

## Does seed-applied Salicylic Acid Affect Rapeseed Germination Performance under Salinity?

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### Abstract

In order to examining of salinity and Salicylic Acid treatment on response of Modena genotype rapeseed (***Brassica napus L***) in seedling stage, an factorial (AS) experiment on base completely randomized design (CRD) with three replications conducted at Seed technology laboratory in Shahed University. Salicylic Acid Hormone at three levels (Control - sprayed with water only, Acid applied at 0.2 and 0.4 mM) and salt stress at the four levels of NaCl by 0, 3, 6, 9 and 12 ds.m<sup>-1</sup> on germination and seedling growth were studied. Germination rate, root and shoot length, radical, plumul fresh and dry weights, root/shoot length ratio (R/S), Germination coefficient, Vigore weight index (VWL) and Vigore length index (VLI) with quality components as protein and protein content were measured. Also results showed that Acid and salt stress applied influenced on some characters such as seedling dry weight, seedling vigor index, length of radicles and plumules and seedling, seed vitality, seedling vigor, at probability level of 0.001 and Number of hard seeds at probability level of 0.05. In general, significant differences were found in seed germination components, physiological processes for rapeseed treated by Acid levels, saline stress and Acid\*saline interaction. Seed vigor, length of radicles, length of seedlings, number of normal seedlings, seedling and seed vigor index were higher in 0.2 mM Acid during experiment. Number of abnormal seedlings and hard seeds were higher in the seeds under 12 ds.m<sup>-1</sup> level of salinity. It seems more number of the seeds that set under saline test, due to tolerance stress, went to obligatory dormancy, and produced more number of abnormal seedlings, too. Significant differences were found in shoot and root length, dry weight shoot and root in levels of Acid in response to saline stress, which 9 and 12 ds.m<sup>-1</sup> level of salinity had the best results. While it is possible that rapeseed's salt resistance is enhanced by acid accumulation, showed dry weight of salt stress in 12 ds.m<sup>-1</sup> and 0.2 mM acid interaction was equal with salinity of 9 ds.m<sup>-1</sup> level. Genotype in consequence of stress was higher in the most characters such as seed vigor, seedling dry weight, number of normal seedlings, seedling vigor index. Under saline stress conditions Modena had longer radicle, more lateral roots and higher proportion of root to plumule. It is necessary for salt tolerance this indicator and thus Modena genotype was more resistance to Saline stress.

**Key words:** Rapeseed, Salinity, Germination, Seedling growth, Salicylic Acid, Vitality

### Introduction

Plants are exposed to many stress factors, such as drought, high salinity or pathogens, which reduce the yield of the cultivated plants or affect the quality of the harvested products. Salt stress, in general, reduces the water uptake capacity of the plant, thus reduces growth rate and metabolic activity. The initial growth reduction could be due to hormonal signals generated by the roots encountering salinity (Munns, 2002). As a more long term impact of salinity, the excessive salt toxicity levels lead to senescence and reduce the photosynthetic capacity due to the closure of stomata and limited carbon dioxide uptake, which cannot sustain proper growth (Zhang 2001; Munns, 2002). In addition, Salinity is known to affect many aspects of metabolism, anatomy and ultra structure of plant cells (Rahman *et al.*, 2000). These reactions are often considered to be adaptive strategies, being helpful to sustain NaCl salinity. Salt stress even delays germination of seeds as well as the final germination percentage (Zeinali *et al.*, 2002). Salt stress causes a number of changes in plant metabolism. Rapeseed is one of the most important oils for human beings and considered to be one of the moderately salt tolerant plants (omid *et al.*, 2010). The aim of subsequent experiments was to study the effects of Salicylic Acid (SAS) or Ascorbic acid (AsA) on seed germination, ultra structure and anatomy of sorghum seedlings grown on nutrient solutions with or without salt supply. **Materials and Methods**

**Plant materials**

Rapeseed (*Brassica napus* (L.) SImo46. var.) Grains were secured from the Agricultural Research Centre, Karaj, Iran.

**Chemicals**

1. SAS was supplied by Sigma Chemical Co., USA and used at the concentration of 2000 or 4000 ppm.
3. NaCl from EL-Gomhoria Co., Egypt and was used at the concentrations of experiment by ppm unit.

**Germination experiment**

The experiment was carried out in the glasshouse of the Agric. Dept., Fac. of Agric., Shahed Univ., Tehran city, Iran during the summer season 2010. Canola cultivar, namely, Option 500 was used in this study.

In order to examine the effect of salinity and Salicylic Acid treatment on the response of Modena genotype rapeseed (***Brassica napus* L**) in seedling stage, a factorial (AS) experiment on a base completely randomized design (CRD) with three replications was conducted at the Seed technology laboratory in Shahed University. Salicylic Acid Hormone at three levels (Control - sprayed with water only, Acid applied at 0.2 and 0.4 mM) and salt stress at four levels of NaCl by 0, 3, 6, 9 and 12 ds.m<sup>-1</sup> on germination and seedling growth were studied. Germination rate, root and shoot length, radical, plumule fresh and dry weights, root/shoot length ratio (R/S), Germination coefficient, Vigore weight index (VWL) and Vigore length index (VLI) with quality components as proline and protein content were measured.

The germination test was conducted on 50-seed samples of each cultivar at 25 °C for seven days on moistened blotter papers. Tests were replicated three times. Only normal seedlings were counted. The accelerated aging test was conducted by aging seeds at 40 °C and 90% RH for 48, 120, and 192 hours using the wire-mesh tray method (McDonald and Phengdranath, 1978). Following incubation, the seeds were germinated at 25 °C for days.

Mean Germination Time (MGT) (Re1), Germination Coefficient (GC1) (Re2), Uniformity of Mean Germination Time (UMGT) (Re3) and Seed Vigour Index (SVI) (Re5) were estimated. In this relation S and D were consist of germinated and cultivated, respectively.

$$GC = \left( \frac{1}{MGT} \right) * 100 \quad (\text{Re2}) \quad \quad MGT = \frac{\sum_{i=1}^{n} NiDi}{\sum_{i=1}^{n} Ni} \quad (\text{Re1})$$

$$UG = \left( \frac{1}{VMGT} \right) * 100 \quad (\text{Re4}) \quad \quad VMGT = \left[ \frac{\sum_{i=1}^n (Di - D_j)}{N} \right]^2 \quad (\text{Re3})$$

$$(\text{Prac Variance Re}) \sigma_j^2 = \frac{\sum_{i=1}^n Di^2 - \left( \frac{\sum_{i=1}^n Di}{N} \right)^2}{N} \quad \quad SVI = MGT * \left( \frac{\sum_{i=1}^n Ni}{\sum S} \right) \quad (\text{Re5})$$

Root length and shoot weight, root and shoot dry weight of seedling, radicle diameter, number of sub-radicle, percentage of germination, germination speed and germination index, germination coefficient, seed vigor and germination rate for all treatments were measured. Data were analyzed using Minitab and SAS program. Multiple range tests were used for mean analysis.

**Result and discussion**

Results showed that Acid and salt stress applied influenced some characters such as seedling dry weight, seedling vigor index, length of radicles and plumules and seedling, seed vitality, seedling vigor, at probability level of 0.001 and Number of hard seeds at probability level of 0.05 (Table 1-4). In general, significant differences were found in seed germination components, physiological processes

for rapeseed treated by Acid levels, saline stress and Acid\*saline interaction. Seed vigor, length of radicles, length of seedlings, number of normal seedlings, seedling and seed vigor index were higher in 0.2 mM Acid during experiment. Number of abnormal seedlings and hard seeds were higher in the seeds under 12 ds.m<sup>-1</sup> level of salinity. It seems more number of the seeds that set under saline test, due to tolerance stress, went to obligatory dormancy, and produced more number of abnormal seedlings, too. Significant differences were found in shoot and root length, dry weight shoot and root in levels of Acid in response to saline stress, which 9 and 12 ds.m<sup>-1</sup> level of salinity had the best results. While it is possible that rapeseed's salt resistance is enhanced by acid accumulation, showed dry weight of salt stress in 12 ds.m<sup>-1</sup> and 0.2 mM acid interaction was equal with salinity of 9 ds.m<sup>-1</sup> level. Genotype in consequence of stress was higher in the most characters such as seed vigor, seedling dry weight, number of normal seedlings, seedling vigor index. Under saline stress conditions Modena had longer radicle, more lateral roots and higher proportion of root to plumule. It is necessary for salt tolerance this indicator and thus Modena genotype was more resistance to Saline stress.

**Table 1.** The influence of different rates of Acid Salicylic (A) on the spring oilseed rapeseed characterizes on Germination phase

Source of variance	D.f	MEAN SQUARE									
		Seed vigour	Germination Coefficient	MGP	Germination percent	Sub lateral Root	Radicle diameter	plomule Dry weight	Radicle Dry weight	Radicle length	plomule length
Acid Salicylic(A)	2	0.503	748.777ns	1.754**	2777.95**	0.962ns	0.146ns	590.24**	15.85**	24.53**	0.62ns
Salinity (S)	4	0.20	476.348**	0.285ns	1109.68**	8.181**	0.200**	682.18**	32.07**	46.25**	22.327**
S*A	8	0.186	583.197ns	0.414ns	2377.95ns	0.536ns	0.055ns	168.13**	2.22ns	7.28**	0.579ns
Error	30	0.176	748.949**	0.226ns	222.93**	0.183**	0.460ns	21.9**	1.92**	0.5**	0.51**

.ns, \* and \*\* : not significant, significant at the 5 and 1 % levels of probability ,respectively.

**Table 2.** Mean comparisons of Germination characters affected by Acid Salicylic (A) on phase (2009-2010)

Acid Salicylic(mM)	Seed vigour	Germ. Coefficient	MGP	Germination percent	Sub lateral Root	Radicle diameter	plomule Dry weight	Radicle Dry weight	Radicle length	plomule length
0	0.63ab	35.22a	1.67b	58.93b	1.26a	1.14a	37.67 b	2.5 b	4.28 Ab	3.77 A
20	0.88a	35.34a	1.43b	51.46b	0.77a	0.97b	32.01c	2.67 b	2.85c	3.38 A
40	0.53b	39.32a	2.1a	77.86 a	.92 a	1.14 a	44.54 a	4.36a	5.4a	3.68 A

Mean followed by the same letters in each column are not significantly different (Duncan multiple rang test 5 %).

**Table 3.** Mean comparisons of Germination characters affected by Salinity (2009-2010)

Salinity Ds.m <sup>-1</sup>	Seed vigour	Germination Coefficient	MGP	Germination percent	Sub lateral Root	Radicle diameter	Dry weight plomule	Radicle Dry weight	Radicle length	plomule length
0	0.58a	44.01a	1.77a	76.88a	1.84a	0.99ab	45.44a	5.77a	6.56a	5.46a
3	0.62a	39.89a	1.70a	68.00ab	2.11a	1.20a	38.46b	4.08b	5.55b	4.65b
6	0.65a	38.34a	1.68a	56.71ab	0.77b	1.19a	41.58ab	3.01bc	4.93b	3.83c
9	0.61a	29.65b	2.01a	58.22bc	0.20c	1.16a	41.74ab	2.26c	2.96c	2.58d
12	0.95a	31.23b	1.52a	47.11c	0.00c	0.87b	23.14c	0.76d	0.87d	1.53e

Mean followed by the same letters in each column are not significantly different (Duncan multiple rang test 5 %).

**Table 4.** Mean comparisons of interaction effects of characters affected by Salinity and Acid Salicylic

Acid Salicylic(mM)	Salinity Ds.m <sup>-1</sup>	Seed vigour	Germ. Coefficient	MGP	Germ. percent	Sub lateral Root	Radicle diameter	plomule Dry weight	Radicle Dry weight	Radicle length	plomule length
0	0	0.58b	42.98abc	1.80bcd	76.00abc	2.40a	1.02abc	48.90ba	4.33bac	6.77bc	6.20a
0.2		0.62b	45.75ab	1.62bcd	73.33abc	1.93abc	0.92bc	43.50abc	6.13bac	5.72dc	5.24ab
0.4		0.53b	43.32abc	1.89bcd	81.33ab	1.20dc	1.02abc	49.63a	6.85ab	7.20b	4.94abc
0	3	0.62b	38.75abcd	1.72bcd	66.67abcd	2.06ab	1.24ab	33.96bdc	3.63abc	5.32de	4.74bcd
0.2		0.74b	37.56abcd	1.36bcd	50.57cdef	1.80abc	1.15ab	29.60dec	2.36bc	2.19gh	4.26bcd
0.4		0.50b	43.36abc	2.04abc	86.67a	2.46a	1.23ab	46.33ab	6.26abc	9.14a	4.96abc
0	6	0.55b	33.38cd	1.86bcd	62.67abcde	1.53bc	1.13ab	25.96def	4.00abc	6.84bc	4.27bcd
0.2		0.62b	33.81bcd	1.68bcd	58.67abcdef	0.06e	1.23ab	29.40dec	2.30bc	3.07fg	3.50de
0.4		0.80b	47.74a	1.49bcd	69.33abcd	0.73ed	1.22ab	3770abcd	3.50abc	4.88ed	3.72edc
0	9	0.58b	31.01d	1.72bcd	53.33bcdef	0.33e	1.42a	38.90abcd	1.00bc	2.02gh	2.54ef
0.2		0.88ab	30.18d	1.49bcd	44.00def	0.06e	0.90bc	39.96abcd	2.23bc	2.72gh	2.40ef
0.4		0.36b	27.76d	2.82a	77.33abc	0.20e	1.17ab	46.36ab	3.56abc	4.15ef	2.81ef
0	12	0.83ab	29.89d	1.26cd	36.00ef	0.00e	0.88bc	17.60ef	0.30c	0.42i	1.08g
0.2		1.56a	29.39d	1.01d	30.67f	0.00e	0.65c	12.20f	0.35c	0.56i	1.50gf
0.4		0.45b	34.42bcd	2.29ab	74.67abc	0.00e	1.07ab	42.63abc	9.13a	1.64hi	2.01gf

Mean followed by the same letters in each column are not significantly different (Duncan multiple rang test 5 %).

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