FULL RESEARCH PAPER

Effects of plant growth-promoting rhizobacteria on nodulation of *Phaseolus vulgaris* L. are dependent on plant P nutrition

Roseline Remans · Anja Croonenborghs · Roldan Torres Gutierrez · Jan Michiels · Jos Vanderleyden

Abstract Several plant growth-promoting rhizobacteria (PGPR) have shown potential to enhance nodulation of legumes when coinoculated with *Rhizobium*. To optimize the efficiency of these *Rhizobium*-PGPR-host plant interactions, unravelling the underlying mechanisms and analyzing the influence of specific environmental conditions is crucial. In this work the effect of four PGPR strains on the symbiotic interaction between *Rhizobium* and common bean (*Phaseolus vulgaris*) was studied under deficient versus sufficient phosphorus supply. It was observed that the effect on nodulation of three out of four PGPR tested was strongly dependent on P nutrition. Further, the use of specific PGPR mutant strains indicated that bacterial indole-3-acetic-acid production (IAA) and 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity play an important role in the host nodulation response, particularly under low P conditions. Moreover, it was shown that the differential response to PGPR under low versus high P conditions was associated with changes in the host hormone sensitivity for nodulation induced under P deficiency. These findings contribute to the understanding of the interplay between *Rhizobium*, PGPR and the plant host under different environmental settings.

Introduction

Legumes capable of symbiotic nitrogen fixation are crucial in agricultural crop production (Smith and Hume 1987; Vance 1997; Pepper 2000). Common bean (*Phaseolus vulgaris* L.) is the most important food legume for direct human consumption in the world (Broughton et al. 2003). In Africa and Latin America, common bean production is often undertaken on small farms using marginal soils low in nitrogen (N) and phosphorus (P) (Graham 1981; Thung 1991) and with minimal technical inputs. Progressive mining of soil nutrients does occur (Franzluebbers et al. 1998), with P second only to N as the most limiting factor for plant growth (Vance et al. 2000). P deficiency specifically is a nutritional limiting factor for efficient nodulation and nitrogen fixation (Christiansen and Graham 2002). N_2 fixation in legumes is a P-requiring process. Nodules of P-sufficient plants have a higher P concentration than shoots and roots, and may contain up to 1.5% of the total plant P (Graham and Rosas 1979; Pereira and

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Bliss 1987, Schulze et al. 2006). Al-Niemi et al. (1997) suggest that nodule bacteroids in beans may be P limited even though the plant is well supplied with P. P-deficient plants exhibit reduced carbohydrate supply to nodules, and are usually restricted in nodule initiation, development and growth, and in nitrogenase activity (Graham and Rosas 1979; Pereira and Bliss 1987; Vadez et al. 1999; Kouas et al. 2005; Schulze et al. 2006).

Simultaneous inoculation with *Rhizobium* and other plant growth-promoting bacteria has shown potential to enhance plant growth, nodulation and nitrogen fixation of several legumes. Examples are numerous. Dual inoculation with *Azotobacter* spp. or *Azospirillum* spp. and *Rhizobium* strains showed a synergistic effect on nodulation, plant growth, yield and N uptake in soybean, clover, common bean and peanut (Burns et al. 1981; Raverker and Konde 1988; Burdman et al. 1997). Similarly, coinoculation with *Pseudomonas* spp. and *Rhizobium* spp. has been reported to enhance nodulation and nitrogen fixation, plant biomass and grain yield in various leguminous species including alfalfa (Bolton et al. 1990), soybean (Dashti et al. 1998), green gram (Sindhu et al. 1999) and chickpea (Goel et al. 2002). Coinoculation with *Bacillus* spp. and *Rhizobium* or *Bradyrhizobium* spp. ameliorated the nodulation and plant growth of common bean and soybean respectively (Srinivasan et al. 1997; Camacho et al. 2001; Bai et al. 2003). The mechanisms used by rhizobacteria to promote nodulation and nitrogen fixation are mostly unknown or remain ambiguous. Increased root development and nutrient uptake induced by plant growth-promoting rhizobacteria (PGPR) and their metabolites (reviewed by Lugtenberg et al. 2002; Persello-Cartieaux et al. 2003) may indirectly contribute to nodulation and nitrogen fixation (Vessey and Buss 2002). Evidence for a more direct effect on nodulation was described by Volpin et al. (1996) and Burdman et al. (1996). They observed that *A. brasilense* increased the exudation of flavonoids, plant signalling molecules crucial in the establishment of the *Rhizobium*-legume symbiosis, in the alfalfa and common bean rhizosphere. The use of a *gusA*-marked *Azospirillum* strain in an *Azospirillum–Rhizobium* coinoculation experiment on white clover demonstrated that *Azospirillum* colonized the tap root, root hairs and sites near or on the nodules (Tchebotar et al. 1998). Taken together with the

observation that non-nodulated plants form more root hairs and lateral roots (Plazinski and Rolfe 1985a), it is suggested that when coinoculated with *Rhizobium*, *Azospirillum* stimulates the formation of epidermal cells that become infected root hair cells, or creates additional infection sites that are later occupied by rhizobia (Plazinski and Rolfe 1985c; Tchebotar et al. 1998). Recently, Thilak et al. (2006) showed that PGPR in conjunction with efficient *Rhizobium* can also affect the growth and nitrogen fixation in pigeon pea by enhancing the occupancy of introduced *Rhizobium* in the nodules of the legume. The nodule occupancy of the introduced *Rhizobium* strain increased from 50% (with *Rhizobium* alone) to 85% in the presence of *Pseudomonas putida*.

Positive effects of PGPR on nodulation, nitrogen fixation and plant growth of legumes have been observed in greenhouse experiments using hydroponic, vermiculite-based and soil-based systems as well as in field experiments (Burdman et al. 1997; Bai et al. 2003; Hamaoui et al. 2001). However, the influence of specific environmental factors on these *Rhizobium*-PGPR-plant interplays has not yet been studied. In the present work, the effect of different PGPR on the *Rhizobium*-bean symbiosis was evaluated under deficient versus sufficient P supply, thereby exploring the potential of *Rhizobium*-PGPR coinoculation to enhance nitrogen fixation under P deficiency. To initiate mechanistic studies of the synergistic effects, wild-type and specific mutants of some of the PGPR tested were used for the first time in *Rhizobium*-PGPR coinoculation experiments.

Materials and methods

Bacterial strains and growth conditions

The strains used in this study are listed in Table 1. *Rhizobium etli* CNPAF512 was grown overnight in liquid tryptone-yeast extract (TY) medium at 30°C or maintained on yeast extract -mannitol (YEM) (Vincent 1970) agar plates (15 g agar l^{-1}). *Azospirillum brasilense* Sp245 was grown overnight in liquid L* broth, which is Luria-Bertani broth medium (Sambrook et al. 1989) supplemented with 2.5 mM CaCl$_2$ and 2.5 mM MgSO$_4$, at 30°C or maintained on yeast extract-peptone agar plates (YEP)

Table 1 Bacterial strains used in this study

Bacterial strains	Relevant characteristics	Reference
Rhizobium etli CNPAF512	Wild-type strain, isolated from *Phaseolus vulgaris* nodules, Brazil	Michiels et al. (1998)
Azospirillum brasilense Sp245	Wild-type strain, isolated from surface-sterilized wheat roots, Brazil	Baldani et al. (1986)
FAJ009	*ipdC* Tn5 mutant of *A. brasilense* Sp245; Km^r	Costacurta et al. (1994)
Bacillus subtilis LMG7135	Wild-type strain, LMG bacteria collection	Chun and Bae (2000)
Pseudomonas putida UW4	Wild-type strain, isolated from the rhizosphere of reeds and selected for ACC deaminase activity, Canada	Glick et al. (1995)
UW4/AcdS$^-$	AcdS, ACC deaminase mutant of *P. putida*, Tc^r	Li et al. (2000)
Pseudomonas fluorescens SBW25	Wild-type SBW25	Rainey and Baily (1996)

(15 g agar l^{-1}). *Bacillus subtilis* LMG7135 was grown overnight in liquid TY medium at 30°C or maintained on TY agar plates (15 g agar l^{-1}). *Pseudomonas* strains, *Pseudomonas fluorescens* SBW25 and *Pseudomonas putida* UW4, were grown in liquid Tryptic Soy Broth (TSB) medium at 30°C or maintained on TSB agar plates (15 g agar l^{-1}). For the genetically modified strains the antibiotics kanamycin (Km) or tetracycline (Tc) were added to a final concentration of 25 and 10 μg ml^{-1} respectively.

Preparation of bacterial inocula

Pure cultures were grown overnight at 30°C in liquid medium (see above). Cells were washed twice with 10 mM MgSO$_4$ and resuspended in 10 mM MgSO$_4$ at a density of 10^7 colony forming units (cfu) ml^{-1} for *Rhizobium* or at a density of 10^9 cfu ml^{-1} for *Azospirillum*, *Bacillus* and *Pseudomonas* strains. *Rhizobium* inoculum contained only *Rhizobium* cells at a density of 10^7 cfu ml^{-1} MgSO$_4$. Coinoculants contained *Rhizobium* cells at a density of 10^7 cfu ml^{-1} and cells of other specified bacteria at a density of 10^9 cfu ml^{-1} MgSO$_4$.

Plant material and growth conditions

Seeds of *Phaseolus vulgaris* cvs BAT477 were kindly provided by the International Centre of Tropical Agriculture (CIAT), Cali, Colombia. A hydroponic system with a perlite-matrix was optimized to grow bean plants under low versus high P conditions in the greenhouse. One litre pots filled with perlite were initially moistened with 250 ml nitrogen-free medium (based on Snoeck et al. 2003). During growth a drip irrigation system maintained the pH (pH 6) and concentration of nutrients in the pots. The high P culture medium contained 100 μM KH$_2$PO$_4$, 1.71 mM K$_2$SO$_4$, 0.48 mM MgSO$_4$, 0.70 mM MgCl$_2$ and 1.06 mM CaCl$_2$. The low P medium was identical to the high P medium apart from the concentration of KH$_2$PO$_4$, which was 1 μM in low P conditions, and the concentration of K$_2$SO$_4$, which was 1.76 mM in low P medium (to maintain equal amounts of potassium under low versus high P conditions). An initial gift of 0.5 mmol KNO$_3$ was added to each pot at sowing.

The seeds of bean cv. BAT477 were surface-sterilized as described previously (Vlassak et al. 1998) and pre-germinated during two days on moist filter paper in the dark at 28°C. One pre-germinated seedling was planted per pot. The seedlings were inoculated with 200 μl inoculum (prepared as described above) containing 10^6 *Rhizobium* cells or 10^6 *Rhizobium* cells and 10^8 cells of *Azospirillum*, *Bacillus* or *Pseudomonas*. The number of cells used for inoculation is based on previous research described by Burdman et al. (1996), Burdman et al. (1997), Hamaoui et al. (2001) and Bai et al. (2002). For each condition 12 plants were used and arranged in a randomised block design. Plants were harvested at 21 and/or 42 days after inoculation (DAI) for determination of nodule number, nodule dry weight, shoot dry weight, root dry weight and NPK contents.

Hormone treatments

Low and high P culture media were supplemented with 0, 10, 100 or 200 nM indole-3-acetic acid (IAA). IAA was solubilised in ethanol at a concentration of 0.1 M before adding to the nutrient solution. IAA was purchased from Sigma Chemicals.

Plant analysis for NPK contents

Total N content (%N) was measured by ignition with a Variomax CN analyser. Total P and K contents (%P, %K) of shoot, root and nodule tissues were measured by Inductively Coupled Plasma—Optical Emission Spectroscopy (ICP-OES, Perkin Elmer, Optima 3300 DV, axial plasma).

Statistical analysis

Significance of difference between inoculation treatments and P levels was determined by the Tukey–Kramer Honestly Significant Difference Test at $P = 0.05$, using the SAS programme (SAS 9.1) (SAS Institute 1996). All experiments were performed at least twice, yielding similar results.

Results

Nodulation and plant growth parameters under deficient versus sufficient P conditions

BAT477 bean plants were inoculated with *Rhizobium etli* CNPAF512 and grown under low and high P conditions in the greenhouse. Plant growth and nodulation parameters were determined at harvest 42 DAI. The results, summarized in Table 2, show that (i) plants grown under low P (1 μM) conditions used in our experimental set-up are deficient in P; (ii) nodulation is more reduced than plant growth under P deficiency and (iii) nodules contain a higher concentration of P than other plant parts under high as well as under low P conditions. These results are consistent with previous observed effects of P deficiency on nodulation and growth parameters of common bean and other legumes.

Effect of PGPR on nodulation and plant growth parameters of bean under low versus high P conditions

BAT477 bean plants were inoculated with *Rhizobium etli* CNPAF512 or coinoculated with *Rhizobium* and other rhizobacteria, *Azospirillum brasilense* Sp245, *Bacillus subtilis* LMG7135, *Pseudomonas putida* UW4 or *Pseudomonas fluorescens* SBW25. For each of these rhizobacteria when used as a single inoculum plant growth-promoting activities have been extensively documented in the literature with various plant species. Similarly as described above, plants were grown under low and high P conditions in the greenhouse and harvested at 42 DAI. The effect of coinoculation on nodule number under high and low P conditions is shown in Fig. 1a, b respectively. In this figure the nodule number for each inoculation treatment is presented as % nodule number of plants inoculated with *Rhizobium* only. Under high P conditions, coinoculation with *Rhizobium* and *A. brasilense* Sp245 or with *Rhizobium* and *B. subtilis* LMG7135 significantly ($P < 0.05$) increased bean nodulation. However, under P deficient conditions, this positive effect of Sp245 and LMG7135 was not observed. On the contrary coinoculation with *A. brasilense* Sp245 resulted in a strong reduction of nodulation under low P. LMG7135 did not affect nodulation significantly under low P. The reverse effect of P nutrition was observed for coinoculation with *Rhizobium* and *P. putida* UW4. UW4 enhanced nodulation strongly under P deficiency but only slightly under P-sufficient conditions. *Pseudomonas fluorescens* SBW25 did not affect nodule number of bean line BAT477 significantly under the conditions tested.

Similar effects of the PGPR strains used were observed on nodule dry weight (data not shown), shoot dry weight (Fig. 1c, d) and root dry weight (Fig. 1e, f) under low versus high P conditions. The similarity in response to PGPR of nodule number, shoot and root dry weight strongly suggests a correlation between nodulation and plant growth parameters. Coinoculation with *Rhizobium* and PGPR did not affect plant NPK concentrations, only total NPK contents (data not shown).

Table 2 Nodulation and growth parameters of BAT477 bean plants inoculated with *Rhizobium etli* CNPAF512 and grown under low P (1 μM KH$_2$PO$_4$) and high P (100 μM KH$_2$PO$_4$) conditions

	High P	Low P	Low P As % of high P
Nodule number	359.2 ± 39	107.5 ± 18	29.8*
Nodule dry weight (mg)	200.4 ± 30.8	25.9 ± 5.6	12.9*
Acetylene reduction Activity (μl ethylene h^{-1})	7.1 ± 1.45	0 ± 0	0*
Shoot dry weight (SDW) (g)	2.06 ± 0.34	0.74 ± 0.18	35.9*
Root dry weight (RDW) (g)	0.51 ± 0.08	0.37 ± 0.05	72.6*
SDW/ RDW	4.04 ± 0.12	2.00 ± 0.09	49.5*
Shoot % N	3.86 ± 0.36	3.31 ± 0.25	85.8
Shoot % C	43.9 ± 0.44	42.4 ± 0.76	96.4
Shoot % P	0.22 ± 0.02	0.08 ± 0.02	36.4*
Shoot N/P	17.53 ± 1.06	41.40 ± 4.45	236.2*
Root % P	0.25 ± 0.03	0.16 ± 0.02	64.0*
Nodules % P	0.46 ± 0.05	0.34 ± 0.04	73.2*
Total N (mg)	146.5 ± 24.91	24.5 ± 6.56	16.7*
Total C (mg)	905.82 ± 121.7	313.64 ± 45.6	34.6*
Total P (mg)	4.53 ± 0.83	0.59 ± 0.12	13.0*

Values are averages of 12 plants ± standard deviations

*$P < 0.05$ Tukey–Kramer for difference between low P and high P values

Differential nodulation and growth response to PGPR under low versus high P is associated with production or conversion of phytohormones by PGPR

To delineate the mechanisms by which PGPR contribute to the differential nodulation response under low versus high P conditions, PGPR mutant strains affected in genes known to be involved in their plant growth-promoting effect were used. Bean plants were inoculated with *Rhizobium*, coinoculated with *Rhizobium* and a wild-type PGPR strain or with *Rhizobium* and a mutant PGPR strain, and grown during 42 days under low and high P conditions in the greenhouse. This allowed us to compare the effect of the mutant and wild-type PGPR strain on nodulation and to evaluate the role of the mutations in the modulation of nodulation.

Figure 2a, b shows that the effect of coinoculation on nodulation was less pronounced when the mutant strain FAJ009 of *A. brasilense* and the UW4/AcdS$^-$ mutant of *P. putida* UW4 were used instead of parent wild-type strains. FAJ009 is an insertion mutant of *A. brasilense* Sp245 in which the indole-3-pyruvate decarboxylase (*ipdC*) gene is

inactivated, resulting in 90% reduction of production of the phytohormone indole-3-acetic acid in FAJ009 as compared to the wild-type Sp245 strain (Costacurta et al. 1994; Vande Broek et al. 1999). UW4/AcdS$^-$ (Li et al. 2000) is an insertion mutant of *P. putida* UW4 in which the gene encoding the enzyme 1-aminocyclopropane-1-carboxylate deaminase (ACCD) is inactivated. The enzyme 1-aminocyclopropane-1-carboxylate deaminase converts ACC, the precursor of the plant hormone ethylene, to alpha-ketobutyrate and ammonium and thereby reduces the production of ethylene. The mutations in FAJ009 and UW4/AcdS$^-$ have been described to be stable without antibiotic selection by Dobbelaere et al. (1999) for FAJ009 and by Hontzeas et al. (2004) for UW4/AcdS$^-$. In the media used no differences in growth rate were observed between wild-type and mutant strains.

Under low P conditions, FAJ009 did not affect nodulation significantly while the Sp245 wild-type strain reduced nodule number by 36% as compared to single *Rhizobium* inoculation. Under high P conditions, FAJ009 enhanced nodule number significantly, but the increase in nodulation was less pronounced compared to coinoculation with the wild-type strain.
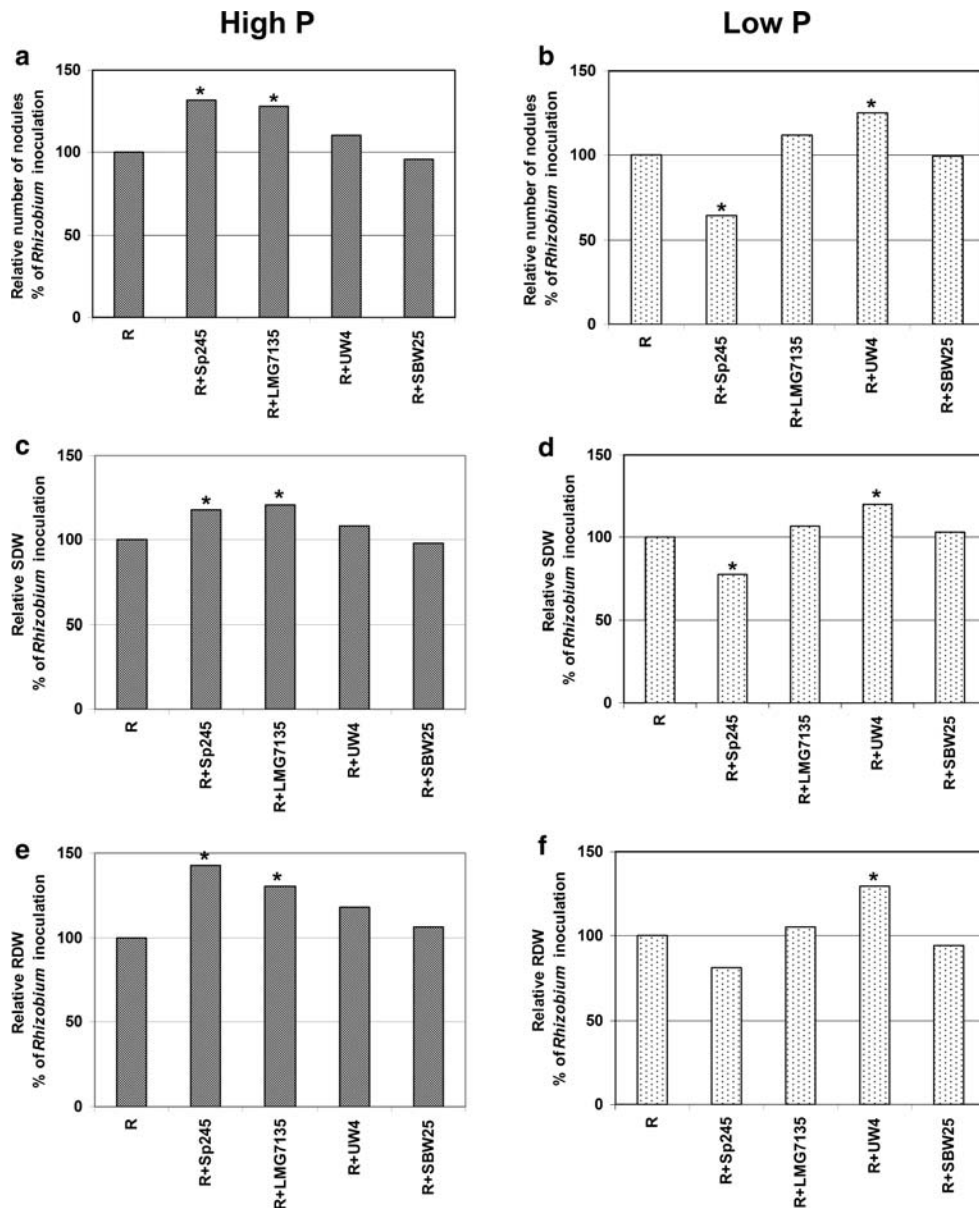
Fig. 1 Effect of PGPR on nodulation and plant parameters under high and low P conditions. Bars represent percentages of (**a**) nodule number per plant under high P; (**b**) nodule per plant number under low P; (**c**) plant shoot dry weight (SDW) under high P; (**d**) SDW under low P; (**e**) plant root dry weight (RDW) under high P (**f**) RDW under low P of BAT477 plants inoculated with *Rhizobium etli* CNPAF512 (R) or coinoculated with *Rhizobium* and *A. brasilense* Sp245 (R + Sp245), *B. subtilis*

LMG7135 (R + LMG7135), *P. putida* UW4 (R + UW4), *P. fluorescens* SBW25 (R + SBW25). 100% represents the average value of plants inoculated with *Rhizobium* alone (see Table 1 for absolute values of nodule number, SDW, RDW). Plants were grown for 42 days under low and high P conditions and harvested for nodulation and plant parameters analysis. 12 plants per condition were used. *$P < 0.05$ Tukey–Kramer. This experiment was performed three times, yielding similar results

Inactivation of the ACC-deaminase in *P. putida* UW4 reduced the positive effect on nodulation of UW4 under low P conditions. Under high P conditions, no

significant differences were observed between single *Rhizobium* inoculation and coinoculation with *Rhizobium* and UW4 or its ACCD minus mutant.
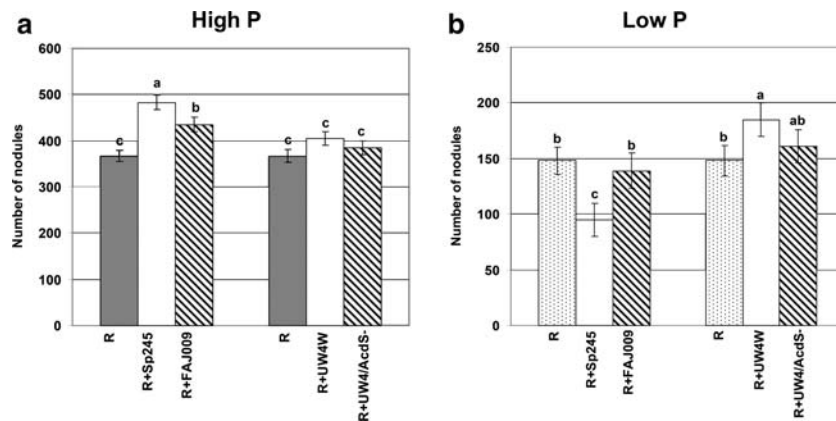
Fig. 2 The effect on nodule number of mutant strains of PGPR compared to the effect of their wild-type strain under (**a**) high P and (**b**) low P conditions. Bars represent average values of nodule number of BAT477 bean plants inoculated with *Rhizobium etli* CNPAF512 (R), coinoculated with *Rhizobium* and the wild-type strain of *A. brasilense* Sp245 (R + Sp245 WT, white bars) or the wild-type strain of *P. putida* UW4 (R + UW4 WT, white bars), or coinoculated with *Rhizobium* and the FAJ009 mutant of Sp245 (R + FAJ009, striped bars) or

the AcdS⁻ mutant of UW4 (R + UW4/AcdS⁻, stride bars). Plants were grown for 42 days under low and high P conditions and harvested for nodulation and plant parameters analysis. 12 plants per condition were used. Error bars represent ±95% confidence intervals (Tukey–Kramer). Letters on top of the bars indicate different classes of statistical differences (Tukey–Kramer). This experiment was performed three times, yielding similar results

Effect of IAA on nodulation under low versus high P conditions

To further unravel the role of exogenous IAA in modulation of nodulation under low and high P conditions, increasing concentrations of IAA were added to the nutrient solution medium of bean plants inoculated with *Rhizobium*. BAT477 plants were grown for 21 days under low and high P conditions and harvested for analysis of early nodulation and plant growth. Figure 3 shows the dose-response curve of nodule number per plant (represented as % of average nodule number at 0 nM IAA) to increasing concentrations of IAA in the nutrient solution. It was observed that IAA at low concentrations enhanced nodule number, while higher concentrations inhibited nodulation under high as well as under low P conditions. These results underline the dose-dependent effect of IAA on nodulation. This is consistent with what has been described previously for other legumes (Van Noorden et al. 2006; Plazinski and Rolfe 1985b, c). Interestingly, nodulation was more responsive to IAA under P deficiency than under P-sufficient conditions. Under low P conditions, a concentration of 100 nM IAA already strongly inhibited nodulation, while the same concentration
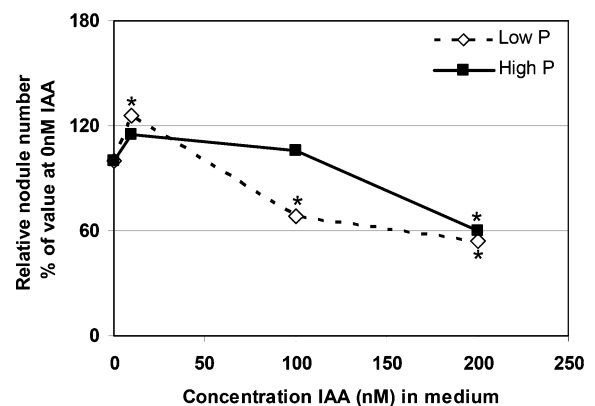

Fig. 3 The effect on nodule number of increasing concentrations of indole-3-acetic acid (IAA) under high P versus low P conditions. Points represent percentages of nodule number of BAT477 bean plants inoculated with *Rhizobium etli* CNPAF512 and grown in the presence of different concentrations of indole-3-acetic acid (IAA) under low P (dashed line) and high P (full line) conditions. 100% represents the average nodule number per plant grown at 0 nM IAA. Under high P 100% is equal to 142 nodules per plant; under low P 100% is equal to 85 nodules per plant. Plants were harvested 21 days after inoculation. IAA was added to the nutrient solution as described in materials and methods. * indicates significant difference ($P < 0.05$ Tukey–Kramer) for absolute values between plants grown in the presence of IAA and control plants grown in the absence of IAA (0 nM). This experiment was performed three times, yielding similar results

of IAA under high P conditions still slightly enhanced nodule number.

Discussion

This study shows that the effect of PGPR on nodulation and plant growth of bean is dependent on plant P nutrition. The increase in nodulation induced by *A. brasilense* Sp245 and *B. subtilis* LMG 7135 observed under high P, was not observed under low P. On the contrary, the positive effect of *P. putida* UW4 was more strongly expressed under P deficiency than under sufficient P supply. These results encourage integration of environmental factors including P nutrition in studies exploring the potential of *Rhizobium*-PGPR coinoculation to enhance nodulation and plant growth.

The difference in plant and nodulation response under low versus high P conditions was most pronounced when plants were inoculated with the combination of *Rhizobium* and *A. brasilense* Sp245. Under high P conditions this combination enhanced nodule number, shoot dry weight and root dry weight significantly, while under low P the same inoculation treatment had a negative effect on these parameters. Intriguingly, using an *ipdC* mutant of *A. brasilense* Sp245 reduced IAA biosynthesis by 90% (Costacurta et al. 1994; Vande Broek et al. 1999); it was demonstrated that the *Azospirillum* IAA production plays a crucial role in the modulation of nodulation, particularly under low P conditions. This was further supported by experiments in which increasing concentrations of IAA were added to the growth media. The results of these experiments confirm the dose-dependent response of nodulation to IAA as described previously (Van Noorden et al. 2006; Plazinski and Rolfe 1985a, b). Low concentrations (up to 100 nM IAA) can enhance nodule number, while higher concentrations inhibit nodulation. Interestingly, Plazinski and Rolfe (1985a, b) described a similar dose-response curve of nodulation in response to inoculation with an increasing number of *Azospirillum* cells on bean plants.

The experiments using increasing IAA concentrations further showed that nodulation was more sensitive to IAA under low P than under high P conditions. Increase in auxin sensitivity under P-deficient conditions has previously been described in *Arabidopsis* (Lopez-Bucio et al. 2002). The use of *Arabidopsis*

auxin-resistant mutants also demonstrated the crucial role of auxin in root morphological adaptations under low P conditions (Lopez-Bucio et al. 2002; Al-Ghazi et al. 2003). Preliminary data (not shown) of free and conjugated IAA concentrations in BAT477 roots under low and high P conditions, show that the amount of IAA present in conjugated form is strongly reduced under P deficiency (Remans et al. unpublished). IAA conjugates play a role in the maintenance of free IAA levels in response to changes in IAA levels (reviewed by Woodward and Bartel 2005), e.g. when exogenous IAA is added, the difference in root IAA conjugate concentrations between low and high P conditions further supports the observation of increased IAA sensitivity under low P deficiency. Since IAA sensitivity and adaptation to P deficiency is strongly dependent on the plant genotype, the response to *A. brasilense* Sp245 may also differ strongly across contrasting bean genotypes.

Apart from changes in host sensitivity to IAA, changes in IAA production rate induced in *A. brasilense* Sp245 under low P conditions, may also contribute to the differential response in nodulation to Sp245 inoculation under low versus high P conditions. It has been described that IAA biosynthesis in *A. brasilense* increases under certain environmental stress conditions, including low pH and carbon stress (Ona et al. 2005). The specific effect of P deficiency on IAA production in Sp245 has not yet been studied. The negative effect of IAA on plant growth and nodulation is often mediated by ethylene (Persello-Cartieaux et al. 2003). Auxin produced by bacteria in the rhizosphere can stimulate the activity of the 1-aminocyclopropane-1-carboxylate (ACC) synthase, an enzyme used by plants for the synthesis of ethylene (Xie et al. 1996). Ethylene has been shown to negatively regulate the plant's response to the rhizobial bacterial signal, Nod factor (Oldroyd et al. 2001; Penmetsa and Cook 1997). The inhibition of Nod factor-induced calcium spiking reflects the suppression of the Nod factor signalling pathway at a very early stage and most probably explains the regulation of nodule number by ethylene. Borch et al. (1999) reported that P-deficient roots of common bean produced twice as much ethylene g^{-1} root dry weight than P-sufficient roots. The increased ethylene production in P-deficient roots may contribute to the reduction in nodulation under low P conditions. Interestingly, the positive effect of coinoculation with *Pseudomonas putida* UW4 on nodulation was most expressed under low P conditions.

This enhancement in nodulation was much less pronounced when an UW4 mutant disabled in ACC deaminase activity was used. These results indicate that bacterial ACC deaminase activity, which reduces ethylene production, may play a crucial role in increasing nodulation and plant growth under P deficiency. Direct evidence for a positive effect of ACC deaminase activity on nodulation of alfalfa has been described previously by Ma et al. (2004). They showed that the expression of an exogenous ACC deaminase gene in *Sinorhizobium meliloti* increases its ability to nodulate alfalfa.

In conclusion, this study has showed that P deficiency alters the effect of PGPR on nodulation and growth of common bean. The differential nodulation response to PGPR under low versus high P conditions is associated with changes in phytohormone balances induced by specific PGPR. Potential to enhance nodulation and plant growth under P deficiency by coinoculation with *Rhizobium* and PGPR does exist, particularly for bacteria possessing ACC deaminase activity. Further research to fine-tune combinations of *Rhizobium*, PGPR and host genotype will ultimately lead to better nodulation and stimulated plant growth under low P conditions.

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