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Variation in Seed Germination, Seedling Growth, Nucleic Acid and Biochemical Component in Canola (*Brassica napus* L.) Under Salinity Stress

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Abstract: A laboratorial study was carried out to investigate the effect of NaCl on seed germination, seedling growth, proline concentration and deoxyribonuclease (DNase II) in canola. Five canola genotypes (Hyola308, Hyola401, Hyola60, Optlon50 and RGS003), were grown in petri dishes containing $S_1 = 0$, $S_2 = 100$, $S_3 = 200$ and $S_4 = 300$ mM NaCl in controlled environment. Results indicated that increased salinity caused a significant reduction in germination. Increase salt concentration also affected the early seedling growth and proline concentration in root and shoot tissues. Among the genotypes, Hyola60 appeared to be more tolerant at germination stage and accumulate the highest proline in the root and shoot as a result of salt stress. Among the genotypes, RGS003 had the highest the activity of DNase II in all of salinity treatments but more sensitive at 300 mM NaCl in germination stage then others.

Key words: Salinity, germination, seedling growth, biochemical component, canola

INTRODUCTION

Salinity is a major abiotic stress reducing the yield of a wide variety of crops all over the world (Tester and Davenport 2003). Salinity impairs seed germination, reduces nodule formation, retards plant development and reduces crop yield. Generally, plants are sensitive to salinity during germination and early seedling development (Maas, 1993), but they might also be more or less sensitive to salinity at later growth stages. The spotty pattern in crop stand at maturity, attributed to salinity, is actually initiated at the time of germination and vegetative growth phases (Maas, 1993). The plants that grow in saline soils have diverse ionic compositions and a range in concentrations of dissolved salts (Volkmar *et al.*, 1998).

Salinity inhibition of plant growth is the result of osmotic and ionic effects and the different plant species have developed different mechanisms to cope with these effects (Munns, 2002). The osmotic adjustment, i.e., reduction of cellular osmotic potential by net solute accumulation, has been considered an important mechanism to salt and drought tolerance in plants. This reduction in osmotic potential in salt stressed plants can be a result of inorganic ion (Na^+ , Cl^- and K^+) and compatible organic solute (soluble carbohydrates, amino acids, proline, betaines, etc.) accumulations (Hasegawa *et al.*, 2000; Grattan and Grieve, 1999). The osmotic adjustment in both roots and leaves contribute to the maintenance of water uptake and cell turgor,

allowing physiological processes, such as stomatal opening, photosynthesis and cell expansion (Serraj and Sinclair, 2002).

Salt resistance in plants is usually quantified in terms of survival rates and/or growing abilities under stress conditions, but it is a complex phenomenon that involves biochemical and physiological processes as well as morphological and developmental changes (Yildiz and Kasap, 2007). Although, the relationship between osmoregulation and salt tolerance is not clear, there is evidence that the osmotic adjustment appears, at least partially, to be involved in the salt tolerance of certain plant genotypes (Richardson and McCree, 1985).

Canola (*Brassic napus* L.) has some potential to cope with the toxicity of salts (Francois, 1984) so it can be successfully grown on salt affected soils. The present study was undertaken to assess the effect of salt stress on different growth attributes of some genetically diverse lines of canola at different growth stages.

Because the canola species used in this study are cultivated under various conditions throughout the world, comparisons between varieties are questionable; nonetheless, identification of varietal differences in response to salinity between some Canola species was the aim of the present study. The differences in nucleic acid content and nucleolytic enzyme activity were determined in order to detect if they had similar responses to salinity stress during their growth. It was also determined if the changes in nucleolytic enzyme activity, especially ribonuclease II, could support the hypothesis

that they could be used as a marker for salt stress. Therefore, the objective of this investigation was to evaluate the effects of salt stress on seed germination and seedling growth, proline concentration and deoxyribonuclease (DNase II) of canola under salinity conditions to better understanding of the mechanisms of salt tolerance in these genotypes.

MATERIALS AND METHODS

An experiment was carried out in a growth room of the Department of agronomy and Plant Breeding, University of Zabol, Zabol, Iran during April-June 2009 to screen 5 canola (*Brassica napus* L.) genotypes viz., Hyola308, Hyola401, Hyola60, Optlon50 and RGS003. These five genotypes are the most popular genotypes which can be cultivated in the north and south of Iran. The experiment was laid-out as a completely randomized factorial design with three replicates. Three hundred seeds of each canola genotype were surface sterilized in 5% sodium hypochlorite solution for 5 min and then carefully rinsed with distilled water to remove the traces of sterilizing agent. There were four different regimes of salt stress i.e., $S_0 = 0$, $S_1 = 100$, $S_2 = 200$ and $S_3 = 300$ mM of NaCl.

The treatment solution in each Petri plate was changed every day by dripping out and adding fresh treatment solution. Germination started after two days of sowing and when the radicle reached up to 5 mm in length a seed was considered germinated. The data for germination was recorded daily up to the end of the experiment. Germination percentage was calculated using the following formula:

$$\text{Germination (\%)} = \frac{\text{No. of germinated seeds}}{\text{Total No. of seeds}} \times 100$$

After fifteen days of the start of the experiment, plant seedlings were removed carefully from the Petri plates and separated into shoots and roots and fresh weights recorded. In this time biochemical components such as proline was determined in root and shoots. The extracts of shoot and root were used to determine proline concentration in canola genotypes (Bates *et al.*, 1973).

For free proline content, leaf samples were homogenized in 5 mL of sulpho salicylic acid (3%) using mortar and pestle. About 2 mL of extract was taken in test tube and to it 2 mL of glacial acetic acid and 2 mL of ninhydrin reagent were added. The reaction mixture was boiled in water bath at 100°C for 30 min. after cooling the reaction mixture, 6 mL of toluene was added and then transferred to a separating funnel. After thorough mixing,

the chromophore containing toluene was separated and absorbance read at 520 nm in spectrophotometer against toluene blank.

Deoxyribonuclease (DNase II) assay: After fifteen days of the start of the experiment, the plants were harvested, Fresh samples were immediately used for the determination of nucleic acid content and enzyme activity. A known weight of the fresh seedling was placed in a mortar and homogenised in 20 mL of distilled water. The filtrate was separated from the residue and used in the study. Estimation of DNase II was carried out by the method of Kunitz (1950), in which 0.5 mL of sample was mixed with 2.5 mL of buffer substrate (pH 5.0) and E/30 sec for 5 or 10 min at 260 nm was measured against a blank. The volume activity was equal to $(3.0 \times 1000) / 0.5 \times \Delta E$ (units mL^{-1} sample).

Statistical analyses: Data quantification and statistical analysis were performed using MS Excel software (Microsoft Excel 2003) and then all data were analyzed with SAS Institute Inc 6.12. Data were first analyzed by ANOVA to determine significant ($p = 0.05$) treatment effects. Significant differences between individual means were determined using Fisher's protected Least Significant Difference (LSD) test. Data points in the figures represent the Mean \pm SE of three independent experiments at least three replications per cultivar per treatment combination each.

RESULTS

Germination percentage and seedling growth: The results revealed that the germination of canola, was strongly affected by all salt treatments. Increased salt concentration caused a decrease in germination. Strong reduction was observed mainly at the higher level of salt concentration compared to control. Lowest germination was observed in case of Hyola308 at high salinity treatments (Fig. 1).

The studies were laid to investigate the influence of salinity on seedling growth of germinating seeds of canola genotypes. The results indicated that emergence of root and shoot delayed as the salt stress increases compared to controls. The continuous increase in length of root and shoot was observed in frequent hours of germination in four canola in control as well as salt treatments. The data on the average length (Fig. 2 and 3) of root and shoot shows that RGS003 genotype had the highest length of root and shoot in control treatment. The results presented in Fig. 2 and 3 indicated that RGS003 had the greatest reduction of shoot length and Hyola60

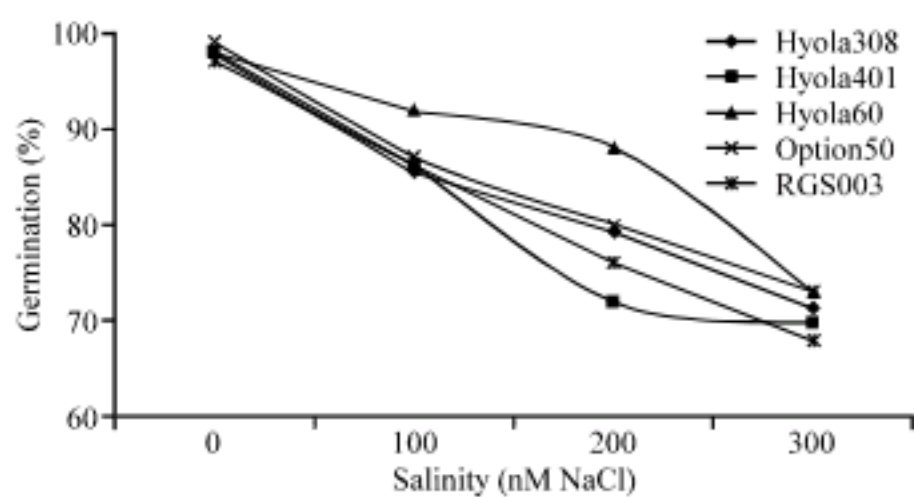


Fig. 1: Effects of salt stress on germination percentage

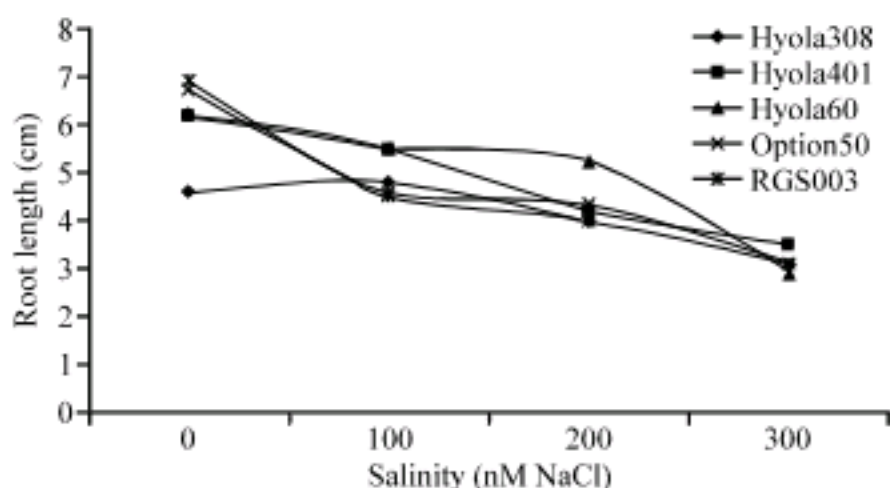


Fig. 2: Effects of salt stress on root length

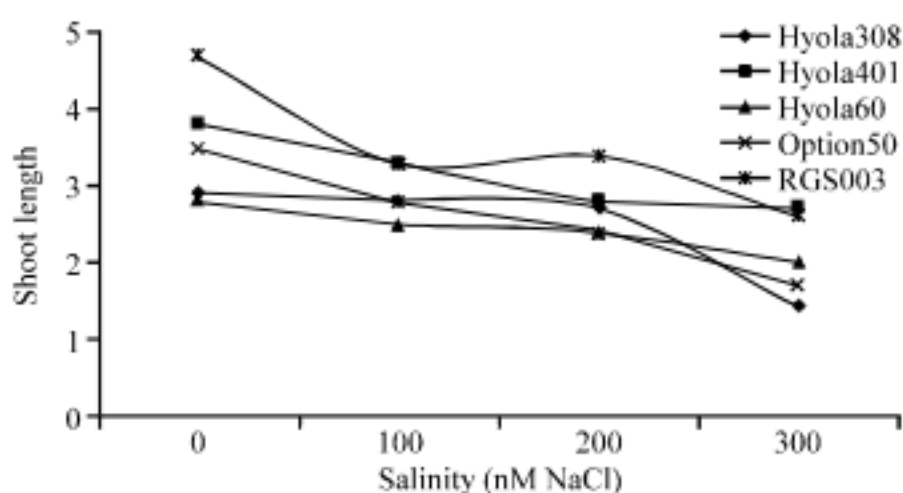


Fig. 3: Effects of salt stress on shoot length

had the highest root length in 300 mM NaCl treatment. Decrease in length of root was more pronounced as compared to shoot in all NaCl salt treatments.

Proline concentration and nucleic acid: With a NaCl concentration of 300 mM, the proline concentration in root and shoot in canola genotypes increased to two-fold values, compared to the control treatment (Fig. 4 and 5) and salinity stress had significantly effect on it. Among the genotypes, Hyola401 and Hyola60 had the maximum concentration of proline in root and shoot in S₃ treatment. In this study root proline content was substantially higher than in shoot.

Salinity disturbed nucleic acid metabolism and caused growth inhibition. Along with increased salinity stress in all of the studied plants, salinity significantly

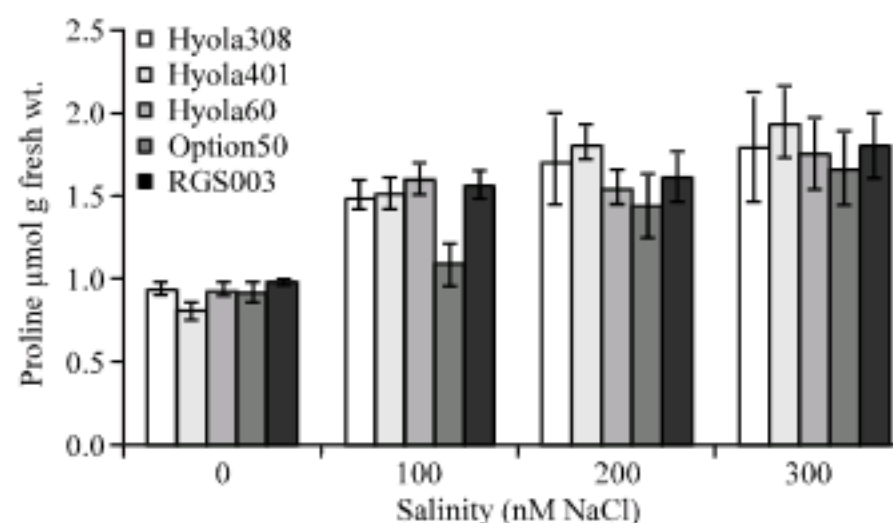


Fig. 4: Effects of salt stress on proline content in root

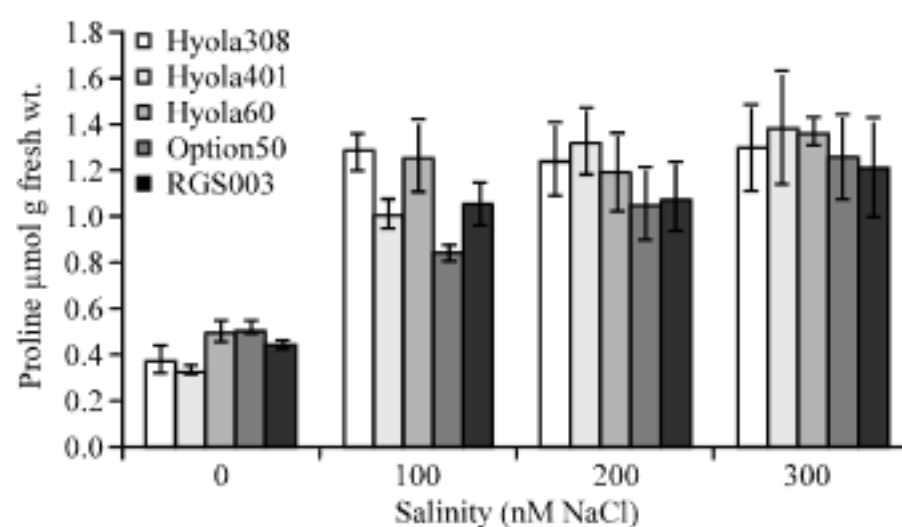


Fig. 5: Effects of salt stress on proline content in shoot

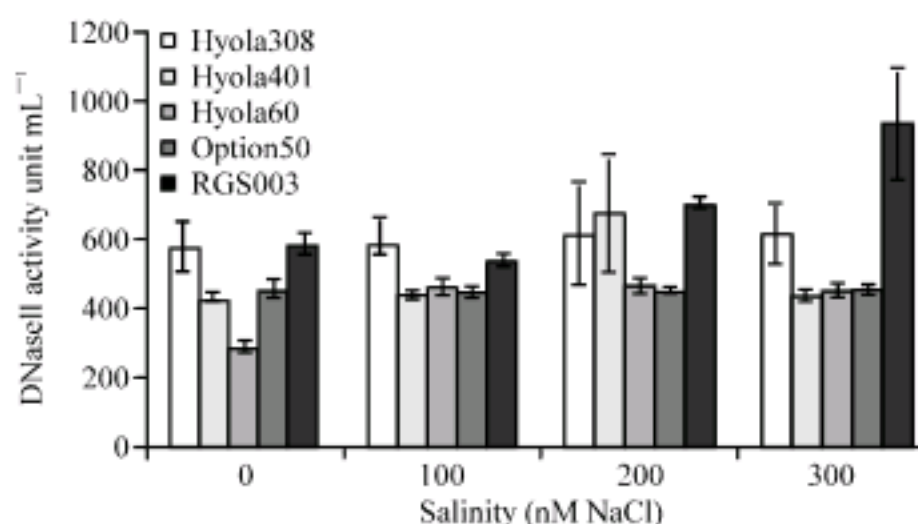


Fig. 6: Effects of salt stress on DNase II activity

affects on nucleic acid metabolism and increased the level of the activity of DNase II (Fig. 6). At the highest concentration NaCl treatment, enzyme activity was significantly enhanced. Among the genotypes, RGS003 had the highest the activity of DNase II in all of salinity treatments and at the S₃ level.

DISCUSSION

Seeds of canola genotypes germinated rapidly in non saline (control) treatment and reached final germination percentage in less than 10 days. Change in salinity significantly affected the final germination of canola seeds. In general, increased NaCl level, led to the

reductions in germination percentage (Fig. 1), germination rate and seedling fresh weight. This can be attributed to prevent of water uptake created by salinity condition. This can be also due to the toxic effects of Na and Cl ions on the germination process. NaCl may be inhibitory to the activities of some enzymes that may play critical roles in seed germination (Khajeh-Hosseini *et al.*, 2003).

Seedling growth was recorded in terms of Shoot/Root length at different levels of NaCl salinity. The increase in NaCl concentrations decreased the shoot and root length of all the canola genotypes. All genotypes responded in same manner to salinity stress. However, the intensity of stress varied with the genotypes. The reduction in root length was greater than shoot length (Fig. 2 and 3). Maximum decrease in root and shoot length at 300 mM NaCl were recorded in Hyola60 and RGS003 genotypes.

Among the genotypes tested RGS003 appeared to be more sensitive at 300 mM NaCl in germination stage than others. Although RGS003 genotype had comparatively low germination at higher salinity levels but performed quiet satisfactorily at seedling stage for activity of DNase II. Among the genotypes, RGS003 had the highest the activity of DNase II in all of salinity treatments and at the S₃ level (Fig. 6). Abo-Kassem (2007) studied effects of salinity: calcium interaction on growth and nucleic acid metabolism in five species of Chenopodiaceae, he found that, the activity of DNase II increased along with increased salinity stress in all of the studied plants. At the highest concentration NaCl treatment, enzyme activity was significantly enhanced (4 times that of the control).

All of the studied plants, showed a progressive increase in DNase II activity with increased salinity. This showed that for plant cells protecting the DNA when under salt stress was a priority, which is confirmed by the data reported by Hasegawa *et al.* (2000), who observed a salinity stress-induced reduction in cell elongation, but no reduction in cell division. Addition of CaSO₄ to NaCl increased DNase I activity in most of the examined plants, whereas NaCl stress reduced DNase- and RNase-specific activity in alfalfa and lentil (Yupsanis *et al.*, 2001).

In this study, in root and shoot of canola genotypes, proline accumulated but in root tissue, accumulation of proline was 2 times higher than that in shoot tissue. Among the genotypes, Hyola401 had the maximum concentration of proline in root and shoot in S₃ treatment than control. In addition, accumulation of solute such as water soluble carbohydrate, polyos and amino acids in tissues is an important adaptation mechanism for plants in response to osmotic stress like water deficit and high salinity levels (Yang *et al.*, 2003).

The results obtained showed that of all genotypes studied, Hyola60 was the most salt-tolerant. It also showed the highest germination percentage and seedling growth (length of root and shoot) in 300 mM NaCl. Solute accumulation such as proline in the root and shoot as a result of salt stress appeared to play an important role in the acclimation of this genotypes to salt stress, suggesting that they could be used as physiological markers during the screening for salt tolerance.

REFERENCES

- Abo-Kassem, E.E.M., 2007. Effects of salinity: Calcium interaction on growth and nucleic acid metabolism in five species of Chenopodiaceae. *Turk. J. Bot.*, 31: 125-134.
- Bates, L.S., R.P. Walderren and I.D. Teare, 1973. Rapid determination of free proline for water-studies. *Plant Soil*, 39: 205-207.
- Francois, L.E., 1984. Salinity effects on germination, growth and yield of turnips. *Hort Sci.*, 19: 82-84.
- Grattan, S.R. and C.M. Grieve, 1999. Salinity - mineral nutrient relations in horticultural crops. *Sci. Hortic.*, 78: 127-157.
- Hasegawa, P.M., R.A. Bressan, J.K. Zhu and H.J. Bohnert, 2000. Plant cellular and molecular responses to high salinity. *Ann. Rev. Plant Physiol. Plant Mol. Biol.*, 51: 463-499.
- Khajeh-Hosseini, M., A.A. Powell and I.J. Bingham, 2003. The interaction between salinity stress and seed vigor during germination of soyabean seeds. *Seed Sci. Technol.*, 31: 715-725.
- Kunitz, M., 1950. Crystalline desoxyribonuclease. I. isolation and general properties. *J. Gen. Physiol.*, 33: 349-362.
- Maas, E.V., 1993. Plant Growth Response to Salt Stress. In: *Towards the Rational Use of High Salinity Tolerant Plants*, Vol. 1. Lieth, H. and A. Al-Masoom (Eds.). Kluwer Academic Publishers, Netherlands, pp: 279-291.
- Munns, R., 2002. Comparative physiology of salt and water stress. *Plant Cell Environ.*, 25: 239-250.
- Richardson, S.G. and K.J. McCree, 1985. Carbon balance and water relations of sorghum exposed to salt and water stress. *Plant Physiol.*, 79: 1015-1020.
- Serraj, R. and T.R. Sinclair, 2002. Osmolyte accumulation: Can it really help increase crop yield under drought conditions. *Plant Cell Environ.*, 25: 333-341.
- Tester, M. and R. Davenport, 2003. Na⁺ tolerance and Na⁺ transport in higher plants. *Ann. Bot.*, 91: 503-527.

- Volkmar, K.M., Y. Hu and H. Steppuhn, 1998. Physiological responses of plants to salinity: A review. *Can. J. Plant Sci.*, 78: 19-27.
- Yang, W.J., P.J. Rich, J.D. Axtell, K.V. Wood, C.C. Bonham, G. Ejeta, M.V. Mickelbart and D. Rhodes, 2003. Genotypic variation for glycinebetain in sorghum. *Crop Sci.*, 43: 162-169.
- Yildiz, M. and E. Kasap, 2007. Comparison of germination responses of cultivated wheat (*Triticum*) and its wild relative (*Aegilops*) species under salinity, temperature and light. *Acta Agron. Hungarica*, 55: 407-415.
- Yupsanis, T., P.S. Kefalas, P. Eleftheriou and K. Kotinis, 2001. RNase and DNase activities in the alfalfa and lentil grown in iso-osmotic solutions of NaCl and mannitol. *J. Plant Physiol.*, 158: 921-927.