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Early seedling growth response of lettuce, tomato and cucumber to *Azospirillum brasilense* inoculated by soaking and drenching

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Abstract

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This study evaluated the effects of three *A. brasilense* strains (i.e. Sp7, Sp7-S and Sp245) on the early seedling growth of lettuce, tomato and cucumber. Seeds were inoculated by soaking and drenching before and after sowing, respectively. Results show that inoculation effect varied greatly with plant species, inoculation methods and PGPR strains which could be dependent on inoculum concentration and IAA (indole-3-acetic acid) production. Generally, the magnitude of inoculation impact on the early growth of vegetables was more pronounced with Sp7-S, followed by Sp245 and Sp7. In particular, Sp7-S and Sp245 strongly enhanced root and shoot growth, germination value and vigour of tomato when inoculated by soaking. Sp245 increased the level of endogenous plant IAA of cucumber and lettuce. Despite the diverse crop responses to inoculation methods, soaking appeared to be a better technique, and majority of the strains demonstrated more consistent beneficial effects on tomato.

Keywords: PGPR; seedling emergence; *Cucumis sativus; Lactuca sativa; Lycopersicon esculentum*

Azospirillum, apart from a general coloniser, is one of the versatile genera of plant growth promoting rhizobacteria (PGPR) (Bashan et al. 2004). Species of this genus were first isolated in the roots of various cereals worldwide, hence most studies have been centred on this group of crops (Barassi et al. 2007). The leading scientific basis for the widespread exploration of *Azospirillum* is based on their ability to fix atmospheric nitrogen to enhance growth and yield (Dobbelaere et al. 2001). Later studies unveiled several mechanisms, including phytohormone production, nutrient solubilisation, Fe sequestration, favouring beneficial mycorrhizal-plant associations and minimising the negative effects of biotic and abiotic stresses (Bashan et al. 2004). The most common morphological effect observed with *Azospirillum* inoculation is enhanced root growth, which could aid plants to take up water and nutrients more efficiently (Barassi et al. 2007). Production of auxin, indole-3-acetic acid (IAA), was linked to such stimulatory effect by *Azospirillum* (Dobbelaere et al. 1999).

The degree of association between PGPR and plant roots is an important determinative factor for growth promotion (Saleh-Lakha, Glick 2006). Ahmad et al. (2011) reported that successful root colonisation

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is the most important prerequisite for PGPR effects. It is often considered that seed inoculation is the first vital step for colonisation process as it offers a great opportunity for bacteria to establish an intimate association with the germinating seed and predisposes the future colonisation. However, the beneficial effects of PGPR inoculation also involve specific strain to a certain crop species or even cultivar, site specificity, modes of inoculation and growing conditions.

Azospirillum inoculation demonstrated beneficial effects on plant growth in various crops including wheat, pearl millet, rice, maize, corn, soybean and sunflower (Pereira et al. 1988; Dobbelaere et al. 2001, 2002; Raj et al. 2003; Cassán et al. 2009; Gholami et al. 2009; Nezarat, Gholami 2009). In vegetables, Rodriguez et al. (2001) reported that inoculation with *Azospirillum* spp. to tomato and pepper seeds improved germination. Barassi et al. (2006) also documented that inoculation with *Azospirillum* sp. to lettuce seeds yielded higher germination than non-inoculated control. Inoculation of *Azotobacter* spp. strains 17 and 20, and *Azospirillum* strains 1 and 23 promoted pepper and maize germination, respectively (Reyes et al. 2008).

Earlier studies with *Azospirillum* and other PGPR introduce bacteria to the seed prior to planting. One of the commonly used approaches is immersion, which requires soaking of seeds in the bacterial suspension for several minutes or hours before planting. The use of aqueous suspension is a common laboratory-based approach for selection and testing bacteria for growth promotion and biocontrol activities (Whipps 1997; Walker et al. 2004). Encapsulation using alginate is another method that introduces PGPR onto the seed surface in the form of alginate beads (Puente, Bashan 1993; Bashan et al. 2002). The practical advantage of alginate-encapsulation over other methods is that bacterial inoculant can be lyophilised and stored at high density for an extended period of time, making this method more attractive for commercial scale (Reed, Glick 2005). In addition, it offers protection for bacteria from the harsh environment by acting as a time-release coating that slowly disintegrates and releases the PGPR to the germinating or growing plant (Bashan et al. 2002). Peat-based formulation of bacterial inoculants is also commonly used with Rhizobial inoculant to coat the seeds in pellet forms for easy sowing in furrows (Bashan 1998). However, there had been several drawbacks of this method such as the nature of peat and its unavailability in many countries (Bashan et al. 2002). In this study, two laboratory-based methods (soaking and drenching) of seed inoculation were used to evaluate the effects of different strains of *Azospirillum brasilense* (i.e. Sp7, Sp7-S and Sp245) on the germination and early seedling growth of lettuce, tomato and cucumber. To our knowledge, little is known about the effect of inoculation with these strains on the early growth of these vegetables.

MATERIAL AND METHODS

General information. Seeds of cucumber (*Cucumis sativus* L., cv. Gremlin), tomato (*Lycopersicon esculentum* L., cv. Grosse lisse) and lettuce (*Lactuca sativa* L. 'Salinas' type) were used. Sterile Petri dish containing one sheet of moistened autoclaved Whatman filter paper No.1 with a 90 mm diameter was used. Petri dishes were partially sealed with parafilm to prevent rapid evaporation. All the materials used in this study were sterilised by either autoclaving or spraying with 80% (v/v) ethanol and 2% (v/v) sodium hypochlorite (NaClO). The set up was replicated 6 times with 10 seeds per replicates and arranged in a completely randomised design. The experiment was conducted in a light and temperature-controlled growing cabinet (Labec Laboratory Equipment, Marrickville, Australia) at the Faculty of Agriculture and Environment, The University of Sydney, Australia.

Seed and inoculum preparation, and inoculation. Seeds were washed with millipore water prior to surface sterilisation. Seeds were then surface-sterilised by soaking in 1% (v/v) NaClO and followed by 70% (v/v) ethanol. Seeds were then washed with autoclaved millipore water to remove the residual bleach and ethanol. Finally, seeds were spread out in a sterile petri dish with dry autoclaved filter paper prior to inoculation.

The inocula of the different strains of *Azospirillum brasilense* Sp7, Sp7-S and Sp245 were provided by Dr. Rosalind Deaker, University of Sydney. Inoculum of each strain was taken from pure culture stored with glycerol at –80°C and streaked onto the nutrient agar containing 15 g agar, 5 g peptone and 3 g beef/l of water and incubated at 28°C for 2 days. A loopfull of the culture was inoculated onto tryptone yeast extract and glucose (TYEG) medium broth, and incubated for 3 days at 28°C with constant agitation. The number of cell forming unit (CFU) was determined following serial dilution and agar plating on nitrogen free broth (NFB) with congo red (Bashan

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et al. 1993). Prior to inoculation, the bacterial cultures were pelleted by centrifugation (4,000 × *g*, 5 min), washed twice with autoclaved 30 mM $MgSO_4$, and resuspended in the same solution.

Seeds were inoculated at population of average Log 9 CFU/ml by soaking and drenching methods. Soaking method was done by soaking or immersing the surface-sterilised vegetable seeds in the bacterial suspension at a volume of 100 µl per seed for 1 hour. This was done at room temperature with constant agitation to allow bacteria bind to the seedcoat and for seed imbibition. Seeds were then sown in the prepared Petri dishes. Drenching method was done by drenching the bacterial suspension at volume similar to other method per seed after sowing. A similar procedure for each method was used for non-inoculated treatments using the $MgSO_4$ solution.

IAA quantification by HPLC and Salkowski colorimetric technique. The IAA concentration in the culture supernatant of *A. brasilense* strains was measured using a spectrophotometer at 535 nm. One ml aliquot of the supernatant was mixed vigorously with 4 ml of Salkowski's reagent containing 150 ml 98% H_2SO_4 (w/w), 250 ml millipore water and 7.5 ml 0.5 M $FeCl_3 \cdot 6H_2O$. The mixture was allowed to stand in the dark at room temperature for 20 min (Dobbelaere et al. 1999; Patten, Glick 2002). Absorbance was measured in triplicate at 535 nm wavelength and the concentrations of IAA were determined based from the standard.

The amount of IAA particularly in the roots was quantified following the method described by Ribaudo et al. (2006) with slight modification. Approximately 1 g of frozen plant root was homogenised with liquid nitrogen. The resulting powder (~500 mg) was dissolved in methanol solution containing methanol and water (4:1, v/v) with polyvinylpyrrolidone and incubated overnight at 4°C. The extract was centrifuged (10,000 × *g*, 10 min) and the supernatant was saved. The solid residue was re-extracted and the two extracts were combined and concentrated using speed vacuum evaporator (UNIVAPO 100ECH; Montreal Biotech Inc., Montreal, CA) until the volume reduced to one-tenth of the initial volume. The pH of the concentrated sample was adjusted to 2.5–3.0 with concentrated acetic acid and partitioned with 1% acetic acid in ethyl acetate (v/v). The acidic ethyl acetate extract was completely dried using speed vacuum evaporator. The dried sample was dissolved in acetic acid, methanol and water (1:10:89, v/v) and filtered with 0.2 µm nitrocellulose membrane into a high-performance liquid chromatography (HPLC) vial. The plant assay sample and IAA standard were resolved in reversed phase C_{18} column with Agilent quad pump HPLC system (Agilent Technologies Inc., Santa Clara, USA). A solvent gradient program was optimised for IAA detection in the presence of 1% acetic acid (v/v). The eluent profile was traced by a dual monitoring system with diode array (282 nm) and fluorescence (Ex 282 nm and Em 360 nm) detectors. The chromatogram was analysed using LC/MS Agilent chemostation software (Agilent Technologies Inc., Santa Clara, USA).

Growing condition and measurements. The petri dishes were placed in the growing cabinet with a constant temperature of 24°C and a daily cycle of 12 h darkness and 12 h light (36 µmol/m^2/s). Daily and final number of seeds germinated were recorded and the germination value was determined using the formula described by Djavanshir and Pourbeik (1976).

At 5th and 7th day from sowing, seedlings were placed in a transparent plastic box with a thin film of water and scanned using a dedicated Desk Scan II scanner (Expression 700, Epson, Nagano, Japan). Scanned images were analysed by WinRhizo Pro V. 2007c (Regents Instrument Inc., Quebec, CA) for shoot and total root length measurement. Vigour index was computed following the formula described by Gamalero et al. (2008).

Roots of 5 randomly selected inoculated seedlings were pound with 1 ml peptone phosphate buffer using autoclaved mortar and pestle. An aliquot of the suspension was plated after a series of dilution in NFB agar medium with congo red (Bashan et al. 1993). After 4 days of incubation at 28°C, the number of CFU with distinctive colour morphology of the test strain were counted and expressed per gram of root fresh weight (Gamalero et al. 2008).

General analysis of variance (ANOVA) was performed using the Genstat® 14th edition software (VSN International, Hemel Hempstead, UK). If interactions were significant they were used to explain the results, and if otherwise main effects were used. Mean differences were determined using the Fisher's protected least significant difference (LSD) ($P < 0.05$).

RESULTS AND DISCUSSION

The main aim of the study was to evaluate the effect of the different strains of *A. brasilense* on ger-

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Table 1. Results of ANOVA of the effect of different *A. brasilense* strains inoculated by soaking and drenching methods on the early seedling growth of vegetables

Crop	Factor	RL	SL	GV	VI	IAA
Lettuce	inoculation method (M)	**	ns	ns	*	ns
	PGPR (P)	ns	ns	**	ns	**
	M × P	**	ns	ns	*	**
Tomato	inoculation method (M)	**	ns	*	**	**
	PGPR (P)	**	**	**	**	**
	M × P	**	**	ns	**	**
Cucumber	inoculation method (M)	ns	ns	*	ns	**
	PGPR (P)	ns	ns	ns	**	**
	M × P	ns	ns	ns	**	**

*significant at $P \leq 0.05$; **highly significant at $P \leq 0.01$; ns – non-significant; RL – root/radicle length; SL – shoot length; GV – germination value; VI – vigour index; IAA – Indole-3-acetic acid

mination and early seedling growth of vegetables using two laboratory-based methods of seed inoculation. The summary of ANOVA results is presented in Table 1. The effects of PGPR and inoculation method on lettuce were noted only on root length and vigour index, and germination value and IAA, respectively. The same effect was observed on their interaction except germination value. In tomato, all factors including interaction showed significant effects on most parameters evaluated except shoot length and germination value that were not affected by inoculation methods and interaction of the two factors, respectively. In cucumber, only germination value, vigour index and the endogenous IAA content were influenced by inoculation method, both PGPR and inoculation method, and both factors and their interaction, respectively.

Characteristics of *A. brasilense* strains

The average number of cells recovered from the inoculated *A. brasilense* strains using two methods was Log 6 CFU/g 6–7 days after inoculation. This result is relatively similar to previous finding with *A. brasilense* Cd reported by Hadas and Okon (1987) who found between 4–5 Log CFU/g from 2-week old vegetables seedlings (i.e. radish, bean, melon and tomato) inoculated at Log 8 CFU/ml. It is known that *Azospirillum* is a non-specific plant-bacterium and persistent root coloniser (Bashan, Holguin 1997; Bashan et al. 2004). Species of this genus have no preference for crop plants or weeds, annual or perennial plants, and can be used for plants that have no previous history with *Azospirillum* (Barassi et al. 2007). This genus is characterised to colonise plant roots externally, living embedded in the mucigel layer in variable numbers (Bashan et al. 1989). Earlier findings showed that some species have the mechanism to colonise the interior part of the roots (Umali-Garcia et al. 1980). For instance, strain Sp245 was demonstrated to penetrate the outer root layers and establish in the intercellular spaces of the root cortex of wheat crop (Schloter, Hartmann 1998).

In terms of the amount of IAA produced by the strains grown in TYEG medium, Sp245 produced the highest of 9.22 µg/ml, followed by Sp7 with 6.61 µg/ml and the least was produced by Sp7-S with 5.93 µg/ml. While *Azospirillum* is known to produce phytohormones (e.g. IAA-auxin, gibberellins), the quantity of production particularly IAA is dependent on the type of culture media and the availability of precursor, tryptophan. For instance, Moghaddam et al. (2012) observed higher amounts of IAA produced by *Azospirillum* spp. isolated from rice rhizosphere reaching up to 678 µg/ml after 48 hr of incubation in a tryptophan-amended substrate. In contrast, Harari et al. (1988) reported high amount of IAA excreted by *A. brasilense* FT-326 up to 36.6 µg/ml grown in tryptophan-free malate synthetic liquid medium. Likewise, Cassán et al. (2009) reported ~13.16 µg/ml of IAA produced by *A. brasilense* Az39 grown in

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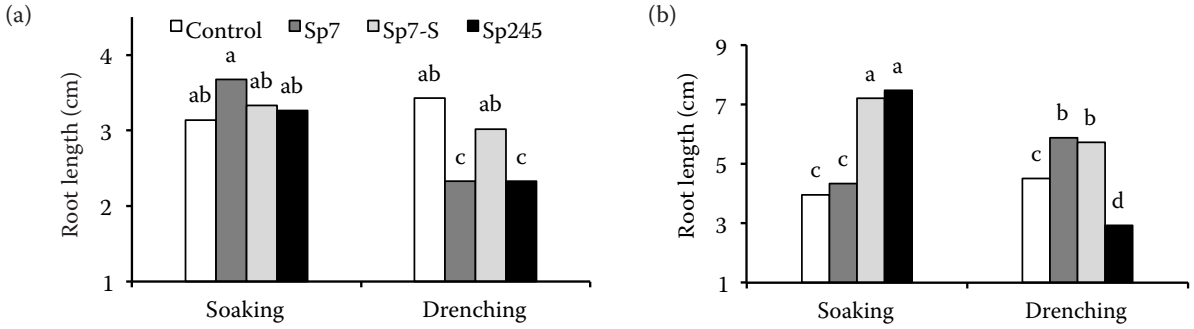

Fig. 1. Interaction effect of the different *A. brasilense* strains and methods of inoculation on total root length of lettuce (a) and tomato (b) 7 DAI, $n = 6$

bars with the same letters are not significantly different, $P \leq 0.05$, LSD; n – number of replications; DAI – days after inoculation

yeast extract-mannitol broth (YEM) without IAA precursor amendment.

Effects of inoculation on root and shoot growth

Inoculation with strains Sp7 and Sp245 inhibited root growth of lettuce when inoculated by drenching while all the strains generated no substantial effect when inoculated by soaking (Fig. 1a). However in tomato, seeds inoculated by soaking with Sp7-S and Sp245 yielded the longest roots, followed by Sp7 and Sp7-S inoculated through drenching and the shortest was produced by Sp245 inoculated by the latter method (Fig. 1b). Likewise, the effect of the strains on the growth of tomato shoot followed a relatively similar trend on their effects on root (Fig. 2). These results suggest that the efficacy of *A. brasilense* to affect early growth varies considerably with crop species, PGPR strains and methods of inoculation. The promotion of root elongation with *Azospirillum* spp. has not always been observed, and their effects appear to be dependent on inoculum concentration and phytohormone production (Morgenstern, Okon 1987). The optimal concentration for promotion and the threshold level of inhibition varies considerably between plant species and bacterial strains. In cereals (e.g. sorghum and wheat), inoculation of high concentration of *Azospirillum* was documented to inhibit root elongation (Harari et al. 1988; Okon, Kapulnik 1986). Hadas and Okon (1987) found that inoculation with *A. brasilense* Cd at 10^8 to 10^9 CFU/ml to tomato inhibits root elongation with deformed root cap. Harari et al. (1988) noted no root growth inhibition of *A. brasilense* FT-326 on wheat seedlings at 10^7 CFU/ml but inhibited sorghum root growth even at lower concentration (10^5). The effects on root elongation at various bacterial concentrations are recognized to be regulated by phytohormones (Hartmann, Baldani 2006; Cassán et al. 2009). In fact, its effect on root growth mimicked with exogenous application of IAA, which is inhibitory at higher level (Morgenstern, Okon 1987; Harari et al. 1988). In the current study, inoculation through drenching with strain Sp245 evidently inhibits root elongation of lettuce and tomato seedlings. Although it was documented that elevated IAA (which this strain had) would normally inhibit root elongation, this reasoning is not applicable because the strain enhanced root and shoot growth of tomato seedlings when inoculated by soaking. Thus, microbial actions or other factors may have influenced seedling growth when inoculated through drenching method. Ha-

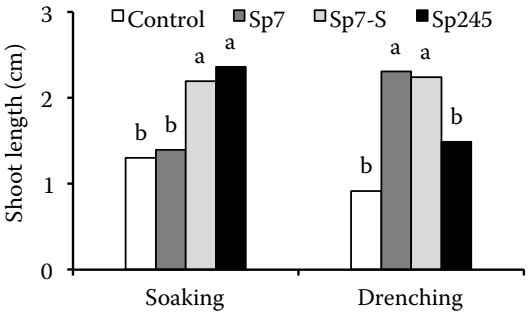

Fig. 2. Interaction effect of the different *A. brasilense* strains and methods of inoculation on shoot length of tomato 7 DAI, $n = 6$

bars with the same letters are not significantly different, $P \leq 0.05$, LSD; n – number of replications; DAI – days after inoculation

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RARI et al. (1988) reported that the inconsistency of the effects of *Azospirillum* on root elongation could be the result of the antagonistic actions of IAA and other growth regulators produced by bacteria at any particular condition. Several studies have shown the beneficial effects of PGPRs are dependent on matching specific strains of PGPR with crop species and even further to specific cultivars, soils, inoculation methods and growing conditions (CHANWAY et al. 1988; NOWAK 1998; ZAHIR et al. 2003).

Apart from IAA, *Azospirillum* spp. are also known to produce other important plant hormones such as gibberellins, cytokinin, polyamines and amino acid that might have been involved to such growth effects (THULER et al. 2003; CASSÁN et al. 2011). Previous studies demonstrated that PGPR-effect at the very early young stage of plant development is due to production of growth substances (LEVANONY 1990; BASHAN, HOLGUIN 1997; BARAZANI, FRIEDMAN 1999; BASHAN, JACOUD et al. 1999; DOBBELAERE et al. 1999; BASHAN et al. 2004). In particular, it was hypothesised that IAA and other growth substances were responsible for this kind of effect on crops such as wheat, canola, sunflower and other cereal species (ABBASS, OKON 1993). VESSEY (2003) also considered phytohormones as responsible in signalling the assimilates partitioning and growth patterns leading to bigger and longer roots, more branched and with greater surface area. In addition, the lengthening of roots leading to increase root surface volume is the most common morphological change with *Azospirillum* inoculation (LEVANONY, BASHAN 1989). VIKRAM et al. (2007) also reported that auxin produced by rhizobacteria could improve strongly root development and may enhance the overall growth performance due to improved plant uptake.

The inoculation with *A. brasilense* and application of synthetic IAA and GA_3 to wheat displayed related effects on the growth pattern of stems and roots (KUCEY 1988). Similarly, ZIMMER et al. (1988) reported that *Azospirillum* inoculation mimicked the effect of exogenous application of IAA on wheat crop. The use of wild *Azospirillum* strain that is capable of producing IAA was also shown to enhance the number and length of lateral roots (GALLI et al. 1988). BOTHE et al. (1992) found that plants inoculated with *A. brasilense* produced more lateral roots with slight increases in root hair formation and dry weight of roots. In contrast, the inoculation with low IAA-producing mutant revealed no effects on the root growth parameters (BARBIERI, GALLI 1993).

Table 2. Germination value of tomato 7 DAI and cucumber 6 DAI as influenced by two methods of inoculation

Inoculation method	Tomato	Cucumber
Soaking	97[a]	125[b]
Drenching	74[b]	181[a]

means in a column with different superscript letters indicate significant differences at $P \leq 0.05$ LSD; $n = 6$; DAI – days after inoculation

Effects of inoculation on germination value and seedling vigour index

The effect of inoculation methods was demonstrated on tomato and cucumber (Table 2). Faster and higher germination in tomato occurred when inoculated by soaking method while drenching for cucumber. In addition, some of the strains affected germination value of both lettuce and tomato irrespective of the inoculation methods used (Fig. 3). In particular, only strain Sp7 inoculation produced better germination value of lettuce while this was enhanced by all strains in tomato. On the other hand, the interaction between different strains and inoculation methods on seedling vigour index were manifested on the three vegetables (Fig. 4). When inoculated by soaking, all strains had no effect on seedling vigour while if drenching was used, the vigour of the seedlings became inferior to those inoculated with Sp7 and Sp245 (Fig. 4a). In tomato, inoculated seedlings by soaking showed superior vigour with strain Sp7-S and Sp245 (Fig. 4b). This result is apparent since these two strains enhanced growth of root and shoot which particu-

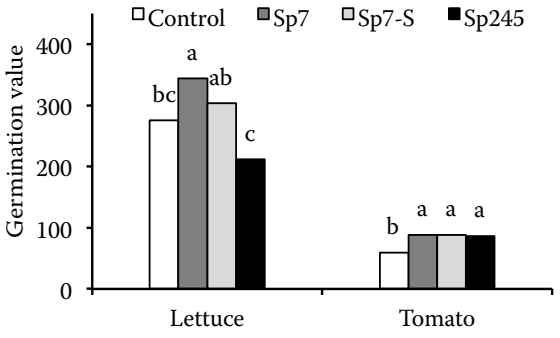

Fig. 3. Germination value of lettuce and tomato 7 DAI as influenced by different *A. brasilense* strains, $n = 6$
bars with the same letters are not significantly different, $P \leq 0.05$, LSD; n – number of replications; DAI – days after inoculation

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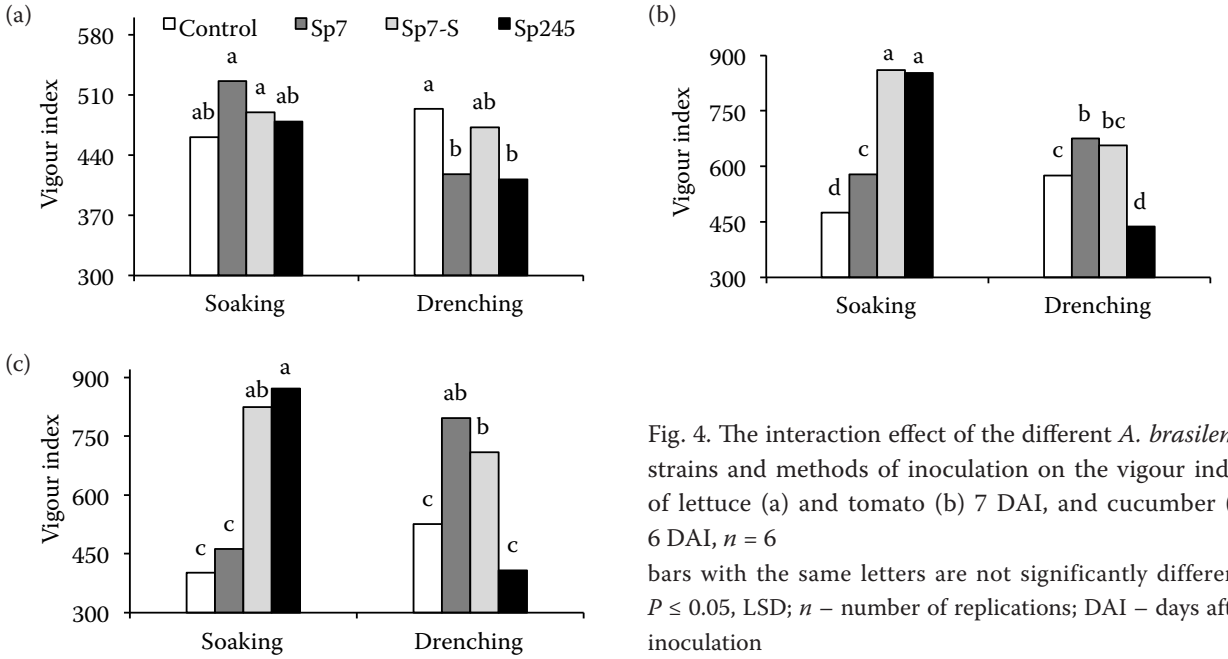

Fig. 4. The interaction effect of the different *A. brasilense* strains and methods of inoculation on the vigour index of lettuce (a) and tomato (b) 7 DAI, and cucumber (c) 6 DAI, $n = 6$

bars with the same letters are not significantly different, $P \leq 0.05$, LSD; n – number of replications; DAI – days after inoculation

larly worked better with soaking method. Likewise, Sp7 also caused moderate improvement of seedling vigour of tomato with drenching method. Superior seedling vigour due to Sp7-S and Sp245, and Sp7 and Sp245 inoculation was manifested in cucumber with soaking and drenching, respectively (Fig. 4c). Generally, across species the results show that some of the strains performed comparatively well when soaking method was used. In addition, Sp245 seemed to be less effective when inoculated by drenching.

In maize, improved germination and vigour was also demonstrated with PGPR-*Azospirillum* inoculation. Likewise, under *in vitro* condition, seed inoculation with some PGPR strains showed better germination, rate of emergence and vigour over non-treated control. Improvement of germination characteristics by PGPR were also reported in wheat (Dobbelaere et al. 2001, 2002), sorghum, pearl millet (Raj et al. 2003), rice (Pereira et al. 1988), maize (Gholami et al. 2009; Nezarat, Gholami 2009), sunflower, corn and soybean (Cassán et al. 2009). As mentioned previously, this PGPR-effect is mainly caused by the production of phytohormones by PGPR particularly auxin and gibberellin (GA) which are known to be involved during seed germination. Taiz and Zeiger (2010) reported that aside from rising of GA activity during germination prior to radicle protrusion, the presence of auxin might also be involved in the synthesis of this hormone since auxin was shown to promote biosynthesis of GA in plants. Thus, auxin might have aided GA biosynthesis and triggered the activity of specific enzymes, i.e., amylase that facilitated the availability of starch assimilation and promoted early and rapid germination (Gholami et al. 2009). In this study, the general improvement of germination vigour of some crops due to inoculation might also be attributed partly to the substantial level of auxin (IAA) synthesised by PGPR that resulted in faster emergence of healthy embryonic growing axis.

Effects of inoculation on the level of endogenous IAA

The effects of the different strains inoculated by soaking and drenching methods on the level of plant endogenous IAA on vegetables are shown in Fig. 5. The IAA content of lettuce was increased more than two folds with Sp7 and Sp245 inoculation using soaking and drenching method, respectively (Fig. 5a). A moderate increase was also noted with Sp245 when inoculated through soaking. In tomato, some of the strains seemed to reduce the amount of endogenous IAA level (Fig. 5b). This was particularly demonstrated with Sp7 and Sp245 when inoculated by soaking, and Sp7 inoculation by drenching. This result somehow contradicts to the previous findings of Ribaudo et al. (2006) who reported enhancement of IAA content of tomato shoots and roots due to *A. brasilense* FT 326 inoculation. However in cucumber, the level of endogenous IAA increased

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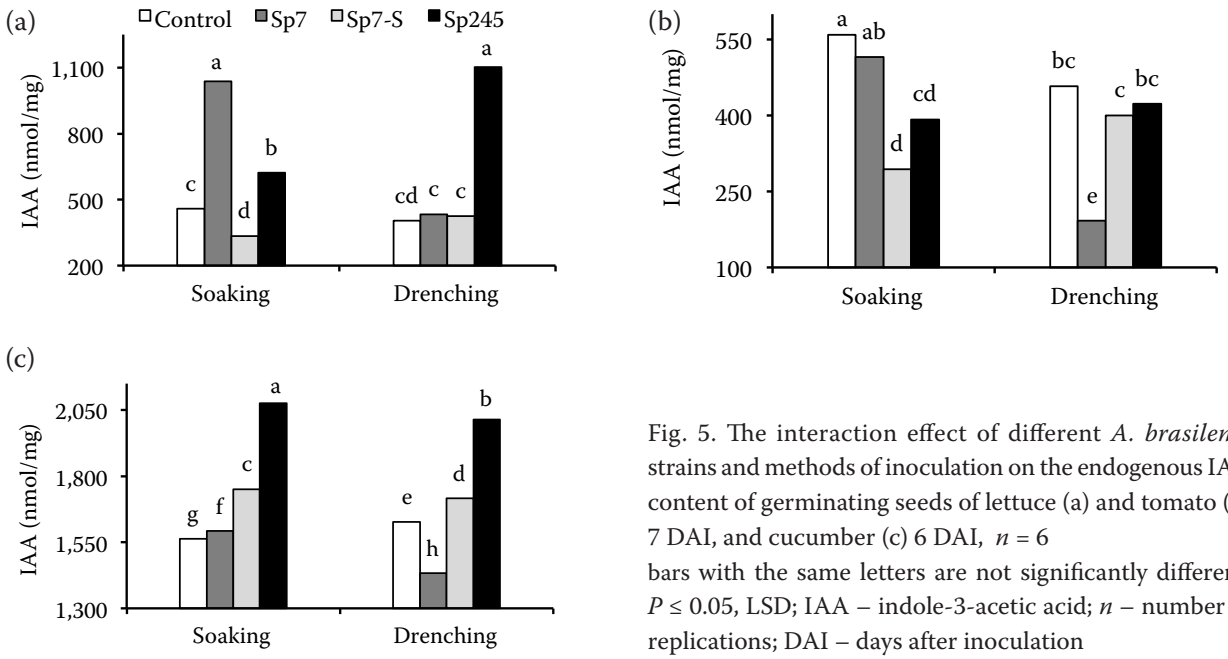

Fig. 5. The interaction effect of different *A. brasilense* strains and methods of inoculation on the endogenous IAA content of germinating seeds of lettuce (a) and tomato (b) 7 DAI, and cucumber (c) 6 DAI, $n = 6$
bars with the same letters are not significantly different, $P \leq 0.05$, LSD; IAA – indole-3-acetic acid; n – number of replications; DAI – days after inoculation

strongly due to inoculation particularly with Sp245 and Sp7-S in both inoculation methods (Fig. 5c). It is evident that Sp245 had the strongest influence on plant IAA content with respect to lettuce and cucumber regardless of the inoculation method used. This might be due to the fact that this strain produced fairly high amount of IAA compared to other strain. Despite varied responses amongst vegetables, the IAA contents of inoculated seedlings of some crops increased considerably in response to *Azospirillum* inoculation. Ribaudo et al. (2006) reported that IAA content in plants increased up to 15 folds with *Azospirillum* inoculation. The elevated auxin level of inoculated plants could have an important implication in the biochemical signaling involved in the growth functioning of the host plant. Thus, it was suggested that the impact of inoculation with *Azospirillum* could be related to their ability to secrete phytohormones, particulary IAA.

CONCLUSION

The magnitude of inoculation impact on early growth of vegetables was more prominent with Sp7-S, followed by Sp245 and Sp7. The inoculation effect varied greatly with plant species, methods of inoculation and PGPR strains that could be dependent on the inoculum concentration and their unique metabolic properties, particularly on the ability to produce phytohormones, e.g. auxins, and other factors that may have influenced early growth and development of vegetables. Generally, soaking method appeared to be a more suitable technique of seed inoculation, and some of the strains demonstrated more consistent beneficial effects on tomato.

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