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Effect of Salinity Stress on Germination and Seedling Properties in Canola Cultivars (*Brassica napus* L.)

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Abstract

Germination and seedling responses of five rape seed cultivars (Elite, Fornax, Licord, Okapi, and SLM046) to salinity stress levels (0 cont, 5, 10, 15 and 20 dSm^{-1}) evaluated in aRCBD base factorial design in three replicates in glasshouse. Increasing Salinity decreased significantly rate and final germination, radicle and plumule length and fresh weight. Decreasing rate was different among cultivars. Salinity and cultivar interaction effect was significant in all attributes. Three parametric logistic regressions fitted the best estimation between germination and salinity levels. The highest and the lowest B coefficient belonged to Elite and SLM046 that shows high and low susceptibility to salinity. Ld 50 threshold for germination for Elite and SLM046 were 10.46 and 23.01 dSm^{-1}, respectively. Tolerance ranking for cultivars was SLM046 > Okapi > Fornax > Licord > Elite. This classification belonged to early season tolerance and it is necessary to study the next growth period for evaluate salinity tolerance rank among cultivars.

Keywords: canola, germination, logistic model fit, salinity stress

Introduction

High salinity is a common abiotic stress factor that seriously affects crop production in some parts of the world, particularly in arid and semi-arid regions (Neumann, 1995). There are about of 8 million hectare agricultural land exposure to salt stress in the world (Munns, 2005). Irrigation with poor quality water is one of the main factors that lead to salt accumulation and the resulting decrease in agricultural productivity. The plant growth is ultimately reduced by salinity stress but plant species differ in their salinity tolerance (Munns and Termaat, 1986).

Saline soils and saline irrigation waters present potential hazards to canola production. Canola (*Brassica napus* L.) is one of the most important oil seed crops in the world and 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 that 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. Germination is one of the most critical periods for a crop subjected to salinity. Soil salinity may influence the germination of canola seeds either by creating an osmotic potential external to the seed preventing water uptake, or the toxic effects of Na and Cl ions on the germinating seeds (Khajeh-Hosseini *et al.*, 2003).

Salt and osmotic stresses are responsible for both inhibition or delayed seed germination and seedling establishment (Almansouri *et al.*, 2001). Germination failures on saline soils are often the results of high salt concentrations in the seed planting zone because of upward movement of soil solution and subsequent evaporation at the soil surface (Bernstein, 1974). These salts interfere with seed germination and crop establishment (Fowler ,1991). Salt stress on seed germination maybe attributed to either osmotic effect (Bliss, Platt-Aloia and Thompson, 1986) and/or to specific ion toxicities to radicle emergence (Hampson and Simpson, 1990a) or seedling development (Hampson and Simpson, 1990b).

Seedling establishment is a critical stage in crop production and considerably depends on biochemical and physiological structures of seed. In order to obtain fast and good establishment of seedling, high vigor seed is needed to provide essential nutrients for seedling until it becomes established and can photosynthesize independently (Derek Bewley and Black, 1994). Seed germination, seedling emergence, and early survival are particularly sensitive to substrate salinity (Baldwin *et al.*, 1996). Successful seedling establishment depends on the frequency and the amount of precipitation as well as on the ability of the seed species to germinate and grow while soil moisture and osmotic potentials decrease (Roundy, 1985).

Germination and seedling characteristics are the most viable criteria used for selecting salt tolerance in plants. Germination percentage, germination speed and seedling growth are most criteria for cultivar selection.

The present study was conducted to evaluate the effect of salinity on seed germination and seedling growth of three canola cultivars under salinity conditions. The primary objective of the present study was to comparison of three canola cultivars to salinity stress levels.

Materials and methods

In order to study effect of salt stress on canola seed germination and primary seedling growth an experiment was conducted in Agricultural Research Center, East Azerbaijan, Iran in 2008. The experimental design was a Randomized Complete Block arrangement in 2×5 factorial with three replications. The first and second factors were canola cultivar (Elite, Fornax, Licord, Okapi, and SLM046) and five salinity levels, respectively. For salt stress induction saline water was collected from a well near Shabestar, Tabriz, Iran. The electrical conductivity of this water was 20 $ds.m^{-1}$. The other characteristics of water are shown in Tab.1.

Other concentrations that is 5, 10, 15 $ds.m^{-2}$ were made by diluting saline water with distilled water an electrical conductivity meter (Jenway,UK) was used for adjusting the EC on 5, 10, 15 $ds.m^{-2}$. The seeds of canola (Brassica napus L. c.v Elite, Fornax, Licord, Okapi, and SLM046) were surface-sterilized for 5 min in sodium hypochlorite solution and then in rinsed with distilled water for 5 min. After sterilization, seeds were transferred in 9^{-cm} sterile petri dishes on a dual filter paper and then were moistened with 5 ml distilled water (control) or saline water solution at 0, 50, 10, 15 or 20 $ds.m^{-2}$. To prevent infection and evaporation of solution, all of the plates were closed with parafilm. All operations were performed under laminar flow. The Petri dishes were incubated in a germinator at 15°C and 12 h illumination. Numbers of germinated seeds were recorded daily and number of final germinated seed was registered when seed germination was stopped for eight days. At end of seed germination test length of radicle, length of seedlings, and their fresh weight were recorded immediately after incubation was terminate. Also final germination percentage (FGP) and germination pace (GP) were calculated by Ellis and Roberts (1980) equation (Equation 1).

Equation 1:

$$GR = \frac{\Sigma N}{\Sigma(n \times g)}$$

Tab.1. Chemical properties of water from Shabestar region

EC $Ds.m^{-1}$	pH	meq.liter					
20	7.9	Carbonate	Bicarbonate	Chlorine	Sulphate	Ca + Mg	Na
		0	3.6	170	26	55	145

Where: GR: Germination pace; n: number of germinated seed on g^{th} day and N: Number of total germinated seeds

Each experiment was conducted two times. The results are mean of data of each test. It is worth noting that, there are not any interaction effect between time of experiment and treatments. Finally, evaluation of salt stress tolerance was defined as the amount of salinity which caused 50 % inhibition of germination. The seed germination percentage was fitted by a three-parametric logistic model (Chauhan and Johnson, 2008) in Sigmaplot software (Ver. 11) and model was defined as equation 2.

Equation 2: $Y = a / [1 + (x + x_{50})^{b}]$

Where: Y: Germination percentage at x salinity stress level; a: Maximum germination percentage in experiment; x_{50}: Necessary salinity level for 50% germination inhibition and b: Slope

All data were analyzed by Sigmaplot software and comparison of means was done by LSD test.

Results and discussion

The results showed that, different levels of salinity have significant effect on canola seed germination. Many researches have been reported similar results (Ashraf and Mcneilly, 1990; Demir, and Aril, 2003; Jeannette *et al.*, 2002; Mauromicale and Licandro, 2002). It observed that, in all of cultivars there was a decrease in germination percentage due to salinity increment and maximum germination percentage was delayed. While in this experiment different cultivars had different response to the salinity (Fig. 1).

Under conditions of the highest salt stress that is 20 $ds.m^{-1}$ Elite cultivar had not any germination after 12 days, while germination of SLM_{046} cultivar was 65% after 4 days.

Also germination percentage of Fornax and Okapi cultivars was less than 25% under conditions of high salinity stress (20 $ds.m^{-1}$) it shows that these cultivars are sensitive to salinity stress. In addition, in similar treatment, Licord cultivar had better germination (by 50%) than above mentioned cultivars. Salinity different levels had significant effect ($p<0.05$) on germination pace of canola cultivars (Fig. 2).

The highest and the lowest germination pace were observed in SLM_{046} and Elite cultivars, respectively. Obviously, decrease in germination pace lead to delay in seedling establishment and as a result canola resistance to unfavorable conditions such as cold will be decreased. According to Ayaz *et al.* (2000), decrease of seed germination under conditions of salt stress is due to occur of some metabolically disorders. It seems that, decrease of germination percentage and germination pace is related to reduction in water absorption into the seeds at imbibitions and seed

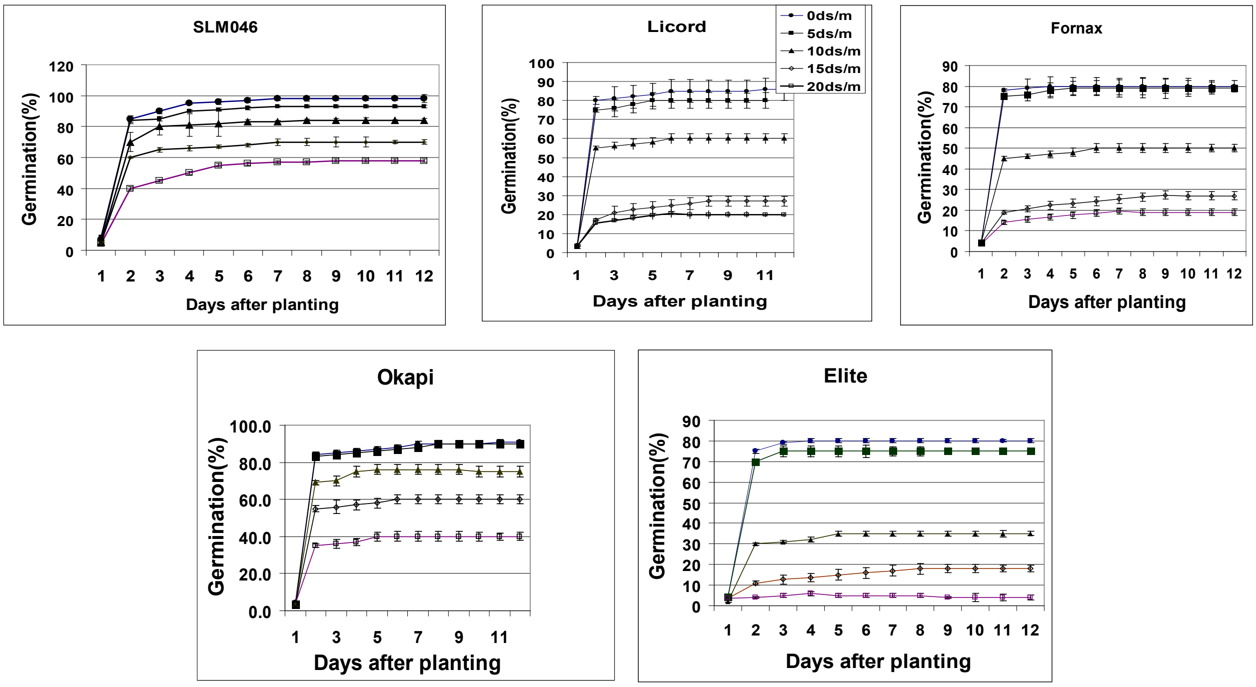


Fig. 1. Effect of different salinity levels on germination percentage of canola cultivars.

turgescence stages (Hadas, 1977). The results demonstrated that, response of radicle length to salt stress was more severe than shoot length(Fig. 3).

It's reported that, radicle and shoot length are important traits in salt stress sensitivity evaluation (Jamil *et al.*, 2006). Decrease of growth in root and stem can be related to NaCl toxicity and disproportion in nutrient absorption by seedlings. According to results of Werner and Finkelstein (1995) salinity decreases water absorption and growth of root and shoot. It's reported that, salinity decreases significantly nutrient absorption and root growth speed (Khan and Gulzar, 2003). Srivastava *et al.* (2004) have been reported that, proteins especially PR 10 protein increases salt resistance in canola varieties at germination stages. In other research, increase of salinity from 6 to 11

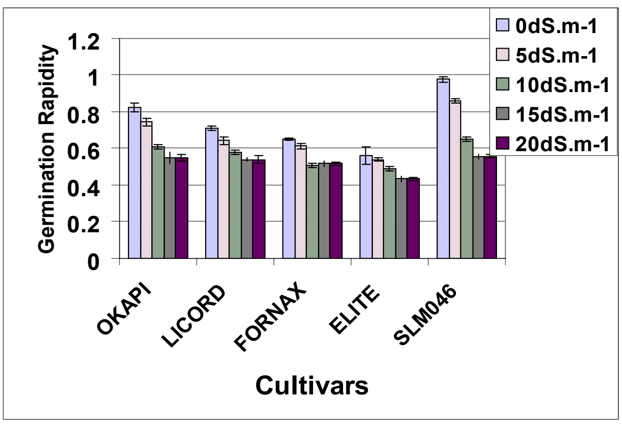


Fig. 2. Effect of different salinity levels on germination pace of canola cultivars(LSD test $p<0.05$)

ds.m[1] decreased canola seed germination by 50% (Francois, 1996). Gulzar and khan (2001) have been reported that, NaCl salinity prevents water absorption by seeds and decreases significantly seed germination percentage and germination pace. In this study, fitted logistic model shows relation between salinity level and seed germination as all of regression models and fitted parameters were significant when this model was used (Tab. 2 and Fig. 4).

According to Koutsoyiannis comments (1973) estimated parameters are reliable when maximum standard deviation is half of numeric value parameter. X50 parameters show salinity intensity as it leads to decrease of germination percentage by 50% and on bas of this model SLM056 cultivar had the highest salinity tolerance at germination stage. Under condition of salinity stress (26.5 $ds.m^{-1}$) SLM056 germination was decreased by 50% while in Elite cultivar observed that, X50 = 14.5 $ds.m^{-1}$ that is this cultivar had sensitivity to salinity stress at germination stage. The b parameter demonstrates slope of germination reduction due to increase of salinity level. The highest slope was related to Elite and the lowest was related to SLM046 and Okapi cultivars. Increase of this slope is demonstrator of salt sensitivity of cultivar. In final according to obtained results and logistic assessments, cultivars were arranged on base of salinity tolerance according to this order;

SLM046 > Okapi > Fornax > Licord > Elite. Ajmal Khan and Weber (2006) found that, salt resistance at germination stage and primary growth of seedling is independent from next growth stages and evaluation of salinity tolerance need more experiment at next growth stages. Khan and Gulzar(2003) found that, presence of NaCl around roots leads to degradation of some protein

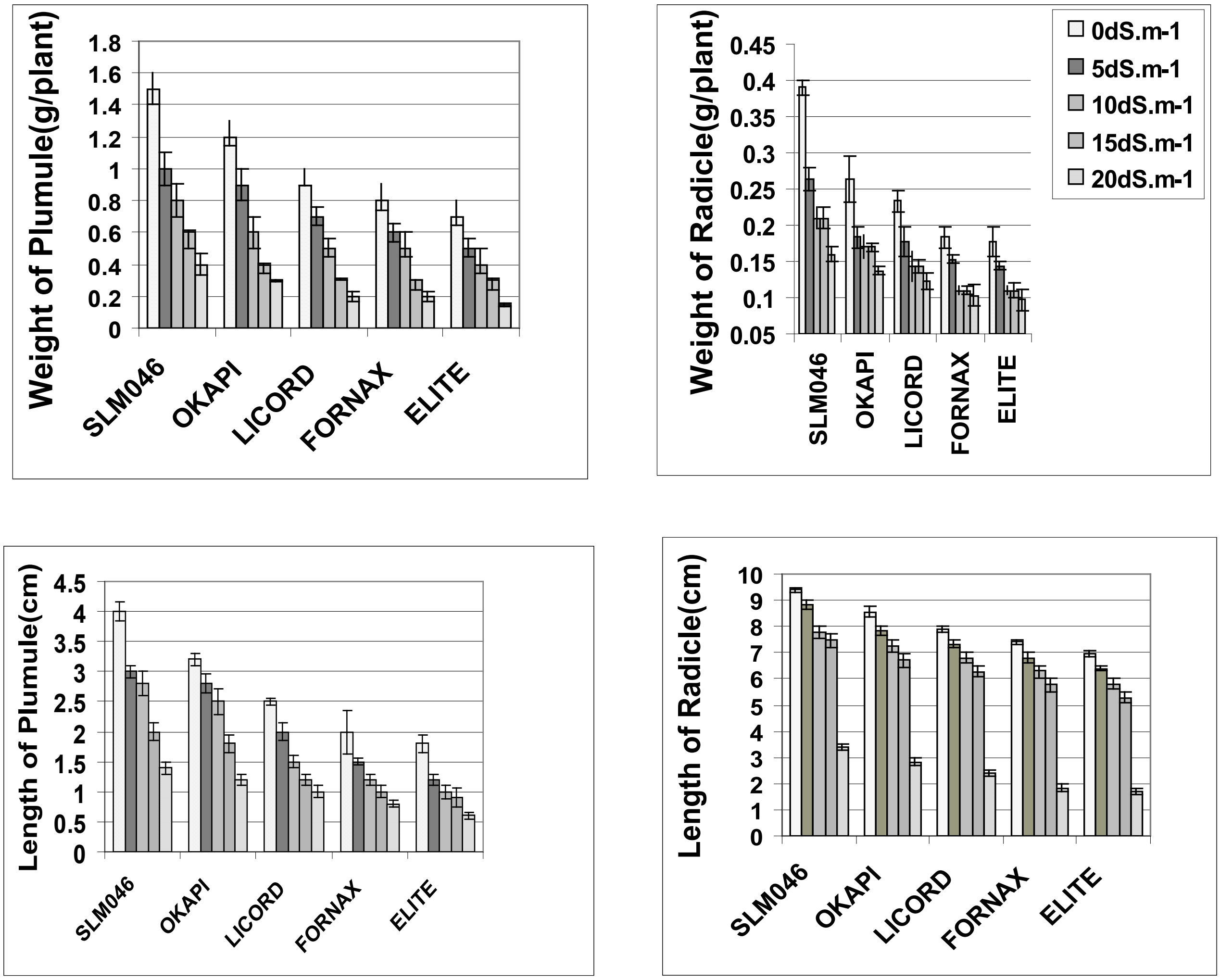
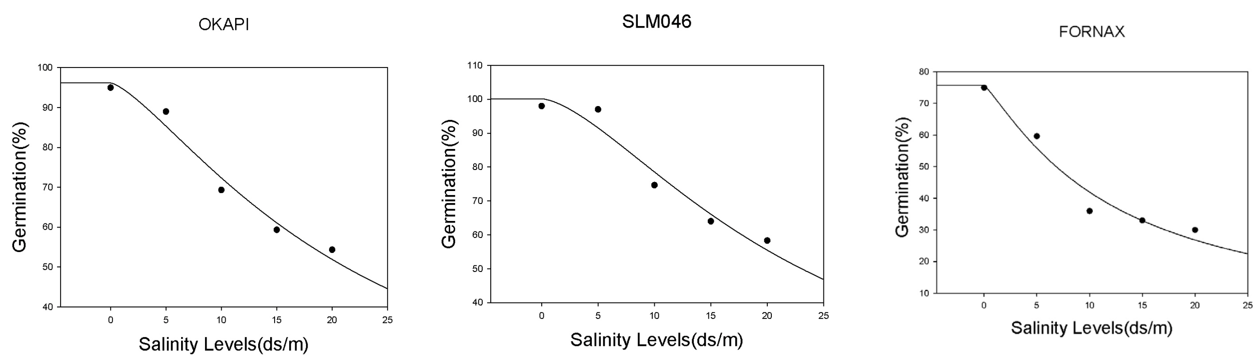


Fig. 3. Effect of different salinity levels on length and fresh weight of radicle and plumule of canola cultivars(LSD test p<0.05)

Tab.2. Logistic regression model coefficients (± SE) shows relations between salt stress and canola seed germination

Cultivars	a	b	X_{50}	R^2
SLM_{046}	110.11±5.35**	1.55±0.51*	23.01±3.57*	0.97**
Okapi	96.17±3.97**	1.37±0.32*	22.45±2.83*	0.98**
Licord	77.82±1.66**	0.93±0.11*	20.57±1.75**	0.99**
Fornax	75.64±5.47**	1.18±0.35*	12.04±2.28*	0.98**
Elite	73.98±4.87**	1.35±0.32*	10.46±1.63*	0.98**

Note:*and** significant at 5% and 1% probability levels, respectively



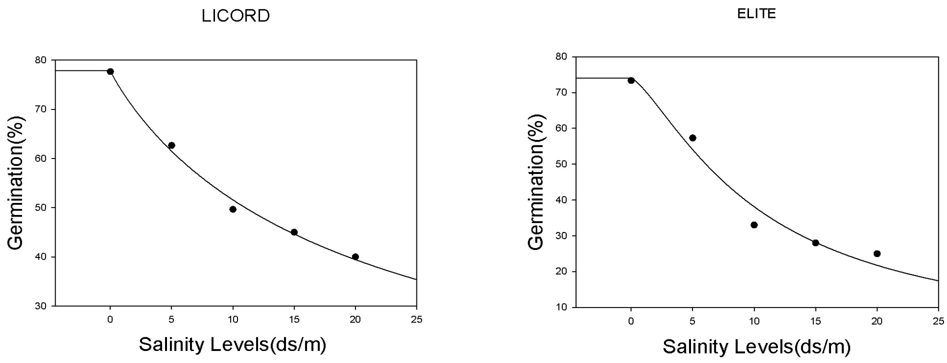


Fig. 4. Effect of different salinity levels on final germination percentage of canola cultivars

involve in root and shoot growth, they have been reported that, some species have specific protein, these proteins have especial structure and salt resistance and germination is increased by this proteins. It seems that, salinity stress affects on seed germination via limitation of water absorption by seeds (Dodd and Donovan, 1999), excessive use of nutrient pool (Bouaziz and Hicks, 1990) and creation of disorders in protein synthesis. It's reported that, salinity stress affects on alpha and beta amylase duration seed germination (Derek Bewley and Black,1994). Obviously, acceptable growth of plants in arid and semiarid lands which are under exposure of salinity stress is related to ability of seeds for best germination under unfavorable conditions, so necessity of evaluation of salt resistance cultivars is important at primary growth stage.

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