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Research article

Effects of plant growth promoting bacteria and mycorrhizal on *Capsicum annuum* L. var. *aviculare* ([Dierbach] D'Arcy and Eshbaugh) germination under stressing abiotic conditions

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ABSTRACT

Capsicum annuum var. *aviculare* to Tarahumara and Papago Indians and farmers of Sonora desert is a promising biological and commercial value as a natural resource from arid and semiarid coastal zones. Traditionally, apply synthetic fertilizers to compensate for soil nitrogen deficiency. However, indiscriminate use of these fertilizers might increase salinity. The inoculation by plant growth promoting bacteria (PGPB) and arbuscular mycorrhizal fungi (AMF) represents an alternative as potential bio fertilizer resources for salty areas. Seeds ecotypes from four areas of Sonora desert (Mazocahui, Baviacora, Arizpe, La Tortuga), in order to inoculate them with one species of PGPB and AMF. Two germination tests were carried out to study the effect of salinity, temperature regime (night/day) and inoculation with PGPB and AMF growth factors measured on germination (percentage and rate), plant height, root length, and produced biomass (fresh and dry matter). The results indicated that from four studied ecotypes, Mazocahui was the most outstanding of all, showing the highest germination under saline and non-saline conditions. However, the PGPB and AMF influenced the others variables evaluated. This study is the first step to obtain an ideal ecotype of *C. a.* var. *aviculare*, which grows in the northwest of México and promoting this type of microorganisms as an efficient and reliable biological product. Studies of the association of PGPB and AMF with the *C. a.* var. *aviculare*-Mazocahui ecotype are recommended to determine the extent to which these observations can be reproduced under field conditions.

1. Introduction

Because Baja California Peninsula and Sonora regions are one of the most arid states of Mexico, with 80 mm average annual precipitation, it lacks surface water resources [1]. 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 increase in the salinity of agriculture soil, which during the last two decades became a major problem in the production of traditional crops in those regions [2–10]. Production alternatives include development of salt tolerant crops, and selection and evaluation of salt tolerant plants that already are adapted to salt flat areas, focusing on those that might make desirable crops [5,11–13].

Capsicum annuum var. *aviculare* called as a Chiltepin (Chiltepin from the Nahuatl Mexican word meaning "flea") is the most chili's extremely hot, measuring between 50,000 and 100,000 [14] Scoville Units. Tarahumara and Papago Indians area and farmers of Sonora desert are the principal people that obtained the fruit of plants that grow in natural conditions and lived from this activity. The last four years the Tarahumara and Papago Indians and farmers are finding the best way to incorporate *C. a. var. aviculare* into traditional agriculture to help support the agricultural economy of those areas affected by salinity. However, although the chiltepin plant produce a high viable seed, the germination and establishment is really poor [15,16]. In the Mexican Sonora state, the chiltepin has a wide distribution along the central part of Sonora state [17,18]. This plant was identified from among many plants

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species tested for possible domestication because of the promise as a new economic resource and possesses a high potential as an agro industrial commodity [17,18]. However, the productivity of *C. a.* var. *aviculare* is limited by a lack of available nitrogen [18,19]. This condition affects its growth and reproduction potential [20,21]. Traditionally, Indians and farmers apply synthetic fertilizers to compensate for soil nitrogen deficiency. However, indiscriminate use of these fertilizers might increase salinity and severely damages soil micro-flora structure and composition [22–25].

Few studies on mycorrhizas diversity within the rhyzosphere of *Capsicum annum* have been published [15,26–30]. In relation to variety *aviculare*, the studies are limited to [31]. It is important to increase the number of known salt tolerant, nitrogen-fixing bacteria and arbuscular mycorrhizal [32,33] as potential bio fertilizer resources for salty production areas. Given the natural biodiversity of Mexico, a similar relevance arises from this work related to the assessment of different responses among the wide number of ecotypes widespread along the northwest Mexico.

On the other hand, it has been proved that several stressing factors (water, temperature, light, and salinity), which regulate seed germination, interact within the soil interface [13,34]. Other variables may co-act with the seasonal variation of temperature to determine the temporal pattern of germination [35]. Osmotic and matric potential of soils narrow the range of temperature that is effective for the germination of seeds [36]. Salinity alone may not be the only critical environmental factor for the germination [37]. Interactions between salinity and temperature occur determining the optimal conditions for seed germination with many plants [38–44]. Halophyte seeds have the ability to maintain seed viability for extended periods of time during exposure to hypersaline conditions and then to start germination when salinity stress is reduced [35,42,43,45–49]. However, halophytes differ in their capacity to grow in varying saline stressing conditions [46]. This variation in recovery responses could be due to the difference in the temperature regime to which seeds are exposed [37,43–45]. The present study describes the effect of the inoculation with the PGPB (*Klebsiella pneumoniae*) and the AMF (*Glomus intraradices*), different levels of salinity and alternating temperature regimes on the seed germination of four non-domesticated ecotypes of *C. annuum* var. *aviculare.*

2. Methods

2.1. Evaluation of inoculants and NaCl on germination and seedling growth of four C. annuum var. aviculare ecotypes

Seeds of four *C. a.* var. *aviculare* ecotypes were collected during the autumn 2007, from natural areas of Sonora, Northwest of Mexico. The collected ecotypes were Mazocahui (Mz), Baviacora (Bv), Arizpe (Az), La Tortuga (Lt). Plants were sifted to separate mature seeds. The remains were cleaned and plant material was dried with sterile paper in order 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; then they were washed three times with sterilized, distilled water and dried with sterile paper. Seeds were inoculated with bacteria treatments according to [50], at a concentration of 10^8 cells mL^{-1}.

According to [51–54] were reproduced *G. intraradices* from sample of AM 120 Granite Seed Company (Lehi, UT 84043) using *Allium schoenoprasum* to obtain enough inoculum. Seeds were inoculated with the AMF-*G. intraradices* treatments according to [50], at a concentration of 120 propagules cm^3.

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, 0.06, or 0.12 M). Germination tests were done inside a growth chamber at 27 °C ± 0.5 °C and 35% ± 1% RH, with continuous white light. 20 mL of the appropriate solution were added every four 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 final percentage germination was measured after 27 days. Germination rate was calculated using the next formula [55]:

$$M = n_1/t_1 + n_2/t_2 + \ldots n_{27}/t_{27}$$

where $n_1, n_2, \ldots n_{27}$ are the numbers of germinated seeds at times t_1, $t_2, \ldots t_{27}$ in days. The hierarchic experiment of a randomised design included three factors (ecotype, inoculation and salinity), with five replicates of 50 seeds. The first factor (ecotype) had four levels (Mz; Bv; Az and Lt); the second factor (inoculants) had three levels: no inoculation, inoculation with the bacterium *K. pneumoniae*, and inoculation with mycorrhiza arbuscular (*G. intraradices*). The third factor had three concentrations of NaCl (0, 0.06, and 0.12 M). The combination of the three studied factors with their corresponding levels yielded 36 treatments. The data for percentage germination were analysed after applying arcsine transformation [56], 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 means of treatments were separated by Duncan's multiple range test at $P = 0.05$. Data were analysed using the Statistical Analysis System [57]. 35 seedlings of each 50-individuals-unit from all treatments were chosen randomly; seedling growth was measured by 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). 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 *C.a.* var *aviculare* was carried out at the conclusion of the study, 27 d after sowing. Seven plants of each treatment (168 seedlings) were washed with sterile, distilled water and introduced during 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 were taken from the bacterial solution of each tube and sowed by dispersion on N-free media OAB in Petri dishes, which were incubated during 24 h at 30 °C for CFU measurement. According to *G. intraradices* verification in root system of *C. a.* var *aviculare,* at the end of the test was probed the presence of the AMF studied, considering the same ones with KOH 10% in warms, and later dyeing with blue Trypan 0.05% in acid lactic and decolourized with glycerol at 50% [52,58]. The roots were mounted on microscope slide and were observed directly to the microscope.

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 [63]. Least significant differences (LSD) between means of treatments were separated by Duncan's Multiple Range test at $P = 0.05$. All statistical tests were done with SAS [57].

2.2. Evaluation of inoculants and NaCl on germination and seedling growth of the superior ecotype (Mazocahui) of C.a. var. aviculare

After the statistical evaluation of first experiment, Mz 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 of