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Original Article

Effects of *Azospirillum* on germination and seedling growth of commercial sweet corn varieties Insee 2 and Hi-Brix 3

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Abstract

Two sweet corn varieties, Insee 2 and Hi-Brix 3 were used to assess the effects of *Azospirillum brasilense* strains (LB1-1, LB1-2, LB1-3, and LB1-4) on seed germination and seedling development under laboratory and greenhouse growth conditions. The highest germination percentage (95%) was observed with the uninoculated (control) seeds in Insee 2 and seeds inoculated with LB1-3 in Hi-Brix3. Insee 2 seeds inoculated with LB1-2 showed the highest germination index (GI) at 62.06% and in Hi-Brix 3, seeds inoculated with LB1-1 had the highest GI at 79.86. The effects of *Azospirillum brasilense* on seedling growth in both varieties of inoculated seeds in all strains were similar to the control in laboratory conditions. All strains had endophytic characters but showed differences in terms of growth. Only 3 treatments (TS2 9 [reference strain], LB1-1, and LB1-4) in the Insee 2 showed endophytic characters in both conditions. The TS29 and LB1-4 showed endophytic characters in laboratory conditions and all treatments had no endophytic characters in the greenhouse conditions in Hi-Brix 3.

Keywords: *Azosprillum*, nitrogen fixation, PGPR, plant growth, sweet corn

1. Introduction

Sweet corn (*Zea mays* L. *Saccarata*) is an economically important food crop. The total export value was more than 207 million USD in 2015. Thailand is the fourth highest exporter of all corn products after the USA, France, and Hungary. Currently, 34,072 ha are used to produce sweet corn especially in the north, northeast, and southern parts of Thailand with an average yield of 10,312 kg ha^{-1} (Office of Agricultural Economics [OAE], 2016). From 2003 to 2007, the cultivation area was about 37,780 ha; however, the yield has decreased 5.23% every year (OAE, 2007). In 2008, the prices of chemical fertilizers and pesticides increased; therefore, the use of alternative fertilizers, such as bio-fertilizers, that contain beneficial bacteria is crucial.

Plant growth-promoting rhizobacteria (PGPR) have gained worldwide importance and acceptance for agricultural benefits. The mechanisms they use to promote plant growth can be classified into four groups: biofertilizers, phytostimulators, rhizoremediators, and biopesticides (Noumavo *et al.*, 2013). Currently, plant inoculation with PGPR is a major asset for biological agriculture. It has also received considerable attention as a way to reduce chemical fertilizer use without affecting crop yield (Puente, Garcia & Alejandro, 2009). Significant increases in growth and yield of agronomical important crops in response to inoculation with PGPR bio-fertilizer have been reported (Asghar, Zahir, Arshad, & Khaliq, 2002). Kloepper, Tuzun, and Kuc (1992) showed that wheat yield increased up to 30%with *Azotobacter* inoculation and up to 43%with *Bacillus* inoculation. In addition,

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Azospirillum inoculation was reported to reduce the use of chemical fertilizers, in particular nitrogen by 20-50% (Attitalla, Alhasin, Nasib, & Ghazali, 2010).

The use of *Azospirillum* spp. emerged among the new technologies for optimizing crop implantation. The main mechanism by which *Azospirillum* enhances plant growth is undetermined. It has been suggested that the hormonal effect is one of the phytostimulators (Puente *et al.,* 2009). Inoculation of plants with *Azospirillum* species could result in significant changes in various growth parameters, such as an increase in plant biomass, nutrient uptake, tissue N content, plant height, leaf size, and root length of cereals under different climatic conditions and may lead to improved crop yields (Bashan, Holguin & de-Bashan, 2004; Noumavo *et al.,* 2013). Thus, it has been shown that *Azospirillum* had potential benefits for agricultural exploitation and could be used as a natural fertilizer (Cakmakc, Aydın, & Sahin, 2006). In Europe, *Azospirillum lipoferum* CRT1 is the most important PGPR used with maize (El Zemrany *et al.,* 2006) as well as in Mexico where *Azospirillum brasilense* UAP-154 and CFN-535 are used under agronomic conditions (Fuentes-Ramirez & Caballero-Mellado, 2006).

The effects of PGPR on seed germination were first reported on *Pseudomonas* spp. isolated from the root (Burr, Schroth & Suslow, 1978). So far, several studies have been carried out to determine the effect of PGPR on plants but very few have involved parameters of seed germination as well as plant growth (Noumavo *et al.,* 2013). Shaukat, Affrasayab and Hasnain (2006) reported that *Azospirillum*, *Psuedomonas*, and *Azotobacter* strains could enhance seed germination and seedling growth. Moreover, Cassán *et al.* (2009) proposed that plant growth regulator compounds produced by *Azospirillum* into the culture medium were responsible for the response in the early developmental stages, such as germination and seedling growth, since they had the first contact between the bacterial formulation and the seed (Puente *et al.,* 2009). Despite these promising growth promoting properties of *Azospirillum* strains, only three reports characterized the isolated strains from the rhizosphere soil of *Zea mays* (Mehnaz, Weselowski, & Lazarovits, 2007). The objectives of this study were to determine the effects of *Azospirillum* isolated from sweet corn on seed germination and seedling development under laboratory and greenhouse growth conditions.

2. Materials and Methods

2.1 Isolation of strain and physiological characterization of the isolates

The bacterial strains were obtained from plant samples of leaves, shoots, and roots of sweet corn in Nakhon Sawan, Lop Buri, Nakhon Rachasima, and Saraburi provinces. Identification of the isolates was carried from the cultural characteristics following Patriquin and Döbereiner (1978) and Döbereiner, Marriel, and Nery (1976). Other tests were performed to determine: (1) the morphological (colony morphology and pigmentation) characteristics; (2) gram staining; (3) utilization of carbon source; (4) indole-3-acetic acid (IAA) production (Salkowski colorimetric technique by Glickmann & Dessaux, 1995); (5) nitrogenase activity (acetylene reduction assay [ARA] method by Hardy, Holsten, Jackson, & Burns, 1968); (6) phosphate solubilization (phospho-molybdate blue color method by Murphy and Riley, 1962 in SRSM broth and SRSM agar plates were used to check halozone and solubilization ability); and (6) genetic characterization (16S rRNA genes). A total of 18 isolates were obtained and their repartitions according to place of origin were as follows: four isolates from Lop Buri; 11 isolates from Nakhon Sawan; and three isolates from Saraburi. All isolates were collected from root samples (Table 1).

2.2 Effects of *Azospirillum* on seed germination and plant growth

The experiment was carried out with four isolates of *Azospirillum brasilense* (LB1-1, LB1-2, LB1-3, and LB1-4). The nucleotide sequence data were reported in the Genbank nucleotide sequence database with the accession numbers KU030838, KU030839, KU030840, and KU030841. *Azospirillum brasilense* strains TS8, TS13, and TS29 (Meunchang, Panichsakpatana, Ando, & Yokoyama, 2004) were used as reference strains. Bacteria were grown in NFb broth containing 0.2 g L^{-1} of yeast extract and 1 g L^{-1} of NH_4Cl at 28 ^oC for 48 h on vigorous shaking at 120 rpm. The resulting bacterial cells, approximately 1×10^7 cfu mL^{-1} determined by a dilution plate count method.

2.2.1 Seed germination and germination index

Corn seeds were prepared using a modified method of Gholami, Shahsavani, and Nezarat (2009). Seed surfaces were sterilized with 1% (w/v) chloramine T for 1 min and rinsed thoroughly in sterile distilled water under vigorous shaking. Ten seeds of each variety were arranged in Petri dishes and sterilized paper towels as germination devices. The seeds were inoculated with 5 mL of each isolate and incubated under dark conditions at 28 ^oC for 48 h. Sterilized distilled water was used for the uninoculated control. Each isolate was tested in duplicate.

$$\text{Germination index (GI, \%)} = \frac{\text{Seed germination (\%)} \times \text{root length of treatment}}{\text{Seed germination (\%)} \times \text{root length of control}} \times 100$$

2.2.2 Plant test

To evaluate their growth promoting abilities, all isolates were inoculated on corn. Two popular varieties of sweet corn in Thailand, i.e. Insee 2 and Hi-brix 3, were used. The seeds were sterilized as described above and incubated at 28 ^oC for 24 h on sterilized filter paper with sterilized distilled water to promote germination. Germinated seeds were placed in a 300 mL glass bottle filled with sterilized sand and a 5 kg plastic pot filled with sterilized soil. The characteristics of the soil that included soil texture (pipette method), pH, organic matter (Walkley & Black, 1943), total nitrogen (Kjeldahl method), available P (Bray II), and exchangeable K (Issac & Kerber, 1971) are presented in Table 2. Prior to inoculation, all isolates were grown on NFb broth as described above and applied to the aseptic corn seeds. A sterilized N-Free nutrients solution (Broughton & Dilworth, 1970) was added to each bottle corresponding to a 60% moisture level. The plants were

Table 1. Summary of plant growth promoting traits analysis.

Isolate	Origins	Part of isolation	IAA production (mgL^{-1})	ARA (nmole C_2H_4 $tube^{-1}h^{-1}$)	Phosphate Solubilization		
					Phospho-molybdate blue color method (mgL^{-1})	SRSM agar plate	
						Halo zone	pH
LB 1-1	LB	R	37.17	135.23	37.00	-	A
LB 1-2	LB	R	23.05	96.67	55.50	-	A
LB 1-3	LB	R	32.99	138.53	65.25	-	A
LB 1-4	LB	R	35.19	176.76	66.00	-	A
NW 1-1	NW	R	12.82	31.95	64.50	-	A
NW 1-2	NW	R	12.56	39.57	71.75	+	A
NW 1-3	NW	R	10.99	35.37	62.50	-	A
NW 1-4 W	NW	R	13.39	32.11	53.75	-	A
NW 1-5	NW	R	12.69	36.26	53.75	-	A
NW 2-1	NW	R	23.65	54.05	54.75	-	A
NW 2-2	NW	R	23.99	41.26	67.25	+	A
NW 3-1	NW	R	14.08	47.79	56.75	-	A
NW 3-2	NW	R	15.75	40.59	65.75	-	A
NW 3-3	NW	R	14.09	41.28	61.50	-	A
NW 3-4	NW	R	11.68	38.59	66.00	-	A
SR 1-3	SR	R	3.25	25.13	64.25	-	A
SR 1-4	SR	R	3.11	24.79	74.25	+	A
SR 1-5	SR	R	15.29	39.89	96.25	-	A
TS13*	CN	Rh	69.00	65.00	28.00	-	A

IAA = indole-3-acetic acid; ARA = acetylene reduction assay; SRSM = synthetic replacement sporulation media; LB = Lop Buri Province, NW = Nakhon Sawan Province, SR = Saraburi Province, CN = Chainat Province; R = roots; Rh = rhizo-sphere soil; A = acid (color of media changed to yellow) where (-) stands for negative test and (+) stands for positive test.

Table 2. Physical and chemical properties of soil before planting.

Properties	Soil sample
Soil texture	Sandy clay loam
pH (soil:water = 1:1)	7.36
Organic matter	15.10 g kg^{-1}
Total nitrogen	0.80 g kg^{-1}
Available P	248 mg kg^{-1}
Exchangeable K	155 mg kg^{-1}

grown in a growth chamber under controlled conditions: 16 h photoperiod at 28 ºC and 8 h of darkness at 18 °C. A sterilized N-Free nutrients solution (Broughton & Dilworth, 1970) was added to each pot corresponding to a 60% moisture level in the first week and sterilized distilled water was used for irrigation during the growth period. Plants were grown under greenhouse conditions. Each isolate was tested on five replications (2 plants/jar and 1 plant/pot)and uninoculated control plants were included according to a completely randomized design (CRD). Three weeks (growth chamber condition) and six weeks (greenhouse condition) later, the endophytic nitrogen fixing abilities were evaluated. The corn roots were washed by tap water several times to remove all bacteria fixed to the root surface. Therefore, the activities were supposed to be mainly induced by the endophytic bacteria in the roots. The ethylene production was determined by ARA according to the method described by Hardy *et al.* (1968). The inoculation effect of each isolate was estimated to determine the relative index of endophytic ARA activity using the following formula:

Relative index of endophytic ARA activity = (Inoculated plants ARA activity) – (Control plants ARA activity)

On the other hand, plant height and root length of each plant were also determined. Dry weight was calculated by drying the plants in an oven at 65 °C until constant weight was recorded.

2.3 Statistical analyses

The collected data were subjected to one-way analysis of variance (ANOVA) using MSTAT statistical software and the significant differences were assessed by Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$ and $P \leq 0.01$ to evaluate the effects of the *Azospirillum* on sweet corn growth.

3. Results and Discussion

3.1 Effects of *Azospirillum* on seed germination

The data revealed that some treatments promoted corn seed germination (Table 3). The highest germination percentage (95%) was observed with the uninoculated (control) seeds in Insee 2 and seeds inoculated with LB1-3 in Hi-Brix3 (Table 3). Moreover, in the case of Insee 2, a germination percentage of 90% was observed when the seeds were inoculated with TS29 and also in Hi-Brix 3 seeds inoculated with LB1-1. The GI is a way to check the effects of inoculation on seed germination by comparison with sterilized distilled water (control). The Insee 2 seeds inoculated with LB1-2 showed the highest GI (62.06%) even though it had a

Table 3. Effects of *Azospirillum* on corn seeds germination and Germination index (GI) of two sweet corn varieties, Insee 2 and Hi-Brix 3.

Treatments	Insee 2		Hi-Brix 3	
	Seed Germination	GI	Seed Germination	GI
	%			
Control	95	-	80	-
TS8	65	25.15	70	19.64
TS13	80	27.52	60	25.94
TS29	90	38.49	65	29.16
LB1-1	75	46.40	90	79.86
LB1-2	80	62.06	75	37.96
LB1-3	85	34.96	95	50.51
LB1-4	65	27.38	90	42.32

germination percentage of 80%. However, seeds inoculated with TS29 ranked second in germination percentage (90%) but had only a GI of 38.49% (Table 3). The Hi-Brix 3 seeds inoculated with LB1-1 had the highest GI (79.86%) but ranked second in germination percentage (90%). Also, the highest germination percentage was obtained from seeds inoculated with LB1-3 but had a GI of only 50.51% (Table 3). Our results are in agreement with Noumavo *et al.* (2013) who found that inoculating maize seed with *A. lipoferum* and a combination of *Pseudomonas fluorescens* and *P. putida* increased seed germination by 98.33% and 100%, respectively.

3.2 Effects of *Azospirillum* on plant growth

The seeds inoculated with all strains had similar plant growth to the uninoculated seeds in both varieties under laboratory conditions (Table 4). Only Insee 2 seeds inoculated with all strains had similar growth to the uninoculated seeds in greenhouse conditions during the experiment (10 to 40 days after seeding [DAS]). All Hi-brix 3 inoculated seeds had similar growth in 20 DAS, while the others were significantly different at 10 DAS, 30 DAS, and 40 DAS. At 10 DAS and 30 DAS, the heights of the plants that were inoculated with LB1-4 were significantly different from the control but at 40 DAS all treatments were similar to the control except for LB1-2 and LB1-3 which were significantly different (Tables 5 and 6). The root lengths under laboratory conditions were significantly different between treatments in both varieties. The greatest root lengths were obtained for LB1-2 treatment in Insee 2 and LB1-3 treatment in Hi-brix 3 (Table 4). Only Hi-brix 3 in greenhouse conditions was not significantly different between treatments in root length (Tables 5 and 6). Plants treated with LB1-1 had the greatest root length followed by LB1-2, LB1-3, and LB1-4 in Insee2. Hi-Brix 3 treated with LB1-2 showed the greatest root length followed by the LB1-4 and TS8 treatments (Table 6). Hence, the Insee 2 seeds treated with LB1-1 and LB1-2 displayed the greatest root length in both the laboratory and greenhouse conditions and the seeds

Table 4. Effects of *Azospirillum* on plant height, root length, dry matter, and nitrogenase activity (ARA) under laboratory conditions.

Treatments	Shoot		Root		Root/Shoot ratio	ARA (nmole C_2H_4 h^{-1} g root dry $weight^{-1}$)
	Dry weight (g)	Height (cm)	Dry weight (g)	Length (cm)		
Insee 2						
Control	0.121 bc	31.8	0.103 c	6.9 b	0.87 bc	0.827
TS8	0.194 a	32.1	0.244 b	13.6 a	1.26 bc	0.480
TS13	0.169 ab	31.2	0.225 b	15.0 a	1.32 bc	0.755
TS29	0.105 c	28.1	0.157 bc	10.4 ab	1.48 b	1.103
LB1-1	0.141 abc	31.5	0.170 bc	11.8 ab	1.21 bc	0.870
LB1-2	0.165 ab	35.0	0.349 a	16.1 a	2.18 a	0.643
LB1-3	0.140 abc	33.7	0.105 c	10.4 ab	0.74 c	0.767
LB1-4	0.174 ab	37.5	0.144 bc	12.6 ab	0.82 bc	1.399
C.V. (%)	18.62	17.94	19.43	19.56	18.22	18.42
Significant	*	ns	*	**	**	ns
Hi-brix 3						
Control	0.151	35.4	0.191 b	10.6 b	1.26	0.804
TS8	0.129	34.6	0.159 b	11.7 ab	1.23	0.540
TS13	0.196	38.4	0.310 a	14.1 ab	1.65	0.743
TS29	0.131	32.7	0.192 b	13.3 ab	1.51	1.047
LB1-1	0.145	30.6	0.218 ab	13.1 ab	1.52	0.665
LB1-2	0.168	30.7	0.224 ab	10.1 b	1.39	0.493
LB1-3	0.142	37.2	0.188 b	15.5 a	1.38	0.660
LB1-4	0.114	32.9	0.116 b	11.4 ab	1.01	1.077
C.V. (%)	14.31	14.04	15.22	18.78	18.72	16.10
Significant	ns	ns	**	*	ns	ns

* = P≤0.05, ** = P≤0.01, ns = non-significant
In a column, the means with different letters are significantly different by DMRT
ARA = acetylene reduction assay

Table 5. Effects of *Azospirillum* on Insee 2 corn plants height, root length, dry matter and nitrogenase activity (ARA) under greenhouse conditions.

Treatments	Shoot					Root		Root/Shoot ratio	ARA (nmole C_2H_4 h^{-1} g root dry $weight^{-1}$)
	Dry weight	Height (cm)				Dry weight	Length		
	(g pot^{-1})	10 DAS	20 DAS	30 DAS	40 DAS	(g pot^{-1})	(cm)		
Control	12.44	43.9	65.1	77.4	102.0	5.18 b	38.5 ab	0.42 d	0.015
TS8	17.53	48.0	74.6	89.8	110.5	12.89 ab	32.7 ab	0.71 cd	0.010
TS13	20.00	42.4	66.1	83.2	102.0	14.92 a	29.3 b	0.75 bcd	0.010
TS29	16.44	44.0	66.5	83.1	101.0	15.22 a	36.9 ab	0.95 abc	0.019
LB1-1	17.48	46.1	73.0	88.3	92.5	19.38 a	48.8 a	1.05 abc	0.016
LB1-2	15.74	43.3	69.9	88.3	106.3	21.13 a	46.3 ab	1.34 a	0.008
LB1-3	17.74	45.4	70.6	87.4	103.5	19.81 a	46.3 ab	1.14 ab	0.010
LB1-4	18.50	48.8	69.3	83.9	105.8	18.55 a	45.8 ab	1.00 abc	0.016
C.V. (%)	18.60	11.20	18.30	14.70	12.90	16.82	18.24	17.53	13.10
Significant	ns	ns	ns	ns	ns	*	**	**	ns

* = P≤0.05, ** = P≤0.01, ns = non-significant
In a column, the means with different letters are significantly different by DMRT
ARA = acetylene reduction assay

Table 6. Effect of *Azospirillum* on Hi-Brix 3 corn plants height, root length, dry matter and nitrogenase activity (ARA) under greenhouse condition.

Treatments	Shoot					Root		Root/Shoot ratio	ARA (nmole C_2H_4 h^{-1} g root dry $weight^{-1}$)
	Dry weight	Height (cm)				Dry weight	Length		
	(g pot^{-1})	10 DAS	20 DAS	30 DAS	40 DAS	(g pot^{-1})	(cm)		
Control	11.57 b	41.4 bc	56.6	61.4 b	84.3 b	5.13 c	35.0	0.45 b	0.014
TS8	18.15 a	44.5 abc	67.6	86.3 a	101.8 ab	12.60 b	41.8	0.69 b	0.007
TS13	16.95 a	39.6 c	60.9	86.0 a	104.3 ab	11.24 bc	39.8	0.68 b	0.012
TS29	15.23 ab	48.3 ab	69.5	83.0 a	101.8 ab	10.69 bc	38.5	0.70 b	0.012
LB1-1	15.00 ab	42.9 abc	61.5	72.3 ab	103.0 ab	22.03 a	31.8	1.499 a	0.012
LB1-2	18.83 a	40.0 c	61.9	85.0 a	107.5 a	15.15 b	46.0	0.80 b	0.011
LB1-3	15.90 ab	42.1 abc	67.0	86.1 a	110.5 a	11.93 bc	38.8	0.79 b	0.010
LB1-4	14.97 ab	48.5 a	70.6	84.0 a	98.8 ab	11.58 bc	44.3	0.79 b	0.008
C.V. (%)	17.76	9.80	18.60	16.40	13.60	15.22	18.11	15.21	10.64
Significant	*	*	ns	*	*	*	ns	*	ns

* = P≤0.05, ** = P≤0.01, ns = non-significant
In a column, the means with different letters are significantly different by DMRT
ARA = acetylene reduction assay

treated with LB1-4 had the greatest height under laboratory conditions (Table 4). A plausible explanation for differential growth promotion of the bacteria could be the composition of the bacterial community depends to some degree on the specificity of particular bacterial genotypes for particular sweet corn cultivar and different plant growth promoting abilities (Reis-Junior, Reis, Silva, & Döbereiner, 2000; Taulé *et al.*, 2011). However, Laslo *et al.* (2012) and Shaharoona, Arshad, Zahir, and Khalid (2006) reported that bacteria isolated from maize rhizosphere had different plant growth promoting and biocontrol activities which support the results from *Azospirillum* used in this study that produced IAA (Table 1) with enhanced shoot and root lengths under control conditions.

3.3 Effect of *Azospirillum* on shoot and root dry weight of corn plant

Significant differences were found between the treatments under laboratory conditions for the shoot and root dry weights of Insee 2. TS8 had the highest shoot dry weight and LB1-2 had the highest root dry weight (Table 4). No significant differences were found between treatments in shoot dry weight for Hi-brix 3 but a highly significant difference was obtained in root dry weight. Seeds treated with TS13 showed the highest shoot and root dry weights (Table 4). Under greenhouse conditions, no significant differences were found in shoot dry weight of Insee 2 or plants treated with TS13 which had the highest shoot dry weight. A

significant difference was obtained between treatments in root dry weight and the highest was obtained from the LB1-2 treatment (Table 5). The shoot and root dry weights for Hi-brix 3 had significant differences between the treatments. Plants treated with LB1-2 had the highest shoot dry weight and LB1-1 had the highest root dry weight (Table 6). Moreover, TS13 treatment in the Insee 2 variety showed the same results in both conditions. A highly significant difference was found between treatments in the root and shoot ratios of Insee 2 in both conditions and the highest was with the LB1-2 treatment (Table 4). Under laboratory conditions, Hi-brix 3 had no significant differences between treatments, whereas under greenhouse conditions significant differences were found. Plants treated with TS13 and LB1-1 had the highest root and shoot ratios under laboratory and greenhouse conditions, respectively (Tables 4 and 6). However, the expression of such bacterial activities under laboratory conditions does not guarantee an association with a host plant (Fuentes-Ramirez & Caballero-Mellado, 2006). On the other hand, Nezarat and Gholami (2009) reported that different PGPR strains improved the shoot fresh and dry weight of maize in axenic and field conditions.

3.4 Effect of *Azospirillum* on nitrogen fixation

Nitrogen fixation is naturally the first major mechanism of action suggested for the enhancement of plant growth by *Azospirillum*. Incorporation of atmospheric nitrogen into the host plants by *Azospirillum* was evaluated mainly by the ARA (which simulates N_2 fixation) (Van Berkum & Bohlool, 1980). Endophytic properties were determined by evaluating the endophytic ARA activity. The ARA values indicated in Table 7 were mainly induced by endophytic bacteria in roots. All treatments had endophytic characters but showed a difference in the growth conditions. In the case of Insee 2, only three treatments (TS29, LB1-1, and LB1-4) showed endophytic characters in both conditions. However, in Hi-Brix 3, only TS29 and LB1-4 showed endophytic characters under laboratory conditions and all treatments had no endophytic characters in the greenhouse conditions (Table 7). The possible explanation of the activity was due to bacterial ARA activity (Table 1). However, endophytic ARA activity of sweet corn plants was detected only in TS29 and LB1-4 inoculated plants in both cultivars. Similar to our results, Rodrigues *et al.* (2008) also reported that *A. amazonense* inoculated on rice had no detectable correlation between the growth-promoting effect and the amount of ARA activity.

The interaction between associative *Azospirillum* and plants can be unstable and good results obtained *in vitro* cannot always be dependably reproduced under field conditions. The variability in the performance of PGPR may be due to various environmental factors that may affect their growth and exert their effects on plants (Saharan & Nehra, 2011). Many studies have also shown that the growth-promoting abilities of some bacteria may be highly specific to certain plant species, cultivar, and genotype (Lucy, Reed & Glick, 2004). Therefore, the endophytic bacteria may be influenced by the host plant and other endogenous organisms. Thus, a way to ensure the stable existence of endophytic bacteria must be developed by taking these influences in consideration.

Table 7. Endophytic ARA under laboratory and greenhouse condition (nmole C_2H_4 h^{-1} g root dry $weight^{-1}$).

Treatments	Insee 2		Hi-Brix 3	
	Laboratory	Greenhouse	Laboratory	Greenhouse
Control	-	-	-	-
TS8	-	-	-	-
TS13	-	-	-	-
TS29	0.276	0.004	0.243	-
LB1-1	0.043	0.001	-	-
LB1-2	-	-	-	-
LB1-3	-	-	-	-
LB1-4	0.572	0.001	0.273	-

ARA = acetylene reduction assay

4. Conclusions

This study confirms the effect of *Azospirillum* on the germination, seedling growth, and nitrogen fixing ability under laboratory and greenhouse conditions in sweet corn varieties Insee 2 and Hi-brix 3 inoculated with seven strains of *Azospirillum*. The highest germination rates were observed in the uninoculated seeds of Insee 2 and LB1-3 inoculated Hi-Brix 3 seeds. Conversely, the highest GI percentage was observed in Insee 2 and Hi-Brix 3 seeds when inoculated with LB1-2 and LB1-1, respectively. The results suggested that inoculated sweet corn seeds with plant growth-promoting *Azospirillum* could promote seed germination and seedling growth in both laboratory and greenhouse conditions. Therefore, use of these *Azospirillum* isolates in sweet corn is an efficient alternative biological fertilizer and could increase sweet corn seed germination and output of corn in the field.

References

Asghar, H. N., Zahir, Z. A., Arshad, M., & Khaliq, A. (2002). Relationship between in vitro production of auxins by rhizobacteria and their growth promoting activities in *Brassica juncea*. L. *Biology and Fertility of Soils Journal, 35*(4), 231-237.

Attitalla, I. H., Alhasin, A. M., Nasib, A. M., & Ghazali, A. H. (2010). Occurrence and microbiological characteristics of *Azospirillum* strains associated with legumenous and non-leguminous plants in AI Jabal Al Akhdar eco-region, Libya. Am-Euras. *Journal Agriculture and Environment Science, 8*(6),617-625.

Bashan, Y., Holguin, G., & de-Bashan, L. E. (2004). *Azospirillum*- plant relationships: Physiological, molecular, agricultural, and environmental advances. *Canadian Journal of Microbiology, 50*(8), 521–577.

Broughton, W. J., & Dilworth, M. J. (1970). Methods in legume-rhizobium technology: Plant nutrient solutions. In P. Somasegaran & H. J. Hoben (Eds.), *Handbook for Rhizobia* (pp. 245-249). Maui, HI: NifTAL Project and University of Hawaii.

Burr, T. J., Schroth, M. N., & Suslow, T. (1978). Increased potato yields by treatment of seed pieces with specific strains of *Pseudomonas fluorescens* and *P. putida*. *Phytopathology Journal*, *68*, 1377-1383.

Cakmakc, R. I., Aydın, D. F. & Sahin, A. F. (2006). Growth promotion of plants by plant growth-promoting rhizobacteria under greenhouse and two different field soil conditions. *Soil Biology and Biochemistry Journal*, *38*(6), 1482–1487.

Cassán, F., Perrig, D., Sgroy, V., Masciarelli, O., Penna, C., & Luna, V. (2009). *Azospirillum brasilense* Az39 and *Bradyrhizobium japonicum* E109, inoculation singly or in combination, promote seed germination and early seedling growth in corn (*Zea may* L.) and soybean (*Glycine max* L.). *European Journal of Soil Biology*, *45*(1), 28-35.

Döbereiner, J., Marriel, I. E., & Nery, M. (1976). Ecological distribution of *spirillum lipoferum* Beijerinck. *Canadian Journal of Microbiology*, *22*(10), 1464-1473.

El Zemrany, H., Cortet, J., Peter Lutz, M., Chabert, A., Baudoin, E., Haurat, J., Moënne-Loccoz, Y. (2006). Field survival of the phytostimulator *Azospirillum lipoferum* CRT1 and functional impact on maize crop, biodegradation of crop residues, and soil faunal indicators in a context of de-creasing nitrogen fertilization. *Soil Biology and Biochemistry Journal*, *38*(7), 1712-1726.

Fuentes-Ramirez, L., & Caballero-Mellado, J. (2006). *Bacterial biofertilizers, PGPR: Biocontrol and Biofertilization* (pp. 143-172). Heidelberg, German: Springer-Verlag,

Gholami, A., Shahsavani, S., & Nezarat, S. (2009). The effect of plant growth promoting rhizobacteria (PGPR) on germination, seedling growth and yield of maize. *World Academy of Science, Engineering and Technology*, *49*, 19-24.

Hardy, R. W. F., Holsten, R. D., Jackson, E. K., & Burns, R. C. (1968). The acetylene-ethylene assay for N_2 fixation: Laboratory and Field Evaluation. *Plant Physiology*, *43*(8), 1185-1207.

Issac, R. A., & Kerbe, J. D. (1971). Atomic absorption and flame photometer: Techniques and uses in soil, plant and water analysis. In L. M. Walsh (Ed.), *Instrumental methods for analysis of soil and plant tissue* (pp. 17-37). Madison, WI: Soil Science Society of America.

Kloepper, J. W., Tuzun, S., & Kuc, J. A. (1992). Proposed definitions related to induced disease resistance. *Biocontrol Science and Technology*, *2*(4), 349-351.

Laslo, E., Gyorgy, E., Mara, G., Tamas, E., Abraham, B., & Lanyi, S. (2012). Screening of plant growth promoting rhizobacteria as potential microbial inoculants. *Crop Protection Journal*, *40*, 43-48

Lucy, M., Reed, E., & Glick, B. R. (2004). Applications of free living plant growth-promoting rhizobacteria. *Antonie Leeuwenhook International Journal of General and Molecular Microbiology*, *86*(1), 1-25.

Mehnaz, S., Weselowski, B., & Lazarovits, G. (2007). *Azospirillum canadense* sp. nov., a nitrogen-fixing bacterium isolated from corn rhizosphere. *International Journal of Systematic and Evolutionary Microbiology*, *57*(3), 620-624.

Meunchang, S., Panichsakpatana, S., Ando, S., & Yokoyama, T. (2004). Phylogenetic and physiological characterization of indigenous *Azospirillum* isolates in Thailand. *Soil Science and Plant Nutrition*, *50*(3), 413-421.

Murphy, J., & Riley, J. R. (1962). A modified solution method for determination of phosphate in natural water. *Analytica Chimica Acta*, *27*, 31-36.

Nezarat, S., & Gholami, A. (2009). Screening plant growth promoting rhizobacteria for improving seed germination, seedling growth and yield of maize. *Pakistan Journal of Biological Sciences*, *12*(1), 26-32.

Noumavo, P. A., Kochoni, E., Didagbe, Y. O., Adjanohoun, A., Allagbé, M., Sikirou, R., . . . Baba-Moussa, L. (2013). Effect of different plant growth promoting rhizobacteria on maize seed germination and seedling development. *American Journal of Plant Sciences*, *4*(5), 1013-1021.

Office of Agricultural Economic. (2016). Thailand foreign agricultural trade statistics 2016. Retrieved from http://www.oae.go.th

Office of Agricultural Economic. (2017). Thailand foreign agricultural trade statistics 2017. Retrieved from http://www.oae.go.th

Patriquin, D. G., & Döbereiner, J. (1978). Light microscopy observations of tetrazolium-reducing bacteria in the endorhizosphere of maize and other grasses in Brazil. *Canadian Journal of Microbiology*, *24*(6), 734-742.

Puente, M. L., Garcia, J. E., & Alejandro, P. (2009). Effect of the bacterial concentration of *Azospirillum brasilense* in the inoculums and its plant growth regulator compounds on crop yield of corn (*Zea mays* L.) in the field. *World Journal of Agricultural Sciences*, *5*(5), 604-608.

Reis-Junior, F., Reis, V., Silva, L., & Döbereiner, J. (2000). Levantamento e quantificaçao de bactérias diazotróficas em diferentes genotipos de cana-de-açúcar (*Saccharum* spp.). *Embrapa Informação Tecnológica Pesquisa Agropecuária Brasileira*, *35*, 985-994.

Rodrigues, E. P., Rodrigues, L. S., de Oliveira, A. L. M., Baldani, V. L. D., Teixeira, K. R. D., Urquiaga, S., & Reis, V. M. (2008). *Azospirillum amazonense* inoculation: Effects on growth, yield and N-2 fixation of rice (*Oryza sativa* L.). *Plant and Soil*, *302*, 249-261.

Saharan, B. S., & Nehra, V. (2011). Plant growth promoting Rhizobacteria: A critical review. *Life Sciences and Medicine Research*, *2011: LSMR-21*, 1-30.

Shaharoona, B., Arshad, M., Zahir, Z. A., & Khalid, A. (2006). Performance of *Pseudomonas* spp. Containing ACC-deaminase for improving growth and yield of maize (*Zea mays* L.) in the presence of nitrogenous fertilizer. *Soil Biology and Biochemistry Journal*, *38*(9), 2971-2975.

Shaukat, K., Affrasayab, S., & Hasnain, S. (2006). Growth responses of Helianthus annus to plant growth promoting rhizobacteria used as a biofertilizer. *International Journal of Agricultural Research*, *1*(6), 573-581.

Taulé, C., Mareque, C., Barlocco, C., Hackembruch, F., Reis, V. M., Sicardi, M., & Battistoni, F. (2011). The contribution of nitrogen fixation to sugarcane (*Saccharum officinarum* L.), and the identification and characterization of part of the associated diazotrophic bacterial community. *Plant and Soil*, *356*, 35-49.

Van berkum, P., & Bohlool, B. B. (1980). Evaluation of nitrogen-fixation by bacteria in association with roots of tropical grasses. *Microbiological Reviews*, *44*, 491-517.

Walkley, A., & Black, I. A. (1934). An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Journal of Soil Science*, *37*, 29-38.