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The Effects of Bio-priming with PGPR on Germination of Radish (*Raphanus sativus* L.) Seeds under Saline Conditions

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Abstract: The present study was conducted to examine the effects of bio-priming with plant growth promoting rhizobacteria (PGPR) on the germination of radish (*Raphanus sativus* L.) seeds under different saline (NaCl) conditions. Three radish cultivars ('Antep', 'Beyaz', and 'Siyah') were used as plant material and 5 bacteria strains (*Agrobacterium rubi* strain A 16, *Burkholderia gladii* strain BA 7, *Pseudomonas putida* strain BA 8, *Bacillus subtilis* strain BA 142, and *Bacillus megaterium* strain M 3) were applied as priming agent. The effect of bio-priming with PGPR on germination percentage under saline conditions varied with bacteria strains and cultivars. Applications of bio-priming with bacteria strains significantly improved the percentage of seed germination under saline conditions. These results suggested that bio-priming with PGPR under saline conditions could be useful to obtain higher seed germination percentages in radish.

Key Words: Bacteria strains, bio-priming, germination, radish, saline stress

BGURB ile Yapılan Bio-Priming Uygulamalarının Tuzlu Ortamlarda Turp (*Raphanus sativus* L.) Tohumlarının Çimlenmesine Etkisi

Özet: Bu araştırma, Bitki Gelişimini Uyaran Rhizosfer Bakterileri (BGURB) ile yapılan bio-priming uygulamalarının değişik tuz (NaCl) konsantrasyonlarında turp (*Raphanus sativus* L.) tohumlarının çimlenmesine etkisini belirlemek amacıyla yürütülmüştür. Çalışmada, bitkisel materyal olarak 3 adet turp çeşidi (*Raphanus sativus* L. cvs. 'Antep', 'Beyaz' ve 'Siyah'), priming ajanı olarak ise 5 adet bakteri izolatı (*Agrobacterium rubi* A 16, *Burkholderia gladii* BA 7, *Pseudomonas putida* BA 8, *Bacillus subtilis* BA 142, *Bacillus megaterium* M 3) kullanılmıştır. Araştırma sonunda, tuzlu ortamlarda bio-priming uygulamasının etkisinin bakteri izolatı ve çeşide göre değiştiği belirlenmiştir. Bio-priming uygulamalarının yüksek tuz konsantrasyonlarında tohum çimlenmesini önemli derecede artırdığı tespit edilmiştir. Bu araştırma sonuçlarına göre, yüksek tuz konsantrasyonlarında turp tohumlarının çimlenme oranının artırılması için Bitki Gelişimini Uyaran Rhizosfer Bakterileri (BGURB) ile yapılan bio-priming uygulamaları önerilebilir.

Anahtar Sözcükler: Bakteri ırkları, bio-priming, çimlenme, turp, tuz stresi

Introduction

Radish is well known as a cool-season vegetable because it does not grow well in hot and dry weather. Although it is generally considered hardy in cold temperatures, its optimum growth temperature is 15-20 °C (Günay, 2005). However, it has been reported that radish is a salt-sensitive crop (Osawa, 1965; Malcolm and Smith, 1971; Shannon and Grieve, 1999). Salinity caused a lack of coordination between cellular expansion and differentiation in radish seedlings; as salinity increased,

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structural and cellular modifications in the form of wall thickening and metabolic aggregates inside parenchyma cells were evident (Scialabba and Melati, 1990). It was also noted that the salinity tolerance levels of radish types differed (Waisel and Breckle, 1987). Salinity slows the germination rate and higher levels of salinity reduce the germination percentage. Low concentrations of salinity affect the germination rate but not the total percentage of seeds germinated. Thus, reported data are dependent upon the time of observation as well as on the germination conditions (Shannon and Grieve, 1999).

In general, salinity affects almost every aspect of the physiology and biochemistry of plants (Cuartero et al., 2005). The need to develop crops with higher salt tolerance has increased greatly within the last decade due to increased salinity problems throughout the world (Sivritepe et al., 2003). There are several priming techniques used for seeds. The most important techniques utilise certain chemicals. Therefore, researchers have begun to use priming including some chemicals such as NaCl (Cayuela et al., 2001; Sivritepe et al., 2003), PEG, KNO_3, and mannitol (Arin and Kiyak, 2003; Nascimento, 2003) priming. Sivritepe and Dourado (1995) reported that priming (osmoconditioning) is a physiological method that improves seed performance and provides faster and synchronised germination. However, bio-priming with different bacteria genera, especially plant growth promoting rhizobacteria (PGPR), has not been evaluated sufficiently with respect to salt tolerance of radish and other vegetables. Thus, the aim of this study was to determine the effect of bio-priming with PGPR on seed germination percentage and rate of different radish cultivars germinated under saline (NaCl) conditions.

Materials and Methods

This study was conducted in growth chambers in 2006, to determine the effect of bio-priming (priming with beneficial micro-organisms that can improve plant performance under stress environments) on the seed germination of radish under saline conditions. Three radish (*Raphanus sativus* L.) cultivars ('Antep', 'Beyaz', and 'Siyah') and 5 bacteria strains (*Agrobacterium rubi* strain A 16, *Burkholderia gladii* strain BA 7, *Pseudomonas putida* strain BA 8, *Bacillus subtilis* strain BA 142, and *Bacillus megaterium* strain M 3) were used in the experiments.

The work included 2 experiments. In the first, we determined saline solution level. With this aim, 5 saline solutions were prepared with distilled water by adding 0, 2, 4, 6, and 8 g l^{-1} of NaCl. The electrical conductivities of these saline concentrations (EC) were 0, 6.3, 9.8, 13.0, and 15.8 dS m^{-1}. At the end of the first experiment, the effects of bio-priming on germination percentage and rate of radish seeds under saline conditions were not statistically significant. Hence, the second experiment was set up with the same plant material and bacteria strains with higher NaCl concentrations.

Bacteria strains were obtained from the culture collection of the Department of Plant Protection, Faculty of Agriculture, Atatürk University, Erzurum, Turkey. They were stored in lauryl broth amended with 30% glycerol at –80 °C prior to use. Bacterial cell suspensions were prepared by first streaking the isolates onto nutrient agar and incubating at 27 °C for 24 h to check for purity, and then transferring single colonies to nutrient agar plates. After 24 h, the bacterial cells were harvested from the plates in sterile distilled water (SDW). The optical density of the suspension was adjusted using a UV-visible spectrophotometer (Shimadzu, Japan, UV 1201, SN A1080) following the method described by Mortensen (1992) to obtain a final density of 10^8 cfu/ml. Seeds of radish cultivars surface-sterilised with 5% sodium hypochloride by shaking for 2 min were soaked in the bacterial suspension amended with 0.2% sucrose to facilitate the adherence of the bacteria to the seeds and incubated at 27 °C in an incubator rotary shaker at 150 rpm for 6 h. After incubation the seeds were air-dried before use.

Germination tests were conducted using 4 replicates of 50 seeds from each cultivar in 9 cm petri dishes. They were placed in a growth chamber where temperature was 20 °C for 10 days (ISTA, 1996). The radish seeds were saturated daily with 5 different NaCl solutions, which were derived from sterile distilled water by adding 0 (control), 5, 10, 15, and 20 g l^{-1} of NaCl in the second experiment. Electrical conductivities (ECs) were 0, 9.44, 18.28, 26.20, and 34.40, dS m^{-1}, respectively. Petri dishes saturated with sterile distilled water and seeds not exposed to bacterial suspensions served as controls.

Visible-radicle protrusion was the criterion of germination (Güvenç, 2002; Güvenç and Kaymak, 2003, 2006). Germinated seeds were recorded and discarded at 24 h intervals over 10 days (ISTA, 1996). The results were expressed as final germination percentage.

Germination rate was calculated according to the following equation: Germination Rate = Germination Percentage in 1st day/1 + ….. + Germination Percentage 10th day/10' of Esechie (1994) and Kaymak et al. (2004).

The experiments were conducted in a randomised complete block design, with each treatment replicated 4 times. The data were subjected to ANOVA and means were compared using Duncan's multiple range test. Arcsine transformation was performed on the percentage data before the statistical analysis. The data were analysed using SPSS 13.0.

Results

The first observation of this study was that increasing NaCl concentration decreased the germination percentage in radish seeds (Table 1). Increasing germination percentage from 0 to 5 g l^{-1} (0 to 9.44 dS m^{-1}) did not change the germination percentage; however, increasing from 10 to 20 g l^{-1} (18.28 to 34.40 dS m^{-1}) decreased the germination percentage significantly more in 'Siyah' than in 'Antep' and 'Beyaz' ($P < 0.01$) (Table 1).

When treatments were compared to each other, it was seen that the effect of bio-priming with PGPR on germination percentage varied with bacteria strains (Table 1). For instance, germination percentage was higher in bio-priming with *Burkholderia gladii* strain BA 7, *Pseudomonas putida* strain BA 8, *Bacillus subtilis* strain BA 142, and *Agrobacterium rubi* strain A 16 than the seed of the control and *Bacillus megaterium* strain M 3 in 'Siyah' when saturated daily with 15 and 20 g l^{-1} NaCl (26.20 and 34.40 dS m^{-1}). Although the seeds of 'Beyaz' and 'Siyah' did not germinate in the control and *Bacillus megaterium* strain M 3, the other bacteria strains increased germination percentage significantly ($P < 0.01$) when saturated with 20 g l^{-1} NaCl (34.40 dS m^{-1}) (Table 1).

Cultivars varied significantly ($P < 0.01$) in their resistance to NaCl. 'Siyah' was more sensitive than 'Antep' and 'Beyaz'. In addition, 'Antep' was the most resistant cultivar in view of the fact that although 'Beyaz' and 'Siyah' seeds did not germinate in the control 'Antep' seeds germinated (33.21%) in the control when saturated with 20 g l^{-1} NaCl (34.40 dS m^{-1}) (Table 1).

The increasing NaCl concentration decreased the seed germination rate in all cultivars (Table 2). Germination rates of 'Antep', 'Beyaz', and 'Siyah' varied from 77.56, 71.94, and 65.86 to 17.29, 13.84, and 5.30, respectively, as NaCl concentration increased from 0 to 20 g l^{-1} (0 to 34.40 dS m^{-1}) (Table 2). The decrease in germination rate associated with NaCl concentration was higher in 'Siyah' than in the other cultivars.

The effect of bio-priming on germination rate changed with NaCl concentration (Table 2). Germination rate in all cultivars was higher in seeds treated with PGPR than in the control. However, the effect of some bacteria strains such as *Bacillus megaterium* strain M 3 on germination rate was similar to the control in 'Beyaz' when saturated with 20 g l^{-1} NaCl (34.40 dS m^{-1}). Similar effects could be seen at 5 g l^{-1} NaCl (9.44 dS m^{-1}) in 'Siyah' primed with all except *Bacillus subtilis* strain BA 142 and at 20 g l^{-1} NaCl (34.40 dS m^{-1}) primed with *Agrobacterium rubi* strain A 16 (Table 2). In general, it was clear that bio-priming with PGPR increased the germination rate according to the control across cultivars; however, the most ineffective bacteria strain was *Bacillus megaterium* strain M 3 (Table 2).

The analysis of variance showed significant differences among the cultivars, NaCl concentrations, and bacteria strains for germination percentage and rate (Table 3). The C × NaCl, C × Bs, NaCl × Bs, and C × NaCl × Bs interactions were significant for germination percentage and rate. Significant C × NaCl, C × Bs, NaCl × Bs, and C × NaCl × Bs interactions for the studied characters demonstrated that the effect of bacteria strains varied considerably in different NaCl concentrations and cultivars. In other words, these interactions also indicated that the performance of cultivars under different NaCl concentrations was affected positively by bacteria strains in terms of germination percentage and rate.

Discussion

The germination of radish seeds was not affected by using the lowest concentration of NaCl (0 and 9.44 dS m^{-1}), which is in accordance with Shannon and Grieve (1999). On the other hand, seeds treated with bacteria strains showed better performance than the control under the 10 g l^{-1} NaCl (18.28 dS m^{-1}). In addition, because of the depressing effect of NaCl (Scialabba and Melati, 1990) seeds saturated with 15 and 20 g l^{-1} NaCl (26.20 and 34.40 dS m^{-1}) had lower germination percentages. In comparison with the control, bacteria strains significantly improved seed germination under high saline conditions. The effect of bio-priming on the

Table 1. The effect of bio-priming on the germination percentage of 3 radish cultivars under increasing NaCl (g l^{-1}) salinity.

Cultivars	NaCl (g l^{-1}) Treatments	0	5	10	15	20	Mean
Antep	Control	88.72 ^{NS}	88.72 ^{NS}	73.40 b**	56.79 c**	33.21 c**	
	B. gladii strain BA7	88.72	88.72	88.72 a	67.40 ab	53.66 a	
	P. putida strain BA8	88.72	88.72	88.72 a	61.15 bc	42.02 b	
	B. subtilis strain BA142	88.72	88.72	88.72 a	63.55 abc	45.87 b	73.77 (A)**
	A. rubi strain A16	88.72	88.72	88.72 a	68.66 a	33.21 c	
	B. megaterium strain M3	88.72	88.72	88.72 a	58.93 c	46.92 ab	
	Mean	88.72 A**	88.72 A	86.17 A	62.75 B	42.48 C	
Beyaz	Control	88.72 ^{NS}	88.72 ^{NS}	83.00 ^{NS}	60.00 b**	1.28 c**	
	B. gladii strain BA7	88.72	88.72	88.72	68.66 b	44.82 b	
	P. putida strain BA8	88.72	88.72	88.72	80.96 a	42.02 b	73.85 (A)
	B. subtilis strain BA142	88.72	88.72	88.72	88.72 a	49.71 ab	
	A. rubi strain A16	88.72	88.72	88.72	67.40 b	54.73 a	
	B. megaterium strain M3	88.72	88.72	88.72	64.70 b	1.28 c	
	Mean	88.72 A**	88.72 A	87.77 A	71.74 B	32.31 C	
Siyah	Control	88.72 ^{NS}	88.72 ^{NS}	83.00 ^{NS}	34.13 b**	1.28 c**	
	B. gladii strain BA7	88.72	88.72	88.72	53.66 a	27.47 a	
	P. putida strain BA8	88.72	88.72	88.72	53.66 a	19.89 b	65.63 (B)
	B. subtilis strain BA142	88.72	88.72	88.72	54.73 a	21.34 b	
	A. rubi strain A16	88.72	88.72	88.72	54.73 a	16.60 b	
	B. megaterium strain M3	88.72	88.72	88.72	39.01 b	1.28 c	
	Mean	88.72 A**	88.72 A	87.77 A	48.32 B	14.64 C	
Mean	Control	88.72	88.72	79.80	50.31	11.92	63.89 D**
	B. gladii strain BA7	88.72	88.72	88.72	63.24	41.98	74.28 AB
	P. putida strain BA8	88.72	88.72	88.72	65.26	34.65	73.21 B
	B. subtilis strain BA142	88.72	88.72	88.72	69.00	38.97	74.83 A
	A. rubi strain A16	88.72	88.72	88.72	63.60	34.85	72.92 B
	B. megaterium strain M3	88.72	88.72	88.72	54.21	16.49	67.37 C
	Mean	88.72 A**	88.72 A	87.23 B	60.94 C	29.81 D	

** Significant at P = 0.01 level

Table 2. The effect of bio-priming on the germination rate of 3 radish cultivars under increasing NaCl (g l^{-1}) salinity.

		NaCl (g l^{-1})					
Cultivars	Treatments	0	5	10	15	20	Mean
	Control	69.30 b**	48.57 b**	45.19 b**	31.72 NS	12.92 c**	
	B. gladii strain BA7	88.72 a	61.16 a	47.13 ab	34.87	22.09 a	
Antep	*P. putida* strain BA8	77.85 ab	61.25 a	57.88 a	31.63	17.58 b	47.61 A**
	B. subtilis strain BA142	79.00 ab	63.62 a	47.44 ab	34.15	19.48 ab	
	A. rubi strain A16	79.58 ab	64.16 a	46.72 ab	33.56	14.18 c	
	B. megaterium strain M3	71.00 b	67.61 a	49.55 ab	33.10	17.46 b	
	Mean	77.56 A**	61.06 B	48.98 C	33.17 D	17.29 E	
	Control	61.77 b**	47.40 bc*	41.07 NS	30.10 b*	1.28 c**	
	B. gladii strain BA7	74.04 ab	50.20 b	43.28	34.06 ab	18.73 b	
Beyaz	*P. putida* strain BA8	70.90 ab	51.38 b	42.59	34.88 a	21.13 a	42.36 B
	B. subtilis strain BA142	70.50 ab	58.37 a	40.88	35.05 a	21.67 a	
	A. rubi strain A16	74.42 ab	52.54 b	42.51	30.32 b	18.91 b	
	B. megaterium strain M3	80.00 a	45.00 c	42.51	34.02 ab	1.28 c	
	Mean	71.94 A**	50.81 B	42.14 C	33.07 D	13.84 E	
	Control	57.14 b**	45.00 b**	38.25 ab*	17.09 c**	1.28 b**	
	B. gladii strain BA7	65.31 ab	45.00 b	42.59 a	24.06 ab	6.86 a	
Siyah	*P. putida* strain BA8	65.77 ab	45.00 b	37.84 ab	24.59 a	7.66 a	35.68 C
	B. subtilis strain BA142	64.05 b	50.62 a	36.66 b	27.09 a	6.97 a	
	A. rubi strain A16	65.28 ab	45.00 b	37.13 ab	25.06 a	7.73 a	
	B. megaterium strain M3	77.61 a	45.00 b	38.24 ab	19.34 bc	1.28 b	
	Mean	65.86 A**	45.94 B	38.45 C	22.87 D	5.30 E	
	Control	62.74	46.99	41.50	26.31	5.16	36.54 C**
	B. gladii strain BA7	76.02	52.12	44.33	31.00	15.89	43.87 A
Mean	*P. putida* strain BA8	71.51	52.54	46.11	30.37	15.46	43.20 AB
	B. subtilis strain BA142	71.18	57.54	41.65	32.10	16.04	43.70 A
	A. rubi strain A16	73.09	53.90	42.12	29.65	13.61	42.47 AB
	B. megaterium strain M3	76.19	52.54	43.43	28.82	6.67	41.53 B
	Mean	71.79 A**	52.60 B	43.19 C	29.71 D	12.14 E	

*Significant at P = 0.05 level, ** Significant at P = 0.01 level

Table 3. Interactions between cultivars, NaCl (g l^{-1}) concentrations, and bacteria strains.

Source of variation	Degrees of freedom	Germination percentage	Germination rate
		F values	
Cultivar (C)	2	337.607**	313.272**
NaCl concentration	4	6113.905**	2686.553**
Bacteria strains (Bs)	5	147.887**	33.303**
C × NaCl	8	172.370**	7.354**
C × Bs	10	19.319**	1.828*
NaCl × Bs	20	46.295**	6.418**
C × NaCl × Bs	40	20.284**	5.417**
Error	180	5.938	10.271
Total	269		

* Significant at P = 0.05 level, ** Significant at P = 0.01 level

germination rate of radish seeds under saline conditions was statistically significant ($P < 0.01$). The significant interactions among Bs, NaCl, and C supported the increasing of germination percentage and rate under saline conditions (Table 3). Shannon and Grieve (1999) reported that salinity slowed the germination rate and at low concentrations the only effect was on germination rate and not total percentage of seeds. The results of our study clearly showed that bio-priming with PGPR improved germination percentage and rate according to the control in spite of the use of high concentrations of NaCl (Tables 1-3). In early experiments, it was reported that NaCl, mannitol, KNO_3, and PEG priming in different vegetable species (Sivritepe et al., 2003; Nascimento, 2003) improved germination percentage and rate under saline conditions. However, although there has been no detailed report about bio-priming with PGPR on seed germination under saline conditions, the positive effect of bio-priming with PGPR for germination percentage and rate can be seen clearly in Tables 1 and 2.

It is well known that growth promotion in response to PGPR inoculation may involve various mechanisms of action. Most PGPR strains may work through multiple mechanisms, which accounts for the observed beneficial effects on plant growth. Many researchers are of the view that a very important mechanism of direct growth promotion may be the production of plant growth regulators by PGPR (Lifshitz et al., 1987; Frankenberger and Arshad, 1995; Arshad and Frankenberger, 1998). For instance, Nelson (2004) noted that PGPR were able to exert a beneficial effect upon plant growth such as increasing the germination rate. Rodriguez et al. (2001) reported that using Azospirillum spp. gave good germination in tomato and pepper seed. Furthermore, Vargas et al. (2001) stated that *Hafnia alvei* strain P3 increased germination by >36.5% compared with the control in lettuce. Similarly, Basavaraju et al. (2002) reported that inoculation of *Azotobacter choococcum* strain C2 significantly increased the germination percentage in radish. Although the above-mentioned studies about the effect of bacteria strains on germination of different vegetable species were conducted under optimum conditions, our study was conducted under saline conditions and may reveal new data about the subject. In conclusion, our study showed that bio-priming with PGPR was more consistent in improving germination percentage and rate of radish (*Raphanus sativus* L.) seeds under high saline conditions.

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