African Journal of Biotechnology Vol. 11(55), pp. 11797-11806, 10 July, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB12.116
ISSN 1684–5315 ©2012 Academic Journals

Full Length Research Paper

Detection of metabolites in Flor de Mayo common beans (*Phaseolus vulgaris* L.) and their response to inoculation with *Trichoderma harzianum*

Hernández-Mendoza J. L.[1], Quiroz-Velázquez J. D.[1], Díaz-Franco A.[2], García-Olivares J. G.[1]*, Bustamante-Dávila A. J.[2] and Gill-Langarica H. R.[1]

[1]National Polytechnic Institute, Center for Genomic Biotechnology, Experimental Biotechnology Laboratory, Blvd. del Maestro esquina Elías Piña, Col. Narciso Mendoza, C. P. 88710, Cd. Reynosa, Tamaulipas, México.
[2]INIFAP, Campo Experimental Río Bravo, Km 61, Carretera Reynosa-Matamoros, Apdo. Postal 172, 88900 Río Bravo, Tamaulipas, Mexico.

Accepted 10 May, 2012

Seed germination involves complex processes where plant growth-promoting substances are released into the growth medium, mainly involving pathways that start in tryptophan (TRP) and end in the formation of indole-3-acetic acid (IAA). Among them, four are known as TRP-dependent and a fifth as TRP-independent. Some compounds were observed during germination of bean seeds that involved at least three IAA synthesis pathways and that kynurenine (KYN) is the first detected metabolite which is found in greater concentration. It was followed by tryptamine (TAM), TRP and IAA. The results of *Trichoderma harzianum* inoculation in greenhouse tests showed variability in Flor de Mayo beans seedlings in response to physiological level and production parameters. The effect of *Trichoderma* in Flor de Mayo common bean showed that strain 802 had a significant effect on the development of the height of bean seedlings, and that the 812 strain showed significant effect on the development of root bean seedlings. Increased growth of stem and root caused by *Trichoderma* strains in Flor de Mayo beans involves beneficial effects of inoculation on plant growth and development parameters and can be taken as they were a measure of survival and development of seedlings.

Key words: Biofertilizers, indole-3-acetamide, tryptophan,indole-3- acetic acid, kynurenine.

INTRODUCTION

The need to increase productivity and quality of agricultural products has led to excessive use of chemical fertilizers, creating serious environmental pollution. The use of chemical fertilizers decreases the activity of nitrogen-fixing bacteria or plant growth-promoting fungi (Aguirre, 2006). The use of biofertilizers is an alternative to maintain high production with low environmental impact (Hermosa et al., 2012). Different soil-borne bacteria and fungi are able to colonize roots of plants and may have beneficial effect on the plant. The ability of microorganisms to stimulate germination and plant development has been adapted *in vitro* and *in vivo* in some commercial agricultural crops and ornamental (Unno et al., 2005; Ayyadurai et al., 2006). The advantages of these interactions are often due to the ability of bacteria and fungi to fix nitrogen, antibiosis, and improved water and mineral nutrition of the plant (Ayyadurai et al., 2006; Mehnaz and Lazarovits, 2006). Microorganisms that produce these beneficial components are often considered as plant growth-promoting rhizobacteria (PGPR) and plant growth-promoting fungi (PGPF), mainly found in the rhizosphere and rhizoplane (Van Wees et al., 2008).

Some species of the genus *Trichoderma* are considered multifunctional plant endophytic symbionts because of the ability to colonize the intercellular space of the root and the multiple beneficial effects on growth of

*Corresponding author: E-mail: jggarcia@ipn.mx. Tel/Fax: (+52)899-9243627.

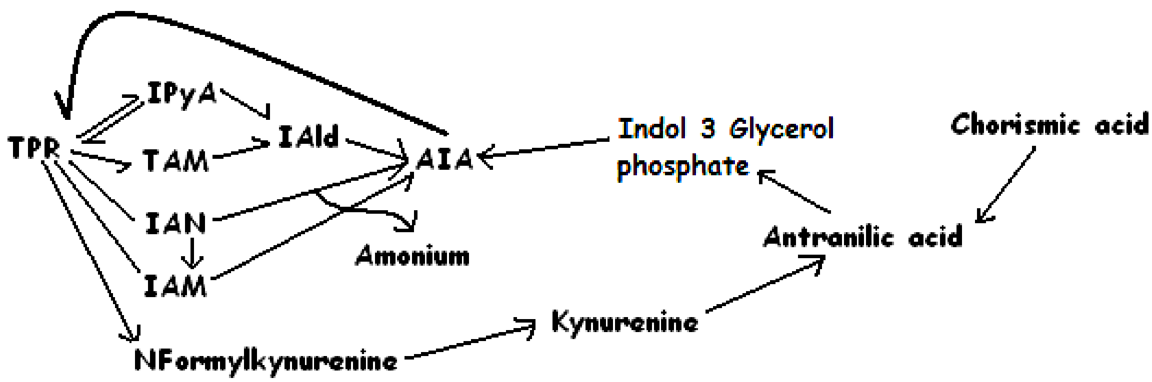

Figure 1. Simplified pathways of IAA synthesis reported in plants, bacteria and fungus. The kynurenine route is called TRP Independent (Zakharova et al., 1999; Carreño et al., 2000; Aguilar et al., 2008; Chang et al., 2003; Hernandez et al., 2010).

plants (Shoresh et al., 2010). The positive effect observed is strongly dependent on the fungal and plant genotype (Tucci et al., 2011) and the physiological status of the plant (Donoso et al., 2008). Several *Trichoderma* strains have the ability to reduce the severity of plant diseases by inhibiting plant pathogens mainly in the soil or on plant roots, through their high antagonistic and mycoparasitic potential (Viterbo and Horwitz, 2010). In addition, several *Trichoderma* strains have been shown to have direct effects on plants, increasing their growth potential and nutrient uptake, fertilizer use efficiency, percentage and rate of seed germination, and stimulation of plant defenses against abiotic damage (Shoresh et al., 2010). Recently, the production of *Trichoderma* related substances of plant growth as indole-3-acetic acid (IAA), which is considered the most important plant growth promoter was reported (Harman, 2006; Samuels et al., 2006). Some pathways routes for IAA synthesis are also reported in plants and microorganisms. Four pathways denominated tryptophan-dependent were broadly studied and one other route called tryptophan independent was reported; this pathways is shown in Figure 1. From TRP, there are four direct routes for IAA synthesis and alternate pathway kinurenina - anthranilic acid (AA). In this pathway, the amino acid (AA) can by formed from chorismic acid.

On the other hand, fungus produces hydrolytic enzymes, secondary metabolites and biologically active substances that can be employed in industry in the degradation of agrotoxins or in agriculture as an antagonist of plant pathogens (Papavizas et al., 1990; Rey et al., 2000). *Trichoderma* produces compounds with antibiotic activity such as gliotoxin and viridine; these metabolites *in vitro* inhibit the germination and root growth in mustard and other dicots, while their effects are smaller in monocots (Jones et al., 1988), as when the pH is near neutrality. Other metabolites produced by *Trichoderma* are viridiol, 9-epi-viridiol and gliovirin. The first has herbicidal activity and its variant, the 9-epi-viridiol has a cytotoxic effect in *Carcinoma epidermoide* cells (Phuwapraisirisan et al., 2006).

The common dry bean (*Phaseolus vulgaris* L.) is of great importance in the Mexican national diet. It ranks second in cultivated area, and is considered an alternative to production within crop rotation (grass-legume). At the end of the crop cycle, this plant provides 30% of the residual nitrogen in the soil (Salinas et al., 2007). Most of the commercial beans grown in Mexico are of the types Azufrado, Bayo, Black, Flor de Mayo, Flor de Junio and Pinto beans (Castellanos et al., 1997). In the central region of Mexico, meanwhile, demand has increased for Flor de Mayo and Flor de Junio beans. Within México, the states with the largest production (from highest to lowest) are Zacatecas (251.8 ton.), Sinaloa (151.3 ton.), Durango (121.5 ton.), Nayarit (77.5 ton.), Chihuahua (75.1 ton. and San Luis Potosi (70.0 ton.), which together account for 68% of total national production (Blair et al., 2011).

However, beans production is affected by several biotic (diseases, pests and weeds) and abiotic factors (soil salinity, temperature and moisture levels) that cause stress on the crop, and thus decreasing its productivity (Singh et al., 2001). So that the aim of this study was to examine the influence of *Trichoderma* strains on Flor de Mayo beans through the analysis of some indicating traits such as germination rate, root elongation, plant height and fresh weight of the seedlings as well as chlorophyll content. Applied aspects of this investigation lie in the field of *in vitro* germination of the bean seeds with symbiotic *Trichoderma* strains in simple medium and then germinated and grown in a greenhouse.

MATERIALS AND METHODS

Germination tests

To evaluate this parameter, bean seeds (10 seeds) were placed in Petri dish plates with 10 ml of water using five plates per replication.

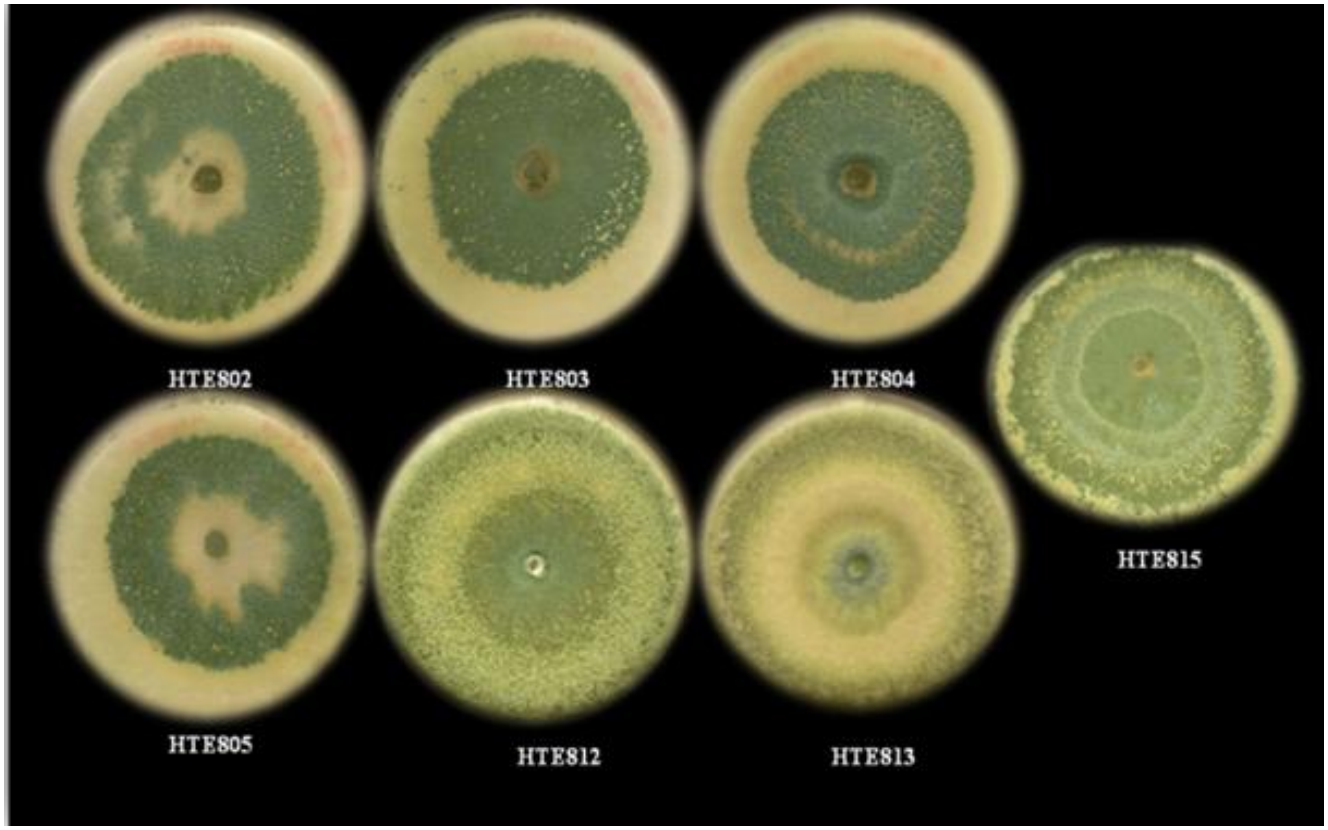

Figure 2. *T. harzianum* colonies showing typical growth and sporulation shapes (Experimental Biotechnology Laboratory CBG-IPN-2010).

The sampling was performed at 24, 48, 72 and 96 h. Fresh weight of the grain seedling was measured, as well as germination rate, embedded volume, dry weight of root, testa and cotyledon.

Determination of metabolites

The supernatant recovered in each sample of leachate was analyzed by high performance liquid chromatography (HPLC; Hewlett Packard model 1100) and compared against kynurenine (KYN), tryptophan (TRP), indole-3-acetic acid (IAA), indol-3-acetamide (IAM) and tryptamine (TAM) standards. Samples were run with a phase composed of 80:20 (phosphate/acetonitrile), at pH 3.1, with an injection volume of 20 µL at 30°C and a wavelength of 220 nm in a column (Ultraspher C18).

Greenhouse experiments

The experiments were conducted at the Center for Genomic Biotechnology of the National Polytechnic Institute in Reynosa, Tamaulipas, Mexico, and were established from 2009 until the fall-winter cycle of 2009 to 2010. The biological material used was the bean variety Flor de Mayo, of the 2008 harvest from Fresnillo, Zacatecas, Mexico.

Biological material

The *T. harzianum* fungi strains were taken from the strain bank of the Experimental Biotechnology Laboratory of the Center for Genomic Biotechnology. These strains were isolated from soil of the northern region of Tamaulipas, México. Strains HTE 802 and HTE 805 showed a green colored colony with a single ring of sporulation. Strains HTE 803 and HTE 804 were green in color, of compact and total growth. Strains HTE 812 and HTE 815 were light-green colored, with several rings of growth, while the strain HTE 813 is yellowish in color with several rings (Figure 2).

For the conduction of tests in greenhouse, *T. harzianum* strains were multiplied in solid substrate (washed and sterilized rice), and incubated for 5 days at 27°C. The number of spores was counted by direct count in a Neubauer chamber. The treatment concentrations were 1×10^6 spores/ml, which were carried out using 6 ml of deionized water in the spore suspension. Only deionized water was used in the control treatment. The bean seeds were placed in a Petri dish plate (50 mm), for 48 h and were planted in 72 cavity plastic trays (KBW of 1.63 × 3.25 cm diameter × depth), prepared with agricultural soil (sandy loam; pH 8) and mixed with agricultural pearl in a 3:1 ratio. They were irrigated according to the crop's water requirements.

Statistical analysis

Data collection was performed at 15 and 40 days after treatment. In each case, the height of the plant was measured (cm), as well as the produced biomass, evaluated as dry weight of root and total dry weight. In addition, the physiological parameter of the chlorophyll index (CI) was evaluated, with a chlorophyll meter (Minolta model SPAD-502) 40 days after the trial was initiated. The obtained data

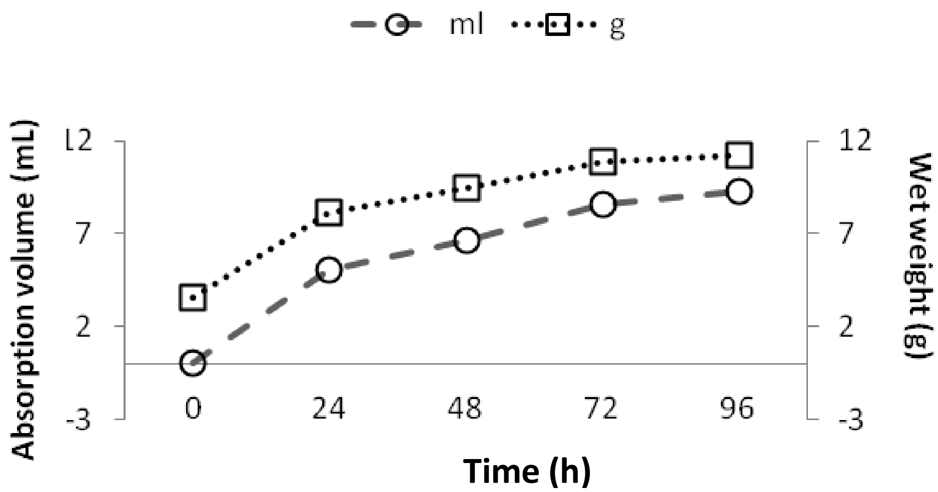

Figure 3. Volume of absorbed water and weight of Flor de Mayo bean seeds at different times.

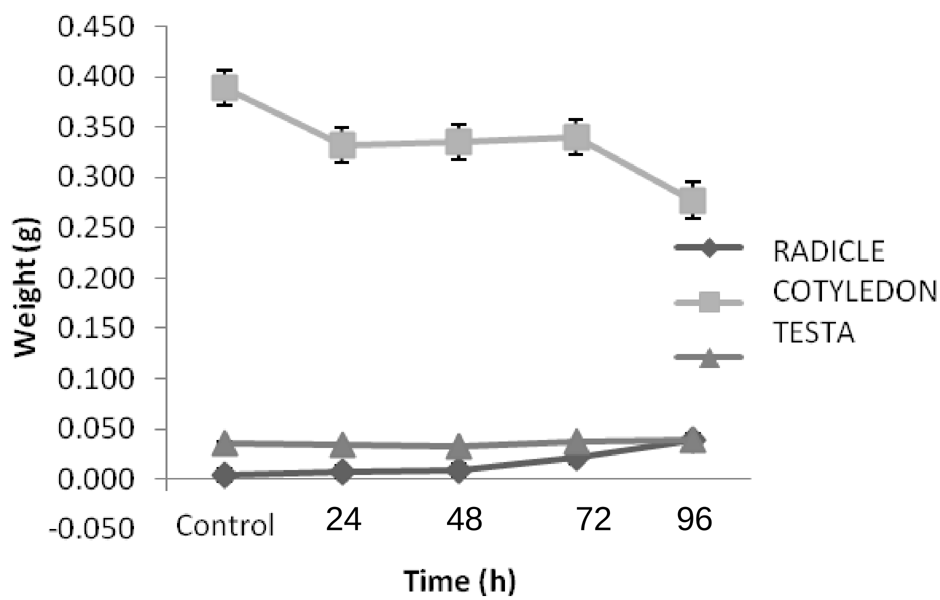

Figure 4. Behavior of Flor de Mayo bean seeds at different times.

was plotted with the program Excel® (2007) and were analyzed with the SAS Institute Inc. (1996) statistical program. The principal components (ACP) was made in order to select the most important and distinguishing variables; for ACP the statistical InfoStat package was used (Di Rienzo et al., 2008).

RESULTS

Leachates

With respect to the phenomena observed during the germination process, it was found that the seed absorbs most of the volume of water during the first 24 h and over 50% of the total retained. Similarly, the wet weight tripled and passed from a starting weight of 3.8 to 11.25 g at the 4th day. On the contrary, after the seed absorbed the water, it dried and it was observed that as time passed, the weight became lower than the starting weight because of the chemical weathering that the seed suffers when substances that acidify it were released into the medium (Figure 3).

With respect to the distribution of the weight of the biomass formed during the germination process, it was observed that most corresponded to the cotyledons which maintain a tendency to decrease in dry weight (Figure 4); and likewise, the testa tends to initially increase its weight, only to decrease it later. Meanwhile, the radicle increased in weight after 72 h, which corresponded with the increase in the percentages in the germination of the seeds, which at every 24 h increased to 10, 60, 78 and 92%, respectively.

Evaluation of metabolites

The leachates obtained from the bean seed during the germination process, released substances linked to plant growth, such as indole-3-acetic acid, the main plant hormone. The presence of TAM, KYN and traces of IAM showed that apparently three synthesis pathways of IAA are involved. Two of them, TAM and IAM, participated in TRP-dependent pathways, while KYN is a less known pathway called TRP-independent. The presence and values of each one of the detected growth-promoting substances vary according to the time of sampling. Thus, in the results obtained at 24 h, the KYN had the least time of retention and was therefore the first metabolite detected. At the same time, it showed the highest concentration with 279 ppm, followed by TAM with 79 ppm, the TRP with 56 ppm and was lesser in IAA with 15 ppm. At 48 h, the metabolites showed the following concentrations; 288 ppm KYN, 29 ppm TAM, 53 ppm TRP and finally IAA with 17 ppm. The highlight at this point is that TAM showed a decrease in the concentration of 50%. Moreover, at 72 h, KYN showed an increase of 34% with 432 ppm, followed by TRP with 67 ppm. As for the IAA and TAM, they were not detected in the third sampling while in the last sampling, at 96 h, a similar behavior to the last sample was observed with 456 ppm of KYN and 62 ppm of TRP; IAA and TAM were not detected (Figure 5a).

In addition, the analysis of principal components showed an association between HTE 802 and HTE 813 strains and seedling height, showing high values of seedling height trait (HTE 802=29.6 cm; HTE 813=27.3 cm); HTE 813 and HTE 815 strains were associated with the total weight, with higher values of total weight trait (THE 813= 1.24 g; THE=1.24 g); HTE 812 strain and the control were associated with the variable root weight, with high values in root weight trait (THE 812=0.40 g; Control=0.39 g). Finally, strain 805 and control was associated with the variable chlorophyll index, with high values of chlorophyll index trait (HTE 805=28.6 Cl; Control=28.9 Cl) (Figure 6).

Greenhouse

With respect to the experiments carried out under greenhouse conditions, where Flor de Mayo bean seeds treated with *T harzianum* were sowed, the results show highly significant differences in plant height, as well as for the physiological parameter for chlorophyll index at 15 and 40 days. Dry plant and root weights differences were only found at 40 days (Table 1). The first two main components of ACP explained 90% of the total variance of the *Trichoderma* effect in Flor de Mayo beans (Table 2).

DISCUSSION

Among plant growth regulators, IAA is the most common natural auxin found in plants and its positive effect on root growth and morphology is believed to increase the access to more nutrients in the soil (Vessey, 2003). In bean seeds, amide conjugates account for more than 80% of the total IAA (Cohen et al., 1990). Our experiments clearly show that bean seeds free IAA synthesized in the first two days of germination and the three and fourth days disappeared. In contrast, Bialek et al. (1991) showed that the levels of both free and amide-conjugated IAA begin to increase after the first day of germination, with dramatic increase observed between the second and third days of seed germination. This increase in the level of IAA parallels the increase in the accumulation of fresh weight in the axes of germinating seedlings. Contrary to this, our experiment show an increase in fresh weight accumulation radicle and testa and decrease in the cotyledons of germinating seedlings.

It is known that TRP considerably stimulates microbial IAA yield *in vitro* (Tsavkelova et al., 2005; Hernandez et al., 2010). Root exometabolites also supply the rhizosphere with TRP, which undergo further conversion into IAA by plant-associated bacteria or fungi (Kravchenko et al., 2004; Kamilova et al., 2006). Our data confirmed abundant level in the four days of germinating seedlings (Figure 4a). Its level of approximately 65 ppm overage was much higher than the level reported by Black and Hamilton (1971) in older bean seedlings (14ug/g) or in *Ricinus communis* (50 µg/g) (Allen and Baker, 1980). The IAA synthesis pathways reported for plants and microorganisms (Hernandez et al., 2010; Zhao, 2010) show that the TRP decomposes to form IAA by various pathways known as TRP-dependent, with the existence of another in microorganisms known as TRP-independent that involves the Kynurenine pathway to pass afterwards by the formation of anthranilic acid (AA) and finally reach the IAA synthesis. In plants, the proposed TRP-independent IAA biosynthetic pathways analyses of TRP biosynthetic mutants in *Arabidopsis* demonstrate that plants also can synthesize IAA without using a TRP intermediate (Normanly et al., 1993; Ouyang et al., 2000). In this case, the IAA are synthetized from the corismic acid between the AA (Hernández et al., 2010). Plants may switch from basal TRP-independent IAA biosynthesis to TRP-dependent pathways during stress, when more IAA may be needed (Ribnicky et al., 2002; Sztein et al., 2002). The indole-3-glycerol phosphate pathway [indole-3-glycerol phosphate to

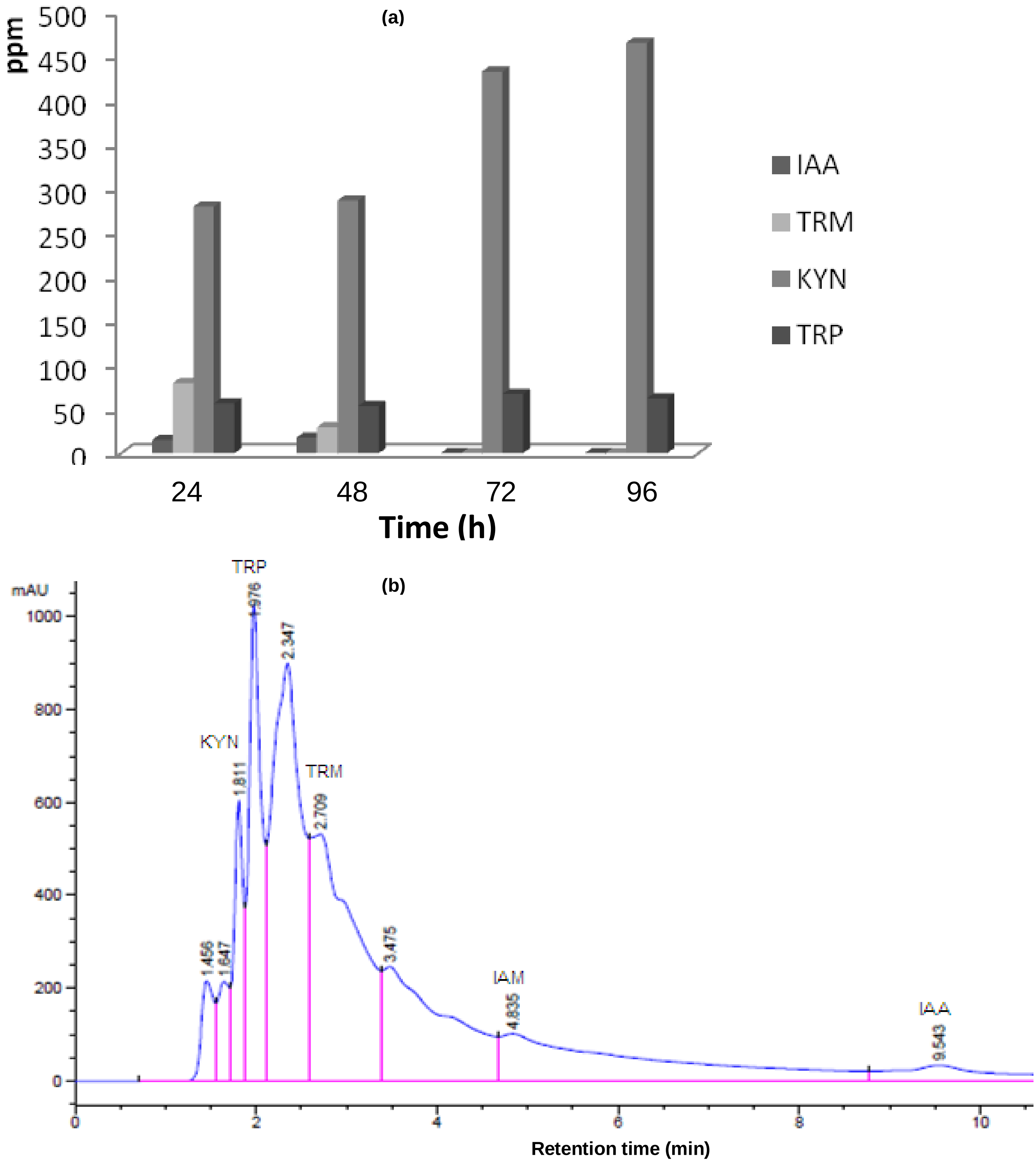

Figure 5. (a) Kinetics of metabolite production during germination of Flor de Mayo beans seeds. **(b)** Chromatogram corresponding to IAA precursor metabolites detected during the germination process of Flor de Mayo beans seeds.

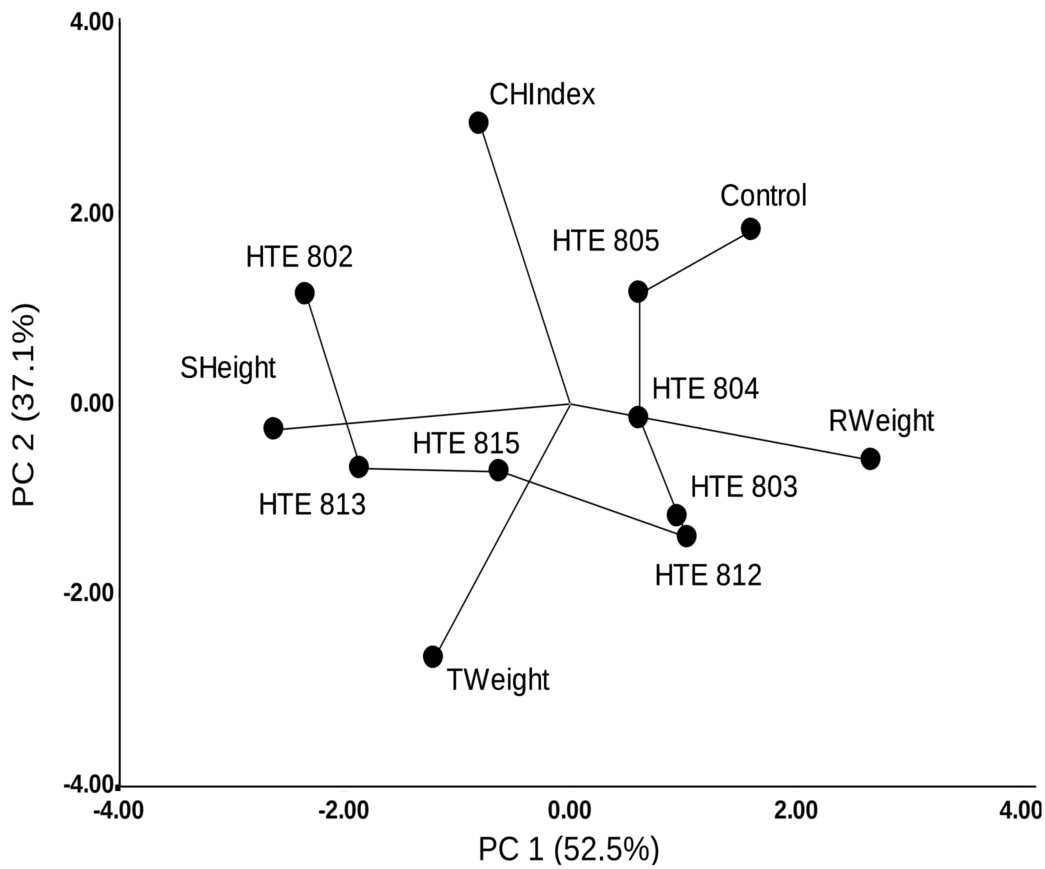

Figure 6. Dispersion *Trichoderma* strains and their association with response traits based on the first two principal components in Flor de Mayo common beans.

Table 1. Mean squares of analysis of variance of Flor de Mayo beans seeds treated with *T. harzianum*.

Source of variation	Seedling height (cm)	Root weight (g)	Plant weight (g)	Chlorophyll Index (CI)
Treatments	270**	0.0066**	0.120**	22.11**
Error	0.00716	0.0071	0.9682	8.68
CV (%)	21	25	28	11

Table 2. Characteristic vectors descriptive value in Flor de Mayo beans seeds inoculated with *Trichoderma* strains.

Principal component	Eigenvalue	Total variance (%)	Cumulative Eigenvalue	Total cumulative variance (%)
1	2.10	53	2.10	53
2	1.48	37	3.58	90
3	0.35	08	3.93	98
4	0.07	02	4.0	100

Indole to IAA] is the hypothetical pathway in plants for the formation of IAA (Cohen et al., 2003; Woodward and Bartel, 2005).

Furthermore, the TRP-dependent pathway of IAA biosynthesis is rich with potential intermediates (Bartel et al., 2001). Enzymes that convert TRP to indole-3-pyruvic acid, tryptamine, and indole-3-acetaldoxime have all been proposed to catalyze initial steps in TRP-dependent IAA biosynthesis in plants (Bartel, et al., 2001). TAM has also been identified in tomato (Cooney and Nonhebel, 1991). The potential importance of the TAM pathway is highlighted by the identification of an *Arabidopsis* flavin monooxygenase-like enzyme that apparently catalyzes the conversion of TAM to N-hydroxyl-tryptamine. These plants accumulate free IAA and display high auxin phenotypes, including long hypocotyls in the light, hookless development in the dark, epinastic cotyledons and leaves and long petioles (Zhao et al., 2001). Our data show abundant level of free TAM in the two days of germinating seedlings and the third and fourth day, the metabolite disappeared (Figure 5a). The four main tryptophan dependent metabolic pathways were showed by Bartel (1997). In regards to the identification of specific metabolites involucres on the biosynthesis pathways of IAA, this study showed that KYN and TRP were detected only in the first two days in all the days. The HPLC analysis showed a lower KYN retention, followed by TRP, then by indole acetamide (IAM), TAM and finally IAA (Figure 5b). So, the enzymes responsible for the synthesis of IAA from both TRP and TAM are active and possibly of IAM. This suggests the existence of the IAM and TAM pathways in the seeds of common bean Flor de Mayo.

On the other hand, root tissues are especially sensitive to fluctuating concentrations of IAA and the development of the root system can be greatly affected by exogenous sources of this plant growth regulator, including microbial (Tanimoto, 2005) while the production of IAA by microorganisms commonly found in the rhizosphere of plants, such as *Pseudomonas* spp. and *Rhizobium* spp. is often associated with their potential to stimulate plant growth (Patten and Glick, 2002). In this context, rhizosphere microorganisms capable of degrading IAA might have a positive effect on plant growth. However, even though Leveau and Lindow (2005) reported the ecological role of such a microorganism, little is known on the specific impact of IAA-degraders on plant growth. For example in Mexico, some of these promoters are reportedly present in native *Azospirillum brasilense* strains (García et al., 2006, 2007; Hernández et al., 2008; Hernández et al., 2010). The native *Trichoderma* strains in Flor de Mayo beans showed variability at a physiological level and in production parameters, which coincide with those reported in the Chihuahua mountains, where the strain HK703 was used to inoculate the common bean variety Pinto Saltillo, and a variability in response to fields treated with *T asperellum* was observed (Jiménez et al., 2010); similar responses was found in *T harzianum* in soybean (*Glycine max* L. Merr) (Corrêa et al., 2007), tomato (*Lycopersicon esculentum* Mill) (Mushtaq, 2011), cabbage (*Brassica oleracea* L.), lettuce (*Lactuca sativa* L.), (Rabeendran et al., 2000) and in corn (*Zea mays* L.) (Mastouri et al., 2010).

Others studies in greenhouse *Trichoderma* increased the rate of germination of maize and seedling growth of both maize and beans (Okoth et al., 2011). The increased root length and collar diameter, stem length and diameter by *Trichoderma* treatment are measures of seedling's survivability and illustrate the direct effect of the fungus on the plants (Okoth et al., 2011). A number of mechanisms for plant growth promotion by *Trichoderma* have been proposed (Harman et al., 2004a). These include production of antibiotics, parasitization of other fungi, and competition with deleterious plant microorganisms. Until recently, these traits were considered to be the basis for how *Trichoderma* exerted beneficial effects on plant growth and development. However, it is becoming increasingly clear that certain strains have substantial direct influence on plant development and crop productivity (Harman, 2006). In our study, all of *Trichoderma* inoculums performed better compared with control and the level of beans growth induced by *Trichoderma* strains was enhanced. Okoth et al. (2011) also showed that *Trichoderma* promoted growth of primary root length and root branching in maize and beans by inducing lateral root growth. In plants, auxins have been demonstrated to initiate lateral root growth (Casimiro et al., 2001) and the observed effects of *Trichoderma* in promoting lateral root development is similar to *in vitro* experiments performed by Hexon et al. (2009) that showed that *Trichoderma* spp. produced indole-3- acetic acid that promoted lateral root formation in *Arabidopsis thaliana.*

The root system is important for plant fitness because it provides anchorage, contributes to water use efficiency and facilitates the acquisition of mineral nutrients from the soil (Lopez et al., 2005a). Increased root size resulted into increased shoot size which translates into increased shoot biomass production, thus indicating a beneficial effect of inoculation on plant growth and development. Our analyzed data showed a significant effect ($p \leq 0.05$) on the four traits analyzed; HTE 802 strain had more plant height (29.6 cm), and average weight of root (0.21 g). Greater weight of roots was shown by strain HTE 812 (0.40 g). Finally, the highest total plant weight was observed in strains HTE 813 and 815. The positive influence of *Trichoderma* on root system architecture would therefore relate to increased yield of plants. *Trichoderma* enhanced root biomass production and increased root hair development (Bjorkman et al., 1998).

Conclusions

Various metabolites related with the growth and the formations of biomass were reported in the germination of Flor de Mayo bean seeds, including TRP, which is considered the main precursor in the synthesis of indole-3-acetic acid. Other metabolites such as TAM and IAM involved in the pathways known as tryptophan-dependent were detected in this study. The inoculation of Flor de Mayo bean seeds with *T harzianum* showed variability in response to physiological and productive traits, high-lighting the strain THE 802 with effect on plant height and strain THE 812 with an effect on root development.

REFERENCES

Aguilar PJJ, Xiqui VML, García GS, Baca BE (2008). Producción de ácido Indol-3- acético en *Azospirillum*. Artículo de revisión. Rev. Lat. Microbiol. 50:29-37.

Aguirre JL (2006). Biofertilizantes Microbianos: Experiencias Agronómicas del Programa Nacional del INIFAP en México. Campo Experimental Rosario Izapa, INIFAP. Libro Técnico No. 2. México, p. 206.

Allen JRF, Baker DA (1980). Free tryptophan and indole-3-acetic acid levels in the leaves and vascular pathways of *Ricinus communis* L. Plant 148:69-74.

Ayyadurai N, Ravindra NP, Sreehari RM, Sunish KR, Samrat SK, Manohar M, Sakthivel N (2006). Isolation and characterization of a novel banana rhizosphere bacterium as fungal antagonist and microbial adjuvant in micropropagation of banana. J. Appl. Microbiol. 100:926-937.

Bartel B (1997). Auxin biosynthesis. Annu. Rev. Plant Physiol. Plant Mol. Biol*.* 48:51-66.

Bartel B, LeClere S, Magidin M, Zolman BK (2001). Inputs to the active indole-3-acetic acid pool: *de novo* synthesis, conjugate hydrolysis, and indole-3-butyric acid b-oxidation. J Plant Growth Regul. 20:198-216.

Bialek K, Michalczuk L, Cohen JD (1991). Auxin metabolism in bean seedlings (abstract No. 496). Plant Physiol. 96:S-77.

Bjorkman T, Blanchard LM, Harman GE (1998). Growth enhancement of shrunken-2 sweet corn when colonized with *Trichoderma harzianum* 1295-22; effect of environmental stress. J. Am. Soc. Hortic. Sci. 123:35-40.

Black RC, Hamilton RH (1971). Indoleacetic acid biosynthesis in Avena coleoptile tips and excised bean shoots. Plant Physiol. 48:603-606.

Blair WM, Diaz ML, Gill LHR, Rosales SR, Mayek PN, Acosta GJ (2011). Genetic Relatedness of Mexican Common Bean Cultivars Revealed by Microsatellite Markers. Crop Sci. 51:2655-2667.

Carreño LR, Campos RN, Elmerich C, Baca BE (2000). Physiological evidence for differently regulated tryptophan-dependent pathways for indole-3-acetic acid synthesis in *Azospirillum brasilense*. Mol. Gen. Genet. 264:521-530.

Casimiro I, Marchant A, Bhalerao RP, Swarup R, Graham N, Inze D, Sandberg G, Caesro PJ, Bennett M (2001). Auxin transport promotes *Arabidopsis* lateral root initiation. Plant Cell, 13:84-852.

Castellanos ZJ, Guzman-Maldonado H, Jimenez A, Mejia C, Munoz-Ramos JJ, Acosta-Gallegos JA, Hoyos G, Lopez-Salinas E, Gonzalez-Eguiarte D, Salinas-Perez R, Gonzalez-Acuna J, Munoz-Villalobos JA, Fernandez-Hernandez P, Caceres B (1997). Habitos preferenciales de los consumidores de frijol común (*Phaseolus vulgaris* L) en Mexico. Arch. Latinoam. Nutr. 47:163-167.

Chang HK, Mohseni P, Zylstra GJ (2003). Characterization and regulation of the genes for a novel anthranilate 1,2-dioxygenase from *Burkholderia cepacia* DB01. J. Bacteriol. 185:5871-5881.

Cohen JD, Bialek K, Slovin JP, Baldi BG, Chen KH (1990). Development of genetic and analytical systems for studies of auxin metabolism. In RP Pharis, SB Rood, eds, Plant Growth Substances 1988. Springer-Verlag, Heidelberg, Germany, pp. 45-56.

Cohen JD, Slovin JP, Hendrickson AM (2003). Two genetically discrete pathways convert tryptophan to auxin: More redundancy in auxin biosynthesis. Trends Plant Sci. 8:197-199.

Cooney TP, Nonhebel HM (1991). Biosynthesis of indole-3- acetic acid in tomato shoots: measurement, mass-spectral identification and incorporation of 2H from 2H20 into indole-3-acetic acid, D- and L-tryptophan, indole-3-pyruvate and tryptamine. Planta 184:368-376.

Corrêa S, Mello M, Zilá RÁ, Leonardo MB, Raquel RP, Diogo G (2007). Cepas de *Trichoderma* spp. para el control biológico de *Sclerotium rolfsii* Sacc. Fitosanidad 11:3-9.

Di Rienzo JA, Casanoves F, Balzarini MG, Gonzalez L, Tablada M, Robledo CW (2008). InfoStat, versión 2008*,* Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina.

Donoso EP, Bustamante RO, Caru M, Niemeyer HM (2008). Water deficit as a driver of the mutualistic relationship between the fungus *Trichoderma harzianum* and two wheat genotypes. Appl. Environ. Microbiol. 74:1412-1417.

García OJG, Moreno-Medina VR, Rodríguez-Luna IC, Mendoza HA, Mayek-Pérez N (2006). Biofertilización con *Azospirillum brasilense* en sorgo, en el norte de México. Agricultura Técnica en México. 32:135-141.

García OJG, Moreno-Medina VR, Rodríguez-Luna IC, Mendoza-Herrera A, Mayek-Pérez N (2007). Efecto de cepas de *Azospirillum brasilense* en el crecimiento y rendimiento de grano del maíz. Rev. Fitotecnia Mex. 30:305-310.

Harman GE (2006). Overview of mechanisms and uses of *Trichoderma* spp. Phytopathol. 96:190-194.

Harman GE, Howell CR, Vitrebo A, Chet I, Lorito M (2004a). *Trichoderma* species opportunistic, a virulent plant symbionts. Nat. Rev. Microbiol. 2:43-56.

Hermosa R, Viterbo A, Chet Ll, Monte E (2012). Plant beneficial effects of *Trichoderma* and of its genes. Microbiology 158:17-25.

Hernández MJL, Moreno-Medina VR, Quiroz-Velásquez JD, García-Olivares JG, Mayek-Pérez N (2010). Efecto de diferentes concentraciones de ácido antranílico en el crecimiento del maíz. Revista Colombiana de Biotecnología 12(1):57-63.

Hernández MJL, Quiroz-Velásquez JD, Moreno-Medina VR, Mayek-Pérez N (2008). Biosíntesis de ácido antranílico y ácido Indol acético a partir de triptofano en una cepa de *Azopirillum brasilense* nativa de Tamaulipas, México. Av. Invest. Agropecuaria 12: 57-67.

Hexon AC, Lourdes MR, Carlos CP, José LB (2009). *Trichoderma virens*, a plant beneficial fungus, enhances biomass production and promotes lateral root growth through an auxin-dependent mechanism in *Arabidopsis*. Plant Physiol. 149:1579-1592.

Jiménez GJC, Herrera MD, Lozano-Jiménez C, Rivera-Ruíz L, Rosales-Serna R, Carrera-Espinoza JM (2010). Genetic diversity in common bean cultivars collected in Chihuahua, México. Annual report of the bean improvement cooperative. Vol. 53.

Jones RW, Lanini WT, JG Hancock (1988). Plant growth response to the phytotoxinviridiol produced by the fungus *Gliocladium virens*. Weed Sci. 36:683-687.

Kamilova F, Kravchenko LV, Shaposhnikov AI, Azarova T, Makarova N, Lugtenberg B (2006). Organic acids, sugars, and L-tryptophane in exudates of vegetables growing on stonewool and their eVects on activities of rhizosphere bacteria. Mol. Plant Microbe Interact. 19:250-256.

Kravchenko LV, Azarova TS, Makarova NM, Tikhonovich IA (2004). The efect of tryptophan of plant root metabolites on the phytostimulating activity of rhizobacteria. Mikrobiologia 73:195-198.

Leveau JHJ, Lindow SE (2005). Utilization of the plant hormone indole-3-acetic acid for growth by Pseudomonas putida strain 1290. Appl. Environ. Microbiol. 71:2365-2371.

Lopez BJ, Cruz-Ramirez A, Perez-Torres A, Ramirez-Pimentel JG, Sanchez-Calderon L, Herrera-Estrella L (2005a). Root architecture In C Turnbull, ed, Plant Architecture and its Manipulation. Blackwell Annual Review Series. Blackwell Scientific, Oxford, pp. 181-206.

Mastouri F, Björkman T, GE Harman (2010). Seed treatment with *Trichoderma harzianum* alleviates biotic, abiotic, and physiological stresses in germinating seeds and seedlings. Phytopathology 100:1213-1221.

Mehnaz S, Lazarovits G (2006). Inoculation eVects of *Pseudomonas putida*, *Gluconacetobacter azotocaptans*, and *Azospirillum lipoferum* on corn plant growth under greenhouse conditions. Microb. Ecol. 51:326-335.

Mushtaq A (2011). Management of *fusarium* wilt of tomato by soil amendment with *trichoderma koningii* and a white. Sterile Fungus Indian J. Res. 5:35-38.

Normanly J, Cohen JD, Fink GR (1993). *Arabidopsis thaliana* auxotrophs reveal a tryptophan-independent biosynthetic pathway for indole-3-acetic acid. Proc. Natl. Acad. Sci. 90:10355-10359.

Okoth SA, Otadoh JA, Ochanda JO (2011). Improved seedling emergence and growth of maize and beans by *Trichoderma harziunum.* Trop. Subtr. Agroecosyst. 13:65-71.

Ouyang J, Shao X, Li J (2000). Indole-3-glycerol phosphate, a branchpoint of indole-3-acetic acid biosynthesis from the tryptophan biosynthetic pathway in *Arabidopsis thaliana.* Plant J. 24:327-333.

Papavizas GC, Roberts DP, KK Kim (1990). Development of mutants of *Gliocladium viren s*tolerant to benomyl. Can. J. Microbiol. 36:484-489.
Patten CL, Glick BR (2002). Role of *Pseudomonas putida* indole acetic acid in development of the host plant root system. Appl. Environ. Microbiol. 68:3795-3801.
Phuwapraisirisan P, Rangsan J, Siripong P, Tin-Pyang S (2006). 9-*epi*-Viridiol, anovel cytotoxic furanosteroid from soil fungus *Trichoderma virens*. Nat. Prod. Res. 20(14):1321-1325.
Rey M, Delgado JJ, Rincón AM, Limón MC, Benítez T (2000). Mejora de cepas de *Trichoderma* para su empleo como biofungicidas. Rev. Iberoam. Micol. 17:531-536.
Ribnicky DM, Cohen JD, Hu W-S, Cooke TJ (2002). An auxin surge following fertilization in carrots: a mechanism for regulating plant totipotency. Planta. 214: 505-509.
Salinas J, Díaz F, I Garza (2007). Labranza y biofertilización como manejo de sostenibilidad en la producción de frijol. Plaza y Valdez. 55: 73-82.
Samuels GJ, Dodd SL, Lu BS, Petrini O, Schroers HJ, IS Druzhinin (2006). The *Trichoderma koningii* aggregate species. Stud. Mycol. 56: 67-133.
Shoresh M, Harman GE, Mastouri F (2010). Induced systemic resistance and plant responses to fungal biocontrol agents. Annu. Rev. Phytopathol. 48:21-43.
Singh SP, Terán H, Gutiérrez JA (2001). Registration of SEA 5 and SEA 13 drought tolerant dry bean germplasm. Crop Sci. 41:276-277.
Sztein AE, Ilic N, Cohen JD, Cooke TJ (2002). Indole-3-acetic acid biosynthesis in isolated axes from germinating bean seeds: the effect of wounding on the biosynthetic pathway. Plant Growth Regul. 36: 201-207.
Tanimoto E (2005). Regulation of root growth by plant hormones—roles for auxin and gibberellin. Crit. Rev. Plant Sci. 24:249-265.
Tsavkelova EA, Cherdyntseva TA, Netrusov AI (2005). Auxin production by bacteria associated with orchid roots. Mikrobiologiia 74:46-53.
Tucci M, Ruocco M, De Masi L, De Palma M, Lorito M (2011). The beneficial effect of *Trichoderma* spp. on tomato is modulated by the plant genotype. Mol. Plant Pathol. 12:341-354.
Unno Y, Okubo K, Wasaki J, Shinano T, Osaki M (2005). Plant growth promotion abilities and microscale bacterial dynamics in the rhizosphere of *Lupin* analysed by phytate utilization ability. Environ. Microbiol. 7:396-404.
Van Wees SCM, van der Ent S, Pieterse CMJ (2008). Plant immune responses triggered by beneficial microbes. Curr. Opin. Plant Biol. 11: 443-448.
Vessey JK (2003). Plant growth promoting rhizobacteria as biofertilizers. Plant and Soil 255:571-586.
Viterbo A, Horwitz BA (2010). Mycoparasitism. In Cellular and Molecular Biology of Filamentous Fungi. Edited by K. A. Borkovich & D. J. Ebbole. Washington: Am. Soc. Microbiol. 42:676-693.
Woodward AW, Bartel B (2005). Auxin: regulation, action, and interaction. Ann. Bot. 95:707-735.
Zakharova EA, Shcherbakov AA, Brudnik VV, Skripko NG, Bulkhin NSH, Ignatov VV (1999). Biosynthesis of indole-3-acetic acid in *Azospirillum brasilense*. Insights from quantum chemistry. Eur. J. Biochem. 259:572-576.
Zhao Y (2010). Auxin biosynthesis and its role in plant development. Annu. Rev. Plant Biol. 61:49-64.
Zhao Y, Christensen SK, Fankhauser C, Cashman JR, Cohen JD (2001). A role for flavin monooxygenase-like enzymes in auxin biosynthesis. Science 291:306-309.