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Effects of Plant Growth Promoting Rhizobacteria on Seed Germination Characteristics of Tomato and Lettuce

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Abstract

Plant growth promoting rhizobacteria (PGPR) represent a wide genera of rhizospheric bacteria which, when introduced in association with the host plant in proper amount, can enhance plant growth and productivity. A series of experiments were conducted to determine the germination responses of tomato and lettuce seeds to PGPR inoculation. Seeds were inoculated with different strains of *Azospirillum brasilense* Sp7, Sp7-S and Sp245, *Herbaspirillum seropedicea* and *Burkholderia phytofirmans* PsJN^T. The results reveal that Sp7-S inoculation yielded better germination rate and total germination of tomato. PGPR-inoculated tomato seeds, except Sp7, produced longer (28%) and heavier (37%) roots with superior vigor. In lettuce, PGPR strains, except *B. phytofirmans* PsJN^T, and Sp7 and *B. phytofirmans* PsJN^T, enhanced germination vigor and length of roots (26%), respectively. The results provide further evidence concerning rhizobacteria importance as PGPR and indicate the potential of exploiting some of these PGPR to further improve vegetable seedling emergence and establishment.

Keywords: *Azospirillum*, PGPR, seedling emergence, vegetable.

Introduction

The use of microorganisms in crop production has been a common practice in recent years. There has been an increasing interest in the interaction between PGPR and plants. PGPR are free living microorganisms that beneficially thrive well around plant roots and even invade root tissues and capable of promoting plant growth (Lee et al., 2010). The major contributions of microorganisms to plant growth include increases in germination rate and percentages, root growth, leaf surface area, water and mineral uptake, tolerance or resistance to stresses, crop yield and delayed plant senescence (Lucy et al., 2004). A diverse group of microorganism genera have been identified including the most common *Azospirillum, Bacillus, Burkholderia* and *Pseudomonas* (Kaymak, 2011).

Azospirillum spp. inoculation to improve yields have been widely explored in cereal crops (Sivasakthivelan and Saranraj, 2013). This is evident because the strains were initially isolated from the rhizosphere of many grasses and cereals worldwide, both in the tropic and temperate climates (Dobereiner et al., 1976). There are, however, few studies done in vegetables, e.g. Rodriguez et al. (2001) reported that inoculation with *Azospirillum* spp. to tomato and pepper seeds improved germination. Barassi et al. (2006) reported higher germination of inoculated lettuce with *Azospirillum* sp. both under normal and saline conditions. *Azotobacter* sp. strains 17 and 20 inoculation promoted pepper germination, whereas the *Azospirillum* strains 1 and 23 improved maize germination (Reyes et al., 2008). Kaymak et al. (2009) reported that bio-priming with *A. rubi* strain A16, *Burkholderia gladii* strain BA7, *P. putida* strain BA8, *B. subtilis* strain BA142, *B. megaterium* strain M3 under saline stress resulted in higher seed germination percentage of radish.

The mechanisms by which PGPR affect plant development are still not clear. However, one commonly proposed mechanism is PGPR-mediated biosynthesis of plant growth regulators including auxins, gibberellins (GA) and cytokinin. Auxins have a role in cell division, extension and differentiation of plant cells and tissues, they can also stimulate seed and tuber germination, increase the rate of xylem and root development both lateral and adventitious roots, and aid biosynthesis of various metabolites (Gamalero and Glick, 2011). The auxin, indole-3-acetic acid (IAA), is commonly produced by about 80% of soil bacteria isolated from the rhizosphere (Patten and Glick, 1996). IAA can promote root growth of intact and detached plant parts (Vessey, 2003). GA is equally important in the synthesis of hydrolytic enzymes during germination that degrade the stored food reserves to support growth of the growing embryo (Taiz and Zeiger, 2010). Increases in growth and yield of agronomically important crops in response to PGPR inoculation have been widely reported (Nezarat and Gholami, 2009), however, timing and mode of application, soil type, crop species and cultivar are all important in determining the degree of response (Nowak, 1998, Zahir et al., 2003). While studies of PGPR on many crop species are frequently reported in the literature, still few investigations have been done on

vegetables. Hence, the aim of the study was to determine the effect of selected PGPR strains on the germination characteristics of tomato and lettuce.

Materials and Methods

Germination and growing condition

Sterile petri dish containing 1 sheet of 90 mm diameter autoclaved Whatman filter paper no.1 was used for seed germination. Each filter paper was moistened with autoclaved millipore water prior to sowing. Petri dishes were partially sealed with parafilm to prevent water loss from evaporation. The set up was repeated three times with 40 seeds per replicates and arranged in a completely randomized design with four replications. The experiments were conducted in the light and temperature-controlled growing cabinet (Labec Laboratory Equipment, Marrickville, NSW, Australia) with constant temperature of 24 °C and a daily cycle of 12 h light and darkness (36 µmol of photons.m^{-2}.s^{-1}). All the materials used in this experiment were sterilized by either autoclaving or treating with sodium hypochlorite (NaClO) and ethanol.

Seed and PGPR preparation and inoculation

Tomato (*Lycopersicon esculentum* L. cv. Grosse lisse) and lettuce (*Lactuca sativa* L. Salinas type) seeds were surface sterilized using 1% NaClO and 70% ethanol. Seeds were then washed with autoclaved millipore water several times to remove the residual bleach and ethanol. Surface sterilized seeds were spread out in sterile Petri dish with autoclaved filter paper for 1 hr prior to inoculation. The inocula of PGPR *Azospirillum brasilense* Sp7, Sp7-S, Sp245, *Herbaspirillum seropedicea* and *Burkholderia phytofirmans* PsJN^T were provided by Dr. Rosalind Deaker, University of Sydney. Inoculum of each strain was taken from a pure culture stored with glycerol in -80°C and streaked onto the nutrient agar containing 15, 5, 3 g.L^{-1} of agar, peptone and beef extract, respectively, of water and incubated at 28°C for 2 days. A loopfull of each culture was transferred separately onto the nutrient broth containing 10 and 6 g.L^{-1} of peptone and beef extract, respectively, of water and incubated for 2 days at 28°C with constant agitation (125 rpm). The number of colony forming unit (CFU) was determined after series of dilution and agar plating. Prior to inoculation, bacterial cultures were pelleted by centrifugation (4000 x *g*, 5 min), washed twice with autoclaved 30 mM $MgSO_4$, and resuspended in the same buffer solution. Seeds were inoculated at population log 10-11 CFU.mL^{-1} by soaking the surface sterilized seeds in bacterial suspension at a volume of 200 µL per seed for 1 h to allow bacteria bind to the seed coat and for seed imbibition. Similar procedure was used for control except seeds were soaked in buffer solution.

IAA quantification

The IAA concentration in the culture supernatant of each strain was measured using a spectrophotometer at 535 nm wavelength. One mL aliquot of supernatant was mixed vigorously with 4 mL Salkowski's reagent (150 mL 98% H_2SO_4, 250 mL distilled water and 7.5 mL 0.5 M $FeCl_36H_2O$) and the mixture was allowed to stand in the dark at room temperature for 20 min (Dobbelaere et al., 1999, Patten and Glick, 2002). Absorbance was measured in triplicate and IAA concentration was determined from the standard curve.

Measurements and analysis

Daily germination was recorded and the germination value was calculated using the formula of Djavanshir and Pourbeik (1976). Root and shoot parts were weighed separately and vigor index was computed using the formula of Gamalero et al. (2008). Germinated seeds were scanned for root and shoot measurement using a Desk Scan II scanner (Expression 700, Epson, Nagano, Japan). The digitized root images were analysed using WinRhizo Pro V. 2007c (Regents instrument Inc., Quebec, Canada). Data were analysed with analysis of variance using Genstat® 14th edition software, VSN International, Hemel Hempstead, UK. Mean differences were separated using least significant difference (LSD) test ($P<0.05$).

Results and Discussion

General observation

The different PGPR strains produced an average of 2.1 µg.mL^{-1} IAA. The result suggests that these PGPR particularly *Azospirillum* possessed intrinsic ability to produce substantial quantities of IAA even without precursor amendment. *Azospirillum* spp. are known for their ability to produce plant hormones such as auxins, gibberellins, cytokinin, polyamines and amino acid in culture (Cassán et al., 2011). These phytohormones can alter plant metabolism and morphology that may lead to better absorption efficiency and as a result, healthier and productive plants (Bashan and de-Bashan, 2010).

Lettuce germinated instantly on the following day while tomato started to germinate on the 3rd day from sowing. Lettuce reached maximum germination six days from sowing whereas tomato reached peak germination after eight days. Plates were partially sealed with parafilm to reduce water loss and prevent contamination. Regardless of the crops, inoculated seeds generally germinated earlier than non-inoculated ones. Among PGPR, seeds inoculated with Sp7-S and Sp245 germinated faster than others. Based on our visual observation all inoculated germinating seeds had more vigorous germination, denser root hairs, and had thicker

and longer roots than control seeds.

Inoculation did not affect tomato and lettuce seed germination (Table 1). However, tomato seeds inoculated with *A. brasilense* Sp7-S strain had faster and higher total germination (Figure 1). Some PGPR also improved the vigor of germinating seeds of two vegetables (Table 2). In particular, superior vigor was noted due to inoculation, except Sp7 and *Burkholderia phytofirmans* in tomato and lettuce, respectively. These results corroborate with the findings of Gholami et al. (2009) who reported improved germination and vigor of maize due to PGPR inoculation.

Table 1. The effect of different PGPR on the germination characteristics of tomato and lettuce seeds.

Crop	FPG	GV	VI	RL	SL	RW	SW
Tomato	ns	*	**	**	ns	*	ns
Lettuce	ns	ns	**	**	ns	ns	ns

Note: * significant, P ≤0.05; ** highly significant, P≤0.01; ns non-significant, P>0.05. FPG: final percentage germination; GV: germination value; VI: vigor index; RL: root/radicle length; SL: shoot length; RW: root weight; SW: shoot weight

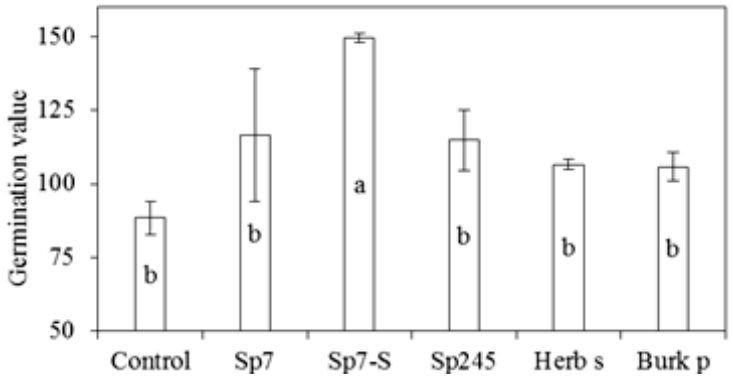

Figure 1. Germination value of tomato as influenced by PGPR; Different letters indicate significant difference (P<0.05). Error bars indicate standard error. Sp7, Sp7-S & Sp245 are strains of *A. brasilense;* Herb s: *Herbaspirillum seropedicea;* Burk p: *Burkholderia phytofirmans* PsJN^{T}.

Table 2. Vigor indexes during germination of tomato and lettuce as influenced by PGPR.

PGPR	Vigor Index	
	Tomato	Lettuce
Control (non-inoculated)	736 ± 46 b	435 ± 9 c
Azospirillum brasilense Sp7	746 ± 33 b	488 ± 14 b
A. brasilense Sp7-S	869 ± 10 a	488 ± 2 b
A.brasilense Sp245	861 ± 41 a	527 ± 18 a
Herbaspirillum seropedicea	946 ± 26 a	524 ± 8 ab
Burkholderia phytofirmans PsJN^{T}	965 ± 44 a	439 ± 15 c
LSD (5%)	110.10	38.18

Note: Means in a column followed by the same letters are not significantly different at 5% LSD

Early seedling growth characteristics

PGPR-inoculated tomato seeds, except Sp7, produced longer and heavier roots compared to non-inoculated ones (Figure 2a and 3). Inoculation increased root length by 28% and root biomass by 37% relative to the control. In lettuce, PGPR-treated seeds particularly Sp7-S, Sp245 and *Herbaspirillum seropedicea* produced longer roots up to 26% over control (Figure 2b). The impact of PGPR on root development may have been due to bacterial phytohormone biosynthesis (IAA) as it has been proposed that PGPR affects early growth stages of plant development due to production of growth substances (Bashan and de-Bashan, 2010).

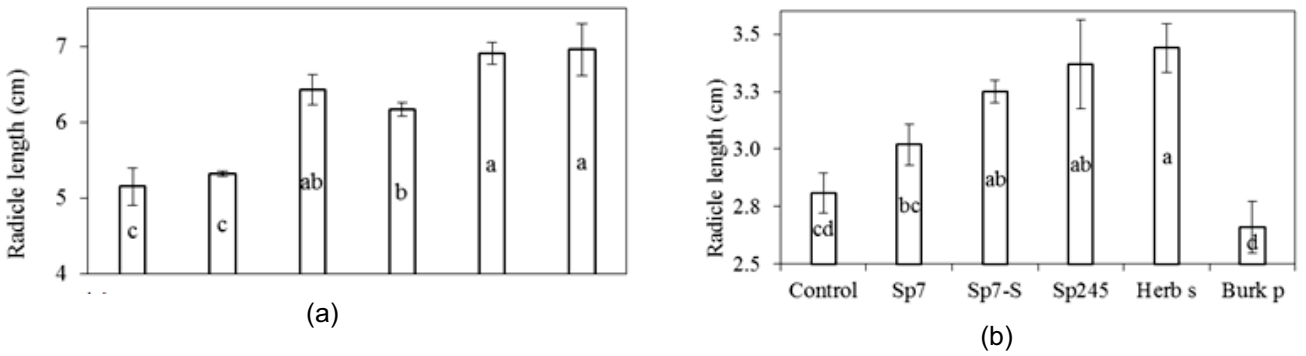

Figure 2. Root length of tomato (a) and lettuce (b) as influenced by PGPR. Different letters indicate significant differences (P<0.05). Error bars indicate standard error. Sp7, Sp7-S & Sp245: strains of *A. brasilense;* Herb s: *Herbaspirillum seropedicea;* Burk p: *Burkholderia phytofirmans* PsJN[T].

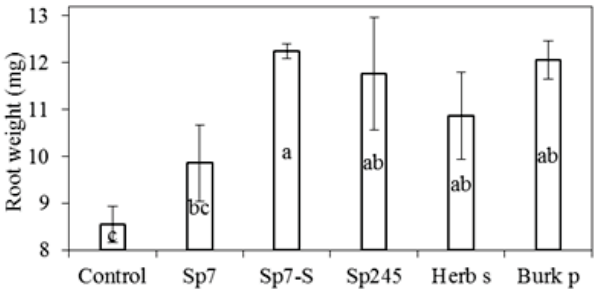

Figure 3. Root weight of tomato as influenced by PGPR; Different letters indicate significant difference (P<0.05). Error bars indicate standard error. Sp7, Sp7-S & Sp245: strains of *A. brasilense;* Herb s: *Herbaspirillum seropedicea;* Burk p: *Burkholderia phytofirmans* PsJN[T].

Several studies reported improvement of seed germination due to inoculation with *Azospirillum* spp. on crops like wheat (Dobbelaere et al., 2001), pear millet (Raj et al., 2003), maize (Zahir et al., 2003), sunflower, corn and soybean (Cassán et al., 2009). In some cases, germinations of PGPR-inoculated seeds were up to 100% greater than controls. This PGPR-effect has been associated with the ability of PGPR to produce auxin and gibberellin (GA) which are seemingly involved in seed germination. Taiz and Zieger (2010) suggested that auxin might aid GA biosynthesis and possibly regulate activity of specific enzymes important germination, i.e., amylase. In addition, Feurtado and Kermode (2007) reported that the enlargement of the cortical cells of the growing axis (e.g. radicle) during germination correlates with the predicted sites for GA production. This supports the view that increased radicle growth potential is regulated by GA and that embryonic GA is released to trigger weakening of the tissues surrounding the radicle. As a result, the significant improvement of vigor of all crops observed in this study could be due to substantial quantity of auxin (IAA) synthesized by PGPR that triggered faster seedling emergence.

All the PGPR used produced IAA in the culture medium at concentrations that might have triggered metabolic activities and physiological changes in the young tissues during seed germination. Similar effects of PGPR, *Azospirillum* on root growth have been observed in wheat, canola, and sunflower and other plant species (Abbass and Okon, 1993, Vikram et al., 2007). Cassán et al. (2009) also speculated that the increases of germination parameter and length of shoot observed are considered a typical GA-like response as this kind of effect mimicked with that of exogenous GA application (Lucangeli and Bottini, 1997).

It has been documented in wheat that inoculation with *A. brasilense* and exogenous application of IAA and GA_3 generated similar effects on the growth pattern of stems and roots (Kucey, 1988). The effects of inoculation of this PGPR can also substitute exogenous addition of IAA as shown in wheat crop (Zimmer et al., 1988). Using wild strain of this PGPR, which is capable of producing IAA, also showed enhancement of the number and length of roots (Galli et al., 1988). However, when using a low IAA-producing mutant, it had no effect on the root growth parameters (Barbieri and Galli, 1993). The increase in mass and length of the growing embryonic axis in this study could be attributed to the differential embryo development induced by bacterial growth regulators during germination which penetrate the seed coat along with water and thereby accelerating root

growth and facilitating absorption of the surrounding substances (Cassán et al., 2009). To date, the production of the phytohormones is the most common explanation for the effects of PGPR at the very early stage leading to better absorption of water and minerals (Bashan and de-Bashan, 2010). Thus, bacterial establishment and initial phytostimulatory effects on plants at early developmental stages such as germination and subsequent growth would play an essential role in achieving good crop stand.

Conclusion

The inoculation of some PGPR strains significantly improved the germination characteristics of tomato and lettuce seeds. The PGPR effects could be attributed to their unique metabolic properties particularly the ability to produce growth regulators (e.g. IAA auxin) and inoculum concentration. The results of this study indicate the potential of exploiting the benefits of some of these PGPR to improve tomato and lettuce seedling emergence and establishment.

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