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ORIGINAL ARTICLE

L-Tryptophan-dependent biosynthesis of indole-3-acetic acid (IAA) improves plant growth and colonization of maize by *Burkholderia phytofirmans* PsJN

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Abstract *Burkholderia phytofirmans* PsJN is a well-known plant growth-promoting bacterium that establishes rhizospheric and endophytic colonization in different plants. PsJN inoculation promotes growth of different horticultural crops. L-Tryptophan (L-TRP) application may further improve its effectiveness, due to substrate (L-TRP)-dependent inoculum (PsJN)-derived auxins in the rhizosphere. In the present study, the substrate (L-TRP)-dependent response of PsJN inoculation to maize growth and auxin biosynthesis was evaluated under pot conditions. In vitro auxin biosynthesis by PsJN was determined in the absence and presence of L-TRP, a physiological precursor of auxins. Surface-disinfected seeds were treated with peat-based inoculum and L-TRP solutions (10^{-4} and 10^{-5} M). Results revealed that L-TRP application and PsJN inoculation, when applied separately, significantly increased the growth parameters of maize compared to untreated control. However, PsJN inoculation supplemented with L-TRP (10^{-5} M) gave the most promising results and significantly increased plant height, photosynthesis, chlorophyll content, root biomass and shoot biomass up to 18, 16, 45, 62 and 55 %, respectively, compared to the uninoculated control. Similarly, higher values of N, P and IAA content were observed with precursor (L-TRP)–inoculum (PsJN) interaction. The inoculant strain efficiently colonized maize seedlings and was recovered from the rhizosphere, root and shoot of plants. The results imply that substrate (L-TRP)-derived IAA biosynthesis in the rhizosphere by PsJN inoculation could be a useful approach for improving the growth, photosynthesis and nutrient content of maize plants.

Keywords *B. phytofirmans* PsJN · L-tryptophan · Precursor–inoculum interaction · Colonization · Maize

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Introduction

Beneficial plant-microbe interactions in the rhizosphere are primary determinants of plant health and soil fertility (Jeffries et al. 2003). The rhizosphere represents a highly dynamic space for interactions between plant roots and beneficial soil microorganisms (Bais et al. 2006). In the rhizosphere, molecular communication between microorganisms and their plant hosts plays a fundamental role in pathogenesis and establishment of beneficial interactions (Mark et al. 2005). Endophytes colonizing plants internally without harming their host may have pronounced positive effects on plant growth and health (Mitter et al. 2013a). Over the years, the utilization of plant growth-promoting bacteria (PGPB), usually rhizosphere bacteria or endophytes, as bio-fertilizers and/or bio-pesticides has received increasing attention and is becoming popular for agricultural production. These microorganisms may not only ensure the availability of essential nutrients to plants, but also enhance nutrient use efficiency (Khalid et al. 2009).

Plant growth regulators (PGRs) play a vital role in controlling plant growth and development. Auxins are an important class of hormones controlling many aspects of root development and architecture, such as primary root growth, lateral root formation, and root hair development (Fukaki and Tasaka 2009). Despite the fact that plants are capable of synthesizing

auxins, they respond to exogenously applied auxins during certain growth phases (Frankenberger and Arshad 1995; Zahir et al. 2010a).

Plant growth-promoting bacteria (PGPB) produce beneficial effects on plant growth through several mechanisms such as nitrogen fixation, improved nutrient uptake, phytohormone production, and induction of systemic resistance (ISR) (Mitter et al. 2013a). It is likely that plant growth-promoting effects exerted by plant-beneficial bacteria are due to the bacterial production of plant hormones such as indole-3-acetic acid (IAA), cytokinins and gibberellins (Bottini et al. 2004; Lugtenberg and Kamilova 2009). About 80 % of bacteria from the rhizosphere are able to produce indole acetic acid (IAA), indicating a possible role in interaction with the plant (Patten and Glick 1996; Spaepen et al. 2007). L-Tryptophan (L-TRP) is considered an efficient physiological precursor of auxins in higher plants, as well as for microbial biosynthesis of auxins (Davies 2004; Khalid et al. 2006). In vitro studies have demonstrated that some microorganisms can produce small amounts of auxins in the absence of L-TRP; however, in its presence, the microbiota produce much greater quantities of auxins (Khalid et al. 2004a; Zahir et al. 2010a). Exogenous application of L-TRP to soils has also been shown to stimulate synthesis of auxins, positively influencing plant growth and development (Khalid et al. 2004b; Zahir et al. 2010a, b).

Burkholderia phytofirmans strain PsJN, an efficient plant growth-promoting bacterium, was isolated from onion roots and reported for growth promotion of horticultural crops, e.g., potatoes, tomato and grapevines (Frommel et al. 1991; Nowak et al. 1995; Ait Barka et al. 2000). In addition, *B. phytofirmans* PsJN colonization enhanced protection against *Verticillium* sp. in tomato (Sharma and Nowak 1998), and *Botrytis cinerea* and *Pseudomonas syringae* in grapevine (Ait Barka et al. 2002; Bordiec et al. 2011). So far, molecular mechanisms responsible for plant growth-promotion in PsJN, such as the reduction of the plant ethylene levels by 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, have been described (Sun et al. 2009). Weilharter et al. (2011) described the presence of two putative IAA synthesis pathways (the indole-3-acetamide and the tryptophan side chain oxidase pathways) in the genome sequence analysis of *B. phytofirmans* PsJN. Very recently, auxin (IAA) production and quorum sensing have been described to be putatively involved in plant growth-promotion and cell-to-cell communication in the efficient colonization of *Arabidopsis thaliana* by strain PsJN (Zúñiga et al. 2013). The present study was conducted to assess whether the amendment of L-TRP and the associated IAA synthesis by strain PsJN affects plant growth and the strain's colonization of maize plants.

Materials and methods

The effect of *Burkholderia phytofirmans* strain PsJN inoculation on the physiology and growth parameters of maize was tested under net-house conditions. We used a genetic variant of the strain *B. phytofirmans* PsJN::*gusA* (Compant et al. 2005) that carries a beta-glucuronidase reporter gene (*gusA*) that allows specific visualization of bacterial cells upon color formation. The genetically engineered derivative strain showed similar behavior in colony and cell morphologies and growth patterns as compared with the PsJN wild-type strain in Luria-Bertani (LB) medium (Compant et al. 2005). Maize (cv. Neelam) seeds were provided by the Maize Research Institute, Ayub Agricultural Research Institute, Faisalabad, Pakistan.

Measurement of auxin production by PsJN

Auxin production by *B. phytofirmans* PsJN, both in the presence and absence of L-TRP (Sigma, St. Louis MO), was determined colorimetrically in terms of IAA equivalents (Sarwar et al. 1992). Two-day old bacterial cultures grown (28±2 °C at 180 rpm) in LB broth supplemented with filter-sterilized 1 % L-TRP solution were harvested by centrifugation. Control cultures, which did not receive L-TRP, were included. Three milliliters of supernatants were mixed with 2 mL Salkowski's reagent (12 g L^{-1} $FeCl_3$ in 429 mL L^{-1} H_2SO_4). Mixtures were incubated at room temperature for 30 min for color development and absorbance was measured at 535 nm using a spectrophotometer (Nicolet Evolution 300 LC, Thermo Electron Corp., Madison, WI). Auxin concentration produced by bacterial isolates was determined using a standard curve for IAA prepared from serial dilutions of 10–100 μg mL^{-1}.

Bacterial inoculum and growth conditions

Inoculum of the *gusA* marked strain PsJN was prepared in 250-mL Erlenmeyer flasks in LB broth containing spectinomycin antibiotic (100 μg mL^{-1}). The broth was inoculated with strain PsJN::*gusA* and incubated at 28±2 °C for 48 h in the orbital shaking incubator (VWR International, GmbH, Austria) at 180 rpm. The optical density of the broth was adjusted at 0.5 and measured at λ 600 nm using spectrophotometer (Gene Quant Pro, Gemini BV, The Netherlands) to obtain a uniform population of bacteria [10^8–10^9 colony-forming units (CFU) mL^{-1}] in the broth at the time of inoculation.

Seed bacterization and phytohormone treatment

The carrier material was collected from the Changa Manga forest soil, Pakistan, sterilized at a pressure of 138 kPa (kilo

Pascal) and temperature of 121 °C for half an hour, and inoculated with broth culture. Peat-based inoculum was incubated at 28±2 °C by adding 10 % sugar solution to increase the microbial populations. For inoculation, the desired suspension of inoculum (10^8–10^9 CFU mL^{-1}; 250 mL kg^{-1} peat) was mixed with sterilized peat and incubated for 24 h at 28± 2 °C before use for seed coating (seed to peat ratio 1.25:1 w/w). Maize seed dressing was prepared with the inoculated peat mixed with 10 % sterilized sugar (sucrose) solution in a 10:1 ratio. In the case of noninoculated control, seeds were coated with the sterilized peat treated with sterilized broth and 10 % sterilized sugar solution. For the phytohormone treatment, maize seeds were treated with L-TRP solutions (10^{-4} and 10^{-5} M)—mixed slurry. For combined treatment, L-TRP solution was mixed with bacterial culture at the time of inoculation.

Net-house experiment

A pot experiment was conducted in the net-house, Soil Bacteriology Section, Ayub Agricultural Research Institute, Faisalabad, Pakistan, to observe the efficacy of PsJN inoculation along with two levels of L-TRP (10^{-4} and 10^{-5} M) for improving growth and photosynthesis of maize. Soil used for the experiment was collected from the field, air dried, thoroughly mixed, passed through a 2-mm sieve and analyzed for various physical and chemical characteristics. The soil was sandy clay loam, having: pH, 7.86; EC, 1.39 dS m^{-1}; organic matter, 0.76 %; total nitrogen, 0.035 %; available phosphorus, 7.76 mg kg^{-1} and extractable potassium, 119 mg kg^{-1}.

Maize seeds were surface-sterilized by dipping in 70 % ethanol for 2 min, and treated with 5 % NaClO for 5 min, followed by washing three times with sterile distilled water (1 min each time). The efficacy of surface sterilization was checked by plating seeds and aliquots of the final rinse onto LB plates where no growth was observed. The experiment contained the following treatments: 1) Control, 2) PsJN inoculation, 3) L-TRP solution (10^{-4} M), 4) L-TRP solution (10^{-5} M), 5) PsJN and L-TRP (10^{-4} M), 6) PsJN and L-TRP (10^{-5} M). Surface-disinfected maize seeds were coated with PsJN/L-TRP treated slurry (described above). For the control, slurry for seed coating was prepared by using sterilized LB broth. Three seeds were sown in each pot containing 16 kg of soil, and thinned to one plant after one week of germination. Pots were arranged in the net-house using a completely randomized design with three replications of each treatment. Recommended doses of N, P, and K fertilizers (160–100-60 kg ha^{-1}) were applied to each pot and equal amount of tap water was applied to the pots whenever needed. The crop was harvested after 45 days and various physiological and agronomic parameters were recorded.

Measurement of physiology and growth parameters

Physiological parameters

Plant physiological parameters of both treated and untreated plants were recorded at midday (between 10:00 and 14:00). A portable infrared gas analyzer [IRGA (CI-340) Germany] was used (at 1,200–1,400 μmol m^{-2} s^{-1} photosynthetic photon flux density) to measure transpiration rate (E), photosynthetic rate (A) and photosynthetically active radiation (PAR). Fully expanded flag leaves were selected for measurements. Leaves were crushed in acetone for determination of chlorophyll content (a and b), and absorbance at λ 645 nm and 663 nm, respectively, was noted on the spectrophotometer after centrifuging at 1,000 rpm for 10 min (Arnon 1949).

Agronomic traits

Plant agronomic parameters such as plant height, flag leaf length/width, shoot biomass and root biomass were recorded after harvesting the maize plants. Plant biomass (above and below ground) was obtained after drying whole plants at 65 °C for 72 h.

Post experiment soil analysis

IAA equivalents from the rhizosphere soil of maize were determined 15 and 30 days after planting (DAP) using Salkowski's reagent as described by Sarwar et al. (1992). Post-harvest soil and plant samples were analyzed for extractable P and soil N (Ryan et al. 2001).

Persistence of *B. phytofirmans* PsJN::*gusA* in the rhizosphere, root and shoot interior

Rhizosphere soil was obtained by agitating roots and sampling the soil still attached to the roots after harvesting. For rhizosphere colonization, soil slurry was prepared by mixing 5 g of rhizosphere soil with 15 mL of 0.85 % (w/v) NaCl solution and agitation (180 rpm) for 30 min at 28 °C. After sedimentation of soil particles, serial dilutions up to 10^{-5} were plated onto selective LB medium containing spectinomycin (100 μg mL^{-1}), 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (XGlcA) (100 μg mL^{-1}), and isopropyl-β-D-galactopyranoside (IPTG) (100 μg mL^{-1}). IPTG was used to induce *gusA* expression, as the strain PsJN was marked with *mTnSSSgusA10*. The plates were incubated at 28±2 °C for 3–4 days and blue colonies were counted to determine the colonization value.

For root/shoot colonization, plant tissues (root/shoot) were surface-sterilized by first dipping in 70 % ethanol for 1 min, and then in 2 % sodium hypochlorite (NaClO) for 5 min, followed by repeated washings with sterile distilled water.

Surface-sterilized root samples (2 g) were homogenized in 10 mL of 0.85 % NaCl solution by using a sterile mortar and pestle. Similarly, 3 g of shoots from each treatment were homogenized in 15 mL of 0.85 % NaCl solution. The homogenized material was placed in a shaker for 30 min at 28 °C. After settling the solid material, serial dilutions up to 10^{-4} were spread on selective LB medium. The plates were incubated at 28±2 °C for 48 h and then transferred to 4 °C for three days. Blue colonies were counted on each plate and colonization was calculated.

Statistical analysis

The data of plant growth parameters and colonization were subjected to analyses of variance. The means were compared by Least Significant Difference (LSD) test ($p<0.05$) to detect statistical significance among treatment (Steel et al. 1997). All of the statistical analyses were conducted using SPSS software version 19 (IBM SPSS Statistics 19, USA). The percent increases in growth parameters as well as colonization were correlated against IAA equivalent using Excel 2010.

Results

Results revealed that exogenously applied L-TRP and PsJN::*gusA* inoculation significantly increased the physiology and growth of maize when tested in separate treatments. Combined application of PsJN::*gusA* inoculation and L-TRP further increased the growth of maize (Tables 1, 2, and 3).

Agronomic parameters

Data in Table 1 show that PsJN::*gusA* inoculation significantly increased the plant height, leaf length and width over the control. PsJN inoculation along with L-TRP further increased the plant height, leaf length and leaf width by 18, 22 and 23 %, respectively, compared with the control (Table 1). The combined treatment of PsJN::*gusA* and L-TRP (10^{-5} M) resulted in a maximum increase, i.e., 55 %, in shoot dry biomass compared to the control. Up to a 33 and 31 % increase in dry biomass over the control was observed by separate application of PsJN::*gusA* and L-TRP (10^{-5} M), respectively. PsJN::*gusA* inoculation increased root biomass up to 44 % compared to the control treatment (Table 1). A maximum increase of 62 % in root biomass was observed by PsJN::*gusA* inoculation and L-TRP (10^{-5} M) treatment compared to the control. A minimum increase in root biomass, i.e., 20 % compared to the control, was observed by L-TRP (10^{-4} M) treatment.

Physiological parameters

Data (Table 1) showed that PsJN::*gusA* inoculation and L-TRP (10^{-5} M) application increased photosynthesis by 11 and 5 %, respectively, compared to the control. Combined application of PsJN::*gusA* and L-TRP (10^{-5} M) gave a maximum increase in photosynthesis (16 %) compared to the control. Application of PsJN::*gusA* alone resulted in a 22 % increase in transpiration rate compared to the control (Table 2). A maximum increase in transpiration (up to 34 %) was observed by PsJN::*gusA* inoculation supplemented with 10^{-5} M L-TRP compared to the control. In the case of photosynthetically active radiation (PAR), a 15 % increase was observed by combined treatment of PsJN::*gusA* and L-TRP (10^{-5} M), compared to the control (Table 2). Separate PsJN::*gusA* inoculation and L-TRP (10^{-5} M) application increased the PAR by 7 and 8 %, respectively, compared to the control. Data in Fig. 1 show that the combination of PsJN::*gusA* and L-TRP significantly increased the chlorophyll (a, b) content of maize plants compared to when applied individually. Again, a maximum increase of 17 and 45 %, respectively, was observed in the combined application of PsJN::*gusA* and L-TRP (10^{-5} M). PsJN::*gusA* inoculation resulted in a 15 and 28 % increase in chlorophyll a and b compared to the uninoculated control.

Table 1 L-tryptophan–dependent response of PsJN inoculation on the growth parameters and photosynthesis of maize

Treatment	Plant height (cm)	Flag leaf width (cm)	Flag leaf length (cm)	Shoot dry biomass (g pot^{-1})	Root dry biomass (g pot^{-1})	Photosynthesis (μmole m^{-2} s^{-1})
Control	70.3 d*	4.53 d	42.83 d	75.7 e	2.31 e	66.20 d
PsJN inoculation	76.0 c	4.87 c	48.17 c	100.3 c	3.32 bc	73.53 b
L-TRP (10^{-4} M)	75.0 c	4.57 d	47.83 c	89.7 d	2.78 d	68.33 cd
L-TRP (10^{-5} M)	75.3 c	4.90 c	48.83 bc	99.5 c	3.15 c	69.83 c
PsJN+L-TRP (10^{-4} M)	79.0 b	5.20 b	49.83 b	109.0 b	3.52 b	73.93 ab
PsJN+L-TRP (10^{-5} M)	83.0 a	5.53 a	52.50 a	117.4 a	3.74 a	76.47 a
LSD	2.94	0.255	1.186	7.165	0.205	2.89

*Means sharing similar letter(s) in a column for each parameter do not differ significantly at $p = 0.05$

Table 2 L-tryptophan–dependent response of PsJN inoculation on the physiology parameters and nutrient concentration of maize

Treatment	Transpiration (mmole m^{-2} s^{-1})	Photosynthetically active radiation (μmole m^{-2} s^{-1})	Plant N-content (%)	Plant P-content (%)	Soil N (%)	Available P (mg kg^{-1})
Control	5.63 d*	670.97 d	1.117 e	0.208 d	0.032 d	7.55 d
PsJN inoculation	6.87 bc	716.53 c	1.177 c	0.225 c	0.035 bc	7.90 bc
L-TRP (10^{-4} M)	6.03 d	705.40 c	1.153 d	0.224 c	0.034 c	7.79 c
L-TRP (10^{-5} M)	6.73 c	721.63 c	1.173 c	0.225 c	0.035 bc	7.92 bc
PsJN+L-TRP (10^{-4} M)	7.30 ab	745.47 b	1.200 b	0.249 b	0.036 ab	8.04 ab
PsJN+L-TRP (10^{-5} M)	7.53 a	770.40 a	1.230 a	0.262 a	0.037 a	8.13 a
LSD	0.469	16.43	0.017	0.010	0.001	0.143

*Means sharing similar letter(s) in a column for each parameter do not differ significantly at $p = 0.05$

IAA biosynthesis and mineral plant and soil analysis

In vitro data of IAA biosynthesis (Table 3) showed that PsJN produced auxin (IAA equivalents) without L-TRP addition; however, IAA equivalents substantially increased when the medium was supplemented with L-TRP. PsJN produced IAA equivalents (11.78±1.99 μg mL^{-1}) when L-TRP was added. Similarly, data of the pot trial (Fig. 2) showed that PsJN::*gusA* inoculation and L-TRP treatment individually increased in vivo IAA concentration in the plant rhizosphere soil compared to the untreated control. PsJN::*gusA* inoculation showed a 22 and 16 % increase in IAA content for 15 and 30 DAP, respectively, compared to the control. L-TRP application increased IAA content up to 39 and 31 % at both 15 and 30 DAP compared to the control. A combined application of PsJN::*gusA* and L-TRP (10^{-5} M) gave the maximum increase in IAA equivalents, which was 55 and 50 % at 15 and 30 DAP, respectively, compared to the control.

Data regarding the plant mineral contents revealed that separate PsJN::*gusA* inoculation or L-TRP increased N and P contents (Table 2). A maximum increase in shoot N and P contents up to 10 and 26 % was achieved with the combined application of PsJN::*gusA* and L-TRP (10^{-5} M). Likewise, PsJN::*gusA* supplemented with L-TRP (10^{-5} M) increased soil N and P contents by 16 and 8 %, respectively, as compared to the untreated control (Table 2).

A significantly positive correlation (*r* values) was observed between soil IAA-equivalent and maize plant growth promotion caused by PsJN inoculation and L-TRP amendment (Table 4).

Enumeration of PsJN::*gusA* in the rhizosphere, root and shoot interior

The inoculant strain efficiently colonized the rhizosphere and interior of maize roots and shoots (Table 3). However, when supplemented with L-TRP, persistence of PsJN::*gusA* increased as compared to inoculation treatment alone in the rhizosphere and plant tissues. In the inoculation treatment, 2.30×10^5 CFU g^{-1} rhizosphere soil, 1.05×10^5 CFU g^{-1} root interior and 8.21×10^3 CFU g^{-1} shoot interior were recovered. However, more CFU of the inoculant strain g^{-1} dry weight were recovered from the rhizosphere, root interior, and shoot interior in the presence of L-TRP (10^{-5} M).

Discussion

Burkholderia phytofirmans strain PsJN is a plant growth-promoting rhizospheric bacterium, which colonizes the rhizosphere and internal tissues of its plant hosts, and promotes growth and yield of different crops through multifarious

Table 3 Persistence of PsJN in the rhizosphere, root and shoot interior of maize plant and in vitro auxin biosynthesis by PsJN

Treatment	Rhizosphere (CFU g^{-1} dry soil)	Root interior (CFU g^{-1} dry weight)	Shoot interior (CFU g^{-1} dry weight)	In vitro auxin production by PsJN (IAA equivalent μg mL^{-1})	
				Without L-TRP	With L-TRP
PsJN	2.30×10^5 b*	1.05×10^5 b	8.21×10^3 d	0.84±0.33†	11.78±1.99
PsJN+L-TRP (10^{-4} M)	7.10×10^5 a	3.95×10^5 b	3.04×10^4 c		
PsJN+L-TRP (10^{-5} M)	9.20×10^5 a	5.83×10^5 ab	4.06×10^4 c		

*Means sharing similar letter(s) in a column for each parameter do not differ significantly at $p = 0.05$

† Data is the average of three replicates±SD

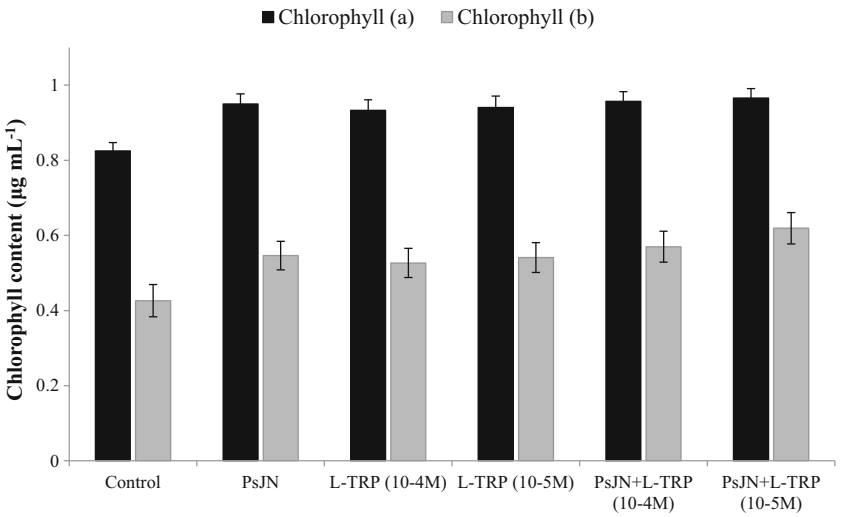

Fig. 1 L-Tryptophan–dependent response of PsJN inoculation on the chlorophyll a and b content of maize plant. Data is the average of three replicate±SE

mechanisms (Sessitsch et al. 2005; Mitter et al. 2013b). In the present study, we demonstrated that L-tryptophan–dependent biosynthesis of IAA by strain *B. phytofirmans* PsJN improves its ability to promote plant growth and colonization.

In the present study, it was observed that PsJN inoculation improved maize plant growth and physiology, which was observed as better survival, root/shoot biomass and nutrient content compared to the uninoculated control (Tables 1, 2, and 3). Increase in the total root system is the most common reported plant response mediated by PGPB inoculation in various plant species (Lucy et al. 2004). Several bacterial mechanisms have been proposed, and hormone production is considered as the most plausible mechanism in controlling root growth and development (Mantelin and Touraine 2004). Production of phytohormones such as auxins in the root zone using L-TRP as a precursor from the root exudates by bacteria is responsible for root architecture (Ludwig-Müller 2011). Bacterial-induced alterations in root architecture might lead to an increase in total root surface area, and consequently, improved nutrient and water uptake, which may have positive effects on plant growth as a whole. This premise is further supported by our results showing that IAA production in strain PsJN is mostly dependent on the presence of L-TRP. When tryptophan was not added, only very low levels of IAA were produced in the culture medium (Table 3). It has been reported that increasing amounts of L-TRP stimulate the secretion of IAA by PGPB, which is responsible for the phytostimulatory effect of bacteria on plants (Omay et al.

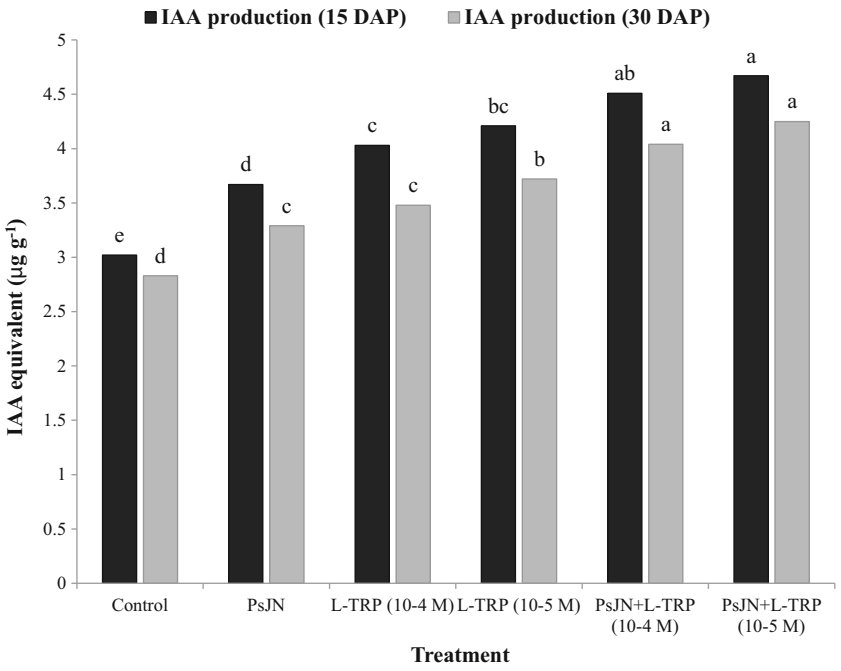

Fig. 2 L-Tryptophan–dependent IAA production by PsJN inoculation in the rhizosphere soil of maize plant [15 and 30 days after planting (DAP)]. Bars sharing similar letters for each parameter do not differ significantly at $p=0.05$

Table 4 Correlation (*r*) between growth parameters of maize and their percent increases and soil IAA equivalent values

Plant parameter	IAA production		IAA production	
	15 DAP	30 DAP	15 DAP	30 DAP
Plant height (cm)	0.90†	0.93†	0.79‡	0.87‡
Flag leaf width (cm)	0.81	0.89	0.78	0.87
Flag leaf length (cm)	0.95	0.95	0.84	0.91
Shoot dry biomass (g pot^{-1})	0.90	0.93	0.74	0.84
Root dry biomass (g pot^{-1})	0.85	0.88	0.61	0.73
Photosynthesis (μmole m^{-2} s^{-1})	0.74	0.79	0.47	0.60
Transpiration (mmole m^{-2} s^{-1})	0.84	0.88	0.64	0.75
PAR (μmole m^{-2} s^{-1})	0.93	0.96	0.85	0.92
Chlorophyll (a)	0.87	0.82	0.61	0.73
Chlorophyll (b)	0.92	0.92	0.76	0.85
Plant N content (%)	0.90	0.93	0.76	0.85
Plant P content (%)	0.89	0.94	0.86	0.92
Soil N content (%)	0.92	0.94	0.77	0.86
Available P (mg kg^{-1})	0.93	0.95	0.79	0.88
Rhizosphere colonization (CFU/ g dry weight)	0.99	0.99	–	–
Root colonization (CFU/ g dry weight)	0.98	0.99	–	–
Shoot colonization (CFU/ g dry weight)	0.98	0.99	–	–

† Correlation coefficient (*r*) between growth parameters/colonization of maize plants and IAA equivalent

‡ Correlation coefficient (*r*) between % increase in growth parameters over control and IAA equivalent

1993). Similarly, our results strongly indicate that the improvement of plant growth and development is, at least partly, due to auxin production, particularly when L-TRP is added. A significantly positive correlation (*r*) was observed between plant growth promotion and soil IAA-equivalent (Table 4). These findings are supported by the work of other researchers who elucidated the effect of PsJN inoculation on the growth and development of various crops (Nowak et al. 1995; Ait Barka et al. 2000).

L-Tryptophan is considered an efficient physiological precursor of auxins, and its exogenous application to soils has been shown to influence plant growth and development positively (Arshad and Frankenberger 1997). As a majority of rhizosphere microflora (≥ 80 %) are able to produce phytohormones, e.g., auxins, that directly enhance plant growth. There is an exciting possibility that most bacteria are capable of producing growth regulators continuously, provided that precursors of phytohormones are available in the rhizosphere (Bais et al. 2006). Because appreciable levels of IAA are not produced unless an external source of tryptophan is supplied to the rhizosphere bacteria, endogenous levels of tryptophan are not sufficient for IAA production. There is likely a high demand for tryptophan by bacteria, as it is used to produce many essential compounds such as proteins and vitamins (Martens and Frankenberger 1993). The different levels of L-TRP were tested separately. A significant increase in plant growth and development was obtained when the L-TRP solution (10^{-5} M) was applied alone and in combination with PsJN; however, L-TRP level at 10^{-4} M gave some nonsignificant results when applied alone as compared with untreated control. In the present study, the exogenously applied L-TRP at 10^{-5} M proved to be more effective in improving the growth parameters of maize compared to 10^{-4} M L-TRP and the untreated control. The effect in modifying plant growth and development observed by L-TRP in our study was concentration dependent. The mechanism of action of L-TRP on plant growth may be attributed to direct uptake of these compounds by plant roots, a change in the rhizosphere microflora discouraging root pathogens, or microbial conversion of other plant-associated microorganisms into metabolites (such as IAA), resulting in a beneficial rhizosphere for plant growth (Sarwar and Frankenberger 1994; Khalid et al. 2006).

In the present study, combined application of PsJN and L-TRP was found to be more effective and showed that maximal increase in plant growth. The importance of auxin production for the ability of bacteria to promote plant growth has been demonstrated through inoculation studies using bacterial mutants (Barbieri and Galli 1993). Fewer studies have described the role of microbial auxin (IAA) in plant growth promotion by using mutants and molecular tools (Patten and Glick 2002; Idris et al. 2007; Spaepen et al. 2008). In the present study, a mutant defective in IAA production, (*iacC*) of this strain *Burkholderia phytofirmans* PsJN, was not used. However, the involvement of auxin in root growth promotion and colonization of *Arabidopsis thaliana* by using a PsJN mutant has been described recently by Zúñiga et al. (2013). Bacterial-induced root growth in the presence of IAA causes several changes in physical and chemical properties of the soil, which can affect the ability of bacteria to colonize the rhizosphere (Table 3). In our study, it is assumed that improvement in the plant growth might be due to L-TRP–dependent IAA biosynthesis by PsJN in the rhizosphere, which might optimize the endogenous suboptimal plant hormone level, or improve mineral uptake by plant roots. Zahir et al. (2010a, b) reported the synergistic response of L-TRP and PGPB inoculation for improving growth and yield of mungbean, as compared to separate application.

PGPB maintain the beneficial association with plants through their roots. Bacteria principally utilize amino acids and other nutrients released from plant roots as exudates. L-tryptophan present in the root exudates or applied exogenously serves as the precursor for IAA biosynthesis (Kulkarni et al. 2013). From the results of present study, it is likely that stimulated bacterial IAA production in the presence of the IAA precursor L-TRP in the rhizosphere (Fig. 2) might be involved in plant growth promotion, suggesting a close

symbiotic relationship between the plants and colonizing PsJN. The ability to colonize plant roots may depend to some degree on the capability of the bacterium to synthesize IAA. Moreover, it has been proposed that bacterial IAA synthesis contributed to enhanced rhizosphere competence and plant interior colonization (Table 3) by stimulation of the release of plant exudates (Lambrecht et al. 2000).

We provide evidence that L-TRP–dependent IAA biosynthesis by *B. phytofirmans* PsJN affects this organism's ability to promote plant growth and colonization of maize plants. Based on our results, we conclude that a combined application of PsJN and L-TRP is more effective to improve plant photosynthesis and biomass than their separate application. Overall, this study implies that the combined application of L-TRP and strain PsJN is an attractive approach for improving the growth and nutrient content of maize plants. The present study describes the response of PsJN inoculation and L-TRP at the early stages of plant development; however, further field investigations are needed to confirm the effect on final yield in a natural soil environment.

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