Salinity Stress Indices of Seed Yield and Nutrient Compositions in Rapeseed (*Brassica napus* L.)

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Received: November 1, 2011 Accepted: November 14, 2011 Published: January 1, 2012
doi:10.5539/ijb.v4n1p154 URL: http://dx.doi.org/10.5539/ijb.v4n1p154

Abstract

Soil salinity is a serious constrain to crop production in many areas of the world. Seven rapeseed genotypes were evaluated at two salinity levels of irrigation water including 0 and 12 ds/m in green house condition based on completely randomized design with three replications. The results showed that the most of the genotypes with the high seed yield in the non saline condition had high amount of seed yield in saline condition. Amount of sodium (Na) and calcium (Ca) were more correlated than potassium (K) with seed yield. Stress tolerance index related to potassium (STI) was significant positive correlated to Yp and Ys which indicating the importance of this stress index for improving seed yield in saline condition.L18 with high efficiency of Ca absorption in saline condition had the highest amount of seed yield in stress condition. STI was more efficient selection than SSI for improving seed yield and the other ions composition. On the basis of factor analysis the shoot ions compositions including Ca and Na are more suitable than K for discriminate the saline tolerant genotypes.

Keywords: Discriminate, Factor analysis, Nutrient compositions, Seed yield

1. Introduction

Plants growing under field conditions are exposed to various environmental factors, which comprise their macro and microenvironment. Any deviation in these factors from the optimal levels is deleterious to plants and leads to stress. Salinity is considered a significant factor affecting crop production and agricultural sustainability in arid and semiarid regions of the world, decreasing the value and productivity of the affected land (Mandhania, et. al., 2010). The loss of farmable land due to salinization is directly in conflict with the needs of the world population. Thus, there is a deliberate need to raise varieties that can, not only withstand high levels of salt but can also maintain optimum yield levels. However, efforts to improve crop performance under salinity have been elusive owing to its multigenic and quantitative nature. This has given an impetus to follow a combinatorial approach employing both conventional and non-conventional strategies to improve salt tolerance (Purty, et. al., 2008). Adverse effect of salinity on plant growth may be due to ion cytotoxicity and osmotic stress. Decrease in uptake of potassium (K) and in this manner decrease in growth at higher sodium (Na) concentration have been reported. Tolerance of oilseed brassicas to salt stress is a complex trait, which is greatly modified by cultural, climatic and biological factors (Kumar, 1995; Minhas, et al., 1990; Ashraf and McNeilly, 2004; Mahmoodzadeh, 2008). 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 product quality (Zamani et. al., 2011). The salinity may reduce the crop yield by upsetting water and nutritional balance of plant (Francois,1994; Islam, 2001). Water availability and nutrient uptake by plant roots is limited because of high osmotic potential and toxicity of Na and chlorine (Cl) ions (Kumar, 1995). Significant variation in seed germination and other growth stages among canola cultivars grown under salinity condition is widely reported by Puppala et al (1999), Mer et. al., (2000), Bybordi, (2010), Tunuturk et. al., (2011) and Zamani et. al., (2011). The differences also included effects of salinity on overall growth, electrolyte leakage, proline accumulation and the K/Na ratio. The amphitetraploids *Brassica* species including *Brassica napus*, *B. carinata* and *B. juncea* are more tolerant to salinity and alkalinity than their respective diploid progenitors such as *B. campestris*, *B. nigra* and *B. oleracea* (Kumar, 1995). In response to salinity stress, endogenous Na concentration increased in the various Brassica genotypes whereas K concentration decreased. Saline soils and saline irrigation waters present potential hazards to canola production. Calcium (Ca) and K ameliorate the adverse effects of salinity on plants (Volkamar, 1998; Amador, 2007; Munnus, 2002). Salinity impairs the uptake up Ca by plants, possibly by displacing it from the cell membrane or in some way affecting membrane function (Lauchli, 1990; Rameeh et. al., 2004). Gorham (1993) claimed that all plants discriminate to some extent between Na and K. Na can be substituted for K for uptake, and it is believed that similar mechanisms of uptake may operate for both ions (He and Cramer, 1992; Porcelli, et. al., 1995; Schorder et.al., 1994). High levels of K in young expanding tissue is associated with salt tolerance in many plant species (Ashaf and McNeilly, 2004; Bandeh-Hagh, et. al., 2008; Mer, et. al., 2000). He and Cramer (1992) reported that Cacould play a regulatory role in the responses of *Brassica* species to saline environments. Thakral et al.(1998) reported positive non-significant correlation between seed yield and K/Na in stress environment in *B. juncea*. Das et al. (1994) claimed that increase in NaCl concentration was associated with increased Na and Cl influx and K efflux in *B. campestris*.

Means for determining the relationship between traits in stress environment, factor analysis can be efficient. The main applications of factor analytic techniques are to define a few factors (less than the number of studied traits) which can be useful for detecting the relationship among studied traits (Johnson and Wichern 1982; Lewis and Lisle 1999). Factor analysis has been used to determine structural factors related to growth traits and yield components in some crops and also it was used for detecting factors relating to environmental stress including drought resistance in *B. napus* (Rameeh et. al., 2004).

Several stress indices have been developed that may be more applicable to work on environmental stress tolerance such as drought tolerance (Cheema et al. 2004; Moghani Nasri, et. al., 2006; Saba, et. al., 2001), salinity tolerance(Rameeh and et al., 2004; Rezai and Saeidi, 2005) and temperature tolerance(Porch and Jahn, 2001). Rosielle and Hamblin (1981) defined stress tolerance (TOL) as the differences in yield between the stress (Ys) and non-stress (Yp) environments and mean productivity (MP) as the average yield of Ys and Yp. Fischer and Maurer (1978) proposed a stress susceptibility index (SSI) of the cultivar. Fernandez (1992) defined a new advanced index (STI= stress tolerance index), which can be used to identify genotypes that produce high yield under both stress and non-stress conditions and geometric mean (GMP). The geometric mean is often used by breeders interested in relative performance since drought stress can vary in severity in field environment over years (Ramirez and Kelly, 1998). The optimal selection criterion should distinguish genotypes express uniform superiority in both stress and non stress environments from the genotypes that are favorable only in one environment. Among the stress tolerance indicators, a larger value of TOL and SSI represent relatively more sensitivity to stress, thus a smaller value of TOL and SSI are favored. Selection based on these two criteria favors genotypes with low yield potential under non-stress conditions and high yield under stress conditions. On the other hand, selection based on STI and GMP will be resulted in genotypes with higher stress tolerance and yield potential will be selected (Fernandez, 1992).

Due to variation of canola cultivars tolerance to salinity in different growth stages, the objectives of the present study were to investigate the effect of salinity stress on the yield and the nutrient compositions (Ca, K and Na) contents in the leaves and shoots under salinity stress and their relation ship to stress indices including SSI and STI in order to obtain suitable criteria for salinity tolerance in rapeseed genotypes.

2. Materials and Methods

This experiment was conducted at Agricultural and Natural Resources Research Center of Mazandran, Sari, Iran in 2010. Seven diverse rapeseed genotypes including three breeding spring lines (L_{14}, L_{18} and L_{111} which are early maturity and high yielding genotypes) and two spring type and early maturity cultivars including RGS003, Hyola401 and also two late maturity cultivars (PF7045/91 and Zarfam) were evaluated at two salinity levels of irrigation water including 0 and 12 ds/m during 2010-2011. A completely randomized design (CRD) with 3 replications was considered for evaluation of genotypes at two separate experiments with 0 and 12 dsm^{-1} salinity levels. The salt solution was prepared by taking NaCl:$CaCl_2$ in the ratio of 1:1 and the electrical conductivity of different salinity levels was adjusted by a direct reading conductivity meter. Soli analysis results are shown in Table 1. The soil belongs to the non-saline soil with a natural reaction and the amount of lime which is relatively high. Levels of nutrients, soil organic matter levels in the medium and other nutrients, including potassium, phosphorus, iron, manganese and copper are desirable. In each plot 10 seeds were grown in separate 8-liter pots and five plants were maintained for evaluating. Electrical conductivities of the saline treatments were increased to the desired levels by incremental additions of the salts over 10-day period to avoid osmotic shock to the seedlings. Plants in all pots were irrigated until saturation, with the excess solution allowed to drain into collection pans. All pots were maintained at farm condition and also they were isolated from raining. The studied traits were seed yield and ion concentrations in shoot including Ca, K and Na. For ions extractions, plant samples were ground by mill and then dried in a furnace at 500ºC for 2 hours. After that, plant samples were added 5 ml of 2M HCl for digestion and then they were filtered and diluted by distilled water. The final volume of each sample was 100 ml. Amount of K and Na of each final sample was measured by flame photometer and Ca was measured by atomic absorption (Isaac and Kerber, 1971). Pearson correlation and factor analysis were applied for all the traits. All studied traits were analysis based on completely randomized and all data were analyzed by SAS software and also means comparison were applied based on least significant difference test (Gomez and Gomez, 1984).

The stress tolerance indices also were determined using the equations including stress intensity: $SI=1-(\mu_s/\ \mu_p)$, tolerance index: $TOL=Xp-Xs$, stress susceptibility index: $SSI=[1-(Xs/Xp)]/SI$, stress tolerance index: $STI=(Xp.Xs)/(\ \mu_p)^2$, mean productivity: $MP=(Xs+Xp)/2$ and geometric mean productivity: $GMP=(Xs.Xp)^{0.5}$, respectively. Xs and Xp are studied traits of all genotypes per trial under stress and non-stress conditions, respectively and also µs and µp are the mean of this trait for all the genotypes per trial under stress and non-stress conditions, respectively.

<Table 1>

3. Results

3.1 Analysis of variance

Significant mean squares of the genotypes effects were determined for seed yield and shoot ions compositions including Ca, K, Na in non saline condition (Yp, Ca-p, K-p and Na-p, respectively) and also in saline condition (Ys, Ca-s, K-s and Na-s, respectively), indicating the significant genetic variation of the genotypes for these traits in two saline conditions. The stress tolerance index (STI) related to Ca, K and Na (Ca-STI, K-STI and Ca-STI, respectively) and also stress susceptibility index (SSI) for Ca, K and Na (Ca-SSI, K-SSI and Na-SSI, respectively) were significant at p=1% probability level (Table 2).

<Table 2>

3.2 Means comparison

The result of genotypes means comparison for seed yield in non saline and saline conditions and Ca, K and Na in non saline and saline conditions is presented in Table 3. The genotypes including Hyola401, L18, L111 had high amount of seed yield in both conditions. In compare to the other genotypes, L18 had less reduction of seed yield in saline condition. The high amounts of STI for seed yield were related to L18 and Hyola401which indicating the high salinity tolerance of these genotypes. The low amounts of SSI which indicating the low susceptibility to salinity stress were displayed by L18 and Sarigol. In compare to non saline condition, Ca was increased in saline condition and its amount in L18 in saline condition was about four times higher than its amount in non saline condition. The high amounts of Ca-STI were related to RGS003 and L14. The lowest Ca-SSI and seed yield were detected for Zarfam. Amount of K was decreased in saline condition and it was varied from 15.73 to 33.39 mg g^{-1} and also from 10.86 to 27.60 mg g^{-1} in non saline and saline conditions, respectively. The high amounts of K-STI which is indicating high amounts of K of genotypes under both stress and non-stress conditions were detected for L111 and Sarigol. Na was decreased more than the other shoot ions in saline condition. The high amounts of Na were detected in Hyola401, L111 and Sarigol in saline condition and also the high amounts of Ca-STI were related to L111 and Sarigol. The lowest amount of Na-SSI was observed in RGS003.

<Table 3>

3.3 Correlation analysis

Significant positive correlation was observed between Y-STI and YS, which indicating of the high amount of STI for seed yield is suitable indicator for high seed yield in saline stress condition (Table 4). Ca-STI was significant positive correlated with Ca-p and Ca-s. Ca-SSI was significant positive correlated with Yp and Ys. Na-SSI was significant positive correlated with Y-STI and Ca-SSI and also was negative correlated to Ca-p. Significant positive correlation was detected between K-STI and K-S.

<Table 4>

3.4 Factor analysis

The result of factor analysis related to shoot ions compositions in non saline and saline conditions and also their associated stress indices is presented in Table 5. The results of factor analysis revealed four factors for the studied traits and their respective salinity stress indices. The eigenvalues for factor 1, 2, 3 and 4 were 6.01, 4.05, 3.26 and 1.51, respectively. The cumulative variation for these four factors was 0.91 and it's portions for factor 1, 2, 3 and 4 were 0.37, 0.25, 0.20 and 0.09, respectively. In factor 1 high coefficients factor loading were related to Yp, Ys, Ca-p, Ca-SSI and Na-SSI. Y-SSI and Ca-s and K-SSI had high coefficients factor loading in factor 2. Na-p, Na-s and Ca-STI had high coefficients factor loading in factor 3. In factor 4 high coefficients factor loading were detected for K-P, K-S and K-STI.

<Table 5>

4. Discussion

Significant differences among the genotypes for Yp, Ca-p, K-p ,Na-p, Ys, Ca-s, K-s and Na-s, and their respective salinity stress indices makes the possible selection the suitable genotypes for improving these traits in non saline and saline conditions. Most of the genotypes with the high seed yield in the non saline condition had high amount seed yield in saline condition. L18 with the highest seed yield in saline condition, low stress intensity and highest STI was more preferable for salinity stress tolerance. The least amount of SSI also was detected for L18, so for seed yield two stress indices including STI and SSI have the same efficiency for recognizing the best genotypes. Reduction of seed yield of brassica due to salinity stress were reported in earlier studies (Francois, 1994; Islam, 2001; Zamani et. al., 2011).

Although Ca was increased in the most of the genotypes in saline condition, but there were significant differences among the genotypes for absorbing of Ca in saline condition. L18 had high efficiency for Ca absorption and its amount of Ca in saline condition was about four times its amount in non saline condition.Ca-s was positive correlated to Ys, so most of the genotypes with the high seed yield in saline condition had high amount of seed yield. Significant positive correlation of Ca-SSI with Ys also indicated the importance of Ca for salinity tolerance. Ca ameliorates the adverse effects of salinity on plants (Volkamar, 1998; Amador, 2007; Munnus, 2002). In earlier study (Rameeh et. al., 2004) was reported that salinity impairs the uptake up Ca by plants, possibly by displacing it from the cell membrane or in some way affecting membrane function.

In compare to non saline condition, K of the genotypes were decreased in saline condition. Gorham (1993) claimed that all plants discriminate to some extent between Na and K. Na can be substituted for K for uptake, and it is believed that similar mechanisms of uptake may operate for both ions (He and Cramer, 1992; Porcelli, et. al., 1995; Schorder et.al., 1994). Sarigol had the highest amount of K in saline condition, so this genotype can be used for improving of the other genotypes for increasing this ion composition. K-STI was significant positive correlated to Yp and Ys which indicating the importance of K for improving seed yield in saline condition. In earlier working (Ashaf and McNeilly, 2004; Bandeh-Hagh, et. al., 2008; Mer, et. al., 2000) were noted the high levels of K in young expanding tissue is associated with salt tolerance in many plant species.

Na in shoot and leaves of the genotypes were increased in saline condition. The genotypes including L18, Hyola401 and L111 with the high seed yield in saline condition had high amount of Na. It seems that high amount of Na in tissue of the high seed plant has important role for osmotic potential adjustment and decreases drought stress effects in saline condition. These results are similar to earlier finding of Tunuturk, et. al. (1995) who reported that in response to salinity stress, endogenous Na concentration increased in the various Brassica genotypes whereas K concentration decreased. Ca-STI was significant positive correlated with the Ca-p and Ca-s, so in tolerant genotypes these two ion compositions were increased simultaneously.

The results of factor analysis in factor 1 revealed that the shoot ions compositions including Ca and Na are more suitable than K for discriminate the tolerant genotypes. In factor 2 also was confirmed the second degree importance of K. On the basis results of factor 3 Na-STI was more correlated than Na-SSI with Na-p and Na-s. In factor 4 was also emphasized the importance of K-STI than K-SSI for discriminating of the genotypes with the high amounts of K-p and K-s.

In conclusion the genotypes had significant variations for the studied traits in non saline and saline conditions and associated stress tolerance indices. Although amount of K related to the genotypes was decreased in saline condition but Ca and Na were increased. Amount of Na and Ca were more correlated than K with seed yield. STI was more efficient selection than SSI for improving seed yield and the other ions composition. On the basis of factor analysis the shoot ions compositions including Ca and Na are more suitable than K for discriminate the tolerant genotypes.

Acknowledgment

The authors wish to thank from Agricultural and Natural Resources Research Center of Mazandaran for providing genetic materials and facility for conducting the experiment.

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Table 1. Some of physicochemical properties of soil sample

Class	(%)			($mg\ Kg^{-1}$)						TNV	OC(%)	PH	Ec
	Clay	Silt	Sand	Cu	Zn	Mn	Fe	K	P	(%)			(dsm^{-1})
Si-C-L	28	56	16	3	0.64	3.1	9	352	9.2	15	1.41	7.3	0.68

Table 2. Analysis of variance for seed yield and Shoot ions including Ca, K and Na and their associated stress indices

S.O.V	df	M.S							
		Yp	**Ys**	**Y-STI**	**Y-SSI**	**Ca-p**	**Ca-s**	**Ca-STI**	**Ca-SSI**
Genotypes	6	2.92**	1.79**	1.74**	2.89**	1224.01**	1453.56**	2.97**	141.16**
Error	14	0.06	0.03	0.03	0.21	41.25	145.35	0.18	9.88
S.O.V	df	M.S							
		K-p	**K-s**	**K-STI**	**K-SSI**	**Na-p**	**Na-s**	**Ca-STI**	**Na-SSI**
Genotypes	6	131.38**	81.61**	0.27**	0.69**	0.14**	19.89**	19.89**	0.74**
Error	14	13.42	10.20	0.03	0.17	0.05	3.65	3.27	0.28

** Significant at p= 1%.

Table 3. Comparison means of genotypes for seed yield and shoot ions including Ca, K and Na and its related stress indices

Genotypes	**Yp** ($g\ pot^{-1}$)	**Ys** ($g\ pot^{-1}$)	**Y-STI**	**Y-SSI**	**Ca-p** ($mg\ g^{-1}$)	**Ca-s** ($mg\ g^{-1}$)	**Ca-STI**	**Ca-SSI**
1-RGS003	0.89de	0.80cd	0.26d	0.27c	72.31a	76.70ab	2.61a	0.48b
2-L_{14}	1.16cd	0.59d	0.24d	1.41b	73.79a	78.10a	2.74a	0.35b
3- Zarfam	0.30e	0.02e	0.01d	2.67a	38.26b	15.80c	0.29b	-3.67b
4-L_{18}	2.52ab	2.47a	2.18a	0.06c	17.77c	65.61ab	0.56b	17.59a
5-Hyola401	3.08a	1.25bc	1.36ab	1.74ab	42.80b	44.25bc	0.89b	0.40a
6-L_{111}	2.30b	1.23bc	1.00bc	1.35b	37.07b	48.12abc	0.84b	1.89a
7-Sarigol	1.60c	1.51b	0.83c	0.11c	39.94b	44.46bc	0.84b	0.77a
Genotypes	**K-p** ($mg\ g^{-1}$)	**K-s** ($mg\ g^{-1}$)	**K-STI**	**K-SSI**	**Na-p** ($mg\ g^{-1}$)	**Na-s** ($mg\ g^{-1}$)	**Ca-STI**	**Na-SSI**
1-RGS003	15.73c	10.86c	0.24c	0.96ab	1.04a	8.70b	5.25abc	0.65a
2-L_{14}	28.39ab	21.87ab	0.85ab	0.77ab	0.94a	7.66b	4.33bc	1.16ab
3- Zarfam	33.20a	15.28bc	0.69b	1.79a	0.77b	7.91b	3.50c	1.37ab
4-L_{18}	24.86abc	19.21abc	0.66bc	0.70ab	0.63c	9.96ab	3.69c	2.29a
5-Hyola401	22.12bc	17.99bc	0.54bc	0.62ab	1.02a	11.15ab	6.65abc	1.48ab
6-L_{111}	33.39a	19.21abc	0.88ab	1.42ab	1.19a	14.52a	9.94a	1.69ab
7-Sarigol	32.14a	27.60a	1.20a	0.45a	1.21a	12.74ab	8.99ab	1.39ab

Means, in each column, followed by at least one letter in common are not significantly different at the 1% level of probability- using Duncan's multiple range test.

Table 4. Correlation among studied traits in rapeseed genotypes at different salinity levels

Traits	Yp	Ys	Y-STI	Y-SSI	Ca-p	Ca-s	Ca-STI	Ca-SSI
Yp	1							
Ys	0.74	1						
Y(STI)	0.86*	0.94**	1					
Y(SSI)	-0.24	-0.72	-0.45	1				
Ca-p	-0.48	-0.60	-0.71	0.05	1			
Ca-s	0.11	0.31	0.13	-0.63	0.52	1		
Ca-STI	-0.31	-0.29	-0.45	-0.23	0.92**	0.80*	1	
Ca-SSI	0.51	0.87**	0.85*	-0.60	-0.56	0.39	-0.21	1
K-p	-0.14	-0.15	-0.12	0.39	-0.40	0.60	-0.52	-0.20
K-s	0.26	0.36	0.24	-0.24	-0.26	-0.06	-0.21	0.11
K-STI	0.06	0.15	0.05	-0.05	-0.29	-0.27	-0.32	-0.05
K-SSI	-0.49	-0.61	-0.46	0.68	-0.07	-0.53	-0.36	-0.37
Na-p	0.10	-0.12	-0.26	-0.14	0.33	0.02	0.21	-0.53
Na-s	0.61	0.47	0.44	-0.24	0.46	-0.20	-0.43	0.09
Ca-STI	0.40	0.20	0.12	-0.20	-0.11	-0.14	-0.16	0.22
Na-SSI	0.61	0.71	0.82*	-0.09	-0.89**	-0.21	-0.71	0.75*
Traits	**K-p**	**K-s**	**K-STI**	**K-SSI**	**Na-p**	**Na-s**	**Ca-STI**	**Na-SSI**
K-p	1							
K-s	0.59	1						
K-STI	0.83*	0.92**	1					
K-SSI	0.39	-0.51	-0.16	1				
Na-p	0.12	0.31	0.34	-0.18	1			
Na-s	0.34	0.44	0.47	-0.13	0.65	1		
Ca-STI	0.32	0.44	0.49	-0.14	0.88**	0.92**	1	
Na-SSI	0.37	0.36	0.33	-0.07	-0.44	0.37	0.02	1

** Significant at p=5% and 1%, respectively.

Table 5. Factor analysis of seed yield and shoot ions including Ca, K and Na and its related stress indices

Traits	Factor1	Factor2	Factor3	Factor4
Yp	**0.74**	0.23	0.44	-0.10
Ys	**0.85**	0.50	0.13	0.08
Y(STI)	**0.95**	0.27	0.09	-0.03
Y(SSI)	-0.29	**-0.81**	-0.14	-0.04
Ca-p	**-0.88**	0.37	-0.02	-0.22
Ca-s	-0.11	0.63	-0.12	-0.22
Ca-STI	-0.67	0.65	-0.10	-0.24
Ca-SSI	**0.82**	0.42	-0.30	-0.02
K-p	0.04	-0.54	0.10	**0.80**
K-s	0.18	0.24	0.24	**0.90**
K-STI	0.08	-0.04	0.25	**0.96**
K-SSI	-0.22	**-0.83**	-0.15	-0.17
Na-p	-0.39	0.13	**0.90**	0.15
Na-s	0.42	-0.06	**0.86**	0.23
Ca-STI	0.05	0.01	**0.96**	0.26
Na-SSI	**0.92**	-0.13	-0.12	0.30
Eigenvalue	6.01	4.05	3.26	1.51
Proportion	0.37	0.25	0.20	0.09
Cumulative	0.37	0.62	0.82	0.91