Effect of NaCl and Incubation Temperature on Seed Germination of Three Canola (*Brassica napus* L.) Cultivars

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Abstract:

This investigation was performed to study the effect of NaCl concentration (50, 100, 150 and 200 mol/m^3 NaCl, in addition to the control, i.e. distilled water) and incubation temperature (15, 25 and 35 ^oC) on seed germination percentage and germination speed of three canola CVs (Al-Serw4, Al-Serw 8 and Pactol). Salinity markedly affected seed germination percentage and speed of germination. The lowest incubation temperature degree (15 ^oC) was the best which recorded the highest seed germination percentage, while the highest incubation temperature degree (35 ^oC) recorded the lowest germination percentage. Meanwhile, the moderate incubation temperature (25 ^oC) was the highest in germination speed. Evaluated canola cultivars significantly differed in speed of germination. Al-Serw 8 recorded the highest germination speed. On the other hand, a slight and insignificant difference was noticed among cultivars in seed germination percentage, however Al-Serw 8 ranked the first in this concern.

Introduction:

Canola (*Brassica napus* L.) is considered a new untraditional oil crop in the Kingdom of Saudi Arabia. Canola oil is now the third largest source of edible oil following soybean and palm oil (Nowlin 1991). This increased demand will undoubtedly promote increased acreage of canola in the world where some soils are prone to become saline (Francois 1994). Saline soils and saline irrigation waters present potential hazards to canola production and expansion.

Germination is the most critical period for a crop subjected to salinity. Germination failures under 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). Seed germination have been reported to decline with

increasing salinity levels (Steppuhn and Wall, 1999 and Puppala *et al.*, 1999 and Houle *et al.*, 2001).

Within canola cultivars, there are cultivar differences in sensitivity to salinity. The genetic role in seed germination resistance to salinity is probably one of the most important advantage that can be used in preeding programs. Significant variation in seed germination between canola cultivars grown under salinity condition is widely reported by Zheng *et al.* (1998) and Puppala *et al.* (1999).

The convenient temperature degree may plays an active role in enhancing canola seed germination. Spring canola is sometimes planted when soil temperatures are below the optimum, causing farmers to have stand losses because of seed rotting in cold soil. Knowledge of the growing-degree hours (GDH) required for emergence of canola from different planting depths could help producers decide when and how deep to plant this crop (Vigil *et al.*, 1997). Canola seeds reached 95% germination within 8 days at 10 ^{o}C and displayed rapid seedling growth (Nykiforuk and Johnson-Flanagan,1994 and Zheng *et al.*, 1994). This was associated with high isocitrate lyase activities and rapid mobilization of total lipid and protein reserves. Low temperature has a deleterious effect on the germination of canola and this may be reflected in a loss or a delay of coordination in the mobilization of reserves (Nykiforuk and Johnson-Flanagan, 1994). Seed germination speed was also reported to be stimulated at 10 ^{o}C (Zheng *et al.*, 1994). However, Puppala *et al.* (1999) reported that Germination was severely limited at 5 ^{o}C.The optimum germination temperature occurred in the 15 to 25 ^{o}C range. They also added that the interactions between cultivar and salinity, cultivar and temperature, and cultivar, salinity and temperature had highly significant effects on canola seed germination

The tolerance of canola to salinity during germination however has not been widely reported. Therefore, the objective of this study was to evaluate the germination response of canola to different levels of salinity, incubation temperatures and cultivars, as well as their interactions.

Materials and Methods:

Seeds of three canola cultivars (Al-Serw 4, Al-Serw 8 and Pactol) were sown on top filter paper in sterilized petri-dishes (7 cm diameter) and wetted with solution of different salinity concentrations (50, 100, 150 and 200 mol/m^3 NaCl), in addition to the control, i.e. distilled water. Petri-dishes were incubated in programmed refrigerated incubators with Sylvania cool-white fluorescent lamps at 15, 25 and 35 ^oC maintained within ±1 °C of target levels. Each treatment was replicated six times in a completely randomized experimental design. Seeds were considered germinated when the radicle was 2 – 3 mm in length. Germination percentage was estimated according to the technique of Standard Germination Test (ISTA, 1993). To estimate germination percentage, germination was counted every two days till 12 days where seeds germinaton were completed.

Germination speed (GS) was calculated according to the formula of McGuire (1962) as follows:

$$GS = \frac{\text{Normal seedlings(No)}}{\text{Days to first count}} + \cdots + \frac{\text{Normal seedlings(No)}}{\text{Days to final count}}$$

Obtained data was subjected to the proper technique of analysis of variance (ANOVA) as published by Gomez and Gomez (1984) and the treatment means were compared using the test of Baysian Least Significant Difference, BLSD, as mentioned by Waller and Duncan (1969). Computations and statistical analysis were done using the facility of computer and SAS software (SAS Institute, 1995).

Results and Discussion :

Salinity concentration effects:

Results of this study reveal that salinity concentrations of media significantly affected seed germination percentage and germination speed (Table 1). It is evident that each increase in salinity concentration after 100 mol/m^3 NaCl was associated with marked reduction in seed germination percentage. There were no significant differences in seed germination percentage between control (seeds wetted with distilled water) and that wetted with solutions of 50 and 100 mol/m^3 NaCl. The reduction in seed germination was obvious when salinity concentration of seeds media

increased to 150 and 200 mol/m^3 NaCl (Fig 1). Similar observations were reported by Maas and Hoffman (1977), Puppala *et al.* (1999) and Houle, *et al.* (2001). Increasing salinity concentration in germination media often cause osmotic and/or specific toxicity which may reduce or retard germination percentage (Waisel, 1972, Mohammed and Sen, 1190 and Basalah, 1991). The response observed with canola up to 100 mol/m^3 was only germination delaying which can be attributed to osmotic effects. Seed germination at 150 mol/m^3 NaCl and more was substantially delayed and reduced. It was quite probable that the delay and reduction in seed germination under these circumstances were equivalently due to osmotic or toxicity effects or a combination of both. Germination speed was significantly affected by media salinity concentration, following the same trend of germination percentage. The faster speed of germination was observed when seeds were wetted with the distilled water. The increase in salinity concentration of media solution resulted in marked decrease in germination speed (Table 2).

Table (1)

Source of variance (S.V), degrees of freedom (D.F), mean squares (M.S) and probability (P) of studied factors for seed germination and germination speed.

S.V	D.F	Seed germination		Germination speed	
		M.S	P	M.S	P
NaCl (A)	4	7303.2	0.0001**	354.0	0.0001**
Incubation Temperature (B)	2	199.5	0.0001**	129.57	0.0001**
Cultivars (C)	2	14.2	0.06 NS	45.92	0.0001**
Interaction (A*B)	8	120.0	0.0001**	30.08	0.0001**
Interaction (A*C)	8	3.9	0.63 NS	8.93	0.0001**
Interaction (B*C)	4	1.71	0.85 NS	4.8	0.067 NS
Interaction (A*B* C)	16	4.25	0.65 NS	5.08	0.070 NS
Experimental Error	225	5.11		2.16	

N.S.: not significant

** : Significant at 0.01% level of probability

Incubation temperature degree effects:

Results indicated that incubation temperature had significant effects on seed germination percentage and germination speed (Table 1). The lowest incubation temperature degree (15 ^{o}C) was the best which recorded the highest seed germination percentage (72.4 %). Raising temperature degree during seed incubation was associated with a significant decrease in germination percentage. The highest incubation temperature degree (35 ^{o}C) recorded the lowest germination percentage (60.4 %), as shown in Fig. (1). Meanwhile, germination speed reached its maximal value (70.3) with the intermediate incubation temperature, 25 ^{o}C (Table 2). The decrease in seed germination percentage under the higher temperature could be particularly attributed to the exposure of seeds during germination to heat which resulted in malfunctioning of the enzymatic system. This situation would lead to limitation in many physiological process vital for seed germination. Similar results were reported by Nykiforuk and Johnson-Flanagan (1994). Furthermore, higher incubation temperature could increase the ions fulxes via cell membrane, particularly Na and Cl which run the risk of toxicity inhibition to the physiological process of seed germination (Salisbury and Ross, 1992).

Canola cultivar differences:

Statistical analysis of data (Table 1) revealed that there were insignificant differences among the three evaluated canola cultivars in seed germination percentage. However, canola cultivars significantly varied in germination speed. The highest germination speed was observed with Al-Serw 8 CV which markedly surpassed both of Al-Serw 4 and Pactool CVs. Meanwhile, there was insignificant difference between Al-Serw 4 and Pactool CVs in germination speed . This probably represents the genetic resistance to salinity of Al-Serw 8 which could be attributed to the condition under which the cultivar was selected. Puppala *et al.* (1999) found varietal variation in germination percentage of canola seeds.

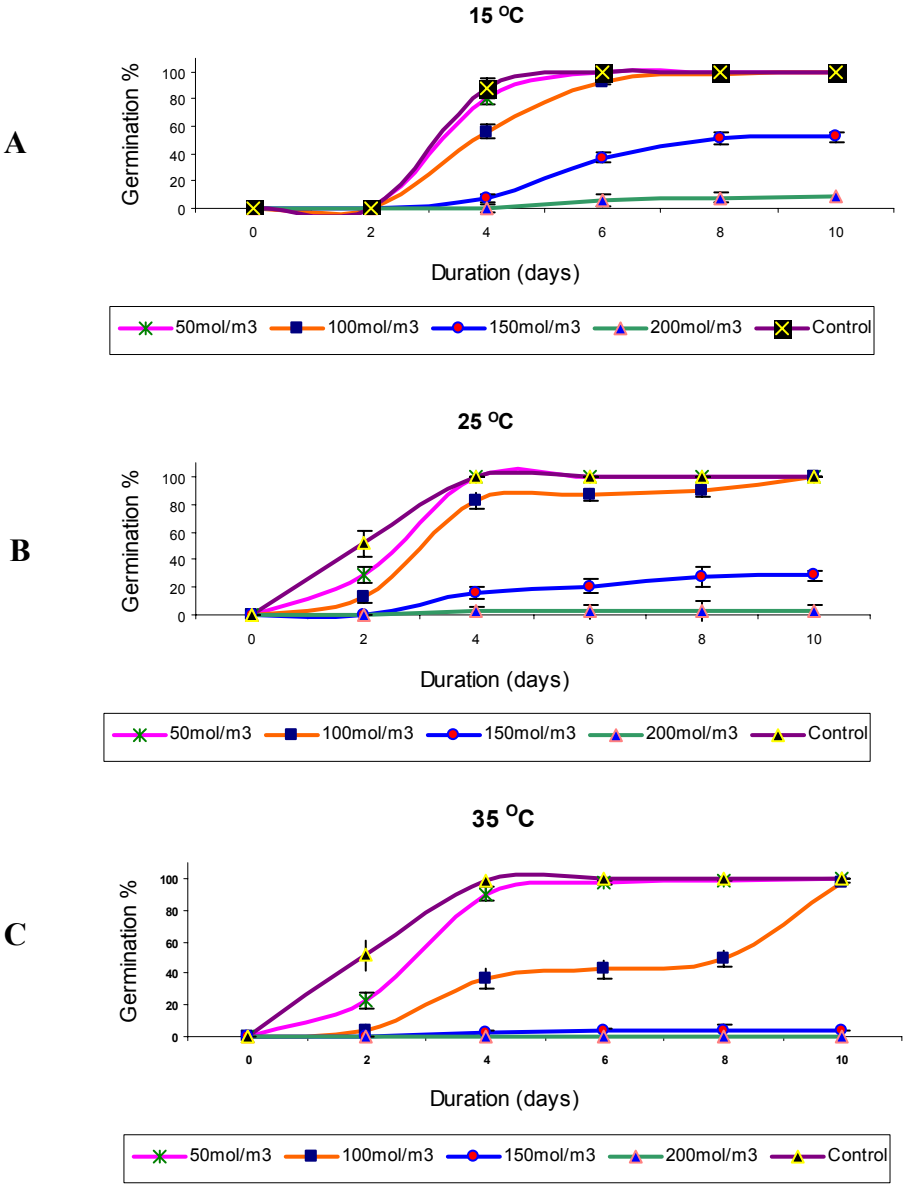


Fig.(1) : Seed germination of canola seeds as affected by salinity concentrations at 15 (A), 25 (B) and 35 (C) ^{o}C incubation temperature, bars are SD values.

Table (2)
Speed of germination of the evaluated three canola cultivars in response to salinity concentration and incubation temperature

Incubation Temperature	Salinity Levels	Cultivar			Mean
		Al-Serw 4	Al-Serw 8	Pactol	
15 °C	5 dSm	74.8	76.2	77.0	76.0
	10 dSm	66.8	71.7	70.3	69.6
	15 dSm	53.3	61.2	58.0	57.5
	20 dSm	30.0	48.7	26.7	35.1
	Control (DW)	77.3	77.7	77.2	77.4
	Mean	60.5	67.1	61.8	63.1
25 °C	5 dSm	83.2	89.3	85.1	85.9
	10 dSm	68.9	84.2	72.5	74.1
	15 dSm	37.5	75.2	84.2	65.6
	20 dSm	36.7	66.7	3.3	35.6
	Control (DW)	87.0	91.3	92.7	90.3
	Mean	62.0	81.3	67.6	70.3
35 °C	5 dSm	78.7	86.2	78.0	80.9
	10 dSm	43.0	60.7	34.7	46.1
	15 dSm	8.3	43.8	0	17.4
	20 dSm	0	0	0	0
	Control (DW)	89.5	92.3	88.2	90.0
	Mean	43.9	56.6	40.1	46.9
Mean	5 dSm	78.9	83.9	80.1	80.9
	10 dSm	58.6	71.1	59.2	63.3
	15 dSm	33.1	60.1	47.4	46.8
	20 dSm	22.2	38.4	10.0	23.6
	Control (DW)	84.6	87.1	86.0	85.9
	Mean	55.5	68.3	56.5	

BLSD 0.05 for: Incub.Temp (A) = 4.3
Salinity Conc. (B) = 5.6
Cultivars (C)= 4.3

Interaction Effects:

The interaction between salinity concentration and incubation temperature had significant effects on seed germination percentage and germination speed. Data graphically depicted in Fig. (2 and 3) show that the highest reduction in seed germination percentage and germination speed were observed as salinity concentration increased (150 and 250 mol/m^3 NaCl) with the highest incubation temperature degree (35^{o}C). Germination speed of the three evaluated canola cultivars significantly varied among them in response to salinity concentrations. Al-Serw 8 was the best in tolerance to salinity, which surpassed the other two cultivars in germination speed under the highest salinity concentrations (Fig. 4). Pactool CV. was the lowest tolerant in this concern. Other interactions did not induce significant effects on germination percentage and germination speed.

In general, it can be stated that canola seeds can be successfully germinated under the incubation temperature ranging from 15 to 35 ^{o}C in case of low concentration of salinity (up to 100 mol/m^3 NaCl). Seed germination percentage and germination speed decreased with increasing salinity concentration levels beyond 100 mol/m^3 NaCl particularly with increasing incubation temperature.

Fig. (2) Germination % in response to NaCl concentration and incubation temperature

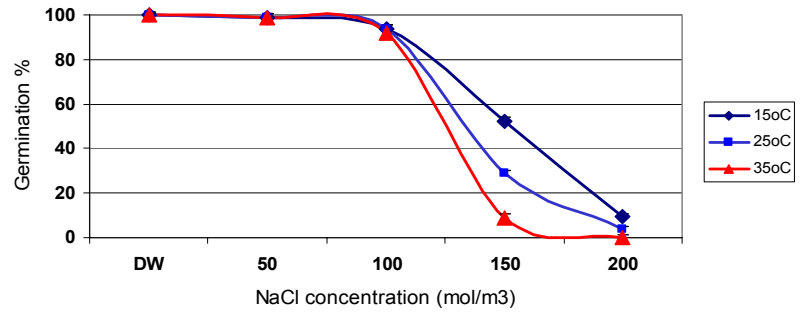


Fig. (3) Germination speed in response to NaCl concentration and incubation temperature

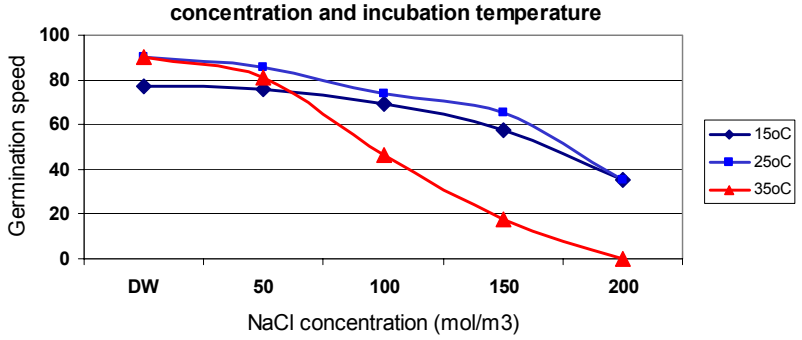
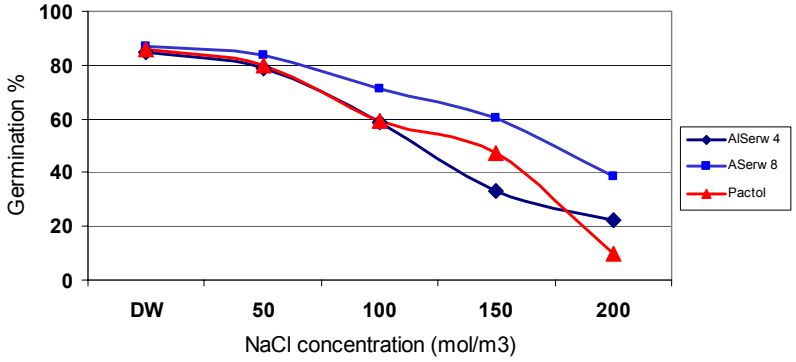


Fig. (4) Germination speed of canols Cvs in response to NaCl concentration



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تأثير كلوريد الصوديوم ودرجة الحرارة على إنبات ثلاثة أصناف من الكانولا

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الأحساء – المملكة العربية السعودية

نفذ هذا البحث لدراسة تأثير تركيز ملح كلوريد الصوديوم (٥٠، ١٠٠ ،١٥٠ ،٢٠٠ مول/م^{٣} ،بالإضافة إلى معاملة المقارنة وهي الماء المقطر) ودرجة حرارة تحضين البذور (١٥،٢٥ ، ٣٥ ^{o}م). على نسبة وسرعة الإنبات لثلاثة أصناف من الكانولا (السرو ٤ ، السرو٨، باكتول). وقد دلت نتائج الدراسة أن تركيزات ملح كلوريد الصوديوم في بيئة الإنبات قد أثرت معنويا علي نسبة وسرعة الإنبات، حيث نقصت سرعة ونسبة الإنبات بزيادة مستويات كلوريد الصوديوم عن ١٠٠ مول/م^{٣} .

سجلت أقل درجة حرارة حضنت فيها البذور (١٥ ^{o}م) أعلى نسبة من الإنبات، في حين سجلت أعلى درجة حرارة (٣٥ ^{o}م) أقل نسبة إنبات. وقد بلغت سرعة الإنبات أقصاها في درجة حرارة ٢٥ ^{o}م .

اختلفت أصناف الكانولا فيما بينها معنويا في سرعة الإنبات ، وقد سجل السرو ٨ أعلى سرعة إنبات بينما لم تختلف نسبة الإنبات معنويا في الأصناف الثلاثة وكان صنف الكانولا السرو ٨ هو الأعلى من حيث نسبة الإنبات.

وبصفة عامة، يمكن القول أن بذور الكانولا يمكنها أن تنبت بنجاح في درجات حرارة تتراوح من ١٥ إلى ٣٥ ^{o}م على أن لا تزيد مستويات الملوحة في بيئة النمو عن ١٠٠ مول/م^{٣} كلوريد صوديوم ، وأن نسبة وسرعة الإنبات تنقص كثيرا بزيادة درجة الحرارة في حالة وجود مستويات عالية من ملح كلوريد الصوديوم (١٥٠ – ٢٠٠ مول/م^{٣} كلوريد صوديوم).