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Phaseolus acutifolius ASSOCIATED WITH *Bacillus amyloliquefaciens* AND *Azospirillum halopraeferens* UNDER SALINITY CONDITIONS

Phaseolus acutifolius EN ASOCIACIÓN CON *Bacillus amyloliquefaciens* Y *Azospirillum halopraeferens* BAJO CONDICIONES DE SALINIDAD

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ABSTRACT

Salinity-tolerant plants offer hope for the future of agriculture by providing solutions to the problems caused by years. Sonora is the most arid Mexican state. The agroindustrial halophytes are an option in dry-arid zones agriculture. In the present study, we evaluated the growth and development under different salinity and field conditions, of two beans (*Phaseolus acutifolius*) genotypes: Indio Yumi, and Navojoa. Seeds were inoculated with plant growth promoting halobacteria, a previously selected and cultivated strain of *Azospirillum halopraeferens* and a native *Bacillus amyloliquefaciens*. Significant differences were observed among them for weight and biomass, as well as biochemical features between the analyzed plant parts. Our findings suggest that a potential yield enhancement and protein production under field conditions can be promoted by the application of the beneficial bacterium *B. amyloliquefaciens* and *A. halopraeferens.* Also, demonstrated the ability of the studied beneficial halobacteria to promote growth and yield of the halotolerant *Phaseolus acutifolius*, a potentially useful finding for the agricultural growers in dry and semiarid zones.

Key words: N_2-fxing bacteria; interaction plant growth promoting halobacteria; salinity tolerance; tepary bean.

RESUMEN

Las plantas tolerantes a la salinidad ofrecen esperanzas para el futuro de la agricultura, al proporcionar soluciones a los problemas causados por años. Sonora, es uno de los estados más áridos de México. Las halófitas agroindustriales son una opción en una agricultura de zonas áridas. En el presente estudio se evaluó el crecimiento y desarrollo de dos genotipos de frijol (*Phaseolus acutifolius*): Indio Yumi y Navojoa. El estudio se llevó a cabo bajo condiciones salinas y de campo; las semillas se inocularon con halobacterias promotoras del crecimiento vegetal, una cepa cultivada y previamente seleccionada de *Azospirillum halopraeferens* y una de *Bacillus amyloliquefaciens* nativo. Se observaron diferencias significativas entre los tratamientos con relación al peso y la biomasa y también para las características bioquímicas de las plantas analizadas. Nuestros hallazgos sugieren que un incremento significativo en el rendimiento y contenido de proteína bajo condiciones de campo puede promoverse mediante la aplicación de las bacterias benéficas de *B. amyloliquefaciens* y *A. halopraeferens*, además de demostrarse la capacidad de las halobacterias para promover el crecimiento y el rendimiento de la halotolerante *Phaseolus acutifolius*, lo cual es un hallazgo potencialmente útil para los productores agrícolas de zonas secas y semiáridas.

Palabras claves: Bacterias fijadoras de N_2; interacción planta y halobacterias promotoras del crecimiento vegetal; tolerancia a la salinidad; frijo tepari.

INTRODUCTION

One of the main approaches to develop crops tolerant to a seawater concentration salinity is to select and assay plants with salt tolerance, focusing on those that might make desirable crops. Tepary beans (*Phaseolus acutifolius* A. Gray) are an economical attractive drought-adapted legume native to the Sonoran desert (Southwestern of USA and Northwestern of Mexico). This species has been used as food source for more than 5,000 years in various regions of America (Nabhan, 1985), and their seeds are characterized by a high content of dietary protein (21.1 to 32.49%) compared with other *Phaseolus* species such as *P. vulgaris* (19.1-29.7%), *P. lunatus* (19.7-24.9), *P. coccineus* (20-27.4%), *P. polyanthus* (21.6-25.6%), *P. filiformis* (24.2%) and *P. angustissimus* (25.9%) (Freeman 1912; Marius *et al.*, 2013). Sonora is one the most arid states of Mexico, with an average annual precipitation of 80 mm, with a lack of surface water sources, such as lakes and rivers. Hence, in this zone, it is of considerable importance to introduce plants with capacity to tolerate salts, such as Tepary beans, which is a potential and alternative resource for local agriculture, due to the prevailing problems to produce traditional crops.

In general, tepary bean yields have been estimated between 200 and 900 kg ha^{-1}. Yields of up to 2000 kg ha^{-1} have been reported (Shisanya, 2002; Ahmad *et al*., 2013) depending on sowing season and appropriate supplementation of nitrogen (N) fertilizers. Several researches have study the nutritional condition and growth of *Phaseolus* spp. however, the quantities of fertilizer as a Nitrogen source are extremely high falling in some adverse pollution effects for other crops (Mota, 1999).

Inoculations of crops with beneficial bacteria such as N_2-fixing bacteria or plant growth pomoting halobacteria are

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an important agricultural way, because they can promote growth and because they eventually play a major role in the nitrogen cycle. The importance to enlarge, introduce, and apply this kind of bacteria with capacity to promote the growth of plants in saline conditions has been growing during the last decade (Hamdi, 1999).

The aim of this study was to evaluate the physiological response of two genotypes of *Phaseolus acutifolius* (Indio Yumi (IY), and NAVO) under field conditions inodulated with *Bacillus amyloliquefaciens* and *Azospirillum halopraeferens*, as an alternative to improveme forage and bean production for the arid and dry region from the northwest region of Mexico.

MATERIALS AND METHODS

Study site

The study was carried out in Santa Ana, Sonora, located in an arid zone of the Mexican State of Sonora, Mexico, at coordinates 30°32′26″N 111°07′14″O. The treatments under study were two genotypes of *Phaseolus acutifolius*: Indio Yumi (IY), and NAVO. The first one was provided by native people (native people "Indios Mayos") located at 27° 21′ 15.12″ N, 110° 2′ 9.6″ W, and the second genotype by Etchojoa farmers located at 26°54′44″N 109°37′53″O in southern Sonora State. Previously to sowing, the wild seed was sifted in order to separate the mature seeds, cleaning them out from dry vegetative material and select the bigger size seeds, uniform color and without apparent mechanical damages. Each one of the Indio Yumi (IY), and NAVO study genotypes were disinfected with a 3% chlorine sodium solution during 30 s. Seeds were washed three times with sterile distilled water and dried with sterile drying paper. After the last wash, we applied a viability test on both genotypes, as suggested by Pérez (1995).

Inoculation of Bacillus amyloliquefaciens and Azospirillum halopraeferans on Phaseolus acutifolius (Indio Yumi =IY, and NAVO)

The bacterium solutions were prepared using a N-free medium with NaCl 0.5 M, media denominated OAB. We adjusted the bacteria suspension with a spectrophotometer (master spectrum FISHER SCIENTIFIC 415) with a wavelength of 540 nm, from a logarithmic phase (14 at 16 h) culture. We diluted the bacterial solution at 1 x 10^9 colony-forming units per mL (CFU/mL). To each media with the bacterial solution we added 0.5 g of seeds (approximately 590 seeds ± 7) according to Carrillo *et al.* (1998). Then, seeds were placed in 1 m^2 growth chambers, containing 7 cm of fine sand and covered with a fine cap (3 mm ± 1) of peat-moss (Sunshine, Sun I Cry Horticulture Canada, Ltd.) at 19 ± 3 °C. Once seeds emergenced, selected seedlings were planted on field, with a 12 seed·m lineal, and 50 cm between rows, from each treatment. For analytical purposes, seedlings were transferred under an experimental array based in a completely randomized design of six treatments and five replicates of 24 seedlings per replicate. The study factors were two genotypes of *Phaseolus acutifolius*: (Indio Yumi (IY), and NAVO), with inoculants based on *Bacillus amyloliquefaciens* and *A. halopraeferens,* and without inoculants. During the development of plants, the correspondence treatments with plant growth-promoting bacteria under study were inoculated in form of beads (spheres) alginate at different vegetative stages. The production of beads alginate was developed according to Carrillo *et al.* (1998), with a cellular density of 1 x 10^9 CFU/mL. The beads inoculation program was applied during the vegetative development of *Phaseolus acutifolius* starting at the seedling phase and was continued during the development of branches, then at the pre-flowering and flowering stages, which means 24, 34, and 44 days after germination, respectively. Inoculation was accomplished placing 1 g of beads with the bacterium within the soil sub-surface, as nearest to the root system of each plant as possible, in every treatment parcel under study.

Sowed and irrigation program

Seeds of the genotypes were deposited on 1 m^2 germination plates containing 7 cm of fine sand substrate. Later the seed was covered with a thin layer (3 mm ± 1) of peat-moss substrate (Sunshine, Sun Gro Horticulture Canada, Limited.). The germinating plates were placed in the open (open sky), during two weeks. The irrigation program used during the emergence development of *Phaseolus acutifolius* was using water with the next characteristics in the first 2 weeks of emergency (pH: 7.00; salinity 0.8 ppm; dS/m 1,194; NO_2 0.108 - 0.114 µm/L; NO_3 87.27-94.17 µm/L). Then, during vegetative development of plants, was applied saline water considering the next characteristics (pH: 8.00; salinity ppm 3.75; dS/m 5.992; NO_2 0.322 - 0.328 µm/L; (NO3) 6.22 – 6.65µm/L).

The soil type of the experimental area was clay loam with 42% sand, 29.62% of silt and 27.18% of clay, salt-free, and organic matter (0.01 %).

A week before of transplanting onto the field, the seedlings were adapted to saline water, increasing the salinity gradually. A sprinkler irrigation system was used in the micro-parcels, applying a volume of 45 ± 5 L per micro-parcel; the time of irrigation was 6 min. In order to know the status of the soil microbiology, we applied a widely used technique for the detection of nematodes according to Thorne (1961), and fungi, according to Manovsky (1982). For this purpose we collected soil samples from the experimental micro-parcels according to SAGAR (1994). Results showed a low population of nematodes, corresponding to the Dorylaimida Order (9 specimens·100 g^{-1} of soil). Fungal microorganisms were found, including *Rhizoctonia* spp, *Alternaria* spp, *Fusarium* spp, and *Aspergillus* spp.

The average monthly values of temperature (°C) during the cropping cycle of *Phaseolus acutifolius* were 23 ± 4 °C for the initial three weeks, 33 ± 4 °C for the next two months, and 40 ± 5 °C for the season corresponding to the final stages of *Phaseolus acutifolius.* At the same time, the mean values of the relative humidity (R.H.) were 25% for the initial one months, and 30 to 35% for the next months (CONAGUA, 2017).

Analysis of variables

The emergence rate and percentage were measured and recorded once the seedlings emerged from the substrate (8 days after sowed). The number of emergenced seedlings was recorded by readings (evaluations) every third day (Emergence Rate), and finally the percentage of emergency (%) was measured after the 12th day of trial. The emergency rate was calculated according to Maguire (1962) by means of the equation: M = n1/t1, n2/t2, …n12/t12; where n1, n2, … n12 are the number of germinated seeds at the time t1, t2, … t12 (in days). The data of emergency percentage were analyzed taking into account a transformation to the arc-sin (Sokal and Rohfl 1988). The emergence rate, which it is the sum of counted emergency seedling per day, was transformed previously for their analysis.

On the other hand, during all the life cycle of plants, 35 plants of each treatment were randomly collected in order to measure plant height, on monthly basis. At the physiological flowering state, the nitrates content in sap ($N\text{-}NO_3$ $mg{\cdot}mL^{-1}$ of sap) was obtained by means of the procedure from Coombs *et al.* (1988). Root length, fresh weight and dry weight by plant were determined only at the flowering stage. The length of the plants and root system were measured using a hand scale micrometer (General, 143, General Tools, Manufacturing Co., Inc. New York, USA). The dry weight was measured once each organ was dried at 110° C during 36 hours. For all the above variables, we considered 5 replicates per treatment. In this stage, we collected 5 plants per treatment, to be sectioned in three parts, obtaining root, stem and above-ground portion. For stem analysis, we considered the first leaves from the plant base, jointly with its lateral branches. The aboveground portion was considered at 30 cm of the final portion from the plant. We assayed proteins, carbohydrates, total lipids, and ashes content for every plant organ. Proteins were assayed by the micro-Kjeldahl method, ashes by difference of weight, burning the sample for 24 h at 500º C, total lipids were assayed according to Barnes and Blackstock (1973). Non-fibrous carbohydrates were determined according to Sato *et al*. (1998): CNF; acid detergent fiber: FDA; Neutral detergent fiber: FDN; Total digestible nutrients: TND; net energy for lactation: ENI.

At the final reproductive cycle, 10 plants per treatment were randomly collected to quantify the variable "seed production per plant" ($g{\cdot}plant^{-1}$). For the variable "seed yield" in $g{\cdot}m^{-2}$, we multiplied in each treatment the average production of seed per plant by the total number of plants that were sown in every micro-parcel. The dry matter was evaluated per micro-parcel (dry matter in $g{\cdot}m^{-2}$). Samples of the harvested seed collected in every treatment were analyzed for protein, humidity, ash and total lipids content.

The previously outlined variables were analyzed applying the procedure of Analysis of Variance (ANOVA), and a test of F to determine the statistical difference (Snedecor, 1956). The data from seeds (dampness, proteins, ashes, and fatty acids) were analyzed after transforming values to arc-sin values (Sokal and Rohfl 1988). The least significant difference was estimated by Duncan's Multiple Range test at P=0.05. The statistical tests were performed through the SAS computer program (SAS, 1996).

RESULTS AND DISCUSSION

Evaluation of the emergence showed that both genotypes took between 8 and 12 days for maximum emergency. The presence of *Bacillus amyloliquefaciens* and *Azospirillum halopraeferans* affected positively the final percentage emergency of the Indio Yumi (IY) genotype (Table 1). This might be a strategy of *Phaseolus acutifolius* to cope with the changing environment of saline soil areas. The final germination percentage seemed unaffected. This variable of emergency in both studied genotypes are explained by the ability of the bacteria to produce or stimulate synthesis of hormones such

Table 1. Growth and yield parameters of Indio Yumi (IY), and NAVO genotypes of *Phaseolus acutifolius* by the effect of *Bacillus amyloliquefaciens* and *Azospirillum halopraeferans* under field conditions.
Tabla 1. Parámetros de crecimiento y rendimiento en los genotipos Indio Yumi (IY) y NAVO de *Phaseolus acutifolius* por efecto de *Bacillus myloliquefaciens* y *Azospirillum halopraeferans* en condiciones de campo.

Plant Genotype	Inoculant [Bacterium]	Emergency (%)	Days after planting (flowering stage)	Fresh weight flowering stage ($g{\cdot}plant$)	NO_3 ($mg{\cdot}mL^{-1}$) flowering stage	Length root (cm)*	Dry matter ($Kg{\cdot}ha^{-1}$)*
Yumi	Control	85.0± 3.2 ab	40± 2	14.22± 3.5 b	21.93± 0.86 d	28.10± 0.2 d	5800± 228 ab
Yumi	*A. halopraeferens*	95.4± 7.6 a	47± 3	17.50± 3.3 b	23.5± 0.43 c	32.56± 0.2 b	6102± 145 a
Yumi	*B. amyloliquefaciens*	93.6± 6.0 a	45± 1	26.69± 2.7 a	28.03± 0.33 a	31.47± 0.2 b	6210± 178 a
NAVO	Control	88.2± 3.5 ab	40± 3	15.18±.3.1 b	20.97± 0.54 e	29.93± 0.1 cd	5945± 210 ab
NAVO	*A. halopraeferens*	97.1± 0.5 a	46± 3	23.64±4.3 a	24.70± 0.23 b	35.27± 0.4 a	6298± 178 a
NAVO	*B. amyloliquefaciens*	92.4± 5 a	47± 3	24.33± 4.5 a	24.51± 0.12 b	30.47± 0.2 bc	6345± 188 a

Means of triplicate values. A letter indicates significant difference. *: in mature plants.
Medios de valores triplicados. Una letra indica una diferencia significativa. *: en plantas maduas.

as indol acetic acid or giberellin (GA_3) (Blackmar and Mallarino, 1996). *Phaseolus acutifolius* has only one opportunity in its annual life cycle for reproduction and population dispersion, tasks that are highly dependent on seed germination and emergency responses. Under natural conditions, *Phaseolus acutifolius* germination occurs like others halophytes when soil or water salinity is reduced (Riehl and Ungar, 1982).

The null inoculation of *Bacillus amyloliquefaciens* and *Azospirillum halopraeferans* in control treatments accelerated flowering stage; the contrary was for those inoculated treatments (Table 1); it took 37 to 50 days to flowering in all treatments. Table 1 shows fresh weight in flowering stage; however, the effect varied in degree with genotype; those treatments inoculated increased with significant differences ($p< 0.05$), sticking out IY genotype + *Bacillus amyloliquefaciens* and NAVO genotype + *Bacillus amyloliquefaciens* and *Azospirillum halopraeferans*. This interaction affected positively in NO_3 content per plant (Table 1). The best values were with the interaction with *Bacillus amyloliquefaciens* and *Azospirillum* with IY and NAVO genotype. The behavior of the non-inoculated genotypes was very similar to that reported elsewhere (Jefferies, 1981; Stumpf *et al*., 1986). The NO_3 concentration in sap with no inoculant was 20 mg mL^{-1} for both genotypes at the flowering stage. However, tratements inoculated, the NO_3 concentration was superior for both genotypes. The probable reason for this is that non-inoculated plants, does not have hormones and nitrogen requirements *vs* those treatments with plant growth promoting bacteria inoculated in this study, which has the opportunity to offer growth promoting substances which altered growth and development (Blackmar and Mallarino, 1996). Our results are evidence that the presence of beneficial microorganisms plays a role in growth and development at different vegetative stages (seedling, prefowering and physiological stage), such as emergency, of diverse halophytes (Bagwell *et al*., 2001).

In relation to root growth in physiological maturity, the NAVO genotype was stimulated with $p< 0.05$, when inoculated with *A. halopraeferens*. The non-inoculated genotypes did not showed significancy with $p< 0.05$. The root growth of NAVO genotype was stimulated 12% when inoculated with *A. halopraeferens,* while *Bacillus amyloliquefaciens* did not show any affect in relation to the controls treatments (Table 1). We cannot explain difference in effect of *A. halopraeferens* and *Bacillus amyloliquefaciens* on *Phaseolus acutifolius* genotypes. However, a similar effect was found in roots of other cultivars, such as sea oats (Will and Sylvia, 1990). The phenomenon seems to be related to the ability of *Azospirillum* spp. and *Bacillus amyloliquefaciens* to produce growth promoting substances.

According the dry matter, the results were no significantly different, however, numerically higher values were to NAVO inoculated with *Bacillus amyloliquefaciens* (6,345 $kg{\cdot}ha^{-1}$), while the lower values were to IY genotype with our inoculation (5,800 $kg{\cdot}ha^{-1}$). Those results are in agreement with the increased shoot growth of sea oats *Uniola paniculata* L. after inoculation with *Bacillus amyloliquefaciens* (Will and Sylvia, 1990).

Clearly, *Phaseolus acutifolius* is a potential crop whose vegetable parts are of special interest. Foliage and total yield are the most important parameter to evaluate, and we found that inoculating with *Bacillus amyloliquefaciens* and *Azospirillum halopraeferans* increased the proximate composition of foliage and seed proximate composition and yield production of both genotypes (Table 2 and 3).

The presence of both inoculants increased crude protein which it is one of the main factors to indicate a good plant as forrajera. The effect varied in degree with genotype; NAVO and IY genotype was stimulated with $p< 0.05$ by *A. halopraeferens* and *Bacillus amyloliquefaciens*, respectively (Table 2). However, *Phaseolus acutifolius* IY genotype was

Table 2. Proximate composition of foliage in Indio Yumi (IY), and NAVO genotypes of *Phaseolus acutifolius* by the effect of *Bacillus amyloliquefaciens* and *Azospirillum halopraeferans* under field conditions.
Tabla 2. Composición próximal del follaje en en los genotipos Indio Yumi (IY) y NAVO de *Phaseolus acutifolius* por el efecto de *Bacillus amyloliquefaciens* y *Azospirillum halopraeferans* en condiciones de campo.

Plant Genotype	Inoculant [Bacterium]	Crude protein %	Crude soluble protein %	Carbohydrates no fibrous %	Acid detergent fiber %	Neutral detergent fiber %	Net energy for lactation Mcal kg-1 MS	Crude protein Kg.ha-1
Yumi	Control	15.4± 1.2 b	43.09± 0.0 c	34.23± 2.3a	45.38± 0.2d	18.10± 0.2d	7.08± 0.1c	947.0±9.2 b
Yumi	*A. halopraeferens*	17.4± 0.6 a	46.79± 0.0 a	37.51± 3.2a	47.57± 0.0c	20.56± 0.2c	6.23± 0.1d	1070.20±25.2 a
Yumi	*B. amyloliquefaciens*	16.6± 0.7 ab	45.55± 0.0 b	36.70± 3.3a	49.76± 0.1a	21.47± 0.2b	9.38± 0.0b	1021±18.2 a
NAVO	Control	15.6± 0.5 b	44.40 ± 0.1 c	35.19± 4.2a	46.47± 0.2d	19.93± 0.1d	7.73± 0.1c	959.49±12.2 b
NAVO	*A. halopraeferens*	17.1± 0.5 a	45.77± 0.0 b	33.65± 3.1a	49.75± 0.0a	22.27± 0.4a	10.74± 0.1a	1051.75±33.2 a
NAVO	*B. amyloliquefaciens*	17.8± 0.5 a	45.23± 0.0 b	34.34± 4.1a	48.87± 0.0b	22.47± 0.2a	9.47± 0.1b	1094.80±35.2 a

Means of triplicate values. A letter indicates significant difference.
Medios de valores triplicados. Una letra indica una diferencia significativa.

Table 3. Proximate composition of seed in Indio Yumi (IY), and NAVO genotypes of *Phaseolus acutifolius* by the effect of *Bacillus amyloliquefaciens* and *Azospirillum halopraeferans* under field conditions.
Tabla 3. Composición próxima de la semilla en los genotipos Indio Yumi (IY) y NAVO de *Phaseolus acutifolius* por el efecto de *Bacillus amyloliquefaciens* y *Azospirillum halopraeferans* en condiciones de campo.

		Seed						
Plant Genotype	**Inoculant [Bacterium]**	**Crude oil (%)**	**Crude Protein (%)**	***Ashes (%)**	**Total Starch (%)**	**Moisture (%)**	**Yield Kg·ha^{-1}**	**P100S2**
Yumi	CONTROL	1.08± 0.04b	22.3± 0.1 c	20.1± 0.1 d	58.0±0.08	9.01±0.12 a	978 ± 13.4 c	13.5± 0.8cd
Yumi	*A. halopraeferens*	1.3± 0.02 a	22.03± 0.1 d	21.6± 0.1 c	60.09±0.04	9.11±0.14 a	1518± 10.8 1	18.9 ± 0.5 a
Yumi	*B. amyloliquefaciens*	1.4± 0.06 a	21.6± 0.7 e	24.5± 0.1 b	60.06±0.10	10.01±0.22 a	1430± 9.0 b	17.9 ± 0.3 b
NAVO	CONTROL	1.07± 0.07 b	19.3± 0.1 f	14.14± 0.1 f	59.05±0.08	9.13±0.11 a	890± 17.01 d	14.3 ± 0.7 c
NAVO	*A. halopraeferens*	1.7± 0.03 a	29.0± 0.1 a	16.3± 0.1 e	60.00±0.03	9.04±0.10 a	1438± 18.1 b	18.7 ± 0.2 a
NAVO	*B. amyloliquefaciens*	1.8± 0.05 a	23.1± 0.1 b	25.4± 0.1 a	60.04±0.05	8.81±0.12 a	1433± 14.4 b	18.8 ± 0.5 a

Means of triplicate values. A letter indicates significant difference. Result expressed on a dry weight basis. Protein: Method of micro-kjeldajl (% N *6.25). Ashes: Determination by difference of weight. Burning at 500º for 24 hrs. P100S2: Weight of 100 seeds in one g.
Medios de valores triplicados. Una letra indica una diferencia significativa. Resultados expresados en peso seco. Proteína: Método de micro-kjeldajl (% N * 6.25). Cenizas: Determinación por diferencia de peso. Ignición a 500º durante 24 hrs. P100S2: Peso de 100 semillas en un g.

stimulated with significant differences by *Bacillus amyloliquefaciens* compared with all treatments. A same behavior was in acid detergent fiber: FDA, Neutral Detergent Fiber: FDN, Total Digestible Nutrients: TND, and Net Energy for lactation: ENI, where NAVO genotype was stimulated with *A. haloraferens* and *Bacillus amyloliquefaciens,* respectively. Similar results were obtained when *A. brasilense* was applied as inoculant to chickpea (*Cicer arietinu*) and faba beans (*Vicia faba*), herbaceus swards and alfalfa (Hamaoni *et al*., 2001) and on various crop plants (Pishchik *et al*., 1996; Kuvtunovych *et al*., 1999; Remus *et al*., 2000).

According the chemical composition of seed, results showed that both inoculants increased concentrations of crude oil, crude protein, ashes, production yield and weight of 100 seeds in g (Table 3). Crude oil component of *Phaseolus acutifolius* was most favored by inoculants with 8 and 7% with *Bacillus amyloliquefaciens* and *Azospirillum halopraeferans* in NAVO genotype, respectively. *Bacillus amyloliquefaciens* and *Azospirillum halopraeferans* increased total protein concentration in the NAVO and IY genotype; more so in the NAVO genotype than in the IY type for both inoculants. On the other hand, total starch were similar for all treatments.

Inoculation of IY *Phaseolus acutifolius* plants, either with *A. halopraeferens* or with *Bacillus amyloliquefaciens*, stimulated total yield. A similar trend was observed for total weight of 100 seeds in g (Table 3). The increment in total lipids, protein and ash, influenced either by *Bacillus amyloliquefaciens* or *A. halopraeferens*, seemed to be related to the increment in total yield and plant biomass, as proven for other microbe-plant associations.

The above results suggest that *Phaseolus acutifolius* growth, during vegetative development, can be promoted by both *A. halopraeferns* and *Bacillus amyloliquefaciens* under the field conditions tested. Similar results were obtained in precise studies with *Zea mays* plants (Blackmer and Mallarino 1996). The authors applied different amounts of NO_3 fertilizer, and the results showed low concentrations of NO_3 in the stem. They concluded that assimilation of the applied nitrogen fertilizer was limited by the growth of the plant in mature stages.

CONCLUSIONS

Phaseolus acutifolius genotypes are affected at some stages by the presence of *Bacillus amyloliquefaciens* and *Azospirillum halopraeferans*. This is the first report in which *Bacillus amyloliquefacien,* and *A. halopraeferens* were used as biofertilizer in the rhizosphere of *Phaseolus acutifolius* under field conditions. It is clear from our data that *Bacillus amyloliquefaciens* and *Azospirillum halopraeferans* stimulated root growth, dry weight, biomass, and yield.

This study implies a promotion of this type of microorganisms as an efficient and reliable biological product for growth enhancement of this kind of plants. Furthermore, studies on association of *Bacillus amyloliquefaciens* and *Azospirillum halopraeferans* with *Phaseolus acutifolius* are recommended to determine the extent to which these observations can be reproduced under field conditions considering the production systems from the farmers.

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