22 Effect by Plant Growth Promoting Bacteria (*Azospirillum halopraeferens* and *Klebsiella pneumoniae*) on Lipid Value in Seed of the Halophyte *Salicornia bigelovii* Torr.

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Abstract

Halophytes species appears on great scale as an alternative for the essential fatty acid production in human nutrition, for that reason, the fatty acid composition receives special importance. *Salicornia bigelovii* is a halophyte that developed in arid and coastal zones like Sonora State and the peninsula of South Baja California, Mexico. *Salicornia* could be an alternate species in the edible oil production. The similar fixation of N_2 by bacteria associated with roots of *Salicornia bigelovii* and halophyte are an important nitrogen source available in coastal ecosystems. The inoculation of *Klebsiellapneumoniae* and *Azospirillumhalopraeferens* was evaluated

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during the vegetative development of *Salicornia bigelovii* under field conditions in two ecotypes (Cerro Prieto = CP and Santa Rosa = SR), having affected positively variable as weight, yields of production and biochemical characteristics and contained total of ashes, protein and total lipids in seed produced. Our results suggest that both ecotypes of *S. bigelovii*, under the conditions field used, can be improved with the application of *K. pneumoniae* and *A. halopraeferens*, showing a potential utility for agricultural producers of coastal semi-arid zones.

1 Introduction

Sustainable agricultural productivity in arid regions includes selection, evaluation and development of salt-tolerant plants, focusing on the desirable crops adapted to dry saline and desert areas (Chatrath et al. 2000; Ungar 2000). Increasing interest on the range of salt levels in soils to enhance the salt-tolerant crops (Stove 1997). Halophytes, *Salicornia bigelovii* particularly (Chenopodiaceae), promise to be a resource in arid coastal areas because they tolerate highly saline conditions (Glenn et al. 1991). *Salicornia bigelovii* was identified among many halophytic species tested for possible domestication, because it is considered as a new promising economical resource of oilseed production (Glenn et al. 1991). *Salicornia bigelovii* is a leafless, jointed and succulent stems that form terminal fruit-bearing spikes, in which seeds are borne (Gallawa 1996). The produced seeds by *S. bigelovii* has a high proportion of unsaturated fatty acid where linoleic acid proportion is 50 %. These important plants could be incorporated into traditional agriculture to help the agricultural economy (Glenn et al. 1995). The state of Sonora and Baja California Sur are two of the driest states of Mexico, with 60 mm average annual rainfall, in addition to lack of water resources. Agricultural activities are dependent on wells. Unfortunately, the extraction of too much water, and inappropriate use of fertilizers has promoted agricultural soil salinization (SARH 1981). Production alternatives include the development of salt tolerant crops and selection and evaluation of salt tolerant plants (Ungar 2000). In the states of Sonora and Baja California Sur, *S. bigelovii* is widely distributed along the coasts. It is possible that this halophyte has commercial value. However, in both states, their productivity is limited by the lack of available nitrogen, a condition that affects the growth and reproduction (Jefferies 1981). The nitrogen-fixing bacteria are endemic ecological-organic alternative for the delivery of this nutrient. Bacteria capable of promoting growth and development in plants; one of the most studied are *Azospirillum halopraeferens,* having halotolerant properties and promote positive outcomes in crop production (Reinhold et al. 1987; Bashan and Holguin 1997). The use of endemic bacteria associated with rhizosphere of plants such as *S. bigelovii* is needed to evaluate its effectiveness as a resource in the vegetative development (Hamdi 1999). For above mention the goal of this investigation was to evaluate the effect of inoculation of *Klebsiella pneumonia* and *Azospirillum halopraeferens* in two ecotypes of *Salicornia bigelovii* (Cerro Prieto = CP and SR = Santa Rosa), recording performance and physiological variables and production of fatty acids in seed, in order to be able to propose (*S. bigelovii* with the interaction of Plant Growth Promoting Bacteria) as an alternative agricultural production in the agricultural sector of dry arid zones.

2 Materials and Methods

2.1 Evaluation of *Salicornia bigelovii* Inoculated with *Azospirillum halopraeferens* and *Klebsiellap neumoniae* Under Field Conditions Establishment

The treatments evaluated in this study were two ecotypes of *Salicornia bigelovii* seeds (ecotype Cerro Prieto = CP and SR = Santa Rosa), from two areas with pure populations of Salicornia. Area 1 is located between Lat coordinates. 31°21′18.82″ N and Long. 113°35′29.41″ W, area 2 is located between Lat. 29°9′26.62″ N and Long. 112°14′40.66″ W, respectively. In both ecotypes the effect of inoculation of the root zone with nitrogen-fixing bacteria was assessed: *Klebsiella pneumoniae* and *Azospirillum halopraeferens* under field conditions. Prior to planting, wild seed was cleaned manually in order to separate the mature seed by wiping dry vegetative material to select larger seeds, uniform color without apparent damage. Subsequently both ecotypes underwent disinfection, performing an immersion in sodium hypochlorite for 30 s at a concentration of 3 % (v/v). They were then washed three times with sterile distilled water. The seeds were then dried on sterile paper towel.

2.2 Inoculation of *S. bigelovii* Seed (Ecotype Cerro Prieto = CP and SR = Santa Rosa) with the Bacteria *Klebsiellap neumoniae* and *Azospirillum halopraeferens*

Bacteria *K. pneumoniae* and *A. haloprefaerens* were independently developed in liquid medium OAB with 0.5 M NaCl. Incubation conditions were continuously stirred (120 rpm) at a temperature of 30 °C, based on recommended by Reinhold et al. (1987). Between 14 and 16 h elapsed (log phase), the culture concentration of each bacteria, according to the following procedure was determined: 1 mL of the culture was poured into a cell for spectrophotometer (master spectrum FISHER SCIENTIFIC 415) taking the reading absorbance at a wavelength of 540 nm against a control medium with liquid OAB without 0.5 M NaCl bacteria. The bacterial suspension was diluted to 1.00 absorbance unit, corresponding to a concentration of 1×10^9 CFU/mL. To each of the crops 0.5 g (590 seeds) were added to each of the ecotypes a Kitazato 50 mL flask were evacuated to 600 mmHg for 5 min in the expression are given by (Carrillo et al. 1998). After this time, the seeds inoculated vacuum infiltration or *Klebsiella pneumoniae* or *Azospirillum halopraeferens* were deposited on 1 m^2 germinating plates containing 7 cm sandy substrate. Then the seed was covered with a thin layer (3 mm ± 1) substrate type peat -moss (Sunshine, Sun Gro Horticulture Canada, Ltd.). The germinating plates were placed in the open (open sky). For 1 month was carried out irrigation with potable water saturation daily. A month after obtaining the corresponding germinated seedlings to each of the treatments were transplanted in field conditions in micro of an area of 1 m^2, in order to avoid mechanical damage to the root system and a possible entry of soil pathogens. Plants (60) were established in each watershed at a distance of 10 cm between plants and 15 cm between rows.

2.3 Inoculation of *Klebsiella pneumoniae* and *Azospirillum halopraeferens*, Solid Route in Alginate Beads in Two Different Vegetative Stages (Seedling, and Flowering-Inoculation of Solid Root System Pathway in Calcium Alginate Beads)

The fertilization program by inoculation via solid in the form of calcium alginate beads during the vegetative growth of *S. bigelovii* was performed by depositing with a spatula, a gram of spheres containing *K. pneumoniae* or *A. halopraeferens*, according to the study treatments in seedling and flowering stages. During the first month of vege-

tative plant development, (germination and seedling development) seedlings were irrigated with potable water and then saltwater. The frequency of irrigation at this stage was every other day, applying 55 ± 5 L/m^2; in these period stages, the irrigation system used was that of flooding. The properties of the fresh water used: electrical conductivity: 1.194 w dS/m; the properties of salt water used: electrical conductivity of 11,230 w dS/m. A week before transplanting, seedlings were adapted to saline water, gradually increasing salinity. After transplantation, the irrigation system that was used in the micro sprinkler type, applying irrigation depth of 65 ± 5 L 30 µm. When finish the growth of *Salicornia* (11 months), 10 plants per treatment were randomly selected to quantify the variable seed production per plant (g/plant). For the variable seed production per m^2 (g/m^2 seed production) of each treatment was determined by multiplying the value obtained from the average seed production per plant by the total number of plants to be planted by watershed. Dry matter produced by watershed (g/m^2 dry matter) was evaluated. In addition, samples of seeds produced in each treatment were analyzed for the quantification of protein, moisture, ash and total lipids. The absolute percentage of fatty acids (palmitic, stearic, oleic, linoleic and linolenic), was analyzed by the technique suggested by Bligh and Dyer (1959) and amended by Arredondo et al. (1997) and Sato and Murata (1988). Analyses of variance were performed as proposed by Snedecor (1956). The least significant difference test was performed by Duncan's Multiple Range ($p > 0.05$ %). Analyses were performed using SAS (SAS 1996).

3 Results and Discussion

Salicornia bigelovii is a halophyte that is emerging for the immediate future as an option to contribute to the agricultural economy with a possible positive impact on the regional development of the agricultural sector of northwestern Mexico (Mota 1980). In addition, the microorganisms with beneficial effects on plantsplay a significant role as potential agents of bio-fertilizers. However, investigations into growth-promoting bacteria and halophyte plants are scarce. Therefore, it is necessary to conduct studies on halophytes and the environment around them in order to extend the range of endemic nitrogen fixing bacteria, to enlarge the knowledge of inoculum density, physiology promoter strains, inoculation into different cultures and ecology rhizosphere level among others. According our study, inoculating *Klebsiella pneumonia* and *Azospirillum halopraeferens* in two ecotypes of *Salicornia bigelovii* (Cerro Prieto = CP and SR = Santa Rosa), the ecotype Cerro Prieto (CP) with *K. pneumoniae* and *A. halopraeferens* was significantly with $p > 0.05$, favored in bloom, fresh and dry weight, root length and content of nitrates (NO_3) in flowering stage. Although *A. halopraeferens* stimulated a greater percentage of flowering in CP ecotype with $p < 0.05$, followed by *K. pneumoniae* compared with uninoculated controls of both ecotypes.

With respect to fresh samples obtained in the flowering stage weight, the results showed significant differences ($p < 0.05$) between treatments under field conditions (SR = 1700 g/plant; CP + *Azospirillum halopraeferens* = 2430 g/plant; CP + *Klebsiella pneumoniae* = 2427 g/plant; SR = 1.428 g/plant; SR + *Azospirillumhalopraeferens* = 1728 g/plant; *Klebsiella pneumoniae* + SR = 1.693 g/plant). In this sense, SR ecotype inoculated with *K. pneumoniae* was stimulated by 200 % while with *A. halopraeferens* the effect was 94 %. However, the CP ecotype inoculated with *A. halopraeferens* and *K. pneumoniae* were stimulated by 900 and 150 %, respectively. In dry weight reached at the flowering stage, the results show that both ecotypes were stimulated by the inoculants, showing *A. halopraeferens* the highest values for both ecotypes (CP = 267 g/plant, CP + *Azospirillum halopraeferens* = 1120.0 g/plant; *Klebsiella pneumoniae* + CP = 673 g/plant; SR = 320 g/plant; SR + *Azospirillum halopraeferens* = 487 g/plant; *Klebsiella pneumoniae* + SR = 865 g/plant).

Regarding the root length in the flowering stage, performing variance analysis significant differences between treatments. The highest val-

ues of growth was CP ecotype inoculated with *A. halopraeferens*, followed by SR with *K. pneumoniae* (CP = 14.23 cm; CP + *Azospirillum halopraeferens* = 26 cm; *Klebsiella pneumoniae* + CP = 15.23 cm; SR = 17.32 cm, SR + *Azospirillum halopraeferens* = 14.45 cm; *Klebsiella pneumoniae* + SR = 20.34 cm).

The nitrate content in control plants of both genotypes showed high values compared with those plants inoculated with N_2-fixing bacteria (CP = 1.3 mg/mL of NO_3/plant, CP + *Azospirillum halopraeferens* = 0.32 mg/mL NO_3/plant; *Klebsiella pneumoniae* + CP = 0.45 mg/mL of NO_3/plant, SR = 1.1 mg/mL of NO_3/plant, SR + *Azospirillum halopraeferens* = 0.35 mg/mL of NO_3/plant, SR + *Klebsiella pneumoniae* = 0.49 mg/mL NO_3/plant). This apparent inhibition in the uptake or nitrate accumulation appear to be controversial. Similar results have been found in studies in maize (Zea mays) (Blackmer and Scheppers 1995; Pérez-Silva 1989), where different levels of fertilizers were applied from N_2, low NO_3 concentrations detected in the sap were being directed towards the formation of biomass. With regard to biomass production, the phenological Salicornia bigelovii response by effect of *Azospirillum halopraeferens* and *Klebsiella pneumoniae* shows that inoculation Cerro Prieto ecotype (CP) with *A. halopraeferens* and *K. pneumoniae* was favored significantly with 718.0 and 677.0 kg/ha respectively. Meanwhile ecotype Santa Rosa (SR) showed values of 549.0 and 576.0 kg/ha as a result of *A. halopraeferens* and *K. pneumoniae*, respectively. The not inoculated control treatments reduced production by up to 50 % less; (CP) with 310.0.0 kg/ha, and ecotype (SR) showed values of 225.0 kg/ha. According the analysis of variance for the proportion of lipid, protein, moisture and ash the treatments showed significant differences. It was found that plants inoculated with N_2-fixing bacteria (Table 22.1), showed higher values compared with controls plants treatments. The higher lipid values were obtained consecutively showing a statistical equality for *K. pneumoniae* + SR treatments , CP + *K. pneumoniae*, *A. halopraeferens* CP + and CP + *A. halopraeferens*. For proteins, CP + *A. halopraeferens* showed higher values followed by the same ecotype inoculated with *K. pneumoniae*. For moisture and ash variable, CP ecotype inoculated with *K. pneumonia* was statistically higher compared to other treatments.

Regarding the proportions of fatty acids, Table 22.2, showed significant differences ($p > 0.05$) between treatments with the content of palmitic fatty acid, when the treatments were inoculated with *K. pneumonia*; however the CP + *A. halopraeferens* ecotype, was the most affected with this acid. Concerning the stearic acid there was not statistical significances between treatments inoculated in both ecotypes of seed, compared with oleic acid. With linoleic acid, CP ecotype and *A. halopraeferens* showed statistically significant. Relative to linoleic acid between treatments there was no significant improvement except without inoculant who proved to lower values ecotypes. This implies the possibility of the possible introduction of beneficial bacteria like *K. pneumoniae* and *A. halopraeferens* as a halotolerant bacteria to be improved with the *S.*

Table 22.1 Effect of N_2-fixing bacteria on the content of lipid, protein, moisture and ash seed present in two ecotypes of *Salicornia bigelovii* under field conditions

Ecotype	Inoculant (Bacteria)	Seed			
		Lipid (mg/g)	*Protein (%)	Moisture (%)	*Ash (%)
Santa Rosa	Control	53.0^{c}	23.3^{c}	4.68^{c}	20.0^{d}
Santa Rosa	*K. pneumoniae*	95.4^{a}	22.4^{e}	4.79^{c}	24.1^{b}
Santa Rosa	*A. halopraeferens*	96.4^{a}	23.0^{d}	4.77^{c}	21.4^{c}
Cerro Prieto	Control	77.0^{b}	20.1^{f}	4.80^{c}	14.0^{f}
Cerro Prieto	*K. pneumoniae*	90.2^{a}	24.1^{b}	6.48^{a}	25.2^{a}
Cerro Prieto	*A. halopraeferens*	99.1^{a}	30.0^{a}	5.85^{b}	16.1^{e}

Means with same literal column indicate no significant differences ($P > 0.05$)
*Results expressed on a dry basis

Table 22.2 Effect of N_2-fixing bacteria on the percentage of fatty acids in seed produced two ecotypes of *Salicornia bigelovii* under field conditions

		Fatty acids (%)				
Ecotype	Inoculant (Bacteria)	Palmitic	Estearic	Oleic	Linoleic	Linolenic
Santa Rosa	Control	16.44^{ab}	0.69^{c}	19.36^{ab}	64.02^{bc}	2.56^{a}
Santa Rosa	*K. pneumonia*	16.44^{ab}	1.18^{b}	16.48^{bc}	66.71^{b}	2.29^{ab}
Santa Rosa	*A. halopraeferens*	10.74^{c}	0.45^{c}	14.30^{c}	75.03^{a}	2.56^{a}
Cerro Prieto	Control	18.85^{a}	2.43^{a}	16.32^{bc}	63.98^{bc}	1.51^{c}
Cerro Prieto	*K. pneumonia*	12.18^{bc}	0.59^{c}	21.59^{a}	66.37^{b}	2.35^{ab}
Cerro Prieto	*A. halopraeferens*	17.78^{a}	0.63^{c}	14.20^{c}	68.12^{b}	2.35^{ab}

bigelovii as a potential halophyte to be introduced in semi-arid areas (Bashan et al. 2000). However, further research is needed on the fate of the inoculations in the rhizosphere of *Salicornia bigelovii* halophyte.

4 Conclusion

The results related with the inoculation of plant growth promoting bacteria's (*K. pneumoniae* and *A. halopraeferens*) with *Salicornia* plants, suggest the feasibility of replacing nitrogen fertilizers, where biomass and seed production values were significant compared with the controls no inoculated. Finally, it is worth mentioning that this type of experimental work contributes to enhancing knowledge in agricultural production alternatives and effects on the application of biofertilizers in new plant materials with productive potential of socio-economic interest to States with problems of water availability good quality, as is the Northwest Mexico.

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