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Screening of Canola (*Brassica napus* L.) Genotypes for Salt Tolerance Based on Early Growth Stage

Irandokht Mansoori*, Hamid Najafe-Zarrini**, Nadali Babaean Jelodar and Ali Pakdin*******

**Ph.D. student of Sari Agricultural Sciences and Natural Resources University of Sari, Iran.*
***Assistant Professor of Sari Agricultural Sciences and Natural Resources University of Sari, Iran.*
****Professor of Sari Agricultural Sciences and Natural Resources University of Sari, Iran.*
*****Assistant Professor, Genetics and Agricultural Biotechnology Institute of Tabarestan (GABIT), Sari Agricultural Sciences and Natural Resources University, Iran.*

(Corresponding author: I. Mansoori)
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ABSTRACT: Soil salinity is a serious constrain to crop production in many areas of the world. In order to study the salinity stress effects resulted from sodium chloride on germination, vegetative growth in 100 rapeseed genotypes, an experiments was carried out in germinator at the Research Station of Sari Agricultural Sciences and natural Resources University. A factorial experiment based on completely randomized design with 3 replications was considered for evaluation of 300 treatments. The first factor of the experiment was 100 canola genotypes to identify genotypes with high or low level of tolerance to salt stress for further studies, and the second factor was salinity stress levels: 0, 6 and 12 dsm^{-1}NaCl. The results showed that the principal components of measured parameters: germination percentage (GP), radicle length (RL) and plumule (stem) length (SL) and plumule fresh weight (PFW) of plumule dry weight (PDW) and radicle dry weight (RDW) was using principal component analysis that determine salt-tolerant and salt-sensitive genotypes. Among 100 canola genotypes, some genotypes 75, 85, 12, 64, 86, 13, 43, 91, 7, 21, 42, 68, 94, 99, 18, 73, 79, 2, 71, 3, 36, 1, 26 and 100) had higher level of salt tolerance and some of them were salt sensitive. The selected genotypes in early screening test for salt tolerance are useful for further evaluations. Results showed that canola genotypes significantly responded to increasing salinity levels. Although, salt stress decreased the germination percentage, shoot and root length and weight in all tested genotypes but maximum reduction in germination was recorded in seeds.

Key words: Genotypes, Germination, plumule length, Root weight, Salinity

INTRODUCTION

Canola (*Brassica napus* L.) is considered as one of the most important oil seed crops all over the world, and even more in Iran, that its production has been notably extended in recent years. Canola seeds contains about 40-50 % oil of high quality for human consumption and the remaining is a high protein meal for livestock feed. Canola oil has the best fatty acid profile of any edible oil. It is characterized by less than 1 % Erucic acid and higher percent of oleic which has been shown to reduce serum cholesterol level. Canola is a moderately salt-tolerant crop, grown mainly for its edible oil. However, its production and quality are greatly reduced by soil salinity (Akbari *et al.*, 2011). A major constraint to seed germination and seedling establishment of canola is soil salinity, which is a common problem in irrigated areas of Iran with low rainfall. This problem adversely affects growth and development of crop, and results into low agricultural production. The most common undesirable effect of salinity on the crop of brassica is the reduction in plant height, size and yield as well as deterioration of the product quality (Zamani *et al*, 2011). There are differences in sensitivity to salinity among canola cultivars (Bybordi, 2010; Tunuturk *et al.*, 2011; Zamani *et al.*, 2011). One of the most sensitive phases of a plant's life to salinity is that of seed germination. Absence of germination in salinity soil is often due to the high concentration of salt in the soil where the seeds are sown. The reason is that the salt solution moves upward, following the evaporation at soil level (Zadeh and Naeni, 2007). Ahmed *et al*, 2010; Bray *et al.*, 2000). The salt disturbs both germination and the plant growth (Bybordi, 2010). The research has shown that in response to soil salinity, seedlings growth, leaves area, root biomass and shoot biomass have all been reduced (Ghazizade *et al.*, 2012).

Although salt stress affects all growth stages of a plant, seed germination and seedling growth stages are known to be more sensitive for most plant species (Ghazizade, *et al,* 2012; Coartero *et al.,* 2006). Furthermore, germination and seedling stage are predictive of plant growth responses to salinity (Coartero *et al.,* 2006).

Therefore, seeds with more rapid germination under salt stress and/or normal conditions may be expected to achieve a rapid seedling establishment and more salt tolerance and hence higher yields (Munns, 2002; Bybordi and Tabatabaei, 2009; Tamartash *et al.,* 2010). Salt tolerance in plants is a complex phenomenon, which depends on a number of inter-related factors based on morphological, biochemical and physiological processes (Ghazizade, *et al.,* 2012). Salinity reduces the ability of plants to take up water, leading to growth reduction as well as metabolic change similar to those caused by the water stress (Bybordi *et al.,* 2010). A high salt concentration in the root affects the growth and yield of many important crops (Taffouo *et al.,* 2006). Salinity may reduce crop yield by upsetting water and nutritional balance of the plant (Khan *et al.,* 2007). Water availability and nutrient uptake by plant roots are limited because of high osmotic potential and toxicity of Na and Cl ions (Bybordi *et al.,* 2010a). The most common adverse effect of salinity on the crop of Brassica is the reduction in plant height, size and yield as well as deterioration of the quality of the product (Ghazizade *et al.,* 2012). Seed germination has been reported to decline with increasing salinity levels (Houle *et al.,* 2001). Within canola cultivars, there are cultivar differences in sensitivity to salinity. The genetic role in seed germination resistance to salinity is probably one of the most important advantages that can be used in breeding programs. Significant variation in seed germination between canola cultivars grown under salinity condition was reported by Bybordi *et al.* (2010). The present study was undertaken to assess the effect of salt stress on some characteristics of 100 canola genotypes such as seed germination, percentage, shoot and root length and weight.

MATERIALS AND METHODS

A. Screening test

The 100 canola genotypes (*Brassica napus* L.) (Table 1) were collected from Oil Seeds Crop Development Center, Sari, Iran. The same size seeds were surface-sterilized for 5 min in sodium hypochlorite solution (10%) and then they were 3-5 times rinsed with distilled water. After sterilization, 10 seeds were transferred into 9 cm sterile Petri dishes on filter paper and then were wetted with 7 ml distilled water (control) or saline water solution at 0, 6 and 12 dsm^{-1}NaCl. To prevent infection and evaporation of solution, all of the plates were closed with parafilm. All operations were performed under laminar flow. The Petri dishes were labeled and incubated in a germinator at 25°C and 25/15 h day/night illumination. Computation of germinated seeds was done daily until the end of the seventh day. After that, five germinated seeds were removed and their morphological traits were assayed.

The germination percentage were calculated using the following formulas (Mostafavi, 2011):

$$GP = SNG/SNO \times 100$$

Where: GC is germination percentage, SNG is the number of germinated seeds, and SNO is the number of experimental seeds with viability (Zadeh, and Naeni (2007).

Table 1: Seed code, name and source of studied case of canola genotypes.

S.No	Seed code	Name	Source
1	ARCB100	Bronowski	Germany
2	ARCB101	Jet Neuf	=
3	ARCB102	Wesroona	=
4	ARCB103	BladKoolVanlo	=
5	ARCB104	Zeus	=
6	ARCB106	Liragold	=
7	ARCB108	Mira	=
8	ARCB109	Tantal	=
9	ARCB110	Leader	=
10	ARCB111	Janetzika	=
11	ARCB112	Kintol	=
12	ARCB113	Gross Luesewitzer	=
13	ARCB114	Magnum	=
14	ARCB115	Gorezanski	=
15	ARCB116	Nugget	=
16	ARCB117	Masora	=
17	ARCB118	Malrias	=
18	ARCB119	Lisandra	=
19	ARCB120	Cobra	=
20	ARCB122	Liberator	=
21	ARCB123	Askaria	=
22	ARCB124	Lord	=
23	ARCB125	Ziho	=
24	ARCB126	GoldgelberZarter Butte	=
25	ARCB127	Kuusika	=
26	ARCB130	Hiroshima Katsuona	=
27	ARCB131	GrnnimSchnee	=
28	ARCB133	Blaze	=
29	ARCB134	BudakalasziFekete	=
30	ARCB135	Bulharska	=

S.No	Seed code	Name	Source
31	ARCB136	Burosemjanajia	=
32	ARCB138	Hei-Ye-mi-tou-gai	=
33	ARCB139	Ib1434	=
34	ARCB140	Ib1632	=
35	ARCB144	Ib1633	=
36	ARCB145	Ib1634	=
37	ARCB146	Ib1635	=
38	ARCB147	Ib1632	=
39	ARCB148	Alaska	=
40	ARCB152	Niro1	=
41	ARCB154	Niro2	=
42	ARCB155	Niro3	=
43	ARCB157	Niro4	=
44	ARCB158	Niro5	=
45	ARCB159	Niro6	=
46	ARCB160	Niro7	=
47	ARCB161	Niro8	=
48	ARCB162	Niro9	=
49	ARCB163	Asko	=
50	ARCB164	Sombuck	=
51	ARCB165	Athiopian	=
52	ARCB166	Topas	Sweden
53	ARCB167	Bele	=
54	ARCB169	Emma	=
55	ARCB170	Falo	=
56	ARCB172	Maleksberger	=
57	ARCB173	Sombuck	=
58	ARCB174	Sumbuck 2	=
59	ARCB176	Chihli	=
60	ARCB178	Lao Tsai	=
61	ARCB179	Zambia	=
62	ARCB180	Athiopian	=
63	ARCB185	ARCB185	Sweden
64	ARCB186	ARCB186	=
65	ARCB187	ARCB187	=
66	ARCB188	ARCB188	=
67	ARCB189	ARCB189	=
68	ARCB190	ARCB190	=
69	ARCB191	ARCB191	=
70	ARCB192	ARCB192	=
71	ARCB193	ARCB193	=
72	ARCB194	ARCB194	=
73	ARCB195	ARCB195	=
74	ARCB196	ARCB196	=
75	ARCB197	ARCB197	=
76	ARCB198	ARCB198	=
77	ARCB199	ARCB199	=
78	ARCB200	ARCB200	=
79	ARCB301	ARCB301	=
80	ARCB202	ARCB202	=
81	ARCB203	ARCB203	=
82	ARCB204	ARCB204	=
83	ARCB208	ARCB208	Netherlands
84	ARCB209	ARCB209	=
85	ARCB210	ARCB210	=
86	ARCB212	ARCB212	=
87	ARCB213	ARCB213	=
88	ARCB214	ARCB214	=
89	ARCB215	ARCB215	=
90	ARCB219	ARCB219	=
91	ARCB221	ARCB221	=
92	ARCB222	ARCB222	=
93	ARCB223	ARCB223	=
94	ARCB225	ARCB225	=
95	ARCB226	ARCB226	=
96	ARCB578	ARCB578	=
97	ARCB759	ARCB759	=
98	ARCB761	ARCB761	=
99	ARCB762	ARCB762	=
100	ARCB763	ARCB763	=

B. Experimental design and analysis of data

The experimental designs were Randomized Complete Block. The treatments were arranged in factorial ones with three replications. The cluster analysis was performed by Ward method using SPSS software version 22 and bipolar decomposition were done using XLSTA software.

RESULTS AND DISCUSSION

Seed germination and establishment are the two critical steps in the life cycle of a crop. The loss of plants leads to reduction in the yield by decrease in plant density. Thus, screening of genotypes at the early stages may be an important criterion for selecting salt tolerant genotypes, thus saving considerable time. However, salt tolerance at early growth stages is not always correlated with that in the following growth stages (Zeng *et al*. 2002; Ferdose *et al.* 2009). In the present investigation, we focused on evaluation of the potential tolerance of canola genotypes to salt stress at early stages of growth.

Results of this experiment showed that, different levels of salinity stress have significant effect on canola seed germination and early seedling growth. which showed that salt stress inhibited root development. This implied that salt stress inhibited cell proliferation in the roots (An *et al.*, 2014). Our results suggested some putative mechanisms to explain the shortened roots caused by salt stress: (1) High concentrations of NaCl in the environment decreased the water potential, which made it difficult for the plant cells to absorb external water (Long, *et al.*, 2013).

Many researchers have been reported similar results (Kandil *et al*., 2012, Kazemi and Eskandari, 2011). A strong correlation was observed between germination percentage and salinity levels. There are various reports, which indicate that salt stress has an inhibitory effect on germination percentage, seedling emergence and seedling growth (Song *et al.,* 2008; Tlig *et al*., 2008; Guan *et al.,* 2009; Ahmad and Khan, 2010). Among the genotype Kunto (68) in terms of germination rate ratio to other varieties was less affected by salinity and genotype PF (98), showed the greatest sensitivity to salt tension. Although salt stress affects all growth stages of a plant, seed germination and seedling growth stages are known to be more sensitive in most plant species (Coratero, 2006). Furthermore, germination and seedling stage is predictive of plant growth responses to salinity (Farhoudi *et al.,* 2015). Therefore, seeds with more rapid germination under salt stress and/or normal conditions may be expected to achieve arapid seedling establishment and more salt tolerance, resulting in good stand establishment and hence higher yields (Song *et al*., 2008). As the results showed, all the studied growth parameters in 12 dsm^{-1}NaCl salinity, had more decrease than 6 dsm^{-1}NaCl. Fordermor the 12 dsm^{-1}NaCl salinity is more appropriate for separation of sensitive from the tolerant genotypes at germination stage.

B. Cluster analysis

Cluster analysis based on salt tolerance indices and seed yield under stress conditions classified the rapeseed cultivars into two groups (Fig. 1, 2). One group consisted of the cultivars which had high values of seed yield as well as reliable salt tolerance. Therefore, this group was known as the salt tolerant one.

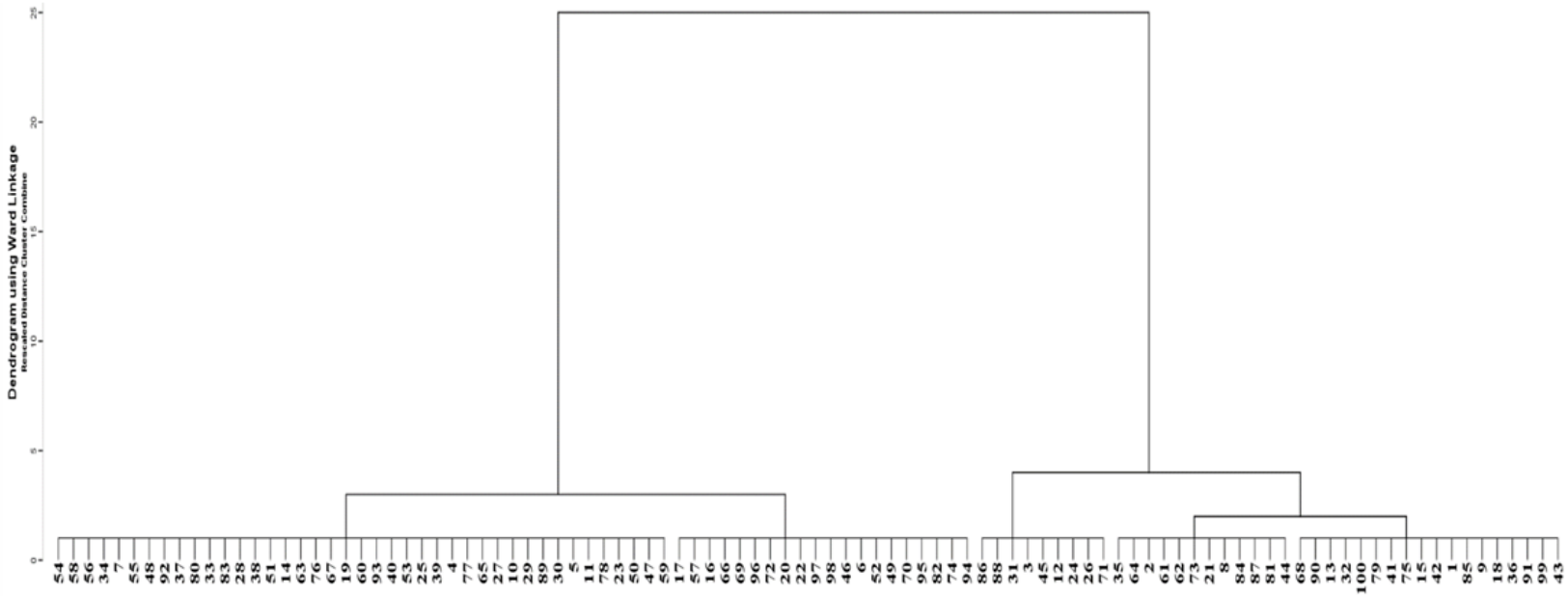

Fig. 1. Dendrogram obtained from cluster analysis of 100 different rapeseed genotypes under stress of 6 dsm^{-1}NaCl using Ward's method.

The cultivars of another group had less stable performance and thus they were considered as salt sensitive rapeseed cultivars. Cluster analysis was also used by several studies to classify genotypes according to their response to salt stress (An. *et al.*, 2014, Agarwal *et al.*, 2013, Long *et al.*, 2013). The results of cluster analysis in 6 $dS.m^{-1}$ salinity, showed that (Fig. 1) canola genotypes were divided into two main groups, 31 genotypes in the first group had higher values of the traits such as germination percentage, root and stem length, fresh and dry weight of root and shoot and were considered as tolerant and 61 genotypes in the other group were considered as sensitive to salinity. Grouping of rapeseed genotypes under stress of 12 $ds.m^{-1}$NaCl (Fig. 2) is shown that in this level of salinity, among 100 tested genotypes, numbers of 31, 100, 26, 1, 36, 3, 71, 2, 79, 73, 18, 99, 94, 68, 42, 21, 7, 91, 43, 13, 86, 64, 12, 85 and 75 were in the first group, and others were in the second group. This grouping showed good agreement with grouping of genotypes at 6 $ds.m^{-1}$NaCl. However, at 12 $ds.m^{-1}$NaCl, fewer genotypes were found in the tolerant group. Suggested that genotypes with high tolerance at both levels of salinity stress, can be studied in a greenhouse in the future.

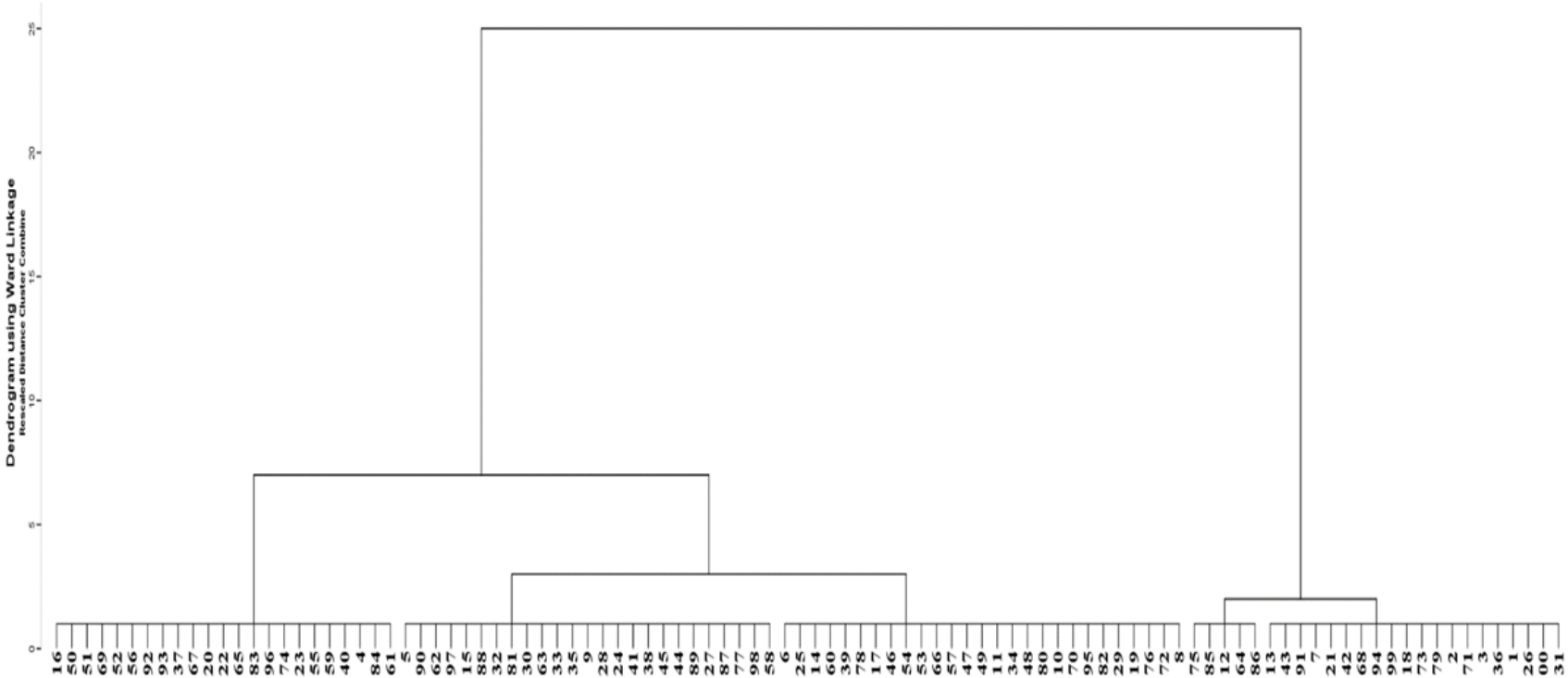

Fig. 2. Dendrogram obtained from cluster analysis of 100 different rapeseed genotypes under stress of 12 dsm^{-1}NaCl using Ward's method.

C. Biplot analysis

In order to confirm the results of cluster analysis andin order to grouping the genotypes based on the results of the main components analysis, the chart of biplot for PC1 and PC2 (Table 2 and 3), was plotted separately at deferent levels of salinity (Fig. 3 and 4). Principal component analysis was performed to provide the combined indicators for selection of the cultivars suitable for both stress and non-stress environments. In these charts, in addition to simultaneous display of more than three variables in a chart, the angle between the graphs can show correlation between variables (Long *et al.*, 2013).

Table 2: Principal component analysis of 100 rapeseed cultivars at 6 dSm^{-1}NaCl.

The main components	Specific values	Percentage of variance	Cumulative variance	GP	SL	RL	SFW	LFW	SDW	RDW
PC1	3.115	44.50	44.50	0.136	0.375	0.481	0.443	0.335	0.430	0.340
PC2	1.076	15.37	59.87	0.472	0.588	0.069	0.312	0.107-0	0.272-	0.533-

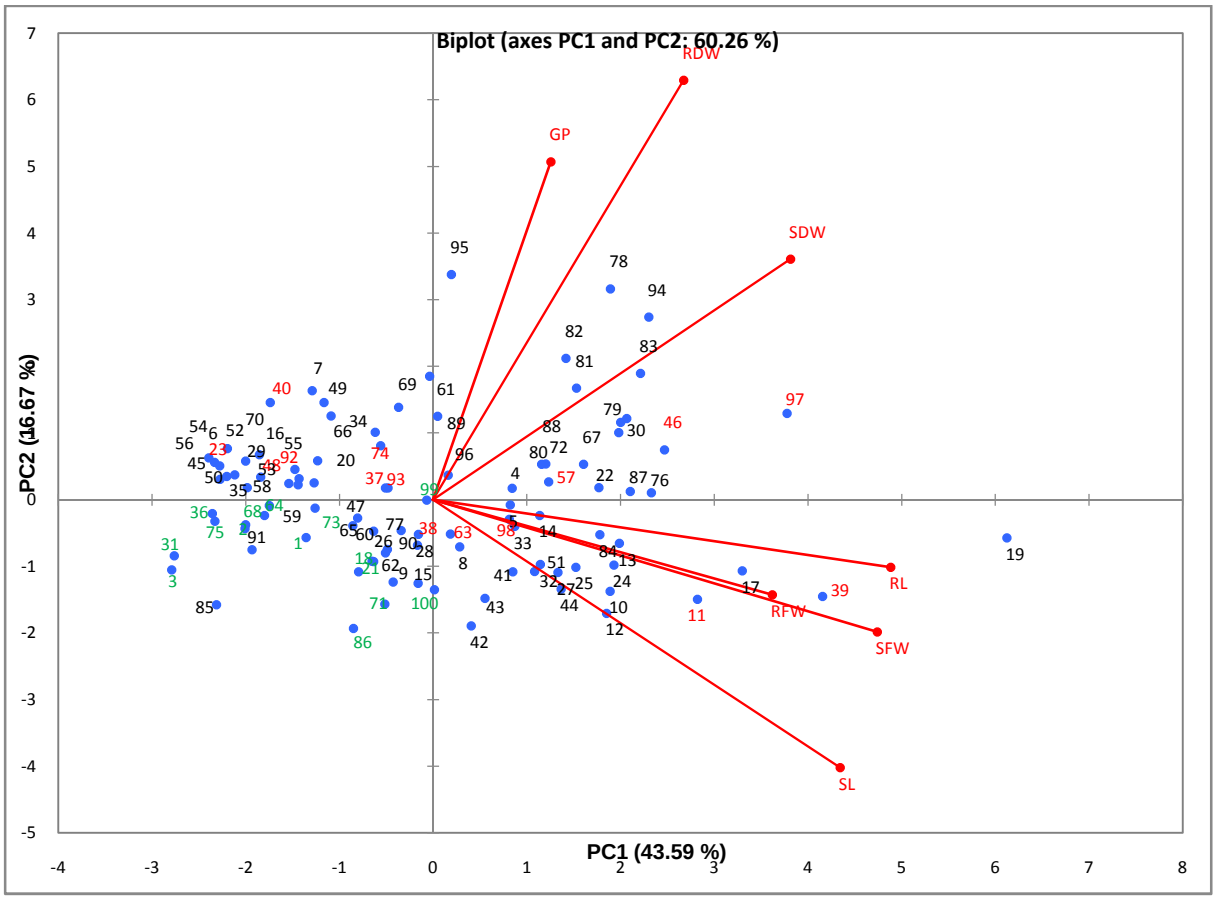

Fig. 3. The biplot diagram based on first and second components for 100 rapeseed genotypes and the indices of salt tolerance in 6 dsm^{-1}NaCl. The genotypes are represented by the numbers given in Table 1.

Table 3: Principal Component analysis of 100 rapeseed cultivars at 12 dsm^{-1} NaCl.

The main components	Specific values	Percentage of variance	Cumulative variance	GP	SL	RL	SFW	LFW	SDW	RDW
PC1	3.051	43.59	43.59	0.124	0.431	0.485	0.470	0.359	0.379	0.265
PC2	1.167	16.67	60.26	0.503	0.399	0.101	0.197	0.141-	0.358	0.625

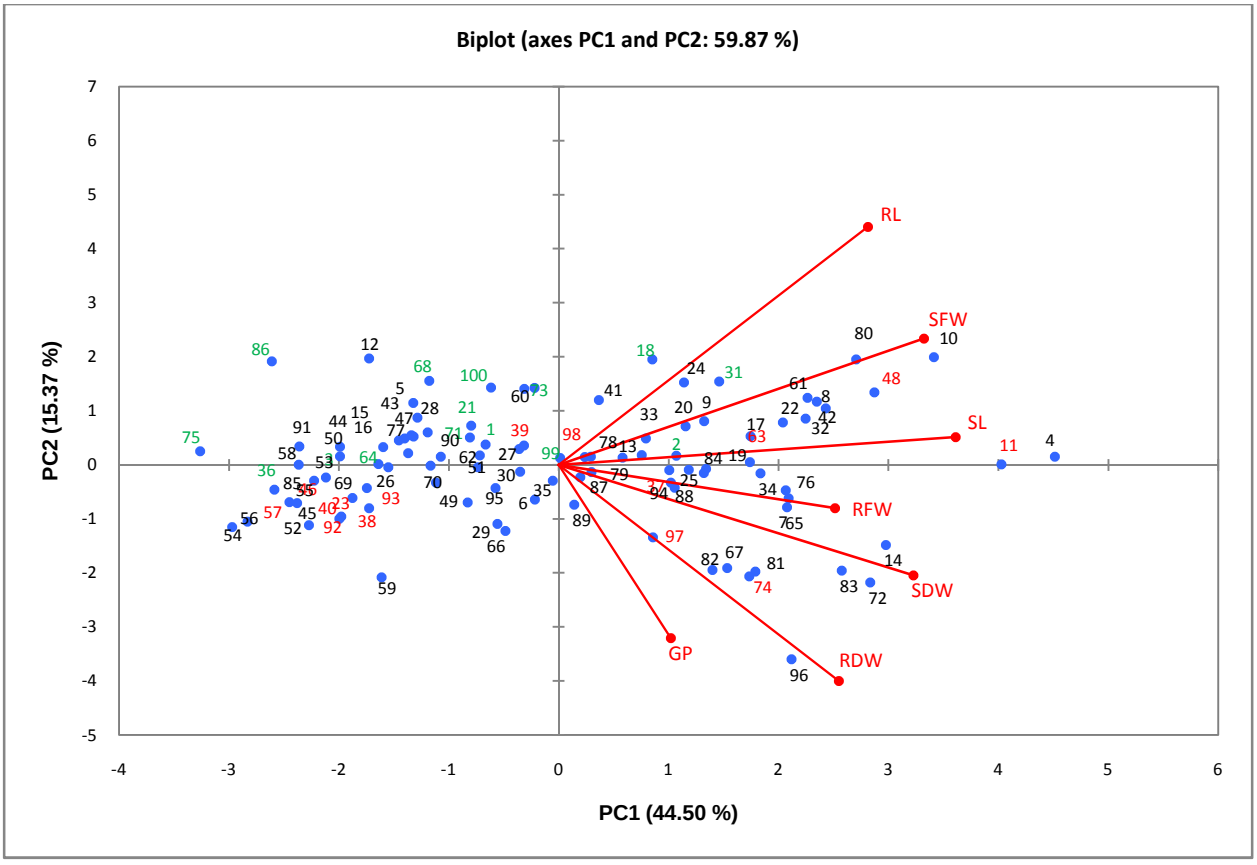

Fig. 4. The biplot diagram based on first and second components for 100 rapeseed genotypes and the indices of salt tolerance in12 dsm^{-1}NaCl. The genotypes are represented by the numbers given in Table 1.

Accordingly, two main components in the biplot diagrams approximately justify 60% of the changes, because these two components justify a significant percentage of the changes, so drawing a biplot based on these two components can be useful. In these charts, since, cosine angle between vectors of two adjective is determined correlation coefficient between them. The correlation coefficient between any two traits is approximated by the cosine of the angle between their vectors (Mostafavi, *et al.*, 2011). The correlation coefficients among the traits indicate that the biplot currently shows relationship among the traits that had relatively large loading on both PC1 and PC2. The biplot of genotypes-adjective is the best way to display the graphic between the traits interactions (Zadeh and Naeni, 2007). The results were generally in accordance with the findings of biplot analysis. At different levels of stress the relationship between traits will also change. In both biplot charts it is shown that germination percentage (GP) and root fresh weight (RFW), to a lesser extent, were able to show diversity among the genotypes. Several reports have used biplot analysis on the basis of the first two principal components for screening salt tolerant genotypes of different crop species (Mozafariyan *et al.*, 2013, Guan *et al.*, 2009, An *et al*., 2014, Agarwal *et al.*, 2013).

Correlation between the studied traits indicates that the positive correlation (r = 0.925**) between plumule length and radicle dry weigh (Table 4). This result confirm that, with accumulation of root dry matter and weight, Water absorption and plumule growth will increase (Bybordi, and Tabatabaei, 2009). There was a positive correlation between GP and RDW (r = 0.935**), GP and SL (r = 0.956**), RL and PL (r = 0.764*) SL and (RDW) and PL (r = 0.925**) and negative correlation between salinity stress and GP (r = -0.910**), RL (r = -0.520*), PL (r = -0.821*), RDW (r = -0.742*) and PDW (r = -11.032). The reductions of fresh weight due to salinity stress have also been investigated by several scientists in several crops in tomato (Mozafariyan *et al.,* 2013) and in *Ocimum basilcum* (Mohammadzadeh *et al.,* 2013) whereas the increase in fresh weight in *Pennisetum alopecuroides* at 100mM salinity has been reported by Mane *et al.,* (2011). The reduction in biomass increased with salinity increasing, because of disturbances in physiological and biochemical activities, under saline conditions is shown by Agarwal *et al*., (2013).

Table 4: A simple correlation between traits of different rapeseed genotypes.

Trait	Salinity	Germination percentage	Radicle length	Plumule length	Radicle weigh	Plumule weight
Salinity	**1**					
Germination percentage	**-0.910***	**1**				
Radicle length	**-0.520**	**0.712**	**1**			
Plumule length	**-0.821***	**0.956****	**0.764***	**1**		
Radicle dryweigh	**-0.742***	**0.935****	**0.685**	**0.925****	**1**	
Plumuledry weight	**-0.032**	**0.234**	**0.574**	**0.490**	**0.312**	**1**

*, **: Significant at the 5% and 1% levels of probability respectively.

CONCLUSIONS

One of the most important constraints to agricultural production in world is abiotic stress conditions prevailing in the environment. Salinity is one of the serious problem especially in the arid and semi-arid region. Furthermore use of tolerant plant in this region is important. The results showed that germination and plant early growth significantly affected by salinity. Plants in response to salinity divided into two, susceptible and tolerated groups. High germination percentage and vigorous seedling growth defines the tolerant genotypes. So the early growth of the plants is effective in the next growth of the plants, it is better to screen for sensitive and tolerant genotypes in the early stages of growth. On the basis of the above findings, it is concluded that germination, emergence, root and shot fresh/dry weight are significant screening criteria for salt tolerance in canola genotypes

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