

Full Length Research Paper

The response and protein pattern of spring rapeseed genotypes to sodium chloride stress

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The research was conducted to investigate the effect of salinity on rapeseed. Furthermore, the protein pattern of genotypes was investigated to support the greenhouse evaluation. Twelve genotypes of rapeseed were treated with zero, 175 and 350 mM of NaCl in hydroponic culture system. Protein pattern of the genotypes were visualized by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) method. Most of the traits were significantly influenced by salinity. Cluster analysis identified Hyola308 and RGS003 as the most tolerant genotypes. Wild cat, Option500, Cracker, SW500, Comet and Olga are clustered together as salinity susceptible group. Twenty five reproducible bands were identified by SDS-PAGE in which fourteen were polymorphic. These results indicate a valuable genetic variability between the genotypes. Accumulation of K⁺ in shoot instead of proline might be a way in which the genotypes perform osmotic adjustment under salinity. By identification of contrasting genotypes, molecular dissection of salinity stress by genomics/proteomics approaches would be amenable in details.

Key words: *Brassica napus*, cluster analysis, physiological characteristics, proline, salt stress, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

INTRODUCTION

Among oilseeds, *Brassica* species were ranked third and colza cultivated worldwide has a special importance in quality oil production. Salinity and heat are the major water shortage environmental stresses limiting corn production and among them, drought and salinity has taken more attention (Ashraf and McNeilly, 1990). Salinity tolerance is a quantitative characteristic controlled by many genes interacting with the environment (Ashraf and Harris, 2004). Breeding programs rely on the identification of a set of traits associated with tolerance to salinity in plant species (Hemantaranjan, 1998). It has been reported that *Brassica* species show different

responses to environmental conditions (Iqbal et al., 2008). Because of the problems related to salinity studies in field experiment, hydroponic system would be suitable option for genotype screening. The indices, such as emergence percentage shoot and root dry weight, leaf related characteristics, flowering and grain yield components are some of the candidate traits that have been proposed to improve the performance of plants subjected to salinity. Leaf area and plant height decrease faster than other morphological traits because accumulating dry matter is hampered by net photosynthesis. Resistant genotypes have tendency to maintain high amount of these variables (Kumar et al., 2009). Electrolytic leakage increases under salinity stress and tolerant genotypes usually indicate lower electrolytic leakage (Sairam et al., 2002). Decreasing the percent of emergence and growth of *Brassica campestris* under

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high concentration of sodium chloride (100 mM) was due to increasing the leakage of metabolites and electrolytes, and the accumulation of sodium chloride was together with the exit of K (Das et al., 1995). It has been reported that there is an indirect relationship between salinity level and water, as well as osmotic potentials, in which, usually part of the reduction in osmotic potential is due to decrease of relative water content (RWC) (Bandeh-hagh et al., 2008). Response to salinity stress and osmotic adjustment due to accumulation of ions and amino acids happens in the cells, which causes absorption of water into the cells and protects cell turgor (Nayyar, 2003).

It has been found that, among organic osmolytes, free amino acids like serine, argenin, valin, losin and Proline have an important role in osmotic adjustment. Proline is more common than other amino acids in plants under stress (Ashraf and McNeilly, 2004), and this amino acid has important role in osmotic adjustment and stabilization of the membrane (Parida and Das, 2005). Salinity tolerant genotypes from *Brassica juncea* accumulated high amount of proline in their leaves compared to the sensitive cultivars (Heidari, 2010), but in *Brassica napus*, the amount of proline in plantlet of sensitive varieties was half to one-third of that in the tolerant varieties (Ashraf and McNeilly, 2004). Absorbing some kinds of ions such as potassium, sodium etc may also cause the osmotic adjustment with less cost (Morant-Manceau et al., 2004). Plants either remove poisonous ions such as sodium and chlore from the leaves in response to salinity (de Lacerda et al., 2005) or accumulate in vacuoles (Parida and Das, 2005). In an experiment (He and Cramer, 1993) two genotypes of *Brassica carinata* and *B. napus*, was irrigated by sea water for a period of 24-days. In the first five days of salinity stress, there was an increment in accumulating Na, Mg and Cl in shoot of both genotypes, but concentration of K and Ca was decreased. On the other hand, accumulating Na, K, Ca, Mg and Cl in the root was influenced by salinity and increased (Ashraf and Ali, 2008). Sodium as a dominant ion under salinity stress has an antagonistic relation with K and Cl, as well as negative association with plant growth. Therefore, if the plants are more active in removing Na⁺, they would be considered more tolerant (He and Cramer, 1992); as a result, the ratio of Na/K in shoot/ root of glycophytic plants is considered as a suitable selection index to identify resistance genotype in breeding for salinity (Ashraf and Orooj, 2006; Grewal, 2010; Kumar et al., 2009).

Toorchi et al. (2009b) reported a known amount of soluble protein content of leaves change in response to abiotic stresses. Agastian et al. (2000) reported that in mulberry, the amount of soluble protein is increased under mild salinity stress, but reduced under high salinity. The SDS-PAGE analysis of proteins in peanut (*Arachis hypogaea*) under NaCl salinity revealed a proteins of 52 and 127 kDa, and repression of a proteins of 38 and 260 kDa (Hassanein, 1999). Apart from the effect of salinity

stress on protein pattern due to its presence and absence, another important effect of salinity stress is a change in the intensity of the different protein bands. Hurkman and Tanka (1988) reported that the effect of salinity on protein pattern in barley was similar under both normal and salinity stress conditions, but the intensity of bands was changed quantitatively. In this research, 12 spring rapeseed genotypes were evaluated at seedling stage using a hydroponic system to investigate the effect of salinity resulted from sodium chloride on *Brassica* species. Furthermore, the protein pattern of genotypes was investigated to support the greenhouse evaluation.

MATERIALS AND METHODS

Twelve spring type genotypes of rapeseed (Olga, Wild cat, Sarigol, Heros, Cracker, Option 500, Comet, Hyola308, Amica, Eagle, SW5001 and RGS003) constituted the plant material for this experiment. Seeds were sterilized and germinated in petri dishes and seven days later these genotypes were arranged in a split plot based on randomized complete block design with three replications. Salinity treatments of zero, 175 and 350 mM of NaCl were imposed to the plants in hydroponic culture system in which they were irrigated four times daily with a modified Hogland nutrient solution. One week after putting the plantlets in the hydroponic system, salinity stress was imposed gradually by adding 50 mM of NaCl per day. Measuring different characteristics were done four weeks after imposing salinity stress (42 days old), just before flowering stage began at the end of seedling stage. Shoot and root dry weight, shoot to root dry weight ratio, leaf area, number of leaves, plant height, root length, shoot to root length ratio, leaf osmotic potential, leaf water potential, electrolytic leakage, relative water content, shoot and root proline content, sodium and potassium content of shoot and root were measured/calculated. These measured/calculated data were analyzed as a split plot experiment based on randomized complete block design with three replications, and mean comparison of genotypes were done by Duncan's Multiple Range Test. The leaf total water potential was measured by pressure chamber (Santa Barbara, CA, USA) using the youngest but well developed leaves. Relative water content was calculated by the following formula using leaf disc obtained from a young leaf of each plant (Morant-Manceau et al., 2004):

$$RWC = \frac{FW - DW}{TW - DW} \times 100$$

Where FW=fresh weight, DW=dry weight, and TW=turgid weight.

Electrolytic leakage was calculated by (Nayyar, 2003):

$$EL = L_1/L_2$$

where L₁ is electric conduction of leaf after putting in the deionized water in 25°C and L₂ is the electric conduction of the autoclaved samples.

Leaf area was measured by Leaf area meter (Model: LI-3100C-LI-COR, Biosciences, USA). Shoot and root dry weights were determined after drying the samples in 75°C for 48 h. Osmotic potential of leaf was measured by micro osmometer. The concentration of proline was measured in the leaf and root after freezing in -80°C by the method of McManus et al. (2000). The amounts of sodium and potassium ions were measured by flame photometer in dried leaf and root samples. Protein pattern of the genotypes were visualized by the method of SDS-PAGE with

Table 1. Analysis of variance for rapeseed genotypes under salinity treatments.

Source of variance	Degrees of Freedom	Mean of square								
		Electrolytic leakage	Shoot proline	Root proline	Na content of shoot	K content of shoot	Shoot Na/K ratio	Na content of root	K content of root	Root Na/K ratio
Replication	2	129.85 ^{ns}	18.091 ^{ns}	0.279 ^{ns}	5.116 ^{ns}	0.093 ^{ns}	0.210 ^{ns}	116.255 ^{ns}	386.62 ^{ns}	0.748 ^{ns}
Salinity (S)	2	19359.52 ^{**}	495.227 ^{**}	181.111 ^{**}	317.567 ^{**}	54.625 ^{**}	15.702 ^{**}	6764.913 ^{**}	59.39 ^{ns}	10.882 ^{**}
Error (a)	4	484.17	14.038	2.838	2.044	1.001	0.106	158.795	111.99	0.343
Genotype (G)	11	199.95 ^{ns}	3.075 [*]	0.675 ^{ns}	0.408 [*]	0.773 ^{**}	0.046 ^{**}	17.587 ^{ns}	35.87 ^{**}	0.039 ^{ns}
G × S	22	178.19 ^{ns}	1.184 ^{ns}	0.573 ^{ns}	0.186 ^{ns}	0.139 ^{ns}	0.028 [*]	9.304 ^{ns}	19.57 ^{ns}	0.020 ^{ns}
Error (b)	66	144.59	1.570	0.485	0.209	0.149	0.016	14.975	13.88	0.025

Source of variance	Degrees of freedom	Mean of square								
		Shoot dry weight	Root dry weight	Leaf area	Number of leaves	Shoot height	Root length	Shoot to root length ratio	RWC	Osmotic potential
Replication	2	0.056 ^{ns}	0.011 ^{ns}	2028.18 ^{ns}	0.308 ^{ns}	137.00 ^{ns}	169.824 ^{**}	6.561 [*]	116.375 ^{ns}	0.105 ^{ns}
Salinity (S)	2	1.785 [*]	0.145 [*]	170871.19 ^{**}	43.817 ^{**}	3692.10 ^{**}	9.243 ^{ns}	38.524 ^{**}	1590.033 ^{**}	13.603 ^{**}
Error (a)	4	0.167	0.017	7738.84	1.626	49.74	3.884	0.418	22.955	0.063
Genotype (G)	11	0.033 ^{**}	0.002 ^{**}	2085.95 ^{**}	0.260 ^{**}	13.63 ^{**}	2.887 ^{ns}	0.314 [*]	17.695 [*]	0.018 ^{ns}
G × S	22	0.007 ^{ns}	0.00072 ^{ns}	318.91 ^{ns}	0.180 [*]	2.89 ^{ns}	2.062 ^{ns}	0.263 ^{ns}	6.561 ^{ns}	0.008 ^{ns}
Error (b)	66	0.005	0.00048	254.90	0.102	2.41	1.807	0.159	9.074	0.013

*Significant at the 5% probability level; ** significant at the 1% probability level; ns: Not significant.

Laemmli (1970) method using Tris-NaCl solution. Using protein banding pattern, RF values were determined for each genotype. A data matrix was prepared taking 0 and 1 value for the absence and presence of a particular protein band. The genotypes were then grouped by Ward and UPGMA clustering algorithm using Jaccard coefficient of similarity under each salinity conditions. To identify the cutting point in the tree dendrograms, discriminant analysis was performed using SPSS 11.5 software.

RESULTS

Significant difference was found between salinity treatments with respect to all the traits except root length and root K⁺ content. The response of genotypes was also different to salinity

treatments, but it does not changed from one genotype to another. On the other word, the salinity × genotypes interactions were not significant for none of the traits (Table 1). The shoot and root dry weight, leaf area, leaf number, shoot to root length ratio, RWC, leaf osmotic potential and shoot potassium content were decreased in response to salinity stress. While electrolytic leakage, shoot and root proline content, shoot and root sodium content, Na⁺/K⁺ ratio in shoot and root were increased under salinity (Figures 1 and 2).

Cluster analysis was carried out using WARD method according to the Euclidean distance on standardized data (Figure 5). Discriminant function analysis was employed to identify the

cutting point in the dendrograms and determination of group number; therefore, the genotypes were divided into four groups (Table 2). Group one includes two genotypes, with higher mean for shoot dry weight, root dry weight, leaf area index, number of leaves, plant height, root length, shoot to root length ratio, leaf water potential, electrolytic leakage, sodium content of shoot and root, potassium content of shoot and root Na⁺/K⁺ ratio. Considering the characteristics, this group can be considered as tolerant genotypes based on the investigated characteristics under salinity stress. Second group, which includes Amica and Eagle has higher mean for the traits, such as shoot and root dry weight, leaf area index, leaf osmotic potential,

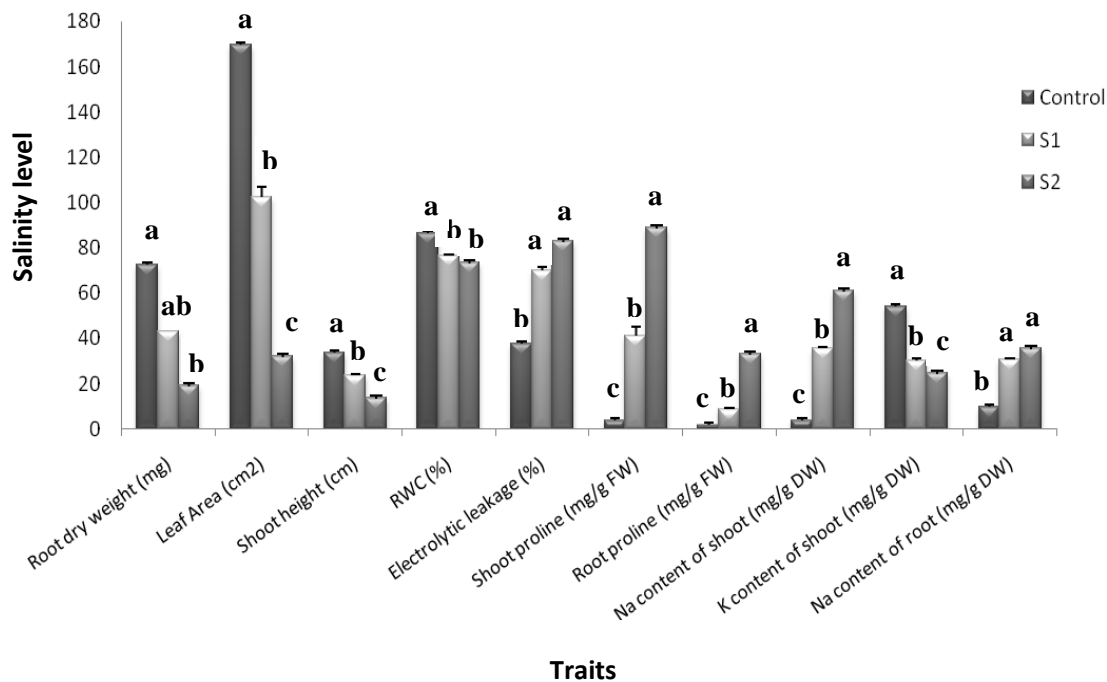


Figure 1. Mean of some traits according to salinity levels.

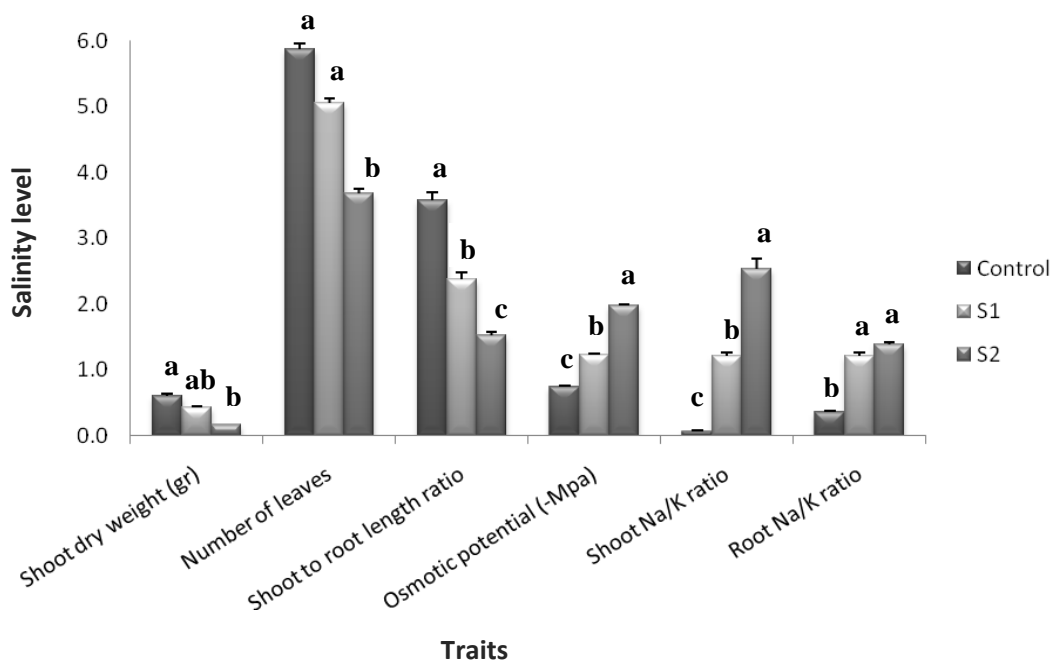


Figure 2. Mean of some other traits according to salinity levels.

electrolytic leakage, shoot and root proline and potassium content of shoot. This group also can be considered as tolerant to salinity stress, but compared with group one, this group includes semi-tolerant genotypes. Group three includes Sarigol and Heros with average shoot and root

dry weight, number of leaves, osmotic potential, shoot to root length ratio and root Na⁺/K⁺ ratio; however, this group has lower mean of leaf area, relative water content and potassium content of shoot. Therefore, group three can be considered as a semi-sensitive group. Cluster 4,

Table 2. Discriminant function analysis to identify the cutting point.

Wilk's lambda	Probability	Number of groups
0.032	0.208	2
0.000277	0.002	3
0.000033	0.001	4

which includes Wild cat, Option500, Crackers, SW5001, Comet and Olga has lower mean with respect to RWC, root proline and most of the other traits and ranked as sensitive genotypes to salinity stress. The genotypes of group one and four is seen to be isodirectional with respect to most of the alleles conferring tolerance to salinity, consequently, selected genotypes from these clusters may be crossed reciprocally to develop a genetic population suitable for QTL mapping.

To explore the protein expression of genotypes to salinity stress, three to four independent samples were taken from each replication of all genotypes under different salinity treatments and subjected to SDS-PAGE. The reproducible bands, which are repeated in all the gels are considered for analysis. Twenty five reproducible bands are recognized in which fourteen were polymorphic (Picture 1). Bands 14, 17, 41, 43 and 49 appeared under control condition in all of the genotypes, but under salinity, the genotypes showed different responses to salinity stress. In contrast, bands 24 and 40 did not appear under control condition in all genotypes, but inductions of salinity caused these bands to appear in some of the genotypes. Band 88 induced in all the genotypes due to salinity stress, but was not seen under control condition. Band 89 was not seen in the stress conditions, but appears in the control condition and some of the genotypes. The bands 3, 38, 69 and 72 showed polymorphism among genotypes, but their expressions did not change as a result of salinity.

This experiment indicated that polypeptides with molecular weight of less than 18.4 kDa are induced in all of the genotypes under salinity stress. It seems these polypeptides have a key role in tolerance to salinity stress in these genotypes. Protein band of 54.9 kDa is also identified, which is significantly influenced by salinity stress. This band is also seen in Olga under severe salinity stress. The expression of bands 46, 57 and 91 with 48.4, 35 and 14.4 kDa is decreased under salinity stress. In Hyola308, bands 3, 14, 38, 41, 43, 49 and 69 are not shown under salinity stress conditions. Band 42 is just seen under severe salinity stress but band 89 is not observed. In SW5001, bands 40, 42, 69, 72 and 88 are induced under salinity stress and but not in control condition.

DISCUSSION

Increment of Na^+/K^+ ratio in shoot and root is because of

the antagonistic relation between Na^+ and K^+ . The presence of Na^+ in the environment abundantly prevents the absorption of K^+ (Bandeh-hagh et al., 2008).

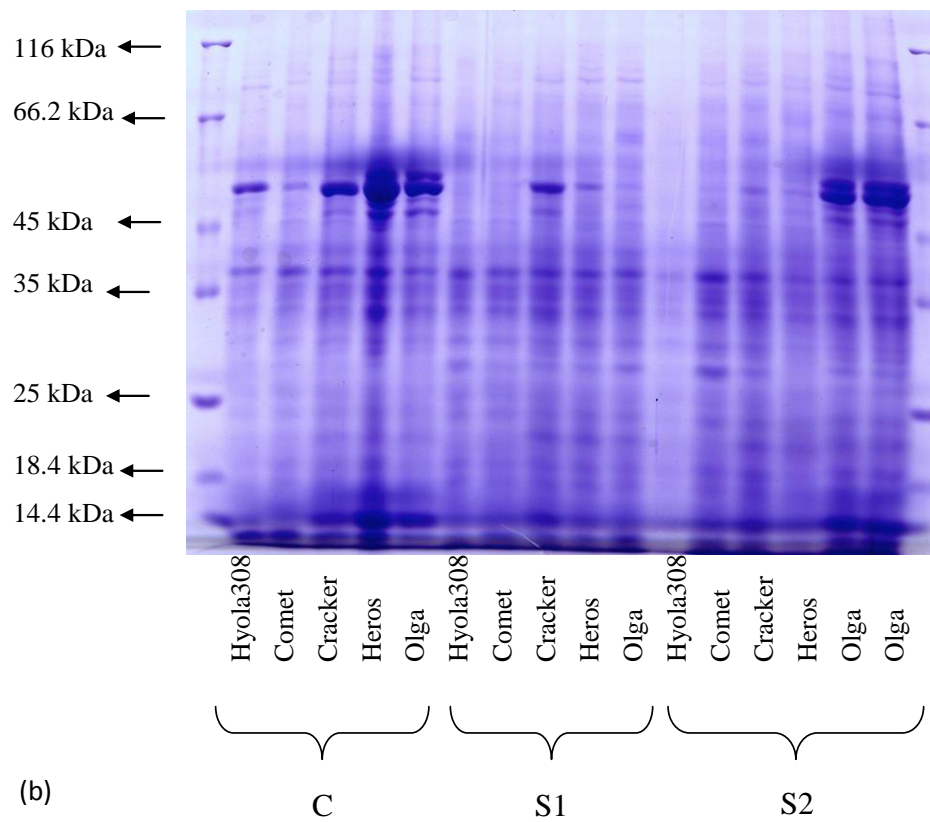
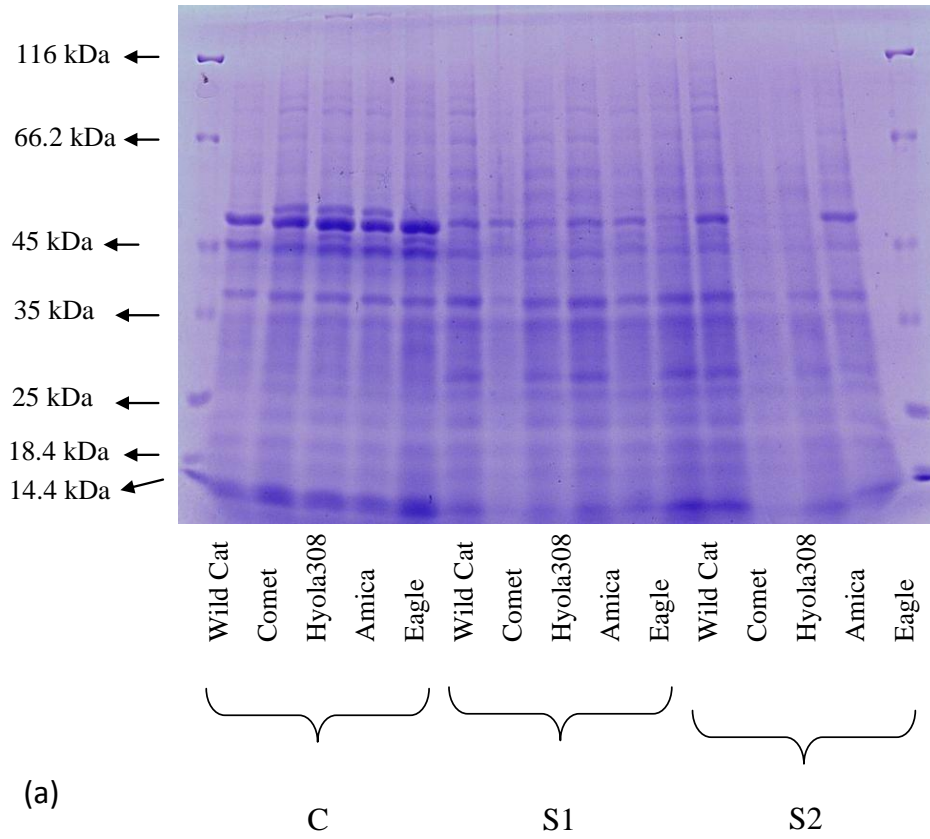
The negative effect of salinity on the growth and the decrease in root weight and plant height were reported earlier (Poljakoff-Mayber and Lerner, 1994). Electrolytic leakage was increased under salinity due to the increment of metabolites and electrolytes leakage in response to accumulation of sodium chloride together with cumulative entering of Cl^- and Na^+ and the exclusion of K^+ (Iqbal et al., 2008). Hemantaranjan et al. (1998) reported that under salinity stress, the developing of leaf area and plant height decreased faster than other morphologic characteristics, and the tolerant genotypes tend to show high amounts of these characteristics. Leaf water potential decreases due to water deficit as a secondary effect of salinity stress (Toorchi et al., 2009a). Significant reduction of osmotic potential was observed in leaves due to salinity stress. It has been reported that proline content of plant tissues increased under salinity as seen in the present experiment (Ashraf and Orooj, 2006).

Hyola308 showed a suitable and highest shoot and root dry weight, leaf area, plant height and root length (Figure 3). Furthermore, it showed the highest RWC, less amount of sodium in shoot, high amount of potassium in shoot and root, and suitable Na^+/K^+ ratio under normal conditions. These results indicate that Hyola308 typically tends to regulate the osmotic pressure by removing sodium ions and absorption of potassium than accumulation of proline. In the case of SW5001 shoot and root dry weight, leaf area, plant height and root length were decreased and its RWC and leaf osmotic potential also was faint than other genotypes. Shoot Na^+/K^+ ratio of the later genotype was the lowest among other genotypes (Figure 4). Interestingly, proline content of SW5001 as a susceptible genotype was more than Hyola308 (Figure 3). It seems that some other non-organic component, such as ions, play critical role in osmotic adjustment of canola genotypes (Ashraf and Harris, 2004; Toorchi et al., 2010).

Cluster analysis using categorical data obtained from SDS-PAGE put Hyola308 and SW5001 in two distinct groups (data not shown). This is in agreement with the result of cluster analysis using physio-morphological traits in which Hyola308 and SW5001 were identified as tolerant and susceptible genotypes.

Conclusions

In conclusion, this experiment indicated significant genetic variability among genotypes, which can be used in breeding programs. Hyola308 is considered as a tolerant genotype by higher amount of potassium in shoot and root and lower Na^+/K^+ ratio in shoot. This implies the fact that Hyola308 is mostly liable to osmotic adjustment



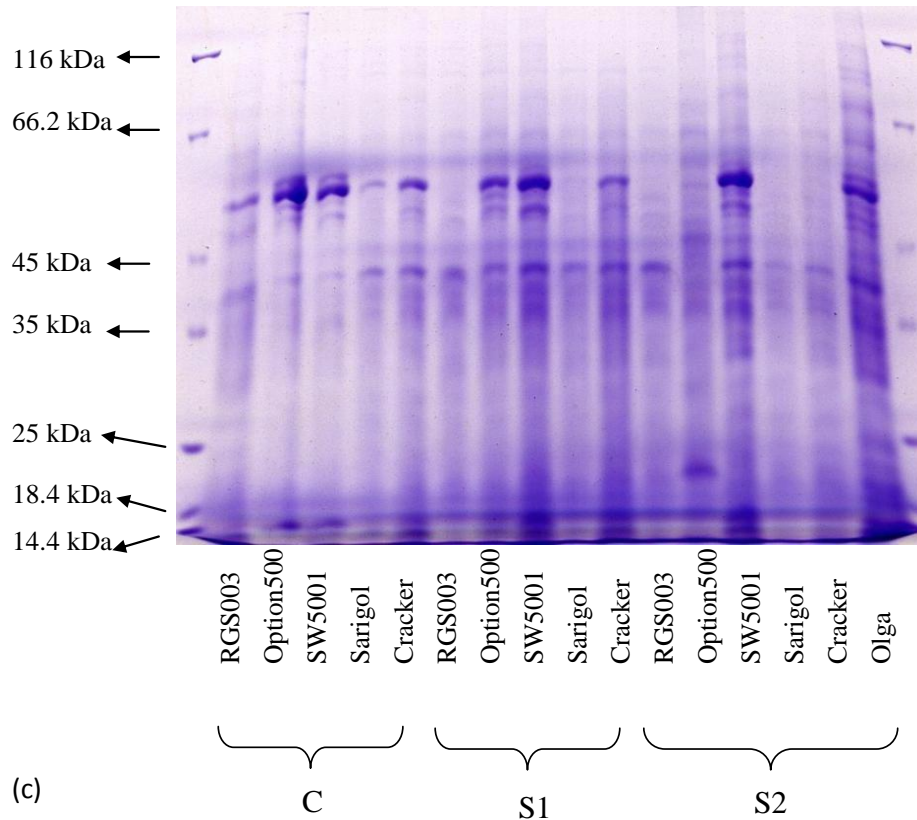


Figure 1. Protein pattern of canola genotypes in different salinity stress using SDS-PAGE.

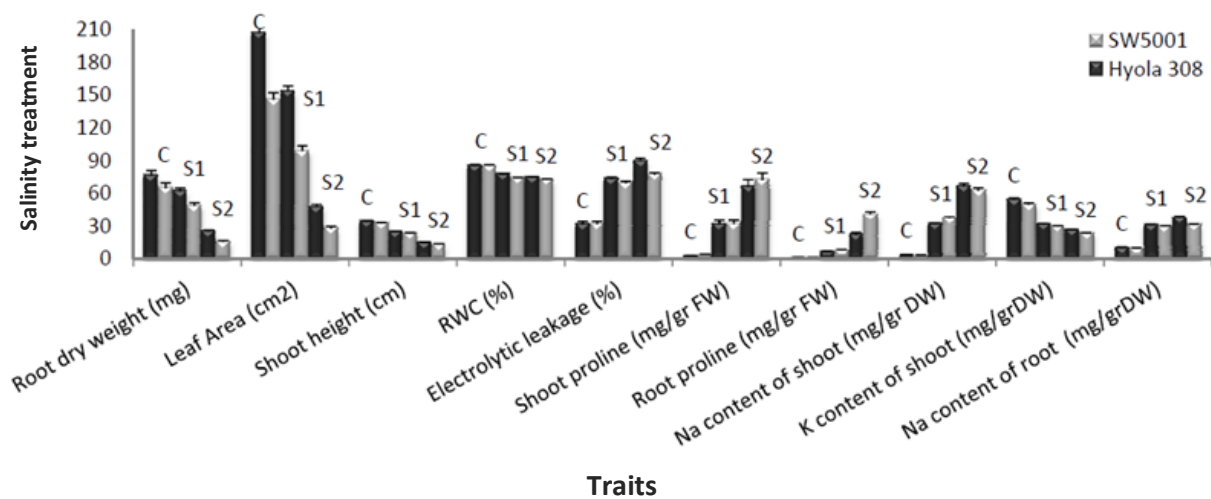


Figure 3. Comparing of Hyola308 and SW5001 in different salinity treatments (C=zero, S1=175 and S2=350 mM of NaCl treatment).

by removing sodium and absorption of potassium than accumulating proline. The reverse is true for SW5001, but with higher amount of proline in the leaf. This implies

that there is no direct relation between the amount of proline and plant tolerance to salinity stress in all the crops. Investigating protein pattern of the genotypes with

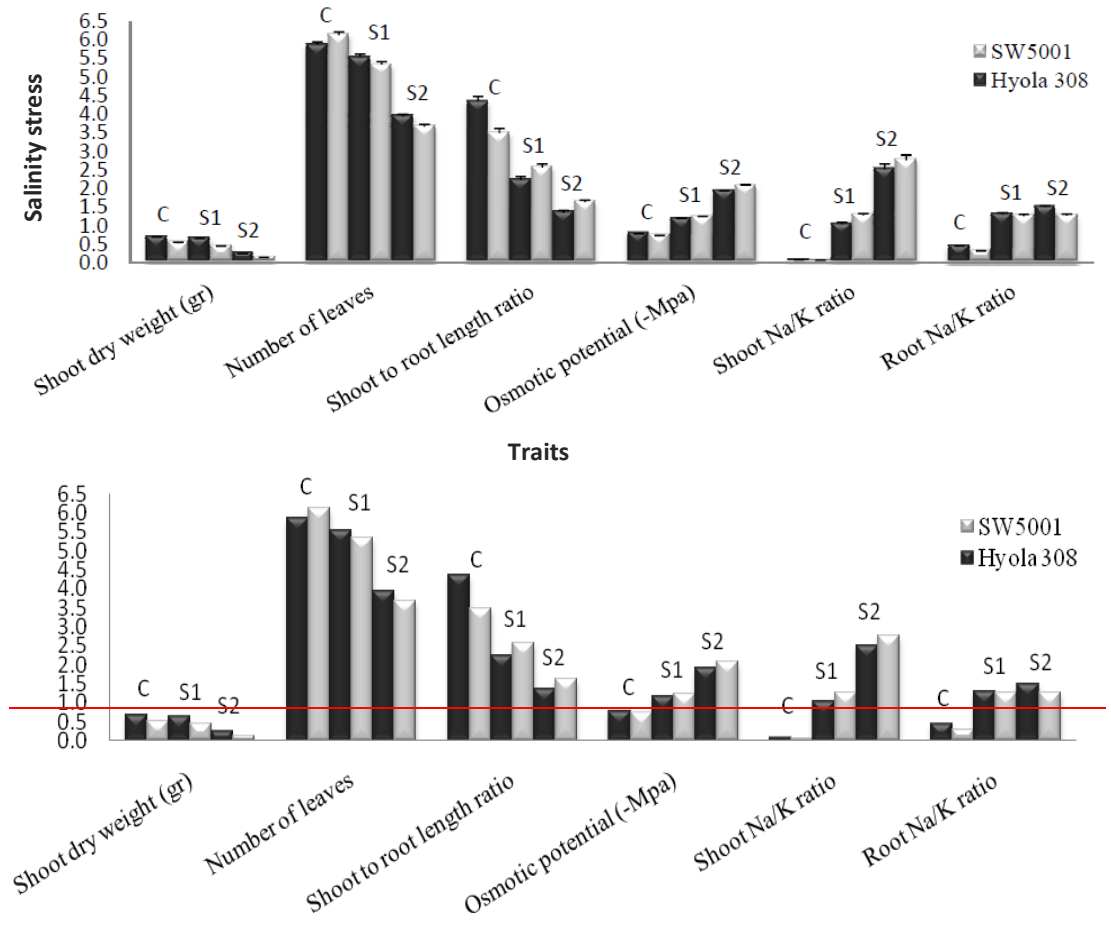


Figure 4. Comparing of Hyola308 and SW5001 in different salinity stress by other traits (C=zero, S1=175 and S2=350 mM of NaCl treatment).

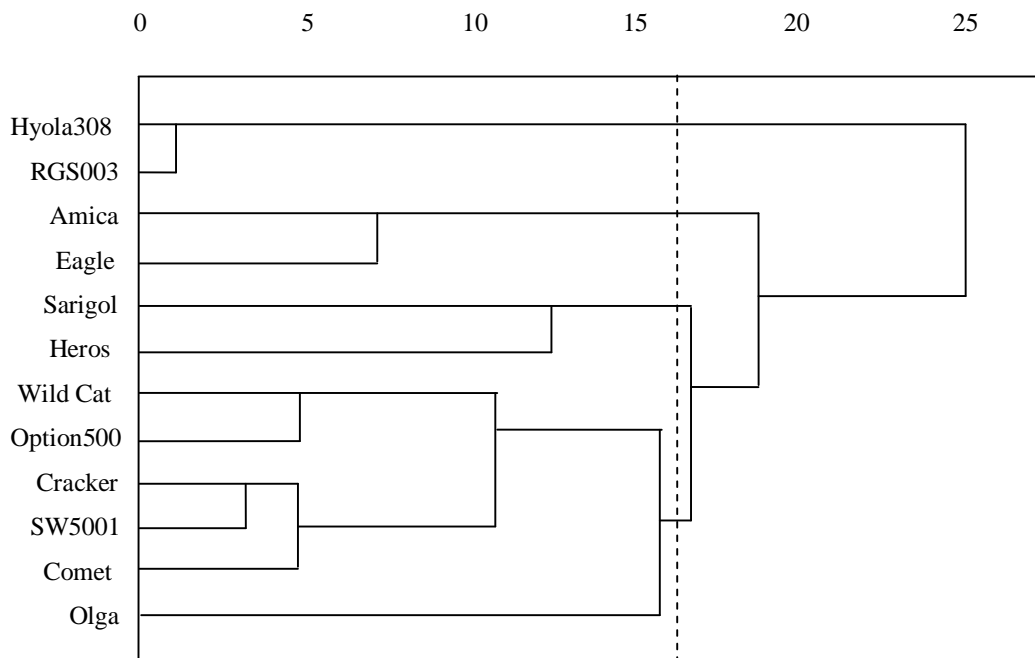


Figure 5. Cluster analysis of rapeseed genotypes based on the studied traits.

SDS-PAGE indicated that protein patterns of genotypes are not similar under different salinity conditions and the behavior of genotypes is changed in response to salinity.

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