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Short Communication

Potentials of *Azospirillum Spp.* for Improving Shoot and Root of a Malaysian Sweet Corn Variety (J 58) under *In Vitro* Condition

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Abstract

Azospirillum is a N_2-fixing bacterium which can improve root, shoot as well as total biomass of different cereals in tropical regions where soil is less fertile and eventually production cost is high. In this proposition, a Malaysian sweet corn hybrid variety (J 58) was inoculated with four *Azospirillum* strains: *Azospirillum brasilense* Sp7, *A. brasilense* UPMB 10, *A. brasilense* CCM and *A. brasilense* B-7C and five replicated trails with four treatments including: (i) without *Azospirillum* and without N (-A-N), (ii) without *Azospirillum* and with N (-A+N), (iii) with *Azospirillum* and without N (+A-N), (iv) with *Azospirillum* and with N (+A+N), were conducted to evaluate the efficacy of *Azospirillum spp*. for *in vitro* sweet corn development by observing the responses of different plant parts, root and shoot to these treatments. In these investigations, *A. brasilense* strains inoculated corn seedlings were formed longer roots compare to control at 7th day. There was no significant difference with N treatments but when N and *Azospirillum* were used together the results were remarkably altered. In case of root number, the highest number of root was observed in *A. brasilense* CCM with N followed by *A. brasilense* Sp7 and *A. brasilense* UPMB 10. Moreover, *Azospirillum* with N performed the best results for lateral and tertiary root formation. The dry weight of roots was also enhanced and the impact was statistically sound. Similar effects were observed in shoot development and total biomass expansion. The highest biomass was obtained from *A. brasilense* CCM with N treatment. On the contrary, the lowest was observed in control, which indicated the efficacy of different *Azospirillum spp.* on major developmental parameters of corn variety in conjunction with nitrogen fertilizer. The gathered information will explore the potential use of *Azospirillum spp.* for high yield of sweet corn in Malaysian tropical environment.

Keywords: *Azospirillum*; Root-shoot development; Biomass; Sweet corn

Introduction

Sweet corn (*Zea mays* L.) is an important and widely used cereal in Malaysia but the production is not satisfactory due to less fertile high land and high production costs. Improved appropriate cultivation practice and use of bio-fertilizer can increase productivity of corn as well as minimize production costs and at the same time avoid environmental hazards as stated by Galal *et al*. (2001). On the other hand, atmosphere contains 78% nitrogen, which may be an important nutrient supplier for maize production through using *Azospirillum* spp. as a diazotrophs that can fix nitrogen as a free-living organism (Ramos *et al*., 2002). *Azospirillum* can improve crop growth utilizing the phyto-hormones that accelerate root growth, water absorption and minerals which finally lead to a larger yield and more productive plants (Molla *et al.,* 2001). Moreover, *Azospirillum* increases root length, appearance, elongation rates and density of root hairs. It also increases root surface area and water uptake rate in most cases. These effects cause roots to reach into deeper layers and take up more water and minerals, thus resulting faster plant growth. Under appropriate agronomic conditions, these processes had showed increase crop yield (Sarig *et al.,* 1984). Though *Azospirillum* is not a principal component of the Temperate Zone (Lindberg and Granhall, 1984), in tropical soil, 10^4-10^7 *Azospirillum* cells has been detected in g^{-1} dry weight of roots. *Azospirillum* exists as groups in grain grasses such as wheat, corn, rice, sugarcane and sorghum (Barber *et al.,* 1976; Bothe *et al.,* 1992). They also mentioned that roots of tropical grasses for example wheat (Pedersen *et al.,* 1978), Maize (Barber *et al.,* 1976) and many other plants and soil can contain *Azospirillum* in larger quantities and they defined *Azospirillum* as a N_2-fixing bacterium.

Research results showed that in tropical Malaysia, *Azospirillum* influenced positive effect on different crops like oil plum (Shamsuddin *et al.,* 1995), vegetable soybean (Molla *et al.,* 2001) and banana (Mia *et al.,* 1999). So, there is a great possibility to use *Azospirillum* as bio-fertilizer for corn production. In these investigations, we tried to identify the effects of different *Azospirillum* strains along with nitrogen fertilizer on *in vitro* sweet corn seedlings growth and development, which will indicate the possibility of *Azospirillum* uses in field experiment.

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Materials and Methods

The research was conducted at Soil Microbiology Laboratory of University Putra Malaysia, Serdang, Malaysia.

Organisms and Growth Environments

Azospirillum strains were grown in 100 mL (Okon media) flask at room temperature for 24 h. For Okon medium preparation, each part of the medium was prepared individually before mixing together. Buffer was prepared separately and added to the medium for bacterial inoculation. All components of the medium were mixed with less than 1 L of distilled water in a beaker and stirred with a magnetic stirrer. The pH was adjusted to 6.8 and after stabilization water was added up to 1 L. After that 100 mL of the medium was poured into each of 10 conical flasks. The conical flask neck was then covered with cotton and wrapped with aluminum foil prior to autoclaving at 121ºC for 20 min. This Okon media (Okon *et al.,* 1977) was used for the bacteria inoculation.

Seed Surface Disinfection

Disinfection of seeds was done according to Neuer *et al.* (1985). Seeds were treated with 5.25% sodium hypochlorite (NaOCl) solution for 10 min then with ethanol for 10 minutes and finally washed with sterile water for 5 to 6 times. Age of seedling was counted according to Langer (1979) instructions and it took 1-2 days for first three seminal roots formation.

Impacts of *Azospirillum* on Development of Root

Corn grain surface were sterilized and the tips were excised then placed in Eppendorf tube. These Eppendorf tubes were then placed in 25 mL sized test tubes containing 10 mL wheat medium (7 gm wheat flour/L) with 3.5 mg $NaNO_3$/L. The test tubes supplemented with 10 micro liter of one day old *Azospirillum* culture with density of 1 x 10^{-9} cfu/mL. All alterations were done in sterile condition.

***Azospirillum* strains:** Four *Azospirillum* strains: *Azospirillum brasilense* Sp7, *A. brasilense* UPMB 10, *A. brasilense* CCM and *A. brasilense* B-7C were used as an inoculums on corn (J 58) seedlings.

Treatments: Four types of treatment were used in this experiment such as (i) Without *Azospirillum* and without N (-A-N), (ii) without *Azospirillum* and with N (-A+N), (iii) with *Azospirillum* and without N (+A-N) and (iv) with *Azospirillum* and with N (+A+N). Nitrogen was used as nitrate @ 0.4 mM/test tube suggested by Abul *et al.* (2001).

Parameters: In this study six different parameters were evaluated, including: (i) Root number were counted visually, (ii) Root length (mm) were measured as suggested by Newman (1966) (iii) Root volume were measured by deducting increased volume through emerging total fresh roots into 30 mL water in 50 mL beaker, (iv) Root weight (fresh and dry in gm), (v) Shoot length (mm) and (vi) Shoot weight (fresh and dry in gm). Six replications were performed in completely randomized design (CRD) and the data were analyzed by using SAS 9.2 (2008) software with Microsoft Excel while DMRT test (p=0.05) were carried out for comparing different treatment means.

Plant biomass: Two plants from each treatment were collected carefully from the test tube, washed with distilled water and fresh weights of both shoots and roots recorded. Then, plant samples were oven-dried at 65°C and dry weights were recorded.

Results

In this research the planned test identified stimulatory impacts of *Azospirillum* on the development of corn seedlings (Root and Shoot), which gave effective outcomes. Distinguishing differences among inoculated crops with and without bacteria in conjunct with and without nitrogen were visible by eyes after 3 days and become clearer at 7th day presented in Fig. 1. Moreover, Fig. 2 exhibited that 7-days-old corn seedlings (inoculated with different *Azospirillum* strains) had formed much longer roots compare to control. But, there was no marked difference between nitrogen treatments (+N/-N). However, the outcomes were dramatically changed when nitrogen and *Azospirillum* were used together (Up to 120% over un-inoculated and up to 50% over N non-treated) in terms of higher root growth *viz.* root mass, root number, root length and shoot growth i.e. leaf area presented on Table 1. Additionally, substantial rise in dry weight of biomass of seedlings were observed. In case of root number, the highest number of root was observed in *A. brasilense* CCM with nitrogen followed by *A. brasilense* Sp7 and *A. brasilense* UPMB 10 (Fig. 3). Under controlled conditions, *Azospirillum* influenced to increase of the lateral and tertiary root formation (Table 2) and *Azospirillum* with nitrogen influenced better shoot development (Fig. 4).

Discussion

Potential effects of *Azospirillum* were observed in root and shoot development as well as biomass production (Table 1) of corn seedlings within 3 days and distinct differences were observed at 7 days (Fig. 1). A stimulatory impact of *Azospirillum* on the development of corn shoot gave impressive results. However, *Azospirillum* with nitrogen performed the best results for shoot development (Fig. 4). Liang and Richards (1994) and Alvarez *et al.* (1996) also observed increased coleoptiles speed of growth in wheat seedlings when they used *A. brasilense*.

When germinated corn seeds were incubated with *Azospirillum* under controlled conditions, the bacteria prompted increase of the lateral and tertiary root formation (Table 2). Perception of roots in sorghum, corn seedling, wheat and other different grasses presented a significant

Table 1: Effects of *Azospirillum* inoculation with nitrogen on *in vitro* corn seedlings development

Strain (*A. brasilense*)	Root number (No.)	Root length(mm)	Shoot length (mm)	Biomass (g plant^{-1})
Sp7	4.8a	28ab	11ab	0.032b*
CCM	4.8a	29.2a	13a	0.044a*
UPMB 10	4.6ab	27.2ab	11ab	0.030bc*
B 7C	4.4ab	24.4c	10b	0.027c*

*Values are marked with same letter(s) is not markedly different at 5% level of DMRT

Table 2: Development of lateral and tertiary roots (No. plant^{-1}) of *in vitro* corn seedlings over time

Strains (*A. brasilense*)	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day
Sp7	X	X	<5	-	-	-	106
CCM	X	X	>5	-	-	-	153
UPMB 10	X	X	<5	-	-	-	87
B - 7C	X	X	X	-	-	-	93
+N	X	X	<5	-	-	-	134
-A-N	X	X	X	X	<5	-	59

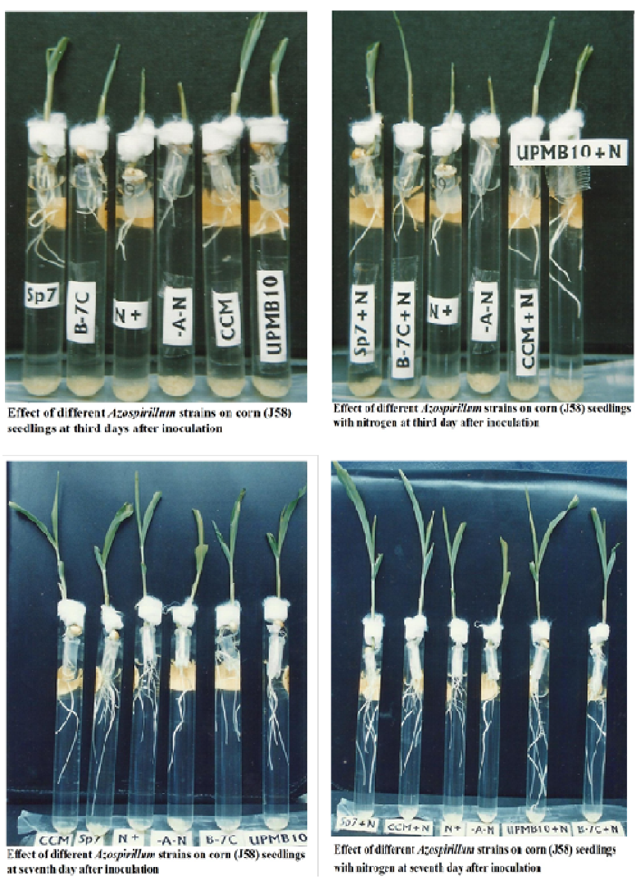

Fig. 1: Effects of *Azospirillum* strains and nitrogen on *in vitro* shoot and root development of 3 and 7 days old J 58 sweet corn seedlings

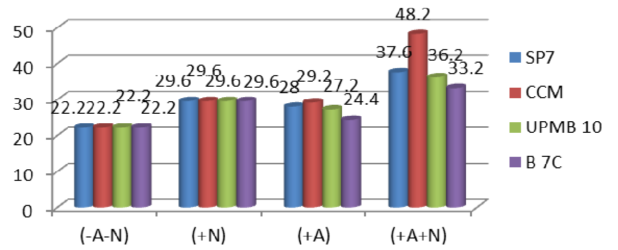

Fig. 2: Influence of different *Azospirillum* strains on *in vitro* total root length (mm) of each corn (J 58) seedlings

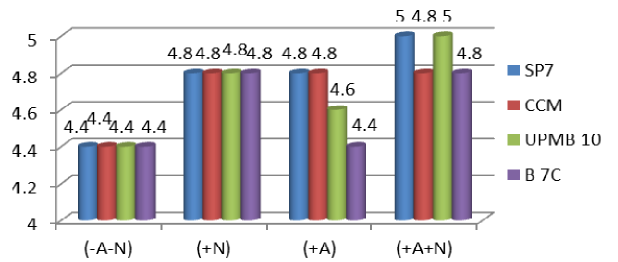

Fig. 3: Influence of different *Azospirillum* strains on root number of each corn
(J 58) seedlings

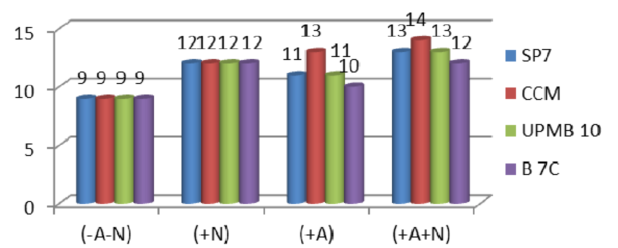

Fig. 4: Influence of different *Azospirillum* strains on *in vitro* shoot length (mm) of each corn (J 58) seedlings

impact of *Azospirillum* inoculation on root area, length and branching. Mature plants, which were inoculated transparently demonstrated an increased branch and improved system of root, whether developed hydroponically in pots or in the field (Sarig *et al.,* 1984; Okon and Kapulnik, 1986). A impact of *Azospirillum* also observed on the development of corn root length and root number (Fig. 2 and Fig. 3).

In the present investigation, a significant influence of *Azospirillum* was observed in the enhancement of root's dry weight. The highest biomass was observed in *A. brasilense* CCM with nitrogen (0.06$_{-g}$ plant^{-1}). On the contrary, the lowest was observed in control (0.02 g plant^{-1}). Similar results were also observed by Ribaudo *et al.* (2006) in tomato and Hadi *et al.* (2012) in black cumin. Reis *et al.* (2008) observed increased dry matter of maize roots inoculated with *Azospirillum* spp. supplemented with the production of growth enhancing substances. In addition, Quadros (2009) were found raised up to 3770 kg ha^{-1} of dry weight of maize shoots after inoculation of seeds by *A. brasilense* compare to control. Our study also supported by the recent observations of Maria *et al.* (2012) who mentioned that shoot dry biomass significantly influenced by the interactions between nitrogen topdressing fertilization and inoculation by *A. brasilense*, which provides developed root system through increasing of density, root hair length, volume and number of lateral roots, resulting greater uptake ability and utilization of water and nutrients.

Okon *et al.* (1983) also studied the inoculation impacts on fixation of nitrogen and yield of *Setaria italic* (foxtail millet) using *Azospirillum spp.* This test also confirmed the positive influences of *Azospirillum* on the development of crops reported by other researchers (Elmerich, 1984; Okon, 1985). Moreover, the present research presented that *A. brasilense* CCM with nitrogen performed excellent results on newly UPM released corn variety (J 58) followed by *A. brasilense* Sp7 and *A. brasilense* UPMB 10.

Conclusion

The importance of *Azospirillum* inoculation with plant, which can improve root growth, shoot development and yield, always demand critical studies. As it was mentioned above, *Azospirillum* with Nitrogen performed the best results for shoot development and total root growth. Among all strains, *A. brasilense* CCM had the best influence on total root length when inoculated with nitrogen. Moreover, *A. brasilense* Sp7 and *A. brasilense* UPMB 10 with nitrogen showed significant impact on the number of roots. Finally, *A. brasilense* CCM with nitrogen showed the dramatic result for shoot length. Thus, *A. brasilense* (Strain Sp7, CCM and UPMB 10) with N may be used as a bio-enhancer and bio-fertilizer for successful cultivation of J 58 in Malaysia.

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