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# The Study of Salinity Effects on germination Components of five Canola Genotype

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**ABSTRACT:** In order to study the effects of different NaCl concentrations on the germination components of canola varieties, five genotypes including Zarfam, Sarigol, Hyola308, SLM046 and RGS003 were compared in a CRD design with three replications under four osmotic potentials (including 0, 4, 8, and 12 ds/m). .The experiment was done at the calture room in guilan university of agriculture at 2014. The results of statistical analysis showed that, all of the studied component including radicle length, shoot length, radicle / shoot ratio, radicle fresh weight, radicle dry weight and etc were significantly affected by salinity, and also showed a decreased pattern with increasing salinity. In the most of components, the highest amount was observed in control and the lowest one was in12 ds/m treatment. The results also showed that for all of studied component, 8 ds/m could be considered as salinity tolerance threshold. The difference between genotypes was significant in many cases, except root length, radicle shoot rate, and shoot growth rate. The interaction between genotype and salinity in radicle growth rate, radicle dry weight, subtraction of dry and fresh weight of radicle and radicle fresh weight were significant. These results revealed that there was a different response to salinity among the studied genotypes.

***Keywords*:** Abiotic stress; Cluster analysis; Salinity; Sodium chloride

## INTRODUCTION

Soil salinity is one of the most important factors limiting production in arid and semi-arid region (Neumann , 1995). Plant growth reduced due to salinity but, plant species are differ in salt tolerance (Munns and Termaat, 1986). Salt tolerance is an important feature of choosing a cultivar varieties for planting in a region exposed to salt stress that must be considered. Plants adapted to the saline conditions with different mechanisms which including developmental and morphological changes, physiological and biochemical processes (Zhu , 2001).High levels of salinity could discontinued due the combined effects of high osmotic potential and toxicity of the particular ion and inhibition the seed germination and seedling growth (Grieve and Suarez, 1997). In particular, seed germination, seedling emergeance and primary survival seeds are sensitive to salinity (Mariko et al, 1992 ; Baldwin et al, 1996). In this regard, salt stress can affect to the seed germination through the osmotic effects . The region with high level of salinity also have face with high temperatures .On the other hand , it has been shown that tolerant of salinity in plant species in germination stage affected by interaction between the temperature and salinity (Delesalle and Blum, 1994 ; Khan ,2000). Leilah (Leila et ai , 2004) in a study on the effects of four salinity levels (50 to 200 mol per cubic meters of sodium chloride) on three varieties of canola showed that the rate and percentage of germination significantly affected by salinity. Otherwise, Puppala in research on canola seed germination was observed that there is an insignificant difference between cultivars in the percentage of germination. A study on salt tolerance of canola seed germination in five salinity levels from 5 /4 to 26/ 4dS/m showed that with increasing

salinity level from 10/ 1to 16/2 dS m, germination was reduced by 40 percent compared to the control treatment (Puppala et al , 1994). Based on their results, canola has had a moderate tolerance to salinity based on the classification by (Maas and Hoffman, 1977). In study of germination component of five varieties of canola(Regent, Ceres, Talaye, Cobra × W. 2 and PF-91/7045) in three levels of salinity, -0/4 and -0/8 MPa showed that the salinity has a significantly affected on germination rate, seedling length and root to shoot ratio but , the effectiveness on traits has not been the same. They showed that the variables of cultivars on seedling growth and root to shoot ratio is also not significant but, the germination rate, uniformity of germination and cumulative percentage of germination is significantly affected (Zenali et al, 2002). Salinity inhibitory on seedling components have also been reported by (Munns and Termaat, 1986). They also emphasis on reducing these components in response to the increased salinity gradient and expressed that in response to increasing intensity of salinity , gradient of decreasing was increase. Although the results of such research in the field of canola provides some general points about this salinity , however, according to the findings data show that the information that is unique to one or more digits, can't be precise criteria for definite decision about the canola of a species to salinity or determine the class of salinity tolerance. Based on available information canola is in the category of salt tolerant plants and 10 ds/m could be considered as salinity tolerance threshold and has been shown that its yield reduced to 50 percent reduced with 14 ds/m of salinity ,but no information has not been published about this plant tolerance to salinity in germination stage (Kafi ,2003). Lack of information in this field on one side and the number of varieties available in provinces on the other side , show the necessity of study these cultivars in response to salinity at the seed germination stage. It's essential to have at least information in this field especially according to acceptable tolerance to salinity , be considered to convert to saline areas marginal land to arable land . Therefore, this study aimed to response germination components of five canola to salinity in and related components was conducted in laboratory conditions.

**Materials and methods**

In order to study the effects of different NaCl concentrations on the germination components of canola varieties, five genotypes including Zarfam, Sarigol, Hyola308, SLM046 and RGS003 were compared in a CRD design with 3 replications under 4 osmotic potentials (including 0, 4, 8, and 12 ds/m). That was recommended varieties in Guilan province. An experiment was conducted in growth champer at Guilan Agricultural University during the Autumn seasons of 2014. Seed disinfected with sodium hypochlorite 5% and then washed every 2 minutes and for 5 times by water distilled water.

For examine the effect of salinity tolerance was accomplished by placing 10 seed samples in 8 by 9 mm plastic petri dishes containing one watman paper and seeds were treated different concentrations of sodium chloride and pH was set on in 5/5 by sulfuric acid and potassium hydroxide.

Seed were germinated under a photoperiod of 16 h light and 8 h dark at 21°C and were irrigated with solution sodium chloride when it get dry and allowed to growth for 14 days. In order to determine the germination percentage in each Petri dishes , number of germinated seeds (with 2 mm root growth) were counted. Shoot and radicle length were measured with caliper each 2 days for 5 times. Then, for measurement of the wet and dry weight, shoot and radicle separated and weight were measured separately.To determine dry weight , shoot and radicle were put in to the foil separately for 48 hours and then were put in the oven with a temperature of 80 ° C. Finally, for analysis of data the average measurement for each 10 seeds were used .Finally for Mean square of studies trait we used ANOVA and for Mean comparison of studies trait we used LSD by the SAS software. For identification resistant and susceptible genotypes, we used cluster analysis for all the traits. For cluster analysis, the distance between the genotype calculated with square Euclidean distance .Different clustering methods, including integration based on the average distance between the groups (UPGMA), nearest neighbors (SL), complete linkage (CL) and Ward's minimum variance (Ward) was done by using SPSS software version 18 .

**Discussion**

The results showed that the lowest germination rate was observed in the EC 12 dS/m. In the most of components, the highest amount was observed in control and the lowest one was in12 ds/m treatment. The means of seed germination percentage reached to 56/2. In this level of salinity Sarigol and Hyola 308 with 76 and 33 percentage have the highest and lowest seeds germination (Figure 1). The highest percentage of germination belonged to control that decreased with increasing of salinity levels. Research showed that the salinity of more than 12 dS/ m is not necessary to study. The results of statistical analysis showed that, all of the studied component including radicle length, shoot length, radicle / shoot ratio, radicle fresh weight, radicle dry weight, shoot dry weight, shoot wet weight , shoot growth rate and radicle growth rate were significantly affected by salinity, and also

showed a decreased pattern with increasing salinity(Figure 2 and 3). These results revealed that there was a different response to salinity among the studied genotypes. The difference between genotypes was significant in many cases, except root length, radicle shoot rate, and shoot growth rate (Table 1). The interaction between genotype and salinity in radicle growth rate, radicle dry weight, subtraction of dry and fresh weight of radicle and radicle fresh weight were significant. These studies indicate that although salinity levels had significant effect on all traits but in more than half of the components, genotype could not to be intensifies or weaken. In comparison to one by one of genotype for root length, not significant different was observed. In shoot lenght, only SLM046 and Hyola308 were significant at 1%. In radicle/ shoot ratio any of the genotypes were not significant.

Hyola308 - Zarfam, and Hyola308 - SLM046 genotypes were significant effect with each other in relation to radicle fresh weight .In root dry weight the difference between genotypes was significant in many cases except Sarigol- Zarfam, RGS003 - Hyola 308, and RGS003- SLM046. In Subtraction of dry and wet weight of Radicle, all the genotypes except SLM046-Zarfam and Hyola308-Zarfam was not significant. In shoot fresh weight compared with each other in half of genotypes were not significant include: Hyola308 - RGS003, Hyola308 _ SLM046, RGS003- SLM0046 , Sarigol- SLM046, Sarigol- Zarfam and also in shoot dry wet,the half of genotype were not significant including; RGS003-Hyola308, Sarigol-Zarfam, SLM046- Zarfam, Zarfam- Hyola308 and SLM046-Sarigol. RGS003-Zarfam , Hyola 308- Sarigol, RGS003-Sarigol, Hyola308-Zarfam and SLM046 - Sarigol the genotype that associated with Subtraction of dry and wet weight of Shoot were not significant .Sarigol-SLM046 were the only genotypes that associated with root growth rate had significantly affected with each other. None of the genotypes associated with the shoot growth rate were not significantly different.

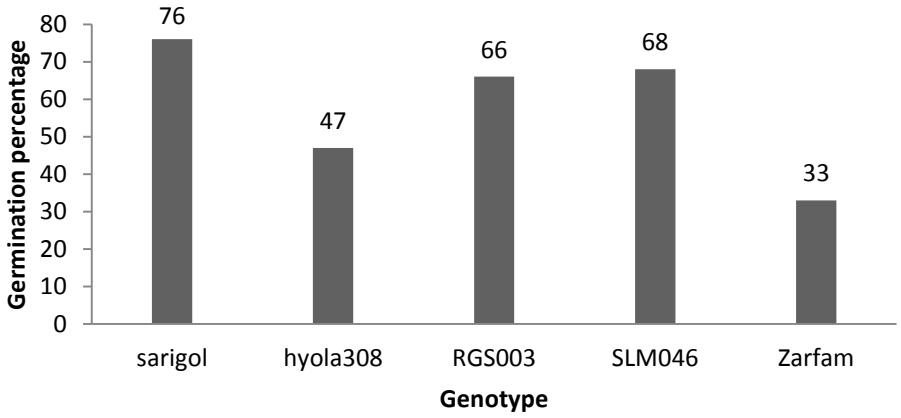


Figure1- Germination percentage in 12 ds/m salinity

**Table 1- - Mean square of studies trait**

| S.O.V | DF | Shoot dry weight (gr) | Radicle dry weight (gr) | Shoot length (mm) | Radicle length (mm) | Radicle growth rate (mm/day) | Radicle/Shoot |
|---|---|---|---|---|---|---|---|
| Genotype | 4 | 0/0001$^{**}$ | 0/00001$^{**}$ | 36/06$^{*}$ | 446/32$^{ns}$ | 8/66$^{ns}$ | 0/006$^{ns}$ |
| Stress | 3 | 0/000005$^{**}$ | 0/0001$^{**}$ | 240/62$^{**}$ | 8/20207$^{**}$ | 33/01$^{**}$ | 0/001$^{**}$ |
| Genotype* Stress | 12 | 0/000001$^{ns}$ | 0/00001$^{**}$ | 18/30$^{ns}$ | 53/1130$^{ns}$ | 7/5$^{ns}$ | 0/007$^{ns}$ |
| Error | 40 | 0/000001 | 0/00001 | 24/57 | 704/83 | 5/8 | 63/2 |
| CV | - | 38/83 | 31/52 | 24/24 | 36/59 | 34/03 | 26/29 |

**,*=Significant at 0.01 and 0.05 levels, ns = non-significant

**Table 1- Mean square of studies trait**

| S.O.V | DF | Shoot weight (gr) | Radicle weight(gr) | shoot growth rate (mm/day) |
|---|---|---|---|---|
| Genotype | 4 | 0/0020$^{**}$ | 0/0001$^{**}$ | 87/37$^{*}$ |
| Stress | 3 | 0/0013$^{**}$ | 0/0012$^{**}$ | 4249/56$^{**}$ |
| Genotype*Stress | 12 | 0/0002$^{ns}$ | 0/0001$^{**}$ | 132/99$^{*}$ |
| Error | 40 | 0/0001 | 0/00004 | 27/80 |
| CV | - | 31/47 | 39/19 | 16/26 |

**,*=Significant at 0.01 and 0.05 levels, ns = non-significant

For obtained an idea of the similarities and differences between genotypes for all traits, cluster analysis in different ways like within groups the average distance between the nearest and farthest neighbor and Ward's minimum variance was done and the clusters were compared with each other. Distance matrix of morphological classification became standardization, and estimated by using the average square Euclidean distance. Since the method of UPGMA get the best result in the Group of varieties studied, only the results of this method were reported. Cutting line was drawn in district of 6, the first group includes the 8 genotypes, second group had 2, third group 3 and fourth and fifth group had respectively w 2 and 3 of genotype. In the first group, three of the four levels of salinity in Sarigol genotypes were together which suggests that had equally affected in terms of morphologically by stress and its function is the same in normal conditions. In the second cluster, RGS003 and Hyola308 were next to each other and shown almost the same reaction in terms morphologically. In the fourth cluster and in the 12 ds/m stress , Sarigol and SLM046 were in the same situation and had similar response. On the other wise, Hyola 308 had the highest dispersion among the genotypes. Based on the results for all components, Sarigol could be considered as resistant genotype and Hyola 308 as susceptible one (Figure 4)**.** To identify resistant and susceptible genotypes based on morphological response, genotype named in four levels of salinity and five class of genotype respectively including; 1, 2 ,3 ,4 and 1, 2, 3, 4 and 5 and to ensure accuracy of the clustering , evaluation of genotypes clustering and the canonical discriminant based on fisher linear discriminant was done and showed that clustering of treatments have been correct .

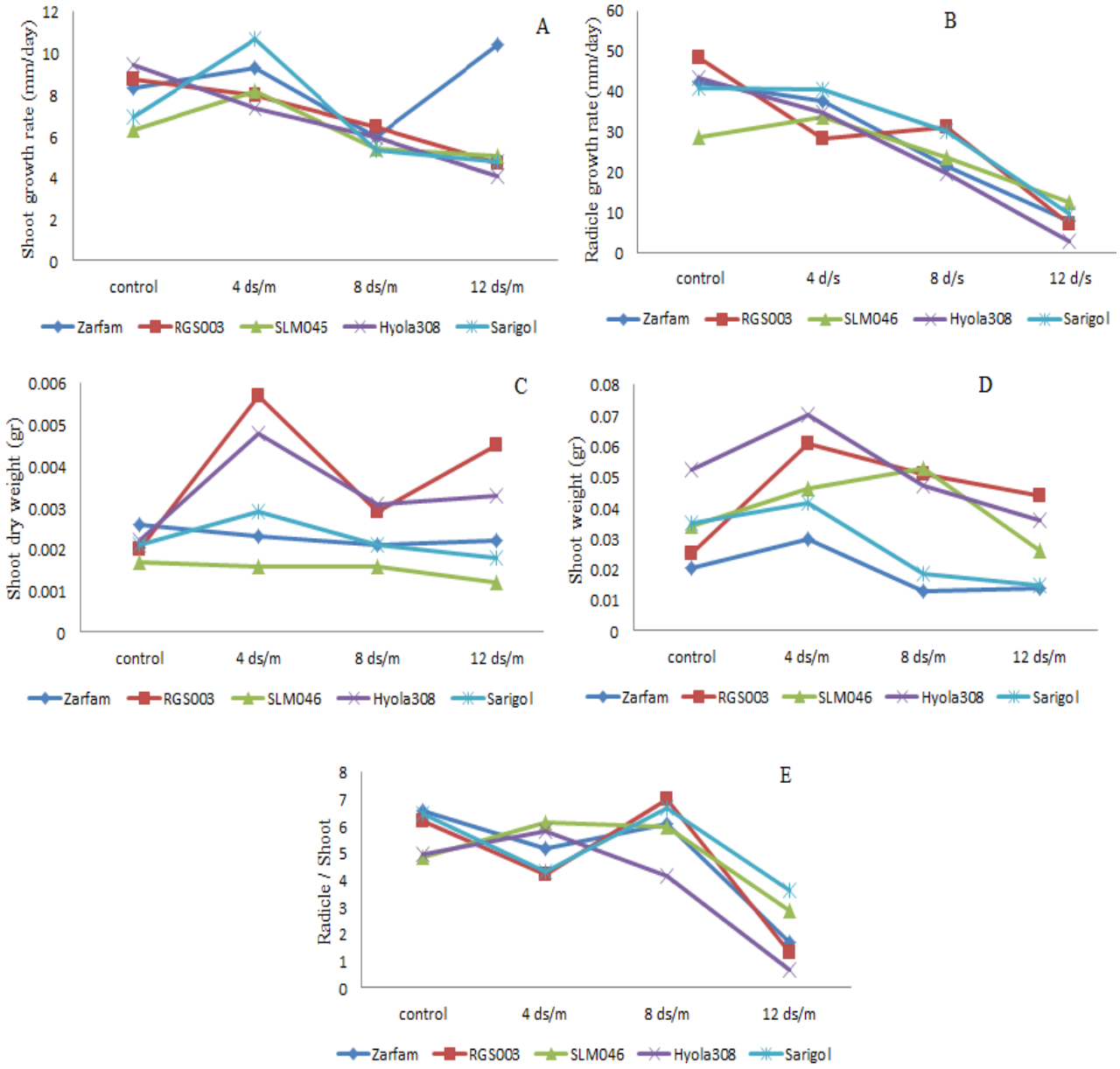


Figure 2-The Change trend of genotype in different level of NaCl salinity ;(A)Shoot growth rate – (B) Radicle-growth rate-(C) Shoot dry weight – (D)Shoot weight – (E) Root / Shoot

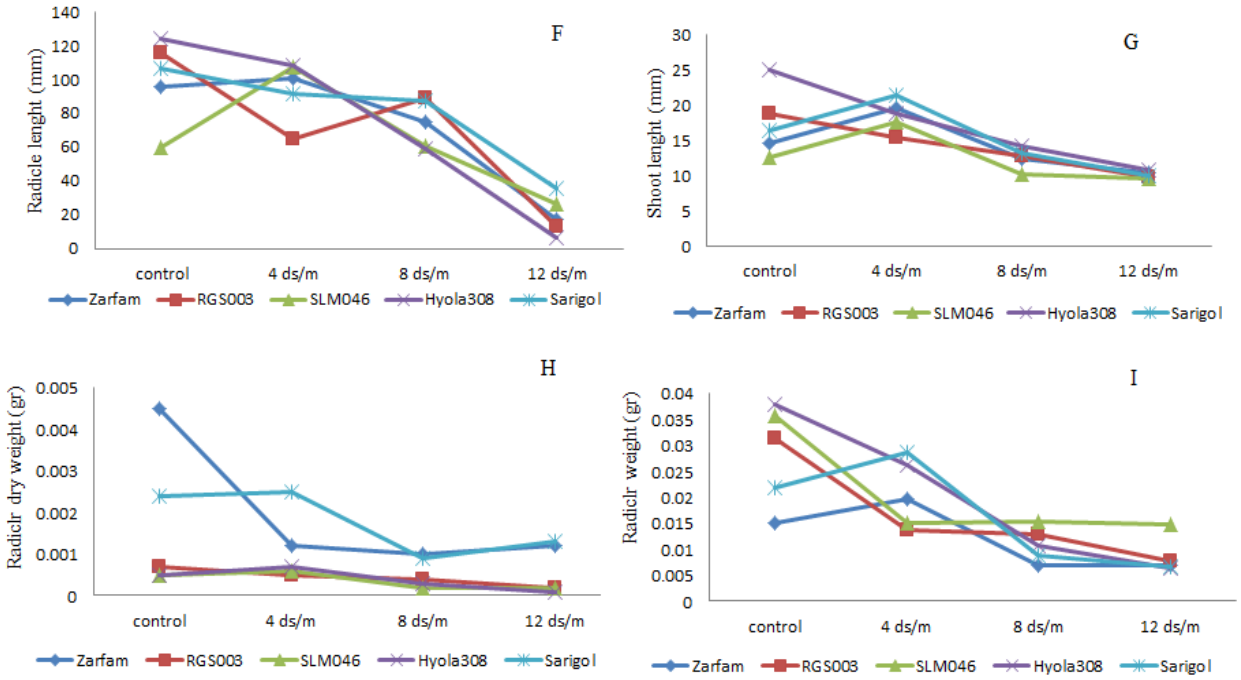


Figure 3- The Change trend of genotype in different level of NaCl salinity ;(F)Radicle lenght – (G) Shoot lenght-(H) Radicle dry weight – (I)Radicle weight

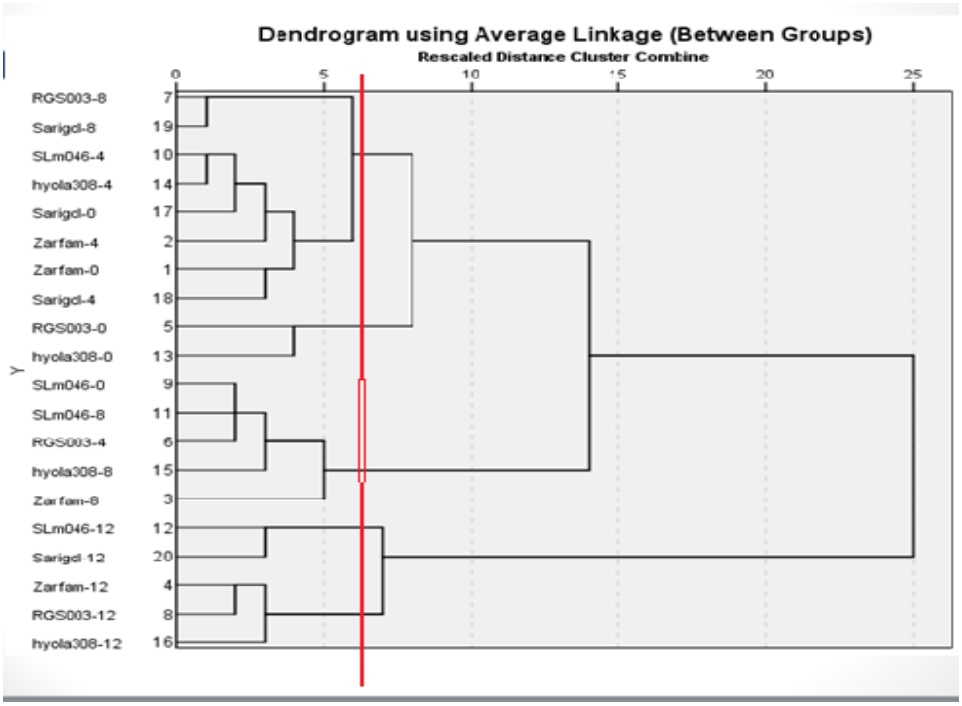


Figure4- Clustering of genotypes based on Euclidian distance and with UPGMA method and criteria for classification based on morphological characters

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