on rapeseed genotypes. Columns with the same letters are not significantly different at ($P>0.05$) levels by Duncan's multiple range test.

Group classification of genotypes can provide useful information like a cross between genotypes in plant breeding programs. Cluster analysis by WARD method according to the Euclidean distance was performed. Discriminant function analysis identified three groups of genotypes (Fig. 2). The first group included Wild Cat, Cracker, Comet, Eagle and Hyola308. The second group had 5 genotypes (Sarigol, RGS003, Heros, Amica and Olga), and the third group included Option500, and SW5001 genotypes. In the first group the average of shoot dry weight, root dry weight, leaf area, number of leaves, shoot height, root length, leaf water potential and electrolyte leakage, were higher than the average of total groups.

A close look at the nature of traits that are above or below of the total group's average revealed that genotypes of this group can be identified as tolerant genotype. The second group in terms of shoot and root dry weight, leaf area, shoot height, root length, RWC, osmotic potential, electrolyte leakage, had a higher average than total group's average. According to the evaluated traits and character of these traits that had a high and low average, this group can be called semi-tolerant. The third group had low average in most of the traits and only in terms of RWC had higher average than the total group average. Thus, it seems that the third group includes susceptible genotypes to salinity.

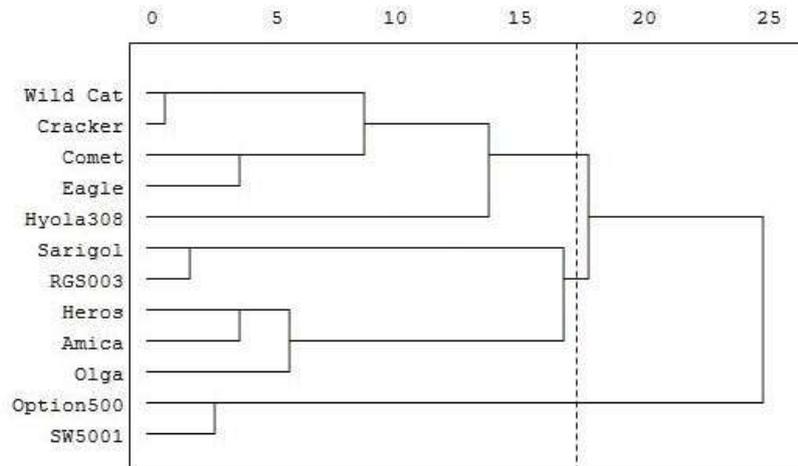


Fig. 2. Cluster analysis of rapeseed genotypes based on WARD method at studied traits.

Conclusion

This experiment showed significant genetic variability among genotypes, which can be used in subsequent researches. Since the genotypes of the first and third groups at the most traits had the most differences, therefore, various varieties can be chosen for breeding programs. Studying the tolerant and susceptible genotypes in this review with molecular methods will provide some useful information about the mechanisms of salt tolerance and will identify the genes in response to the salinity.

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