J. Agronomy & Crop Science **193**, 167—176 (2007) doi:10.1111/j.1439-037X.2007.00254.x
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ISSN 0931-2250

Salinity Stress

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Germination of *Salicornia bigelovii* Ecotypes under Stressing Conditions of Temperature and Salinity and Ameliorative Effects of Plant Growth-promoting Bacteria

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With 3 tables

Received May 31, 2006; accepted November 14, 2006

Abstract

Salinity is a major stress condition. *Salicornia bigelovii* is a valuable edible halophyte, considered to be a promising resource for cultivation in arid coastal zones. Its productivity depends on the supplementary provision of nitrogen, for which an option is chemical fertilization. Nevertheless, indiscriminate use of chemical fertilizers contributes to the problem of increased salinity. The inoculation of plant growth-promoting bacteria (PGPB) represents an alternative. Seed ecotypes from four coastal areas [Santa Rosa Chica, Santa Rosa Grande, Santa Cruz and Cerro Prieto (CP), Sonora, México] were collected, in order to inoculate them with two species of PGPB (*Azospirillum halopraeferens* and *Klebsiella pneumoniae*). Two germination tests were carried out to study the effect of salinity, temperature regime (night/day) and inoculation with PGPB on germination (percentage and rate), plant height, root length and biomass produced (fresh and dry matter). In the first test, all four ecotypes were considered, whereas in the second test only the CP ecotype was involved because it was found to be the outstanding ecotype in the previous test. Results showed inhibition of germination when salinity was higher in all ecotypes except CP. The CP ecotype showed a decrease of seed germination with an increase in NaCl concentrations at all temperatures tested. However, when it was inoculated with both PGPB, the germination percentage was influenced.

Key words: *Azospirillum halopraeferens* — ecotypes — *Klebsiella pneumoniae* — plant growth-promoting bacteria — *Salicornia bigelovii* — salinity — seed germination — temperature regime

Introduction

Because Baja California Peninsula and Sonora State are two of the most arid states of Mexico, with 80 mm average annual precipitation, they lack surface water resources (Rueda-Puente et al. 2004). Agricultural activities are dependent on groundwater from wells. Unfortunately, water extraction, in excess of the rate of replenishment, and inappropriate use of fertilizers have caused increases in the salinity of the soil, which during the last two decades has become a major problem in the production of traditional crops in those regions [Secretaria de Agricultura, Ganadería y Desarrollo Rural (SAGAR) 1981, Salim 1989, Shalaby et al.

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1993, Ashraf and Fatima 1994, Flowers and Yao 1995, Sangakkara et al. 1995, Rashid et al. 1999, Abdullah et al. 2001, Flowers 2004]. Production alternatives include development of salt-tolerant crops, and selection and evaluation of salt-tolerant plants that are already adapted to salt flat areas, focusing on the desirable crops (Ashraf and McNeilly 1988, Ashraf and O'Leary 1996, Abdus et al. 1999, Chatrath et al. 2000, Ungar 2000).

Halophytes, particularly *Salicornia bigelovii* (Chenopodiaceae), are promising plant resources in arid coastal zones because of their tolerance to highly saline conditions (Glenn et al. 1991). These potentially important plants might be incorporated into traditional agriculture to help support the agricultural economy of those areas affected by salinity (Glenn et al. 1994, 1999). Elsewhere, actually there is an increasing interest about the remediation effects of plant halotolerants on a range of salt levels in soils (Stove 1997). In Baja California and Sonora State of the Mexican Peninsula, *S. bigelovii* has a wide distribution along the coasts (Troyo-Diéguez et al. 1994, Glenn et al. 1995, Bashan et al. 2000). This plant was identified from among many halophyte species tested for possible domestication because of its promise as a new oilseed resource (Jefferies et al. 1981, Rey et al. 1990, Glenn et al. 1991, 1999). It is a facultative halophyte that possesses a high potential as an agro-industrial commodity (Glenn et al. 1991). It is a leafless, annual halophyte with green, jointed and succulent stems that form terminal fruit-bearing spikes, in which seeds are borne (Glenn et al. 1991, Gallawa 1996). However, the productivity of this halophyte is limited by a lack of available nitrogen (Jefferiers 1977, Loveland and Ungar 1983). This condition affects its growth and reproductive potential (Jefferiers 1977, Loveland and Ungar 1983). Traditionally, farmers apply synthetic fertilizers to compensate for soil nitrogen deficiency. However, indiscriminate use of these fertilizers might increase salinity and severely damage soil microfloral structure and composition (Kapulnik et al. 1981, Banwari and Rao 1990, Akhavan et al. 1991, Nahid and Gomah 1991).

Few studies on bacterial diversity within the rhizosphere of salt marsh plants have been published, with the most studied plant being *Spartina alterniflora* (Lovell et al. 2000). Recent molecular biology studies on *Spartina* rhizosphere flora suggest a diazotrophic assembly and an apparently large number of unclassified micro-organisms (Lovell et al. 2000, Nielsen et al. 2001). In relation to *Salicornia* spp., studies are limited to the mycoflora of *S. europaea* (Ito et al. 1999) and to a previous study carried out by Rueda-Puente et al. (2003). It is important to increase the number of known salt-tolerant, nitrogen-fixing bacteria (Hamdi 1999, Whipps 2000) as potential biofertilizer resources for saline areas. Given the natural biodiversity of Mexico, a similar relevance arises from this study related to the assessment of different responses among the number of ecotypes widespread along the north-west Mexican coastline.

On the other hand, it has been proved that several stress factors (water, temperature, light and salinity), which regulate seed germination, interact within the soil interface (Ungar 1995, 2000). Other variables may co-act with the seasonal variation of temperature to determine the temporal pattern of germination (Khan and Gul 1998). The osmotic and water potential of soils narrow the range of temperature that is effective for the germination of seeds (Hegarty 1978). *Salicornia* spp. are highly salt-tolerant annuals but vary in their response to salinity (Ungar 1977, Philipupiya and Ungar 1984). *S. europaea* showed a 10% rate of germination at 5% (860 mM) NaCl (Ungar 1967a), *S. bigelovii* had a 63% germination at 8% (1376 mM) NaCl (Rivers and Weber 1971) and, according to Chapman (1974), *S. stricta* was reported to have 10% germination at 10% (1720 mM) NaCl. Salinity may not be the only critical environmental factor for the germination of annual halophytes (Khan and Ungar 1998a). Interactions between salinity and temperature occur, determining the optimal conditions for seed germination of halophytes (Ungar 1962, Rivers and Weber 1971, Philipupiya and Ungar 1984, Badger and Ungar 1989, Keiffer and Ungar 1995, Khan and Ungar 1996, 1998b). Philipupiya and Ungar (1984) found that *S. europaea* had optimal germination (43%) at 5–15 °C in 860 mM NaCl, when compared with a value $< 2\%$ at other temperatures. Halophyte seeds have the ability to maintain viability for extended periods of time during exposure to hypersaline conditions and then to start germination when salinity stress is reduced (Khan et al. 1976, Woodell 1985, Keiffer and Ungar 1995, Khan and Ungar 1996, 1997, 1999, Khan and Gul 1998, Gul and Weber 1999). However, halophytes differ in their capacity to grow in varying saline stress conditions (Woodell 1985). This variation in recovery responses could be due to the difference in the temperature regime to which seeds are exposed (Khan et al. 1976, Khan

and Ungar 1996, 1998a,b). This study describes the effect of the inoculation of two plant growth-promoting bacteria (PGPB) (*Klebsiella pneumoniae* and *Azospirillum halopraeferens*), different levels of salinity and alternating temperature regimes on the seed germination of four non-domesticated ecotypes of *S. bigelovii*.

Materials and Methods

Evaluation of inoculants and NaCl on germination and seedling growth of four *S. bigelovii* ecotypes

Seeds of four *S. bigelovii* ecotypes were collected during autumn 2003 and 2004, from salt flats of Sonora, northwest of Mexico. The collected ecotypes were Santa Rosa Chica (SRCH), Santa Rosa Grande (SRG), Santa Cruz (SC) and Cerro Prieto (CP). Plants were sifted to separate mature seeds. The remains were cleaned and plant material was dried to select the largest seeds with uniform colour and with no visible damage. Seeds of each ecotype were disinfected by immersion in sodium hypochlorite (3% active chlorine) for 30 s; they were then washed three times with sterilized, distilled water. Seeds were inoculated with bacterial treatments according to Carrillo et al. (1998), at a concentration of 10^8 cells ml^{-1}. Germination tests were performed in sterilized Petri dishes, each with a cotton layer substrate (150 × 15 mm) covering the bottom of the dish. Dishes were moistened with uniform amounts of NaCl solution (0, 0.25 or 0.5 M). Germination tests were performed inside a growth chamber at 27 ± 0.5 °C and 35 ± 1% relative humidity (RH), with continuous white light (Environmental Chamber, Biotronette® Mark III, Melrose Park, IL, USA); 20 ml of the appropriate solution was added every 4 days to each dish. Seeds were considered germinated when the radicle was at least 2 mm long. The number of germinated seeds was recorded daily (germination rate), and the final percentage of germination was measured after 27 days. Germination rate was calculated using the formula described by Maguire (1962):

$$M = \frac{n_1}{t_1} + \frac{n_2}{t_2} + \cdots + \frac{n_{27}}{t_{27}}$$

where n_1, n_2,…,n_{27} are the numbers of germinated seeds at times t_1, t_2,…,t_{27} in days. The hierarchic experiment of a randomized design included three factors (ecotype, inoculation and salinity), with five replicates of 50 seeds each treatment. The first factor (ecotype) had four levels (SRCH, SRG, SC and CP); the second factor (inoculants) had three levels (no inoculation, inoculation with the bacterium *K. pneumoniae*, and inoculation with *A. halopraeferens*). The third factor had three concentrations of NaCl (0, 0.25 and 0.5 M). The combination of the three factors studied with their corresponding levels yielded 36 treatments. The data for percentage germination were analysed after applying arcsine transformation (Sokal and James 1988), with three-way analyses of variance (ANOVA). Germination rates, which were sums of germinations per day, were not transformed before analysis. Least significant differences among mean values of treatments were separated by Duncan's multiple range test at $P = 0.05$. Data were analysed using the Statistical Analysis System (SAS 2001). Thirty-five seedlings of each 50-individual unit from all treatments were chosen randomly; seedling growth was measured by recording dry and fresh weights on the 27th day. Root length and height were measured with a digital calliper (General No. 143; General Tools Manufacturing Co., Inc., New York, USA). Dry weight was determined after drying each organ in a forced air dryer at 110 °C for 36 h.

Quantification of bacteria adhering to the root system of *S. bigelovii* was carried out at the conclusion of the study, 27 days after sowing. Seven plants of each treatment (168 seedlings) were washed with sterile, distilled water and introduced for 1 min into Eppendorf tubes with sterile water. Tubes were agitated for 1 min to detach bacteria from the roots. Three samples of 100 µl each were taken from the bacterial solution of each tube and sowed by dispersion on N-free media in Petri dishes, which were incubated for 24 h at 30 °C for colony-forming unit (CFU) measurement.

Dry and fresh weights, root length, root height, and CFUs were analysed by three-way ANOVA; then an F-test was applied to determine statistically significant differences (Snedecor 1956). Least significant differences (LSD) between mean values of treatments were separated by Duncan's multiple range test at $P = 0.05$. All statistical tests were performed with SAS (SAS Institute 2001).

Evaluation of inoculants and NaCl on germination and seedling growth of the superior ecotype (CP) of *S. bigelovii*

After the statistical evaluation of the first experiment, the CP ecotype was selected as the most outstanding, and then a new experiment was carried out with this ecotype: seeds were separated from the inflorescence, and then stored at 4 °C. Seed germination was assayed after sterilization with four consecutive immersions for 1 min in 75% ethanol, 10 min in 65% Clorox® (final concentration – 3.41% sodium hypochlorite) and 30 s in 75% ethanol. Seeds were washed three times with sterilized, distilled water, and then inoculated with bacterial treatments, and exposed to NaCl solutions as in the first experiment. Seeds were considered to be germinated with the emergence of the radicle. To determine the effect of temperature on germination of the CP ecotype, alternating temperature regimes of (night–day) 5–15, 10–20, 15–25, 20–30 and 25–35 °C were used based on a 24-h cycle of 12 h (growth chambers with Sylvania cool white fluorescent lamps (Sylvania® Cool White, Danuers, MA, USA), 25 µmol m^{-2} s^{-1}, 400–750 nm). The number of germinated seeds was recorded daily (germination rate), and the final percentage of germination was determined after 27 days. Germination rate was calculated using the Maguire (1962) formula mentioned above.

The hierarchic experiment of randomized design related three factors (inoculation, temperature regime and salinity), with five replicates of 50 seeds. At this second experiment, the first experimental factor (inoculants) had three levels:

without inoculants, with inoculant *K. pneumoniae*, and *A. halopraeferens*. The second factor had three concentrations of NaCl (0, 0.25 and 0.5 M), and the third factor had five temperature regimes (5–15, 10–20, 15–25, 20–30 and 25–35 °C, based on a 24-h cycle of 12 h). The combination of these three factors yielded 45 treatments. The data for percentage germination, germination rates, root length, height, and dry and fresh weights were registered and evaluated as previously mentioned in the first experiment. Data were analysed using the SAS (SAS Institute 2001).

Results

Evaluation of germination and seedling growth of four *S. bigelovii* ecotypes

After inoculation with PGPB during the germination stage, results showed significant differences among treatments (Table 1). When salinity was higher, inhibition of germination was observed in all ecotypes except CP, which showed the highest values in both germination percentage and rate, under all applied salinities, with and even without PGPB. Seeds of SRCH, SRG and SC ecotypes with both PGPB germinated 10% more at 0 M NaCl than 0.25 and 0.5 M NaCl. *K. pneumoniae* and *A. halopraeferens* were unaffected below 0.25 M and 0.5 NaCl, respectively, when assayed on CP. According to the germination rate results, the CP ecotype showed a total germination 10 and 12 days after sowing at 0, 0.25 and 0.5 M NaCl. However, CP inoculated with both PGPB, showed the highest values under 0, 0.25 and 0.5 M NaCl.

Both PGPB influenced plant growth of all ecotypes, during the early seedling stage. PGPB influenced plant growth, with significant differences among treatments recorded for plant height, root length, and fresh and dry weight (Table 1).

Evaluation of PGPB inoculants, NaCl levels, and temperature regimes on germination and seedling growth of CP ecotype

Optimal germination of the CP ecotype in 12/12 h light/dark conditions occurred in 0 M NaCl at temperatures 20–30 and 25–35 °C (Table 2), when it was inoculated with both PGPB. Seed germination was decreased or delayed with an increase in NaCl concentrations at all temperatures. A delay in germination was more evident at 5–15 °C than at the other temperature regimes, with or without inoculation of both PGPB (Table 2). Change in temperature regimes significantly affected the germination of the CP ecotype seeds. At the highest temperature regime (25–35 °C), seeds in non-saline conditions reached about 94% germination rate compared with a value $< 10\%$ of germination at the lowest temperature regime, 5–15 °C (Table 2). However, when it was inoculated with both PGPB, the germination percentage was high, reaching 97% and 99% for *A. halopraeferens* and *K. pneumoniae*, respectively. The calculated germination rate decreased when the salinity increased. The highest rate of germination was obtained at the 25–35 °C temperature regime and the lowest value at the 5–15 °C regime. Different inoculants, temperatures and several concentrations of salinity individually, and their interaction, significantly affected the rate of germination of the CP ecotype seeds (Table 3).

Discussion and Conclusions

Salicornia bigelovii includes different ecotypes which grow along the most saline coastlines of the north-west of México, producing seeds during September and October. Seeds germinated during February to April when the seawater salinity concentration is decreased by the rains commonly occurring from December to January. Our laboratory studies indicated that of the four ecotypes studied, CP was the most outstanding, showing the highest germination under saline and non-saline conditions. However, PGPB influenced germination rate and seedling growth, plant height, length of the root system, and fresh and dry weight with significant differences between treatments (Tables 1 and 2). Our results related to plant–bacterium associations agree with the findings of Baldani and Dobereiner (1980), and Arsac et al. (1990), who indicated that results depend on strain–host plant specificity. However, the results disagree with those of Okon and Labandera (1994), who stated that the effect is not strain-dependent among different plant species.

Low temperatures delayed germination but with an increase in temperature there was a substantial improvement in germination. CP was capable of germinating under 0.5 M NaCl at 15 °C constant temperature, hence showing to be more tolerant to salinity. Seed germination was more successful at higher alternating temperature regimes when we used the PGPB. Our results indicated the ability of CP to germinate in high salinity, in agreement with the results of Khan et al. (1976), and Khan and Gul (1998). Ungar (1967b), Rivers and Weber

Table 1: Effects of *A. halopraeferens* and *K. pneumoniae* on mean values for germination (%), root length, plant height, fresh and dry weights of seedlings of *S. bigelovii* ecotypes (Santa Rosa Chica, Santa Rosa Grande, Santa Cruz, Cerro Prieto) under three concentrations of NaCl

Ecotype	Bacterial inoculant[1]	NaCl concentration (M)	Germination (%)	Plant height (cm)	Root length (cm)	Fresh weight (mg)	Dry weight (mg)
SRCH	Control	0	40.57 f	17.65 ef	24.05 d	0.0242 g	0.0066 a
SRG	Control	0	31.83 ij	10.85 h	15.60 f	0.0052 ll	0.0038 d
SC	Control	0	29.72 ij	08.35 hi	13.50 g	0.0130 j	0.0032 de
CP	Control	0	90.00 ab	48.10 a	61.55 a	0.0310 e	0.0034 d
SRCH	*K. pneumoniae*	0	39.85 fj	16.70 f	23.70 d	0.0274 fg	0.0032 de
SRG	*K. pneumoniae*	0	37.94 g	13.30 g	20.15 e	0.0104 l	0.0022 f
SC	*K. pneumoniae*	0	36.67 g	12.40 g	20.05 e	0.0266 g	0.0020 f
CP	*K. pneumoniae*	0	92.00 a	49.25 a	62.50 a	0.0200 j	0.0022 f
SRCH	*A. halopraeferens*	0	55.55 d	25.65 c	37.10 b	0.0226 i	0.0036 d
SRG	*A. halopraeferens*	0	49.80 e	17.00 ef	28.05 c	0.0114 k	0.0030 de
SC	*A. halopraeferens*	0	33.73 h	09.80 h	16.00 f	0.0056 ll	0.0010 g
CP	*A. halopraeferens*	0	93.00 a	48.70 a	60.70 a	0.0246 g	0.0028 e
SRCH	Control	0.25	29.64 ij	09.75 h	13.70 g	0.0296 f	0.0026 e
SRG	Control	0.25	23.77 l	06.10 j	09.00 hi	0.0290 f	0.0034 d
SC	Control	0.25	19.82 ll	03.60 l	05.75 j	0.0126 j	0.0016 f
CP	Control	0.25	90.00ab	46.85 b	61.90 a	0.0470 c	0.0044 c
SRCH	*K. pneumoniae*	0.25	43.80 f	19.50 l	27.15 c	0.0384 d	0.0056 b
SRG	*K. pneumoniae*	0.25	25.50 k	06.35 j	10.30 h	0.0264 g	0.0028 e
SC	*K. pneumoniae*	0.25	30.35 ij	07.15 j	11.15 h	0.0216 i	0.0024 ef
CP	*K. pneumoniae*	0.25	93.00 a	46.35 b	62.05 a	0.0632 a	0.0052 b
SRCH	*A. halopraeferens*	0.25	56.61 d	25.45 c	36.30 b	0.0288 f	0.0042 c
SRG	*A. halopraeferens*	0.25	21.10 l	04.00 l	06.50 j	0.0108 l	0.0020 f
SC	*A. halopraeferens*	0.25	22.06 l	04.75 l	07.65 ij	0.0120 j	0.0024 ef
CP	*A. halopraeferens*	0.25	93.00 a	46.25 b	62.25 a	0.0562 b	0.0064 a
SRCH	Control	0.5	17.58 m	04.10 l	05.55 j	0.0256 g	0.0036 d
SRG	Control	0.5	14.09 n	01.85 m	02.95 k	0.0048 ll	0.0022 f
SC	Control	0.5	16.16 mn	02.30 ll	03.95 k	0.0136 j	0.0030 de
CP	Control	0.5	87.26 c	45.00 b	60.70 a	0.0594 b	0.0056 b
SRCH	*K. pneumoniae*	0.5	48.72 e	21.55 d	30.60bc	0.0216 i	0.0042 c
SRG	*K. pneumoniae*	0.5	34.55 h	12.35 g	18.35 d	0.0242 g	0.0048 bc
SC	*K. pneumoniae*	0.5	34.24 h	10.00 h	16.70 f	0.0024 m	0.0010 g
CP	*K. pneumoniae*	0.5	91.00 ab	48.40 a	62.25 a	0.0256 g	0.0044 c
SRCH	*A. halopraeferens*	0.5	41.76 f	16.30 f	24.10 d	0.0386 d	0.0050 b
SRG	*A. halopraeferens*	0.5	25.70 k	06.45 j	10.00 h	0.0232 h	0.0030 de
SC	*A. halopraeferens*	0.5	25.89 k	05.60 k	10.05 h	0.0128 j	0.0022 f
CP	*A. halopraeferens*	0.5	91.00 ab	46.40 b	62.15 a	0.0390 d	0.0060 a

SRCH, Santa Rosa Chica; SRG, Santa Rosa Grande; SC, Santa Cruz; CP, Cerro Prieto; control, without inoculants.

Mean values followed by the same letter are not significantly different at $P = 0.05$.

Comparisons were made within columns using Duncan's multiple range test. Values are mean of five replicates.

[1]Bacterium (1×10^8 CFU ml^{-1}).

(1971), Chapman (1974), Khan (1991), and Khan and Gul (1998) reported maximum salinity tolerance ranges from 200 to 1720 mM NaCl in halophytes such as *S. bigelovii*, *S. europaea*, *S. stricta*, *Cressa cretica* and *Suaeda moquinii*. These reports indicate that CP ecotype could be classified as the most salt tolerant during germination.

The seeds of the CP ecotype were able to germinate in high salinity (0.5 M NaCl) under laboratory conditions and may be included among those halophytes which have the ability to withstand high salinity stress during germination. On the other hand, when CP seeds were inoculated with PGPB, *K. pneumoniae* and *A. halopraeferens*,

Table 2: Effects of *A. halopraeferens* and *K. pneumoniae* on mean values for germination (%), root length, plant height, fresh and dry weights of seedlings of *S. bigelovii* ecotype CP under three concentrations of NaCl at temperatures of 5–15, 10–20, 15–25, 20.30 and 25–35 °C

Ecotype	Bacterial inoculant[1]	Temperature regimens (°C)	NaCl concentration (M)	Germination (%)	Plant height (cm)	Root length (cm)	Fresh weight (mg)	Dry weight (mg)
CP	Control	5/15	0	82.2 g	36.10 e	49.35 f	0.0122 fg	0.0024 bc
CP	*K. pneumoniae*	5/15	0	83.2 g	37.25 d	51.30 e	0.0146 ef	0.0014 c
CP	*A. halopraeferens*	5/15	0	85.0 f	36.70 e	49.50 f	0.0178 cd	0.0018 c
CP	Control	5/15	0.25	81.2 g	34.85 ef	49.70 f	0.0380 bc	0.0034 b
CP	*K. pneumoniae*	5/15	0.25	82.4 g	34.35 ef	50.85 e	0.0542 a	0.0042 ab
CP	*A. halopraeferens*	5/15	0.25	85.0 f	34.25 ef	51.05 e	0.0472 ab	0.0054 a
CP	Control	5/15	0.5	76.8 i	33.00 f	48.50 f	0.0504 a	0.0046 ab
CP	*K. pneumoniae*	5/15	0.5	77.2 i	36.40 e	51.05 e	0.0166 d	0.0034 b
CP	*A. halopraeferens*	5/15	0.5	78.8 hi	34.40 ef	50.95 e	0.0300 bc	0.0050 a
CP	Control	10/20	0	84.2 f	37.10 d	49.35 f	0.0220 c	0.0024 bc
CP	*K. pneumoniae*	10/20	0	85.2 f	37.10 d	53.77 d	0.0128 fg	0.0014 c
CP	*A. halopraeferens*	10/20	0	86.6 e	35.90 ef	51.97 e	0.0184 cd	0.0018 c
CP	Control	10/20	0.25	83.2 g	34.40 ef	49.70 f	0.0380 bc	0.0034 b
CP	*K. pneumoniae*	10/20	0.25	84.4 f	34.55 ef	53.32 d	0.0542 a	0.0042 ab
CP	*A. halopraeferens*	10/20	0.25	87.0 de	33.05 f	53.52 d	0.0472 ab	0.0054 a
CP	Control	10/20	0.5	78.8 hi	36.65 e	48.50 f	0.0504 a	0.0046 ab
CP	*K. pneumoniae*	10/20	0.5	79.2 h	35.50 ef	53.52 d	0.0166 d	0.0034 b
CP	*A. halopraeferens*	10/20	0.5	80.8 h	38.17 d	53.42 d	0.0300 bc	0.0050 a
CP	Control	15/25	0	86.2 e	47.50 b	49.35 f	0.0220 c	0.0024 bc
CP	*K. pneumoniae*	15/25	0	87.2 de	48.65 ab	58.83 c	0.0130 f	0.0014 c
CP	*A. halopraeferens*	15/25	0	88.6 f	48.10 ab	57.03 c	0.0184 cd	0.0018 c
CP	Control	15/25	0.25	85.2 e	46.25 bc	49.70 f	0.0380 bc	0.0034 b
CP	*K. pneumoniae*	15/25	0.25	86.4 d	45.75 c	58.38 c	0.0542 a	0.0042 ab
CP	*A. halopraeferens*	15/25	0.25	89.0 h	45.65 c	58.58 c	0.0472 ab	0.0054 a
CP	Control	15/25	0.5	80.8 g	44.40 c	48.50 f	0.0504 a	0.0046 ab
CP	*K. pneumoniae*	15/25	0.5	81.2 g	47.80 b	58.58 c	0.0166 d	0.0034 b
CP	*A. halopraeferens*	15/25	0.5	82.8 c	45.80 c	58.48 c	0.0300 bc	0.0050 a
CP	Control	20/30	0	90.2 c	48.50 ab	49.35 f	0.0220 c	0.0024 bc
CP	*K. pneumoniae*	20/30	0	91.2 c	49.65 a	63.80 b	0.0146 ef	0.0014 c
CP	*A. halopraeferens*	20/30	0	92.4 d	49.10 a	62.00 b	0.0156 e	0.0018 c
CP	Control	20/30	0.25	89.2 c	47.25 b	49.70 f	0.0380 bc	0.0034 b
CP	*K. pneumoniae*	20/30	0.25	90.4 bc	46.75 bc	63.35 b	0.0542 a	0.0042 ab
CP	*A. halopraeferens*	20/30	0.25	93.0 f	46.65 bc	63.55 b	0.0472 ab	0.0054 a
CP	Control	20/30	0.5	84.8 f	45.40 c	48.50 f	0.0504 a	0.0046 ab
CP	*K. pneumoniae*	20/30	0.5	85.2 e	48.80 ab	63.55 b	0.0166 d	0.0034 b
CP	*A. halopraeferens*	20/30	0.5	86.8 b	46.80 bc	63.45 b	0.0300 bc	0.0050 a
CP	Control	25/35	0	94.2 ab	49.00 a	49.35 f	0.0220 c	0.0024 bc
CP	*K. pneumoniae*	25/35	0	97.0 a	50.15 a	66.80 a	0.0130 f	0.0014 c
CP	*A. halopraeferens*	25/35	0	99.0 bc	49.60 a	65.00 a	0.0192 cd	0.0018 c
CP	Control	25/35	0.25	93.4 b	47.75 b	49.70 f	0.0380 bc	0.0034 b
CP	*K. pneumoniae*	25/35	0.25	95.8 a	47.25 b	66.35 a	0.0542 a	0.0042 ab
CP	*A. halopraeferens*	25/35	0.25	99.2 c	47.15 b	66.55 a	0.0472 ab	0.0054 a
CP	Control	25/35	0.5	90.6 c	45.90 c	48.50 f	0.0504 a	0.0046 ab
CP	*K. pneumoniae*	25/35	0.5	91.6 b	49.30 a	66.55 a	0.0166 d	0.0034 b
CP	*A. halopraeferens*	25/35	0.5	94.4 a	47.30 b	66.45 a	0.0300 bc	0.0050 a

CP, Cerro Prieto ecotype.
Mean values followed by the same letter are not significantly different at $P = 0.05$.
Comparisons were made within columns using Duncan's multiple range test. Values are mean of five replicates.
[1]Bacterium (1×10^8 CFU ml^{-1}).

Table 3: Results of two-way ANOVA carried out on factors of salinity, thermoperiods, bacterium and their interactions

Independent variable	Salinity	Thermoperiod	Bacterium	Salinity × thermoperiod	Bacterium × salinity	Bacterium × thermoperiod	Bacterium × thermoperiod × salinity
Percent germination	0.000000	0.000000	0.000000	0.732949	0.042608	0.267727	1.000000
High plant	0.000000	0.000000	0.019353	0.307684	0.028302	0.906244	0.861132
Root length	0.181597	0.000000	0.000000	1.000000	0.001779	0.000000	1.000000
Fresch weigh	0.000000	0.962887	0.000000	0.996498	0.000000	0.981934	0.998899
Dry weight	0.000000	1.000000	0.000001	1.000000	0.000000	1.000000	1.000000

Numbers are F-values, $P = 0.05$.

germination, plant height, root length, fresh and dry weight variables, were found to be increased.

Seed germination of CP was affected by change in temperature. CP germinated earlier at higher temperatures where maximum germination occurred under non-saline and saline conditions, but the lower temperatures delayed germination. These results are similar to the findings reported for the Great Basin desert species, cited by Khan and Weber (1986), to results observed for *S. pacifica* var. *utahensis* and *Allenrolfea occidentalis* (Gul and Weber 1999), and to the findings cited for *Triglochin maritima* (Khan and Ungar 1999). However, halophytes from subtropical maritime deserts of Pakistan, e.g. *Haloxylon recurvum* (Khan and Ungar 1996), *Zygophyllum simplex* (Khan and Ungar 1997) and *Arthrocnemum macrostachyum* (Khan and Gul 1998) had the highest germination at lower temperatures.

All the ecotypes studied and especially CP were benefited by the inoculation of *A. halopraeferens* and *K. pneumoniae*. Similar results were obtained for other plants and beneficial micro-organisms (Rozema 1975, Puente et al. 1999, Goodfriend et al. 2000). Although assays were carried out with other plants and other beneficial micro-organisms, some inhibitive effects on germination were observed (Díaz et al. 2001). However, other studies reported positive effects from this kind of micro-organisms (Arsac et al. 1990, Puente and Bashan 1993), which are also in concordance with our results. The positive effects of bacteria on the experimental CP ecotype possibly suggested the production of plant growth-promoting substances, which are often reported to be responsible for enhancement of plant growth (Arsac et al. 1990, Haahtela et al. 1990, Turyanitsa et al. 1995, El-khawas and Adachi 1999).

This study is the first step to obtain an ideal ecotype of *S. bigelovii*, which grows in the northwest of México. This study also promotes this type of micro-organisms as an efficient and reliable biological product for growth enhancement of halophyte plants. Furthermore, studies of the association of *A. halopraeferens* and *K. pneumoniae* with the *S. bigelovii*–CP ecotype are recommended to determine the extent to which these observations can be reproduced under field conditions.

Acknowledgements

We thank the Consejo Nacional de Ciencia y Tecnología (CONACyT), specifically the program 'Consolidación Institucional Convocatoria 2004-CONACYT' of 'Apoyo Complementario para la Consolidación Institucional de Grupos de Investigación' (Repatriación, Retención, Descentralización y Profesores Visitantes; code 040147). Thanks are due to the Universidad de Sonora. This study is part of the mega-project named: '*Salicornia bigelovii* with the Interaction of Plant Growth-promoting Bacteria: As a Model of Interaction Plant–Microorganisms for Arid Zones', Code: CD-DCACA-0904-14. Thanks are also due to Dr Carlos Mota Urbina, because he was the first person to introduce us to halophyte studies.

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