



## Canola seed germination and seedling growth in response to saline condition and bio-priming

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### Abstract

Seed priming, as a low-cost method, is a technique commonly used to increase germination percentage especially under unfavorable conditions. The objective of this study was to evaluate the effects of bio-priming on the germination of canola (*Brassica napus* L.) seeds under saline (NaCl) conditions. Seeds of canola var. Hyola 401 were inoculated with plant growth-promoting microorganism (PGPM) including bacterium *Bacillus subtilis* strain Ham and fungus *Trichoderma harzianum* strain bp4. The primed seeds were subsequently exposed to four levels of salinity (0, 5, 7.5, and 10 dS.m<sup>-1</sup>). Results showed that application of priming with PGPM significantly improved the percentage of seed germination, root length and seedling vigor index under saline conditions. Results of this study may provide useful information concerning reduction of undesirable effects due to salinity on canola seed germination which leads to increase of the rate of deployment of canola in areas that are facing salinity of soil or irrigation water.

**Keywords:** bio-priming; canola; germination; plant growth-promoting microorganism; saline stress

**Abbreviations:** GP: germination percentage; PGPM: plant growth-promoting microorganism; PGPR: plant growth-promoting rhizobacteria; RL, root length; RW: root fresh weight; ROS: reactive oxygen species; SL: shoot length; SVI: seedling vigor index; SW: shoot fresh weight; UG: uniformity of germination

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### Introduction

Canola seed (*Brassica napus* L.) is the third major world source of edible seed oil after soybeans and palm (Singh and Sharma, 2016). Abiotic stresses such as salinity always limit

growth, distribution, and production of crops especially in arid and semi-arid regions. According to a recent estimate, 1128 million ha of global land is affected by salinity and sodicity (Akhtar et al., 2015). Salinity is a stable stress and thus, plants will be permanently exposed to it during their growth period. Seed germination as the most important stages of plant growth is also affected

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by salinity. Increased Na<sup>+</sup> uptake in plants under salinity disturbs those metabolic processes that require low Na<sup>+</sup> and high K<sup>+</sup>, Ca<sup>2+</sup> or both for normal functioning (Akhtar et al., 2015). Seghatoleslami (2011) found that salinity stress significantly decreased shoot length, speed of germination, and seedling vigor index (SVI) in *Satureja hortensis*, shoot weight in *Cynara scolymus*, and SVI in *Cichorium intybus*.

Seed priming is one of the common techniques that are used to increase germination percentage (GP) especially under unfavorable conditions. This technique also reduces the physiological heterogeneity in the seed masses. Water absorption by seeds sown in the soil is a time-consuming process. Seed priming can effectively reduce this time leading to faster germination and consequently stronger crops (Hosseini and Koocheki, 2007). Seed priming is a low-cost and low-risk method therefore, it is used as a common strategy to increase the percentage, speed, and uniformity of the germination and also to improve the quantity and quality of the plant performance under adverse environmental conditions. This strategy also increases the resistance of plants to salinity stress (Riazi and Sharif Zade, 2009). Bio-priming is one of the seed priming methods that uses biological substances to increase plant growth and activate resistance mechanisms.

It has been reported that application of bio-fertilizers as well as inoculation with plant growth-promoting rhizobacteria (PGPR) can effectively make seeds and shoots more resistant to salinity (Ramamoorthy et al., 2000). PGPRs are bacteria inhabiting around/on the root surface and are directly or indirectly involved in promoting plant growth and development via production and secretion of various regulatory chemicals in the vicinity of rhizosphere (Ahmad and Kibret, 2014). Inoculation of plant tissues with PGPRs induces cell division leading to special structures due to increase in the amount of cytokinin hormone. Increase of cytokinins in root and leaf in turn leads to increase of lateral roots and chloroplasts, respectively. Consequently, leaf chlorophyll content and photosynthetic enzymes are synthesized more quickly (Sakakibara, 2006).

The fungus *Trichoderma harzianum* is a biological control organism against a wide range of

soil-borne pathogens and has plant growth promoting capacity (Ozbay et al., 2004). Singh et al. (2007) suggested that *Trichoderma* fungus increases the solubility of phosphorus and micronutrients and availability of these nutrients for plants.

The objective of this study was to evaluate the effect of bio-priming with plant growth-promoting microorganism (PGPM) including bacterium and fungus on germination indices of canola (Hyola 401) under salinity stress conditions.

## Materials and Methods

### Experimental set up and studied factors

The experiment was conducted in 2015 at Shahed University in the form of factorial based on completely randomized design with 3 replications.

Studied factors included bio-priming with PGPM at four levels of control, inoculation with *Bacillus subtilis* strain Ham, inoculation with *Trichoderma harzianum* strain bp4, and inoculation with both *subtilis* and *T. harzianum* app. The second factor was applying salinity stress at four levels of 0 (control), 5, 7.5, and 10 dS.m<sup>-1</sup>.

Bacteria and fungi used in this study were purchased from Soil and Water Research Institute, Karaj, Iran. The canola seeds var. Hyola 401 were prepared from Seed and Plant Improvement Institute, Shahid Fahmideh Blvd., Karaj, Iran.

First, seeds were disinfected with sodium hypochlorite 10% for 3 minutes and then rinsed with distilled water. The seeds were consequently inoculated with PGPM and were kept at 4 to 10° C for 24 hours. Then, 20 seeds were inoculated with PGPM and sown on a sterilized Whatman paper in each petri dish and were exposed to 4 levels of salinity stress as explained above. The petri dishes were sealed with Parafilm. From the second day and at a specified time, the number of germinated seeds was daily counted for 10 days. In each petri dish, seeds with a root length greater than 2 mm were considered as germinated according to

Table 1

Analysis of variance of traits related to germination and growth indices of canola (*Brassica napus* L. var. Hyola 401) seeds primed (inoculated) with *Bacillus subtilis* and *Trichoderma harzianum* as affected by saline conditions

Source of Variation	DF	RL	GP	SVI	Mean Squares				
					UG	SL	RW	SW	
Salinity (S)	3	91.61 **	11533207.86 **	0.2626 **	12693.35 n.s	9.58 **	0.0413 **	0.1167 **	
Priming (P)	3	5.06 n.s	3979269.16 n.s	0.0272 *	81609.63 *	6.05 **	0.0026 *	0.0061 *	
S×P	9	57.02 **	9913073.08 **	0.0352 **	103460.82 n.s	3.09 n.s	0.0013 n.s	0.0069 n.s	
Residual	16	26.48	1720332.01	0.0078	126420.44	4.52	0.00061	0.0089	
C.V%		16.50	7.65	3.93	12.06	3.44	8.96	11.11	

\* and \*\* mean significant differences at the 0.05 and 0.01 probability levels, respectively and n.s means non-significant.

Table 2

Mean comparisons of interactive effect due to seed priming (inoculation with *Bacillus subtilis* bacteria and *Trichoderma harzianum* fungi) and salinity stress (NaCl) on some germination and growth indices of canola (*Brassica napus* L. var. Hyola 401)

Salinity Stress (dS.m <sup>-1</sup> )	Priming Inoculated with	Germination Percentage	Root Length (mm)	Seedling Vigor Index
Control	Control	96.07±1.84 abcd	11.53±0.19 a	15.47±0.15 a
	<i>T. harzianum</i>	87.79±1.68 ef	7.6±0.24 cdef	10.99±0.33 b
	<i>B. subtilis</i>	88.81±2.14 cdef	10.78±0.26 ab	14.66±0.03 a
	<i>B. subtilis</i> + <i>T. harzianum</i>	90.16±0.16 bcdef	8.47±0.31 bcde	11.07±0.13 b
5	Control	95.67±1.39 abcd	10.52±1.23 abc	14.39±1.53 a
	<i>T. harzianum</i>	99.01±3.13 a	7.65±1.55 cdef	10.95±0.88 b
	<i>B. subtilis</i>	94.44±2.33 abcde	9.93±0.99 abcd	14.35±1.12 a
	<i>B. subtilis</i> + <i>T. harzianum</i>	89.44±0.56 bcdef	8.67±0.02 abcde	10.86±0.02 b
7.5	Control	89.83±1.83 bcdef	7.92±1.07 bcde	11.56±0.94 b
	<i>T. harzianum</i>	93.17±1.28 abcdef	8.01±0.13 bcde	11.38±0.59 b
	<i>B. subtilis</i>	96.67±3.33 ab	4.65±2.33 fg	8.83±2.45 bcd
	<i>B. subtilis</i> + <i>T. harzianum</i>	96.31±1.19 abc	7.27±0.18 def	10.93±0.21 b
10	Control	85.9±2.56 f	3.61±0.33 g	6.31±0.56 d
	<i>T. harzianum</i>	93.94±0 abcde	7.48±1.15 cdef	9.63±0.34 bc
	<i>B. subtilis</i>	88.48±4.11 def	5.92±0.2 efg	8.16±0.42 cd
	<i>B. subtilis</i> + <i>T. harzianum</i>	96.67±3.33 ab	4.73±0.07 fg	6.52±0.17 d

Means that do not share a letter are significantly different

International Seed Testing Association (ISTA, 2016). After 10 days, shoot length (SL), shoot fresh weight (SW), root length (RL), and root fresh weight (RW) were measured.

### Calculated germination indices

Germination percentage (GP) was calculated according to Ranal et al. (2009). Seedling vigor Index (SVI) and the coefficient of uniformity of germination (UG) were calculated using formulas proposed by Abdul-Baki and Anderson (1973) and Ranal and Santana (2006), respectively. Analysis of variance was done using the SAS software (SAS 2003). Charts were drawn using Microsoft Excel (2013) software.

## Results

### Analysis of variance

Result derived from analysis of variance of the studied traits are shown in Table 1. According to this table, salinity significantly affected all traits ( $p < 0.01$ ) except for uniformity of germination. Also, bio-priming with PGPM had a significant effect on seedling vigor index, uniformity of germination, shoot length, root fresh weight, and shoot fresh weight. The interaction of salinity by priming was significant for RL, GP, and SVI. Therefore, we focused on interaction effect only for these three traits (Table 2).

## Effect of Salinity

Mean comparison of traits under the impact of salinity stress levels has been presented in Fig. I. Based on the results, no statistically difference was observed for shoot length at 0, 5, and 7.5  $\text{dS}\cdot\text{m}^{-1}$  levels indicating the relative tolerance of canola to the mentioned stress levels. However, SL drastically decreased at 10  $\text{dS}\cdot\text{m}^{-1}$  of stress level and dropped to 2.53 cm which was equal to 33 percent reduction, as compared with control (Fig. I). Moreover, a relative increase was observed for SL at 7.5  $\text{dS}\cdot\text{m}^{-1}$ . The data presented in Figure (I) revealed that shoot fresh weight and root fresh weight also reduced along with increase in the salinity concentration from 5 to 10  $\text{dS}\cdot\text{m}^{-1}$ . The lowest RW (0.01g) and SW (0.09g) were observed at salinity level 10  $\text{dS}\cdot\text{m}^{-1}$  showing a reduction up to 78 percent for RW and 61 percent for SW, as compared with control.

## Effect of inoculating with PGPMs

In this study, inoculation of canola seeds with *B. subtilis* and *T. harzianum* resulted in increased shoot fresh weight, root fresh weight, shoot length, and the uniformity of germination (Fig. II). Also, the ANOVA table shows that priming had no significant effect on RL and GP. The highest value for SW (0.21 g), RW (0.058 g) as also for SL (3.9 cm) was obtained when the seeds were inoculated with *T. harzianum* rather than *B. subtilis*. This result revealed that growth promotion due to inoculation with fungi was higher than that of bacteria (Fig. II). Compared with control, the observed increase due to seed inoculation with *T. harzianum* for SW, RW, and SL was equal to 20, 28, and 40 percent, respectively (Fig. II).

Based on the findings presented in Figure (II), priming with PGPM, regardless of the type of the microorganism, significantly increased uniformity of germination as compared with control (Fig. II). The best result concerning the UG was achieved when the seeds were primed with a combination of *B. subtilis* and *T. harzianum*.

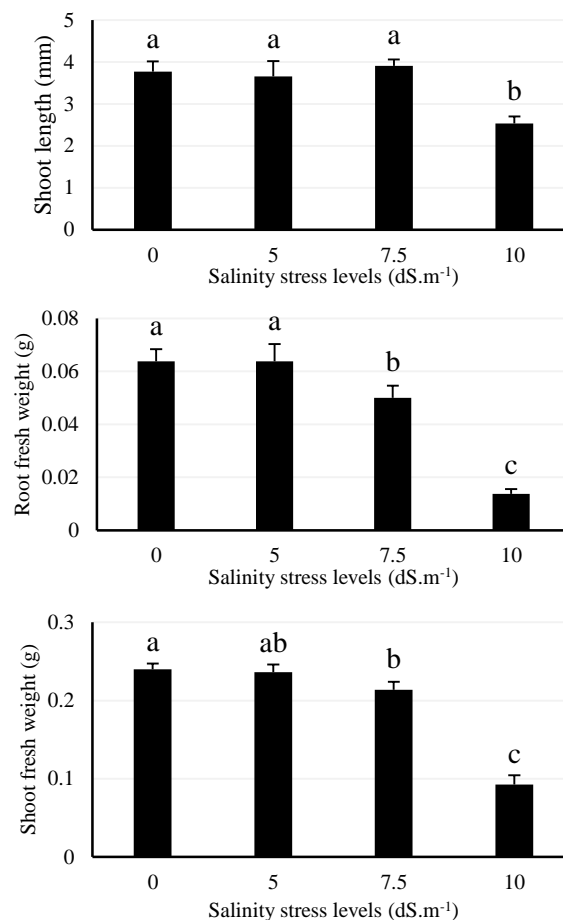


Fig. I. Effect of salinity stress (NaCl) on some germination indices of canola (*Brassica napus* L. var. Hyola 401); means that do not share a letter are significantly different.

## Interactive effect

Based on the results presented in Table 2, despite the increase in stress severity, seed priming could remarkably reduce the adverse impact of salinity on seed germination percentage of canola. The highest value for GP was observed at the salinity level of 5  $\text{dS}\cdot\text{m}^{-1}$  ( $99.01 \pm 2.13$ ) where the seeds were inoculated with *T. harzianum* which was 3.34% higher than that of the control (Table 2). Interestingly, at 10  $\text{dS}\cdot\text{m}^{-1}$  of salinity level, seed inoculating, regardless of the kind of PGPM, could noticeably improve GP in comparison with the control (Table 2). At 10  $\text{dS}\cdot\text{m}^{-1}$  of salinity level, the recorded GPs were 93.94, 88.48, and 96.67 for seeds inoculated with *T. harzianum*, *B. subtilis*, and both *B. subtilis* and *T. harzianum*, respectively which was 8.04, 2.58 and 10.77 percent more than that of control,

respectively. Results suggested that priming could reduce damages caused by salinity stress on the germination percentage of canola seeds. Therefore, with the aim of increasing the rate of deployment of canola in saline soils, which is of high importance in sustainable agriculture, seed priming with bacteria and fungi may be used as recommended in the present study.

## Discussion

### Effect of Salinity

As one of the main environmental limiting factors, salinity has a negative impact on growth and yield of plants. Results of the present work showed that canola could tolerate a salinity stress up to  $7.5 \text{ dS.m}^{-1}$ . However, most of the studied traits drastically decreased when the stress severity raised up to  $10 \text{ dS.m}^{-1}$ . Likewise, studies have shown that salinity has had a negative effect on barley (*Hordeum vulgare* L.) (Huang and Redmann, 1995), tepary bean (*Phaseolus acutifolius*) (Goertz and Coons, 1991), and tomato (*Solanum lycopersicum*) (Foolad, 1996).

In this study, salinity stress had a more negative effect on RW than SW which was in contrast with the results reported by Jamil et al. (2005). Previous researchers have shown that reduction in RW and SW was mainly due to ionic effect especially the proportional increase in  $\text{Na}^+$  concentration. Moreover, the presence of  $\text{Na}^+$  and  $\text{Cl}^-$  ions in the cells may induce changes in protein activity because ions affect the structure of the hydration water which surrounds the protein molecule (Waisel, 1972).

Under the impact of salinity, increased ions ( $\text{Cl}^-$  or  $\text{Na}^+$ ) disrupts the osmotic balance leading to a toxic effect. This phenomenon was the main reason for failure observed in GP. Salinity stress inhibits activities of some enzymes involved in seed germination process. For example,  $0.33 \text{ m}$  of  $\text{NaCl}$  inhibits activities of malate and glucose-6-phosphate dehydrogenase up to 66% and 60%, respectively (Flowers, 1972).

In this study, salt stress remarkably inhibited root development showing inhibition occurred in root cell proliferation. As shown by Long et al. (2015), there are some mechanisms in this regard such as high concentrations of  $\text{NaCl}$  in

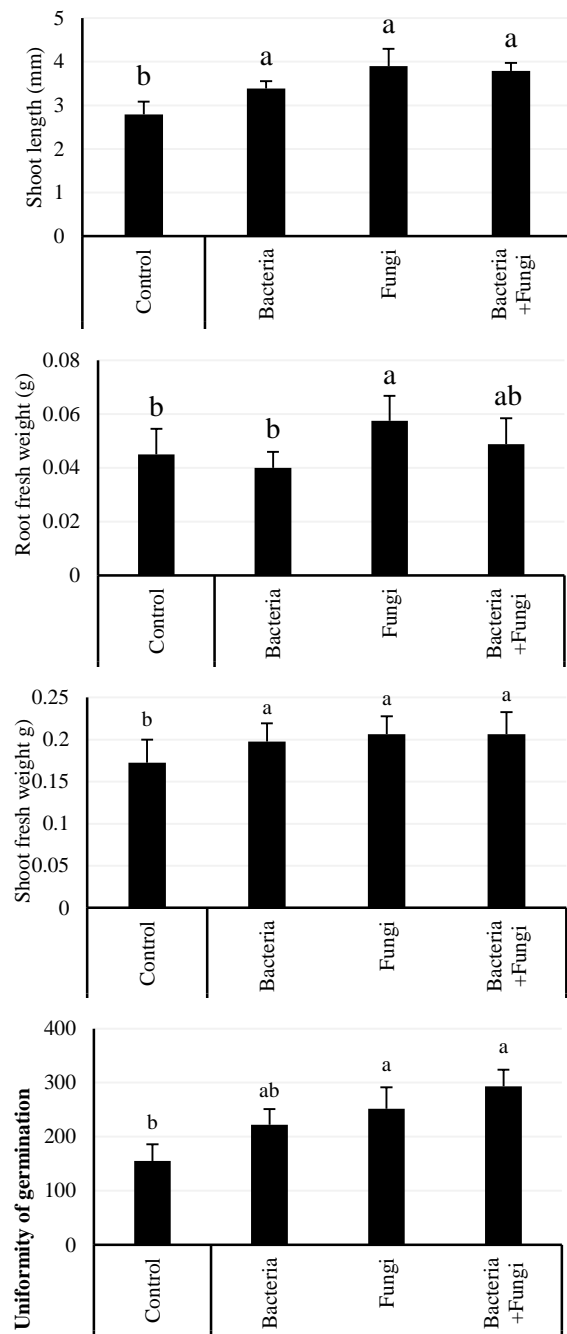


Fig. II. Effects of inoculation of canola seeds (*Brassica napus* L. var. Hyola 401) with growth promoting microorganism of *Bacillus subtilis* and *Trichoderma harzianum*; means that do not share a letter are significantly different.

the environment leading to a decrease in water potential. Consequently, plant cells faced with some difficulties to absorb external water. Although the expression of some genes involved in the proline synthesis pathway (such as Pyrroline-

5-carboxylate synthase) could balance the water potential inside and outside the cells; however, this change could not help cells absorb enough water to perform related substrate synthesis for cell development. Moreover, salt stress induces reactive oxygen species (ROS), which leads to secondary oxidation stress, disturbs cellular redox homeostasis, and damages cell components and structures (Shalata and Neumann, 2001). Under salinity stress, high levels of ROS generated in root cells damage the existing active cells, which have a negative effect on cell proliferation. In addition, salinity stress negatively affects cell wall reconstruction and re-synthesis, which would interfere with roots cell expansion and division (Long et al., 2015). Similar to root length, stem length was also significantly reduced under salinity stress. It seems that similar to root length, the osmotic differences were the main cause of such phenomenon.

### Effect of priming with PGPMs

Due to the increasing demand for edible oils and the growing problem of saline arable lands, the use of bio-priming can be a strategic approach to cope with this problem. Based on the findings presented in Fig. (II), priming with PGPM regardless of the type of the microorganism, significantly increased UG in comparison with control (Fig. II). Such an outcome has been also mentioned in the research by other investigators (Ghorbanpour and Hatami, 2014; Ansori et al., 2015). Studies have shown that growth promotion in response to inoculation with PGPMs may involve various mechanisms. However, production of plant growth regulators by PGPM has been the most important mechanism (Kaymak et al., 2009). For example Sakakibara (2006) reported that inoculation of plant tissues with PGPRs induces cell division leading to special structures due to increase in the amount of cytokinin hormone. Increase of cytokinins in root and leaf in turn leads to increase of lateral roots and chloroplasts, respectively. Consequently, leaf chlorophyll content and photosynthetic enzymes will synthesized more quickly. Research has shown that PGPMs were able to promote seed germination and growth indices in tomato and

pepper (Rodriguez et al., 2001), lettuce (Díaz et al., 2001), and radish (Basavaraju et al., 2002).

### Interactive effect

The data presented in Table 2 indicate that all studied traits reduced with increase in NaCl concentration. However, priming increased their values in the presence of salinity stress. At the salinity level of 10 dS.m<sup>-1</sup>, inoculation of canola seeds with *B. subtilis* and *T. harzianum* increased SVI up to 52% and 29%, respectively as compared with control. It seems that, a significant increase in GP compared to control was the main reason for this phenomenon. Although, root length decreased at 7.5 and 10 dS.m<sup>-1</sup> of salinity, the GP was still considerably high for the seeds inoculated with *T. harzianum* (Table 2). As shown in Table 2, seed inoculation with fungi promoted GP and at the same time reduced the RL. One possibility could be that increase of seedling density as a result of increased GP reduced RL because in the presence of higher seed germination, the total amount of water absorbed by the seedlings would be higher resulting in reduced amount of available water. Thus, less available water along with salt treatment reduced the root length.

In conclusion, findings of the present work showed that bio-priming with PGPM was an effective technique to improve germination percentage and rate of canola (*Brasica napus* L. var. Hyola 401) seeds under saline conditions. PGPR refers to bacteria inhabiting around/on the root surface that promote plant growth and development. In this study a fungus strain was also applied; therefore, we used the term plant growth-promoting microorganism (PGPM) rather than PGPR. Moreover, most of studies about promotion due to bio-priming with PGPM have been conducted under normal conditions while we have investigated the effect of PGPM under salinity stress. Therefore, results of this study may provide useful information concerning reduction of undesirable effects due to salinity on canola seed germination.

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