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Study of canola (*Brassica napus* L.) genotypes for salt tolerance at germination stage via multivariate analysis

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Abstract

This study was conducted to evaluate the effect of salt on the germination and early vegetative growth of canola genotypes. In order to an experiment was performed as factorial form under completely randomized design (CRD) with 3 replications. Genotype factor was contains of 12 genotypes and 6 levels of salt (control, -3, -6, -9, -12 and -15 bar) with NaCl. Results of correlation showed that seedling length had the most positive and significant correlation with index seed vigour (r=0.97**). Factor analysis based on principal component analysis showed that three principle components had eigen values more than 1 and together accounted for 88% of the variability of original data under salt stress. The first component named seedling characteristics and index seed vigour. The second component named mean germination time, plumule length and wet weight of seedling. The three component named germination characteristics and dry weight of seedling. Based biplot, canola genotypes to three groups was divided. The first PCA separated Hyola401 in the extreme horizontal ends of the biplot, Hyola401 expressed as the tolerant genotype in the right and genotypes of H6661 and Q6503 as the most sensitive genotypes in the left side of the biplot.

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Introduction

Salinity, whether from soil or water affects plant growth and development due to salt-induced water deficit, low uptake and accumulation of essential nutrients, and high accumulation of toxic ions such as Na^+ and Cl^-. All these factors cause changes in a wide variety of physiological and biochemical processes such as photosynthesis, protein synthesis and nucleic acid metabolism (Ashraf, 2004; Munns, 2005).

Alternatively, selection and breeding of cultivars tolerant to salinity is a feasible and economical approach for utilizing salt affected soils (Munns *et al.*, 2006). However, the success of this approach depends on the presence of genetic variation in the gene pool of a species.

Canola (*Brassica napus* L.) is one of the most important sources of vegetable oils and protein-rich meals worldwide. Canola ranks third in global production of oilseed crops and fifth among economically important crops following wheat, rice, maize, and cotton (FAOSTAT, 2011). Availability of genetic diversity and genetic variation is the heart of any breeding program which plays a critical role in developing well-adapted and improved varieties.

It is, therefore, important to develop new canola genotypes with high genetic capacity to tolerate salt stress. The first important step in breeding new genotypes with high salt tolerance is to have a useful and substantial genetic variation in tolerance to salinity stress. Breeders seek to develop and identify cultivars that are more tolerant towards salinity and water stress (Janmohammadi *et al.,* 2008).

Germination and seedling characteristics are the most viable criteria used for selecting salt tolerance in plants. Germination percentage, germination rate and seedling growth are most suitable criteria for selection of cultivars. One of the commonest experiments in germination of the seeds is the application of NaCl. Seed response to salinity can be simulated by NaCl induced ionic stress in the germination experiments. Ionic stress is caused by a toxic accumulation of NaCl in plant tissues. Germination rates decrease with an increase in NaCl concentration (Murillo-Amador *et al.,* 2002).

Selection of a salt tolerant genotype based on germination and seedling growth under controlled conditions is simple, quick, precise and not time consuming. Consequently, this research focused on grouping of canola genotypes for salt tolerance based on germination and seedling growth.

Materials and methods

Experimental treatments and experimental design

Effect of salt stress induced by different osmotic potential levels (control, -3, -6, -9, -12 and -15 bar) NaCl treatments on germination and early seedling development of 12 canola genotypes (Hyola401, H1432, H1750, H6463, H6528, H6610, H6661, H6729, T2522, T98002, T98007 and Q6503) was studied. This investigation was performed as factorial experiment under completely randomized design with three replications at Seed Laboratory, Islamic Azad University, Shoushtar Branch, Iran in the year 2013.

Germination test and studied traits

The selected seeds of each genotype were first sterilized in sodium hypochlorite (1.5%) solution and then washed and the washed twice in deionized distilled water. Then petri-dishes containing one layer filter paper were moistened by respective prepared salt solutions. Thereafter, 25 seeds of each genotype were soaked in these petri-dishes and then kept in an incubator (40% relative humidity) at 25°C. Daily germination rate was measured and filter papers were replaced, when needed. Similarly, respective salt solutions were added when required. Seeds were considered germinated when the emergent radicle reached to 2 mm length. After 7 days, germination percentage was measured by ISTA (1996) standard method. By the end of the 7^{th} day, the germination percentage, mean germination time, germination rate, radicle length, plumule length, seedling length, radicle/plumule length ratio, dry weight of seedling, wet weight of seedling, dry/wet weight ratio of seedling and index seed vigour were also measured.

Statistical analysis

For statistical analysis, the data of germinating percentage were transformed to $\arcsin\sqrt{\frac{X}{100}}$. Analysis of the variance, correlations and factor analysis based on principal component analysis using Minitab software was carried out. Variables assigned to different components and independent component with regard to the coefficient value after operating Varimax took turns. Component coefficient greater than 0.3, regardless of its mark as a significant component for any independent components was considered.

Results

Analysis of variance

Results of ANOVA showed significant differences among different levels of salt stress and interaction between salt stress and genotypes for all traits. In the genotypes had significant differences for all traits except seedling length (Table 1).

Table 1. Analysis of variance on mean of squares of studied traits canola genotypes under salt stress.

Source of variance	Df	Germination	Mean germination time	Germination rate	Radicle length	Plumule length	Seedling length
Salt levels	5	34539.765**	7.505**	3.862**	782.808**	90.628**	1380.005**
Genotypes	11	315.717**	0.858**	0.062 **	3.938**	0.930**	3.677 ns
Salt×Genotypes	55	210.556**	1.084**	0.037**	2.862**	0.562**	3.508**
Error	144	47.602	0.022	0.003	1.459	0.209	2.127

ns and**: non significant and significant at P=0.01 level.

Continued table 1.

Source of variance	Df	Radicle/plumule length ratio	Wet weight of seedling	Dry weight of seedling	Dry/wet weight ratio	Index seed vigour
Salt levels	5	56.201**	20201.784 **	9.718 **	0.222**	1101.37**2
Genotypes	11	2.710**	770.782 **	5.944 **	0.054**	3.464*
Salt×Genotypes	55	1.316**	131.292 **	0.923 **	0.037**	3.571**
Error	144	0.395	24.411	0.153	0.004	1.746

**: significant at P=0.01 level.

Simple correlation

Simple correlation coefficients between studied traits are illustrated in Table 2. Results showed that germination percentage had positive and significant correlation with germination rate. Mean germination time had positive and significant correlation with plumule length and dry weight of seedling. Germination rate had positive and significant correlation with wet weight of seedling. Radicle length had positive and significant correlation with seedling length, radicle/plumule length ratio, index seed vigour and dry/wet weight ratio of seedling. Seedling length had positive and significant correlation with radicle/plumule length ratio, index seed vigour and dry/wet weight ratio of seedling. Radicle/plumule length ratio had positive and significant correlation with index seed vigour. Index seed vigour had the most positive and significant correlation with dry/wet weight ratio of seedling.

Sharma *et al.,* (2013) with study mustard (*Brassica juncea*) genotypes reported a highly positive correlation between germination percentage with all germination traits viz. speed of germination, germination index, mean germination time and relative germination rate both in control and salt treatment. Similar trends occurred for speed of germination, germination index and mean

germination rate. Under salt condition, radicle/plumule ratio showed high positive correlation with germination percentage, speed of germination, germination index, mean germination time and relative germination rate. Radicle length showed highly negative correlation with salt tolerance index for radicle length under control but positive correlation with salt treatment. A similar trend was observed for plumule length and tolerance index for plumule length. Dry matter of the seedlings had positive correlation with tolerance index for dry weight under salt condition.

Table 2. Correlation coefficients of studied traits canola genotypes under salt stress .

Traits	1	2	3	4	5	6	7	8	9	10
1.Germination (%)	1									
2.Mean germination time (day)	0.452	1								
3.Germination rate (number in day)	0.732**	0.371	1							
4.Radicle length (cm)	0.196	0.086	0.024 -	1						
5.Plumule length (cm)	0.056	0.604*	0.190	-0.333	1					
6.Seedling length (cm)	0.192	0.365	0.039	0.862**	0.180	1				
7.Radicle/plumule length ratio	0.204	-0.078	0.057	0.848**	0.555-	0.579*	1			
8. Index seed vigour	0.267	0.427	0.017-	0.842**	0.178	0.974**	0.525**	1		
9. Wet weight of seedling (mg)	0.522	0.259	0.703*	-0.428	0.447	-0.210	-0.461	-0.224	1	
10. Dry weight of seedling (mg)	0.099	0.654*	0.051-	0.241	0.493	0.465	-0.103	0.526	-0.213	1
11. Dry/wet weight ratio	-0.114	0.423	-0.328	0.596*	0.194	0.706**	0.271	0.757**	-0.560	0.797**

Principal component analysis

Factor analysis based on principal component analysis showed that three principle components had eigen values more than 1 and together accounted for 88% of the variability of original data under salt stress condition (Table 3). In the first component 41% of all variation data determined. Also, radicle length, seedling length, radicle/plumule length ratio, index seed vigour and dry/wet weight ratio of seedling positive factor coefficients were shown (Table 3). With attention to significant traits in the first component, this component named seedling characteristics and index seed vigour. Furthermore, with attention to be desirable of traits with positive factor coefficients, to be high first component should be considered. In the second component 27% of all variation data determined. Also, mean germination time, plumule length and wet weight of seedling had positive factor coefficients were shown (Table 3). Therefore, named mean germination time, plumule length and wet weight of seedling. With attention to be undesirable of trait mean germination time with positive factor coefficient, to be low second component should be considered. In the three component 20% of all variation data determined. Also, germination percentage and germination rate positive factor coefficients and dry weight of seedling negative factor coefficient was shown (Table 3). Therefore, named germination characteristics and dry weight of seedling.

Principle component analysis which facilitates selection of genotypes especially when there are many genotypes to be selected and many traits to be involved was used to determine whether there was any structure associated with germination indices and salt tolerance or not. Principal component analysis (PCA) has been widely used in the studies of variability in germplasm collections of oilseeds under salt stress such as canola (*Brassica napus* L.) (Abbaszadeh *et al.,* 2012) and linseed (*Linum usitatissimum* L.) (Kaya *et al.,* 2012), under drought

stress such as sunflower (*Helianthus annuus* L.) (Ghaffari *et al.*, 2012) and safflower (*Carthamus tinctorious L.*) (Zareie *et al.*, 2013) and under normal condition such as soybean (*Glysin max* L.) (Rameeh, 2010).

Biplot display

Biplot basis on the first and second components in salt stress condition (Fig. 1) canola genotypes to three groups was divided. This plot showed that the first PCA separated Hyola401 in the extreme horizontal ends of the biplot, Hyola401 expressed as the tolerant genotype in the right because in region with high first component and low second component were located. The tolerant genotype was differentiated with the high values of a cluster of traits like that radicle length, seedling length, radicle/plumule length ratio, index seed vigour and dry/wet weight ratio of seedling. These traits had high correlation with each other.

Table 3. Results of principal component analysis all traits studied in canola genotypes under salt stress.

Traits	Components		
	1	2	3
Germination (%)	0.083	0.359	0.428
Mean germination time (day)	0.210	0.432	-0.117
Germination rate (number in day)	-0.038	0.403	0.411
Radicle length (cm)	0.411	-0.155	0.263
Plumule length (cm)	0.036	0.431	-0.356
Seedling length (cm)	0.440	0.047	0.082
Radicle/plumule length ratio	0.417	-0.241	0.286
Index seed vigour	0.449	0.065	0.051
Wet weight of seedling (mg)	-0.204	0.448	0.198
Dry weight of seedling (mg)	0.301	0.217	-0.363
Dry/wet weight ratio of seedling	0.411	-0.030	-0.290
Eigen value	4.4720	3.0066	2.1554
Relative variance	0.407	0.273	0.196
Cumulative variance	0.407	0.680	0.876

But, genotypes of H6661, T98007, T2522 and Q6503 in region with low first component were located. Therefore, this genotypes were high sensitive to salt stress; especially, genotypes of H6661 and Q6503 as the most sensitive genotypes in the left side of the biplot.

Genotypes of H1432, H1750, H6528, H6463, H6610, H6729 and T98002 in other group and in region with high second component located. Therefore, these genotypes, with attention to results of principal components analysis were semi-sensitive to salt stress.

The use of biplot display in selecting tolerant genotypes has already been used by Kaya *et al.,* (2012) in linseed (*Linum usitatissimum* L.) under salt stress, Ghaffari *et al.*, (2012) in sunflower (*Helianthus annuus* L.) and Zareie *et al.*, (2013) in safflower (*Carthamus tinctorious L.*) under drought stress.

Discussion

With attention to results of correlation, traits of radicle length, seedling length, radicle/plumule length ratio, index seed vigour and dry/wet weight of seedling had positive and significant correlation with other and were located in first component that 41% of all variation data determined and with attention to be desirable of this traits, to be high first component should be considered, therefore this traits are the best germination indices and growth seedling for salt tolerance in canola genotypes.

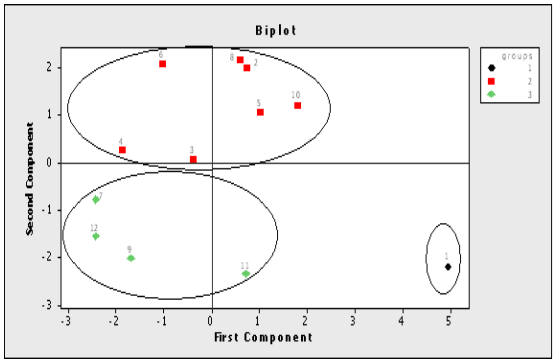

Fig. 1. The biplot display of canola genotypes on the first and second components in salt stress condition (1: Hyola401, 2:H1432, 3:H1750, 4: H6463, 5: H6528, 6: H6610, 7: H6661, 8: H6729, 9: T2522, 10: T98002, 11: T98007, 12: Q6503).

Based biplot, canola genotypes to three groups was divided. The first PCA separated Hyola401 in the extreme horizontal ends of the biplot, Hyola401 expressed as the tolerant genotype in the right and genotypes of H6661 and Q6503 as the most sensitive genotypes in the left side of the biplot. Valuable plants from the most promising materials could be used for future activities in our canola breeding programme.

These results can be related to some earlier studies in which genotypes identified as salinity tolerant at the earlier growth stages showed tolerance when tested at the later growth stages. Although, a considerable magnitude of variation for salt tolerance was observed in the 12 genotypes of canola while screening them at germination stages, further studies need to be carried out to assess whether the lines marked as salinity tolerant at the initial growth stages maintain their degree of salt tolerance when tested as adult plants.

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