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Effect of nodulating bacteria on the seed germination of *Capsicum* spp.

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The genus *Burkholderia* includes different species with biotechnological characteristics of great interest such as the degradation of xenobiotics, biological control, nitrogen fixation in free-living and symbiotic and phytohormone production. The diversity and versatility of this group of species is largely unexplored, including the production of secondary metabolites, which may have important practical applications. Therefore, the aim of this study was to evaluate the effect of seed germination of genotyes jalapeño and manzano peppers, inoculated with nodulating strains *Burkholderia sabiae* and 5B1. The outcome measures were percentage, index and coefficient of emergence. The results show that there is an increase in seed germination of aproximately 100% chili peppers jalapeño and 50% in manzano, inoculated with strain *B. sabiae*. Besides the increase in germination of seeds by *B. sabiae* strain, another feature of great biotechnological interest in this strain was also detected, which is the production of indoles. Based on these results, it is suggested that this strain can be used as an alternative for production of biofertilizers

Key words: Capsicum, germination, seeds, *Burkholderia*.

INTRODUCTION

The cultivation of *Capsicum* commonly known as pepper and one of the most important crops have increased due to demand in Mexico for the multiple uses of the fruits and it is naturally processed. It is also used as a condiment or as raw material for obtaining oleoresin dyes and industrial purposes as well as being a dietary source of antioxidants due to the content of flavonoids, phenolic compounds, carotenoids, ascorbic acid, vitamin A and capsaicinoids (Matsufuji et al., 1998; Osuna-García et al., 1998; Lee et al., 1995; Howard et al., 2000). The center of origin of *Capsicum* spp. is South America. The number of wild species comprising the genus *Capsicum* is between 20 and 30. Others authors mentioned about of 27 or 30 (Eshbaugh, 1982; Loaiza-Figueroa et al., 1983; Hernández et al., 1999; Moran et al., 2004) and they are only four or five domesticated species of *Capsicum* grown in the world. Mexico is the domestication center of five species: *Capsicum annuum* var. *annuum*, *Capsicum chinense*, *Capsicum pubescens*, *Capsicum* var *baccatum pendulum*, and the semi-domesticated *Capsicum frutescens*, and *Capsicum annu*um var. *glabriusculum* (Loaiza-Figueroa et al., 1983; Moran et al., 2004). In Mexico, there are wild populations of *C. annu*um and *C. frutescens*, which show great morphological and genetic variability (Hernández et al., 1998), and the species *Capsicum ciliatum* and *Capsicum lanceolatum*. *C. ciliatum* is found throughout the country except the Northwest, while *C. lanceolatum* has been reported only in the states of Chiapas and Veracruz (Hernandez-Verdugo et al., 1998).

The inoculation of seeds or seedlings with microbial inoculants has been adopted as a method for modifying microbial populations around crops plants to promote both development and yield. The stimulation of seedling

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development by bacteria has also been attributed to the production of biological active compounds. A significant increase in grain yield was also recorded in rice plants inoculated with plant growth promoting bacteria (Mantelin and Touraine, 2004; Mishra et al., 2006; Yang et al., 2009; López-Bucio et al., 2007). Similarly, inoculation of rhizobia to rice produced significantly higher roots and shoots biomass; increased their photosynthetic rate and accumulated higher levels of indoleacetic acid and gibberellin; phytohormones that regulator the growth. Plant growth promoting bacteria (PGPB) are bacteria that improve plant growth when introduced onto seeds, seed pieces, roots or into soil. The PGPB improve plant growth by one or more mechanisms: direct stimulation of plant growth by the production of phytohormones, vitamins and siderphores; enhancement of nutrient uptake; suppression of plant pathogens and/or induction of resistance in plant hosts against pathogens (Dakora and Phillips, 2003; Dobbelaere et al., 2003; Persello-Cartieaux et al., 2003; Mayak et al., 2004; Alikhani et al., 2006). *Burkholderia* species are characterized by their versatility, and by their ubiquity and diversity in both niches and environments. Most of the species belonging to the emerging beneficial-plant-environmental (PBE) *Burkholderia* group share important features, which provide them with advantages in their association with plants and with their immediate environments. The diversity and versatility of this group of species is largely unexplored, and this includes their production of secondary metabolites, which might have important practical applications (Suárez-Moreno et al., 2012).

Although β-rhizobia are particularly associated with the genus *Mimosa* and some related genera, they also nodulate several agriculturally important papilionoid legumes, including common bean (*Phaseolus vulgaris*) and honeybush tea (*Cyclopia* spp.), thus raising the possibility that they could be used as agricultural inoculants when their particular characteristics (e.g., tolerance to extreme pH, high salt tolerance) make them more suited to specific environments, such as in Morocco (Talbi et al., 2010) and the South African Cape (Elliott et al., 2007; Gyaneshwar et al., 2011). Therefore, the aim of this study was to evaluate the effect of seed germination of genotypes jalapeño and manzano peppers, inoculated with nodulating strains *Burkholderia sabiae* and 5B1.

MATERIALS AND METHODS

The experiment was conducted in the Biotechnology and Microbiology Laboratory, Universidad Tecnológica de la Selva, Chiapas, México. The cultivar *Capsicum* spp. used in this study was genotypes manzano and jalapeño. The seeds were collected from wild genotypes of *Capsicum* spp. in the regions of Palenque and Ocosingo, the State of Chiapas, México.

Plant growth promoting bacteria

Nodulating bacteria *Burkholderia sabiae* was provided by Ph.D Paulina Estrada de los Santos, from the Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, México. 5B1 strain was isolated from a legume native forest area of Chiapas. The design of the experiment was completely randomized with three replications (Table 1).

Table 1. Effect of inoculation of two strains nodulating bacteria, on the percentage of seed germination of chili, genotypes jalapeño and manzano after 14 days of inoculation.

Treatment	Germination after 14 days (%)	
	Jalapeño	Manzano
Control	35.0b	46.7b
Burkholderia sabiae	75.0a	68.5a
5B1	36.7b	50.0b

Means having different letters are significantly different at 5% level of significance.

Indol-acetic acid determination

Strains *B. sabiae* and 5B1 were evaluated based on production of indoleacetic acid, which were brown in NFB medium liquid for 18 h at 200 rpm, the inoculated cultures were adjusted to an optical density (OD) 0.2, and inoculated on media Jain and Patriquin culture with and without tryptophan. Once inoculated, they were incubated at 30°C for 24 and 48 h at 200 rpm. Subsequently, aliquots of 600 µl culture media were centrifuged at 5000g for 5 min. The presence of indol-3-acetic acid (IAA) in the supernatant was assayed according to the standard method modified from Rahman et al. (2010) in which the presence of the hormone in the culture reacts with Salkowski reagent.

Inocula preparation

Both bacterial strains were grown in peptone yeast extract (PY) and nutrient broth. Exponentially growing cells in shaken broth culture were inoculated. Chili pepper seeds were surface sterilized using etanol at 70% an Erlenmeyer flask and were treated with 7% sodium hypochlorite for 5 min followed by six times washing with sterile water. After that, the seeds were soaked in various nodulating bacteria. Seeds soaked in normal broth were treated as control. Twenty (20) seeds were inoculated, and the controls was put in sterilized Petri dishes containing agar 1% and kept at 24°C for 14 days.

Seedling emergence test

After soaking, the air-dried seeds were used for germination and the seedling percent emergence was calculated with the following formula:

$$\text{Emergence } \% = \frac{\text{Number of emerged seedlings}}{\text{Number of seeds sown}} \times 100$$

Germination speed

Copeland (1976) considers both vigor index and coefficient of

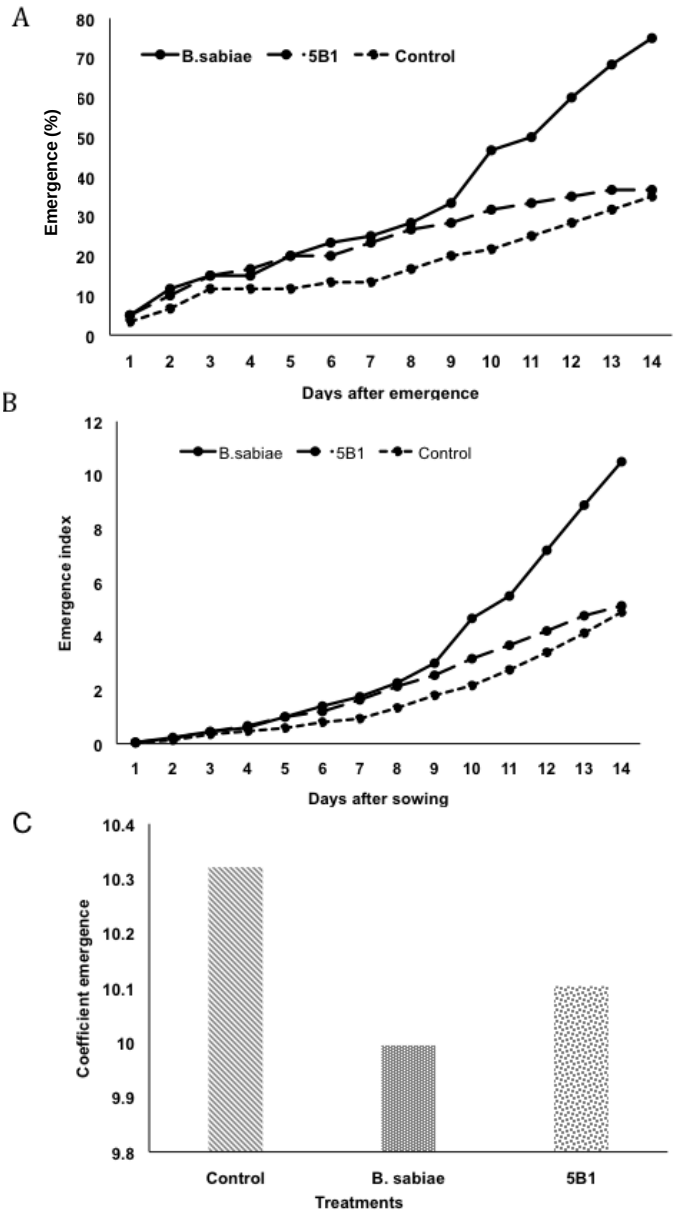

Figure 1. Effect of inoculation of *B. sabiae* and 5B1 bacteria on A, Percentage emergence; B, Emergence index; C, Coefficient emergence of seed germination of chili genotype jalapeño.

germination as measures for speed of germination. The germinated seedlings were counted at an interval of 24 h for 14 days and the speed of germination of seed was monitored. Coefficient of emergence and index emergence were calculated using the following formula (Copeland, 1976):

$$\text{Coefficient of emergence (\%)} = \frac{100(A_1+A_2+A_3+\text{----------}+A_X)}{A_1T_1+A_2T_2+\text{----------}+A_XT_X} \times 100$$

Where, A= number of seed germinated, T = time corresponding to A and x = numbers (1, 2, 3 ...nth).

$$\text{Emergence index} = \frac{\text{Ti Ni}}{\text{S}}$$

Where, Ti = ith number of days after sowing, Ni = ith number of seeds emergence, and S = total number of seed used.

Statistical analysis

Treatments were arranged in a randomized design. The analysis of variance and the LSD were calculated by using SAS package, Version 9.0 (SAS Institute Inc., 2006). Means were compared using the Tukey test at 5% level of significant.

RESULTS

Test germination

The effect of the germination of seeds of two genotypes of native peppers from the forest region of Chiapas, inoculated with strains of nodulating bacteria was evaluated (Figure 1A). Jalapeño genotype had the highest percentage of germination when seeds were inoculated with *B. sabiae* (75%), while strain 5B1 germination percentage was 36.7% (Figure 1A).

The statistical analysis for the comparison test of means by Tukey (5%) showed a significant difference in the treatment of *B. sabiae* with respect to control (Table 1). However, the results of the effect on germination of chili seeds genotype manzano, show that the germination percentage is 68.5% with *B. sabiae* strain (Figure 2A), whereas the percentage germination of seeds inoculated with 5B1 strain was 46.7% (Figure 2A), the statistical analysis for the comparison test of means by Tukey 5% showed a significant difference in *B. sabiae* treatment as compared to the control.

These results present a very similar behavior of *B. sabiae* and 5B1 in the germination of the two genotypes jalapeño and manzano (Table 1).

DISCUSSION

As shown in the results, there was an increase in the percentage of germination of the seeds of genotypes manzano and jalapeño, when inoculated with the strain of *B. sabiae*, the increase in the percentage of germination is a typical response of phytohormones gibberellins. Other parameters evaluated to determine the rate of germination of the seeds of *Capsicum* spp., was the effect of nodulating bacteria with germination coefficient (Figures 1B and 2B) and germination rate (Figures 1C and 2C).

As seen in the results, both parameters indicate that *B. sabiae* improves the speed of germination of chili seeds genotypes jalapeño and manzano. Although both strains are nodulating bacteria they both have an effect on the germination of seeds of *Capsicum* spp., which is a cha-

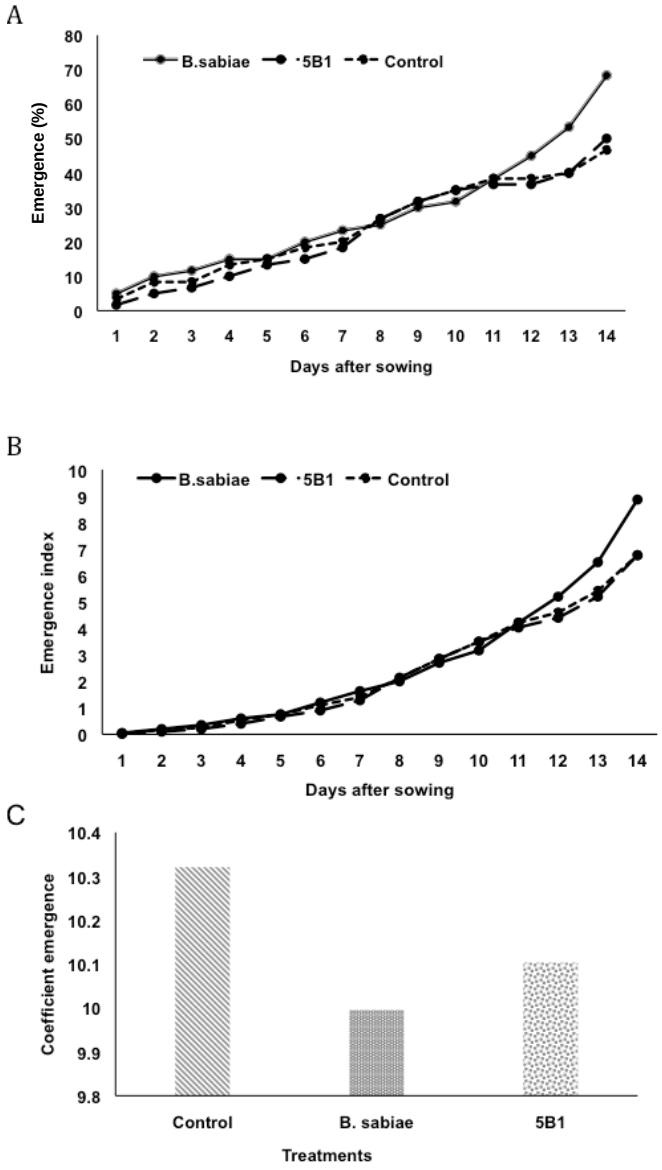

Figure 2. Effect of *B. sabiae* and 5B1 bacteria inoculation on seed. A, Percentage emergence; B, Emergence index; C, Coefficient emergence of germination of chili genotype manzano.

characteristic of great biotechnological interest, because they not only have a beneficial effect through the biological nitrogen fixation in symbiosis with nodulating legumes, but also stimulate germination in plants that do not form nodules as it is the case of *Capsicum* spp.

The effect on the seed germination could be due to a mechanism similar to that described in the rhizobia, they are released into the environment nod factors for leguminous plants, which stimulate the formation of nodules; but for non-legumes it has a stimulating effect on the germination of seeds. This affect rhizobia to stimulants the germination of seeds of leguminous plants that seems to be very similar in nodulating bacteria of *Burkholderia* genus (Antoun et al., 1998; Zhang and Smith, 2001; Dakora and Phillips, 2002; Smith et al., 2002). A fact of great importance is the production of indoles by *B. sabiae* (Data not show), as well as the ability of this strain to stimulate the germination of seeds of non-leguminous plant. The results of this study suggest that nodulating bacteria of *Burkholderia* genus increase the seed emergence and seedling vigor in seeds of *Capsicum* spp.

Also, it constitutes an economic methodology that can be used to optimize the germination rate, uniformity, and final percentages, features that directly affect crop production (Di Barbaro et al., 2005). With these characteristics and the results obtained, we proposed that this strain can be used as an alternative for biotechnological production of biofertilizers.

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