Ions uptake, yield and yield attributes of rapeseed exposed to salinity stress

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Abstract

Soil salinity is a serious constrain to crop production in many areas of the world. A pot experiment was carried out with rapeseed genotypes in order to investigate the effects of salinity stress on yield associated traits and shoot ions composition. Eight rapeseed genotypes were evaluated in three salinity levels of irrigation water including 0, 6 and 12 dsm⁻¹. A factorial experiment based on completely randomized design with 4 replications was considered for evaluation of 24 treatments. Significant mean square of the salinity levels, genotypes and salinity × genotypes interaction effects were exhibited for seeds per pod, pods per plant, seed yield, Ca, K and Na, indicating significant differences of salinity levels and genetic differences for these traits. Due to significant correlation of seed yield with seeds per pod and pods per plant these traits can be considered as indirect selection criteria for seed yield improving. Among shoot ions compositions, K had significant positive correlation with seed yield, therefore this ion can be considered as good indicator for seed yield improving at saline environment. The genotypes including Hyola401, LRT1 and DSM12 had high amounts of seed yield at 12 dsm⁻¹ of salinity level and were considered as tolerant genotypes.

Keywords: correlation, experiment, rapeseed, shoot ions, seed yield.
1. Introduction

Salinity stress is a main environmental constraint to crop productivity in the arid and semiarid regions of the world. Soil salinity greatly decreases the productivity of economically important plants including Brassica specious. Saline environments influence the plant growth in different methods such as reduction water uptake, an increase of ions to toxic levels, and a reduction of nutrient accessibility. Two clear ways were emphasized for decreasing salinity effects on crop productions: reclamation of salt-affected soils by chemical amendments, and alternatively, the saline soils can be used to grow salt-tolerant plants (Ashraf and McNeilly, 2004). High salt stress also disrupts homeostasis in water potential and ion distribution. This distraction of homeostasis take places at both the cellular and the whole plant levels (Tunurturk et al., 2011). Tolerance of oilseed brassicas to salt stress is a complex characteristic, which is greatly modified by cultural, climatic and biological factors (Minhas et al., 1990; Kumar, 1995; Mahmoodzadeh, 2008). The amphitetraploids Brassica species including Brassica napus, B. carinata and B. juncea are more tolerant to salinity and alkalinity than their relevant diploid progenitors such as B. campestris, B. nigra and B. oleracea (Kumar, 1995). Canola (B. napus L.) is one of the most important oil seed crops in the world, and even more in Iran, that its production has been notably extended in recent years. 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 (Islam, 2001; Zamani et al., 2011). There are differences in sensitivity to salinity among canola cultivars (Puppala et al., 1999; Bybordi, 2010; Tunurturk et al., 2011; Zamani et al., 2011). Calcium (Ca) and potassium (K) ameliorate the adverse effects of salinity on plants (Volkamar, 1998; Munnus, 2002; Amador, 2007). 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 sodium (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; Schorder et al., 1994; Porcelli et al., 1995). High levels of K in young expanding tissue is associated with salt tolerance in many plant species (Mer et al., 2000; Ashaf and McNeilly, 2004; Bandeh-Hagh et al., 2008). Closely associated to salt exclusion and its relationship to salt tolerance is the regulation of ion selectivity, in particular the role of Ca/Na and K/Na favoritism in salt tolerance (Sharma and Gill, 1994; Volkamar et al., 1998). Ca could play a regulatory role in the responses of brassica species to saline environments (He and Cramer, 1992). 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.

The objective of the present study was to investigate the effect of salinity on yield associated traits and also shoot ions compositions and their relation to seed yield in order to obtain suitable criteria for salinity tolerance in rapeseed genotypes.
2. Materials and Methods

In order to study salt stress effect on canola seed yield, yield components and also shoot ions composition, an experiment was conducted in Agriculture and Natural Resources Research Center of Mazandran, Sari, Iran, during 2010-11. The experimental design was a completely randomized design (CRD) arrangement in $3 \times 8$ factorial with four replications. Eight diverse rapeseed genotypes (as first factor) including six breeding lines (KRN3, NDK6, KRN1, KRN2, DSM12 and LRT1) and two cultivars (Zarfam and Hyola401) were studied in three salinity levels of irrigation water including 0, 6 and 12 dsm$^{-1}$ as second factor. 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. Soil analysis results are shown in Table 1. The soil belongs to the non-saline soil with a neutral 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 planted in separate 10-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 seeds per pod, pods per plant, seed yield, Ca, K and Na. For ions extractions, plant samples were ground by mill and then dried in a furnace at 500°C 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 was detected for all the traits. All the traits were analyzed based on factorial experiments based on completely randomized and means comparison were done based on least significant difference test (Gomez and Gomez, 1984).

Table 1. Some of physicochemical properties of soil sample.

| Class | (% ) | (mg kg$^{-1}$) | TNV (%) | OC (%) | pH | Ec (dsm$^{-1}$) |
|-------|------|---------------|---------|--------|----|----------------|
| Si-C-L | 26   | 56            | 18      | 2.5    | 0.65 | 3.2            |
|       |      |               |         | 3.2    | 8   | 321            |
|       |      |               |         | 7.8    | 13  | 1.34           |
|       |      |               |         |        | 7.2 | 0.65           |

3. Results and discussion

3.1. Salinity levels effects on the traits

Significant mean squares of the salinity levels were determined for seeds per pod, pods per plant, seed yield, Ca, K and Na, indicating significant differences of these traits at three salinity levels (Table 2). Seed yield of the genotypes were decreased at high salinity levels. Pods per plant varied from 22.94 to 13.71 at 0 and 12 dsm$^{-1}$ of salinity levels, respectively (Table 3). The mean of the genotypes was decreased about half its control amount at 12dsm$^{-1}$ of salinity level. Due to increasing salinity levels, Ca was increased and its amount at 12dsm$^{-1}$ of salinity level was classified as separate group from the other salinity levels. Since Ca is an integral component of cell wall as well as it is usually bound to the exterior surface of the plasma wall.
membrane, it plays a vital role in maintaining the integrity of both cell wall and membrane. Kopittke et al. (2007) was of the view that presence of high quantity of Ca in the cell wall is important so as to provide the plasma membrane with a reasonable amount of Ca to keep its structure intact. Amounts of K were reduced at high salinity levels. The K content in plant tissues represents the main cation in plant cells, and it is a main component of the cell osmotic potential. One of the primary plant responses to salinity is the decrease in K concentration in plant tissues and thus the replacement of K by Na may lead to nutritional imbalances. Both of these ions might compete for entry into plant root cells. This competition can have significant negative effects on plant growth in saline soils, where concentrations of Na often exceed those of K (Tuneturk et al., 2011). Due to increasing of salinity level, Na was increased and its amount at 12 dsm⁻¹ of salinity level was three times of its control amount. 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). 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; Schorder et al., 1994; Porcelli et al., 1995).

Table 2. Summary of analysis of variance for yield components, seed yield, Ca, K and Na in rapeseed genotypes.

| S.O.V       | df  | Seeds per pod | Pods per plant | Seed yield (g plot⁻¹) | Ca (mg g⁻¹) | K (mg g⁻¹) | Na (mg g⁻¹) |
|-------------|-----|---------------|----------------|-----------------------|-------------|------------|-------------|
| Salinity (S)| 2   | 517.52**      | 709.59**       | 21.58**               | 484.00**    | 1211.33**  | 1487.06**   |
| Genotypes(G)| 7   | 36.45**       | 685.72**       | 7.38**                | 1322.24**   | 206.33**   | 19.30**     |
| S×G         | 14  | 12.28**       | 92.74**        | 0.43**                | 278.06**    | 38.43**    | 10.17**     |
| Error       | 72  | 1.22          | 6.38           | 0.11                  | 38.44       | 10.42      | 2.22        |

**: Significant at the 1% levels of probability.

Table 3. Mean comparison of yield components, seed yield, Ca, K and Na for salinity levels based on least significant differences (LSD) test in rapeseed genotypes.

| Salinity levels(dsm⁻¹) | Seeds per pod | Pods per plant | Seed yield (g plot⁻¹) | Ca (mg g⁻¹) | K (mg g⁻¹) | Na (mg g⁻¹) |
|------------------------|--------------|----------------|-----------------------|-------------|------------|-------------|
| S1= 0                  | 13.46a       | 22.94a         | 2.84a                 | 28.98b      | 27.53a     | 6.327c      |
| S2= 6                  | 7.33b        | 19.94b         | 1.62b                 | 28.58b      | 20.86b     | 13.02b      |
| S3= 12                 | 5.89c        | 13.71c         | 1.28c                 | 35.51c      | 15.24c     | 19.96a      |

Means, in each column, followed by at least one letter in common are not significantly different at the 1% level of probability.
3.2. Genetic variations of the genotypes

Significant mean square of genotypes at 1% probability level for seeds per pod, pods per plant, seed yield, Ca, K and Na, indicating significant genetic differences of the genotypes for these traits. Seeds per pod ranged from 6.07 to 11.25 related to NDK6 and LRT1, respectively (Table 4). The genotypes including LRNT1, DSM12, Hyola401 and KRN3 with high amounts of seeds per pod were suitable genotypes for improving this trait. The genotypes including Hyola401, LRT and KRN1 had high amounts of pods per plant. Due to significant correlation of seed yield with seeds per pod and pods per plant (0.78** and 0.80**, respectively), these traits can be considered as indirect selection criteria for seed yield improving (Table 6). Seed yield of the genotypes was varied from 0.68 to 3.09 g plot⁻¹ related to Hyola401 and KRN2, respectively. The genotypes including Hyola401, LRT1 and KRN1 with seed yield of 3.09, 2.78 and 2.30 g plot⁻¹, respectively were detected as suitable genotypes for this trait in saline environment. Ca was ranged from 20.19 to 47.77 mg g⁻¹ related to KRN3 and KRN2, respectively. Non significant correlation between Ca and seed yield, indicated that although Ca had ameliorate effects on salinity stress (Volkamar, 1998; Munnus, 2002; Amador, 2007) but in this study it had not important role for seed yield improving of the genotypes. Significant positive correlation was determined between K and seed yield, therefore this ion can be considered as good indicator for seed yield improving at saline environment. Similarly, in earlier studies (Mer et al., 2000; Ashaf and McNeilly, 2004; Bandeh-Hagh et al., 2008) were notified the important effect of K for salinity tolerance. Na was varied from 11.47 to 15.07 mg g⁻¹ related to KRN2 and KRN1, respectively. Due to significant negative correlation between Na and seed yield, the genotypes with low amounts of Na will be preferable for salinity tolerance.

Table 4. Mean comparison of yield components, seed yield, Ca, K and Na for eight rapeseed (Brassica napus L.) genotypes based on least significant differences (LSD) test.

| Variety | Seeds per pod | Pods per plant | Seed yield (g plot⁻¹) | Ca (mg g⁻¹) | K (mg g⁻¹) | Na (mg g⁻¹) |
|---------|--------------|----------------|----------------------|------------|------------|------------|
| 1-KRN3  | 9.63bc       | 19.00c         | 1.50d                | 47.77a     | 13.07d     | 13.00bc    |
| 2-NDK6  | 6.87e        | 12.11d         | 1.64cd               | 36.23b     | 22.11b     | 11.89c     |
| 3-Zarfam| 8.33d        | 13.34d         | 1.42d                | 44.59a     | 22.78b     | 12.29bc    |
| 4-KRN1  | 8.96cd       | 23.41b         | 2.30b                | 24.4cd     | 23.06b     | 15.07a     |
| 5-KRN2  | 6.07e        | 8.72e          | 0.68e                | 20.19d     | 22.03bc    | 11.47c     |
| 6-DSM12 | 10.33ab      | 17.67c         | 1.89c                | 23.94cd    | 27.49a     | 14.63a     |
| 7-LRT1  | 11.25a       | 25.22b         | 2.78a                | 23.52cd    | 18.56c     | 13.59ab    |
| 8-Hyola401| 9.73bc      | 31.46a         | 3.09a                | 27.54c     | 20.60bc    | 12.88bc    |

Means, in each column, followed by at least one letter in common are not significantly different at the 1% level of probability.
Table 5. Correlation among the studied traits in rapeseed genotypes at different salinity levels.

| Traits                        | Seeds per pod | Pods per plant | Seed yield (g plot⁻¹) | Ca (mg g⁻¹) | K (mg g⁻¹) | Na (mg g⁻¹) |
|-------------------------------|---------------|----------------|-----------------------|-------------|------------|-------------|
| 1- Seeds per pod              | 1             |                |                       |             |            |             |
| 2- Pods per plant             | 0.54**        | 1              |                       |             |            |             |
| 3- Seed yield                 | 0.78**        | 0.80**         | 1                     | -0.13       | -0.21      | -0.24       |
| 4- Ca                         | -0.13         | -0.21          | -0.24                 | 0.56**      | 0.17       | 0.43*       |
| 5- K                          | 0.56**        | 0.17           | 0.43*                 | -0.39       | 1          | -0.69**     |
| 6-Na                          | -0.73**       | -0.27          | -0.52**               | 0.17        | -0.69**    | 1           |

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

3.3. Salinity × genotypes interaction effects on traits

Significant interaction effects of salinity levels × genotypes for all the traits indicated that the trends of variation of these traits among the genotypes were different at the salinity levels. Water availability and nutrient uptake by plant roots is limited because of high osmotic potential and toxicity of Na and Cl ions (Kumar, 1995), therefore due to increasing salinity levels, most of the yield associated traits were decreased. The means of interaction effects of genotypes × salinity levels for the studied traits are presented in Figure 1 to 6 and also in Table 6. Seeds per pod varied from 10.69 to 16 in non saline condition for KRN3 and LRT1, respectively and also it was ranged from 3.23 to 9.45 related to KRN1 and KRN3, respectively at 12 dsm⁻¹ of salinity level (Figure 1). Most of the genotypes with high number of seeds per pod at control condition (0 dsm⁻¹) had high amounts of this trait at the other salinity levels (Table 6). Seeds per pod had less variation for three salinity levels in KRN3. The high amounts of pods per plant were detected for KRN1, LRT1 and Hyola401 at 12 dsm⁻¹ of salinity level (Figure 2), therefore these genotypes can be considered for improving this trait at saline condition. The highest seed yield at control and 12 dsm⁻¹ of salinity level was belonged to Hyola401 and followed by LRT1 and DSM12 had high amounts of this trait at 12 dsm⁻¹ of salinity level (Figure 3). Due to salinity increasing, Ca was increased in most of the genotypes and KRN3, Hyola401 and NDK6 had high amount of Ca at high salinity levels (Figure 4). The concentration of K reduced in the most of the genotypes at high salinity levels and the genotypes including DSM12, NDK6 and Zarfam had high means value of K at highest salinity level (Figure 5). Na was increased in all of the genotypes at high salinity levels and the genotypes including NDK6, Zarfam and KRN2 with low amounts of Na at 12 dsm⁻¹ of salinity level were detected as suitable genotypes. Agricultural productivity is severely affected by soil salinity, and the damaging effects of salt accumulation in agricultural soils have influenced ancient and modern civilizations. The detrimental effects of salt on plants are a consequence of both a water deficit that results from the relatively high solute concentrations in the soil and a Na specific stress resulting from altered K/Na ratios and Na ion concentrations that are inimical to plants. The alteration of ion ratios in the plant is caused by the influx of Na through pathways that function in the acquisition of K (Rameeh et al., 2004; Tunuturk et al., 2011).
Table 6. Mean comparison of yield components, seed yield, Ca, K and Na for eight rapeseed (Brassica napus L.) genotypes based on least significant differences (LSD) test.

| Variety       | Seeds per pod | Pods per plant | Seed yield (g plot⁻¹) | Ca (mg g⁻¹) | K (mg g⁻¹) | Na (mg g⁻¹) |
|---------------|---------------|----------------|-----------------------|-------------|------------|-------------|
| 1-S0 × KRN3   | 10.69de       | 26.92bc        | 2.45cd                | 45.50abc    | 15.88h-k   | 8.63hi      |
| 2- S0 × NDK6  | 12.12cd       | 21.44e         | 2.57cd                | 42.36bc     | 27.89bc    | 6.04ij      |
| 3- S0 × Zarfam| 13.82bc       | 22.85cde       | 2.43cd                | 52.63ab     | 28.08bc    | 5.99ij      |
| 4- S0 × KRN1  | 13.06c        | 21.70de        | 3.25                  | 16.92fg     | 34.29a     | 6.19ij      |
| 5- S0 × KRN2  | 10.66def      | 11.40f         | 1.33b                 | 17.88efg    | 32.78ab    | 6.11ij      |
| 6- S0 × DSM12 | 15.45ab       | 13.59f         | 2.58cd                | 19.36efg    | 32.85ab    | 6.66ij      |
| 7- S0 × LRT1  | 16.00a        | 28.37b         | 4.01a                 | 23.01efg    | 23.09c-g   | 5.80j       |
| 8- S0 × Hyola401| 15.91a      | 37.28a         | 4.14a                 | 14.15g      | 25.40cde   | 5.26j       |
| 9-S1 × KRN3   | 8.75efg       | 18.96e         | 1.08ghi               | 42.45bc     | 16.20h-k   | 11.72fg     |
| 10- S1 × NDK6 | 4.86hij       | 9.85fg         | 1.40fg                | 22.27efg    | 19.83e-j   | 12.17efg    |
| 11- S1 × Zarfam| 6.24hi       | 10.90fg        | 1.06ghi               | 38.79cd     | 22.73c-g   | 13.36ef     |
| 12- S1 × KRN1 | 10.18def      | 26.40bcd       | 2.24de                | 27.81def    | 20.37d-i   | 14.58e      |
| 13- S1 × KRN2 | 4.32ij        | 10.12fg        | 0.47ij                | 24.16efg    | 22.35c-g   | 10.48gh     |
| 14- S1 × DSM12| 8.78efg       | 20.74e         | 1.33fgh               | 27.98def    | 25.91cd    | 14.56e      |
| 15- S1 × LRT1 | 8.62fg        | 26.28bcd       | 2.87bc                | 23.48efg    | 18.51j     | 13.79ef     |
| 16- S1 × Hyola401| 6.91gh      | 36.31a         | 2.49cd                | 21.73efg    | 21.00h     | 13.50ef     |
| 17-S2 × KRN3  | 9.45ef        | 11.12f         | 0.99ghi               | 55.36a      | 7.15l      | 18.65cd     |
| 18- S2 × NDK6 | 3.62j         | 5.04h          | 0.96ghi               | 44.04abc    | 18.59f-j   | 17.48d      |
| 19- S2 × Zarfam| 4.93hij      | 6.29gh         | 0.76hi                | 42.34bc     | 17.53g-j   | 17.52d      |
| 20- S2 × KRN1 | 3.65j         | 22.12de        | 1.42fg                | 28.47de     | 14.53ijk   | 24.84a      |
| 21- S2 × KRN2 | 3.23j         | 4.65h          | 0.26j                 | 18.53efg    | 10.96kl    | 17.83d      |
| 22- S2 × DSM12| 6.75gh        | 18.69e         | 1.76ef                | 24.50efg    | 23.70c-f   | 22.67ab     |
| 23- S2 × LRT1 | 9.13ef        | 21.00e         | 1.48fg                | 24.09efg    | 14.06jk    | 21.17bc     |
| 24- S2 × Hyola401| 6.37hi       | 20.78e         | 2.66bcd               | 46.74abc    | 15.41h-k   | 19.88cd     |

Means, in each column, followed by at least one letter in common are not significantly different at the 1% level of probability.
**Figure 1.** Seed per pod in eight rapeseed genotypes at the different salinity levels.

**Figure 2.** Pods per plant in the eight rapeseed genotypes at the different salinity levels.

**Figure 3.** Seed yield of the eight rapeseed genotypes at the different salinity levels.
**Figure 4.** Ca in eight rapeseed genotypes at the different salinity levels.

**Figure 5.** K in eight rapeseed genotypes at the different salinity levels.

**Figure 6.** Na in eight rapeseed genotypes at the different salinity levels.
In general salinity levels had significant effects on the traits and due to increasing of salinity levels, yield and yield components and also K were decreased. With increasing salinity levels, Ca also had lowest variations among the genotypes and Na was increased in all of the genotypes. Significant correlation of seed yield with seeds per pod and pods per plant indicating these traits can be considered as indirect selection criteria for seed yield improving. Significant positive correlation was also exhibited between K and seed yield, therefore this shoot ion can be considered as good indicator for seed yield improving at saline environment. Due to existing significant negative correlation between Na and seed yield, the genotypes with low amounts of Na at saline condition will be preferable.

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