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Evaluation of commercial arbuscular mycorrhizal inoculants

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[1]Institut Sénégalais de Recherches Agricoles, Centre d'étude Régional pour l'Amélioration de l'Adaptation à la Sécheresse, BP 3320, Thiès, Sénégal; [2]Tropical Soil Biology and Fertility, c/o ICIPE Duduville Campus, Off Kasarani Road, PO Box 823-00621, Nairobi, Kenya; [3]Université Cheikh Anta Diop de Dakar, Faculté des Sciences et Techniques, Département de biologie végétale, BP 5005 Dakar, Sénégal; [4]Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada, 960 Carling Ave., Ottawa, Ontario, Canada K1A 0C6; [5]Kenyan Agricultural Research Institute, PO Box 107, Kitale, Kenya; and [6]Centre International de Recherches Agronomiques pour le Développement, UMR Eco&Sols (CIRAD-IRD-INRA-SupAgro), Land Development Department, 2003/61 Paholyothin Road, Lardyao Chatuchak, Bangkok 10900, Thailand.
Received 18 December 2012, accepted 15 July 2013.

Faye, A., Dalpé, Y., Ndung'u-Magiroi, K., Jefwa, J., Ndoye, I., Diouf, M. and Lesueur, D. 2013. **Evaluation of commercial arbuscular mycorrhizal inoculants**. Can. J. Plant Sci. **93**: xxx–xxx. In order to improve the use of commercial inoculants, 12 arbuscular mycorrhizal fungi (AMF) inoculants were evaluated in a two-step experiment under greenhouse conditions using maize. First, commercial mycorrhizal inoculants were propagated in a trap pot culture experiment under sterilized sand to evaluate their potential for maize (*Zea may* L.) root colonization as compared with an indigenous soil inoculum and to survey the AMF species present in the products. Three inoculants significantly increased root colonization levels compared with a soil inoculum. Instead of 12 declared AMF species, 13 fungal strains were extracted from the pot culture survey, including five undeclared species, while four declared species did not produce spores. In a second experiment, commercial products were inoculated into soil to assess their impact on maize growth and yield. Six weeks after planting, seven inoculants increased root colonization levels compared with control soil, while only three inoculants increased slightly the shoot biomass of maize plants. These experiments highlight the need to pre-evaluate commercial mycorrhizal inoculants on a selected crop and regional soil before launching large-scale field use.

Key words: Commercial inoculants, maize, spore population, Kenya

Faye, A., Dalpé, Y., Ndung'u-Magiroi, K., Jefwa, J., Ndoye, I., Diouf, M. et Lesueur, D. 2013. **Evaluation of commercial arbuscular mycorrhizal inoculants**. Can. J. Plant Sci. **93**: xxx–xxx. Afin d'optimiser l'usage des mycorhizes en agriculture, douze inoculants commerciaux de champignons mycorhiziens arbusculaires (AMF) ont été évalués en serres sur le maïs. Dans un premier temps, les inoculants furent propagés en pots sur sable stérilisé afin d'évaluer leur potentiel sur la colonisation racinaire du maïs par rapport à celui d'un sol agricole du Kenya et d'inventorier les espèces AMF présentes dans les inoculants. Trois inoculants augmentèrent le taux de colonisation racinaire comparé au sol agricole. Treize espèces AMF furent isolées des inoculants dont 5 non déclarées. Quatre des 12 espèces annoncées n'ont pas sporulé. Dans une seconde expérience, les inoculants furent utilisés en combinaison avec le sol agricole afin d'évaluer leur impact sur le rendement du maïs. Six semaines après le semis, 7 inoculants augmentèrent le taux de colonisation racinaire par rapport au sol témoin alors que 3 inoculants entraînèrent une légère augmentation de la biomasse aérienne. Ces évaluations démontrent la nécessité d'effectuer une pré-évaluation des inoculants commerciaux sur une culture et un sol donnés avant de les implanter à grande échelle.

Mots clés: Inoculants commerciaux, maïs, population sporale, Kenya

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Competing interests: The authors declare that they have no competing interests. The mention of commercial products in this publication does not imply any endorsement by the authors. Other products of similar quality exist in world markets. The products mentioned in this publication are for illustrative use only. No financial support from commercial companies has been received by the authors.

Arbuscular mycorrhizal symbiosis is a mutualistic association between the majority of terrestrial plants and microscopic fungi of the phylum *Glomeromycota*. As obligate symbionts, the arbuscular mycorrhizal fungi (AMF) rely on their host plant to obtain carbon and to complete their life cycles. In return, functional mycorrhizal symbiosis can improve plant growth, health and tolerance to biotic and abiotic stressors (St-Arnaud and

Abbreviations: **AMF**, arbuscular mycorrhizal fungi; **PGPR**, plant growth promoting rhizobacteria

Elsen 2005; Subramanian and Charest 2009) by facilitating plant access to more soil mineral nutrients (Smith and Read 1997; Gahoonia et al. 2005). It is assumed therefore, that the judicious use of these natural inoculants can reduce the need to amend soil with chemical fertilizers, thus increasing the viability of sustainable agriculture (Gemma et al. 1997).

Over the past few decades, companies throughout the world have manufactured and commercialized AMF inoculant using either a single AMF species or mixtures of AMF species that may include plant growth promoting rhizobacteria (PGPR) or other symbiotic and/or biocontrol fungi (Gianinazzi and Vosátka 2004). Industrial manufacturing of AMF as crop inoculants is relatively new and, despite the practical demonstrations of their efficiency, their adoption by crop producers has been slow, most likely due to the quality and efficiency of marketed products. One of the main issues with the use of commercial AMF inoculants in agriculture is related to their performance under specific local conditions. Native AMF species are often considered more mutualistic than non-native ones (Lambert et al. 1980; Henkel et al. 1989; Calvente et al. 2004; Oliveira et al. 2005; Querejeta et al. 2006). Gaur et al. (1998) and Rowe et al. (2007) compared local field-collected strains and foreign commercial inoculants and found great variability of commercial inoculant performances. Corkidi et al. (2004) showed that half of 10 commercial inoculants failed to form mycorrhizal associations. Such negative results have been thought to be caused by low adaptation of exotic AMF inoculants to local edaphic conditions such as soil nutrient concentrations and other abiotic factors (Schreiner 2007). In addition, soil inoculation with exotic AMF strains has been suspected to be potentially dangerous by influencing the soil microbial composition and structure (Mummey et al. 2009) and disturbing the indigenous microbial community (Faye et al. 2009). Since AMF inoculants are expected to be an important component of the upcoming new green revolution (Frazer et al. 2009), whether native AMF are more effective symbionts than non-native ones must be evaluated on a case-by-case basis (Tarbell and Koske 2007). On the other hand, arguments used by inoculant producers to promote their products, deal with (i) the potential of inside microbial isolates and mycorrhizal growth helpers to increase plant yield and protection, (ii) the microbial propagule density within the inoculant and, (iii) the diversity of species used (Tarbell and Koske 2007; Wiseman et al. 2009). Arbuscular Mycorrhizal inoculant efficiency, though in conformity with the previous cited criteria, remains largely dependent upon local environmental factors such as soil mineral content, inoculant mycorrhizal efficiency with the cultivated crop, and niche competition with indigenous strains (Jefwa et al. 2009). Thus, evaluation of the bio-fertilizing potential of exotic strains must take into account their adaptability to local soil and cultivated crops.

This study focuses on (i) assessing AMF commercial inoculants' infectivity and surveying AMF species effectively present in tested inoculants in a sterile substrate and, (ii) evaluating mycorrhizal inoculants performances in a soil from a monocrop cultivated field of maize (hybrid 513).

MATERIALS AND METHODS

Evaluation of Commercial AMF Inoculants in Sterile Sand Pot Culture

Experimental Design

A trap culture experiment was established in a greenhouse at the Tropical Soil Biology and Fertility, International Center for Tropical Agriculture (TSBF-CIAT) located at the World Agroforestry Centre in Nairobi, Kenya, to test 12 commercial AMF inoculants. According to the manufacturer's specifications, microbial composition and species concentration varied between inoculants, but included at least one AMF species, with or without plant growth stimulators such as phosphorus solubilizing bacteria, N_2 fixers and PGPR (Table 1). A Kenyan soil from the Chuka Region classified as a Humic Nitisol (Food and Agriculture Organization 2006) was collected at a depth of 0–20 cm during cultivation of maize with no recent fertilizer or organic matter inputs. Soil was sieved, homogenized and characterized (Table 2). Sand was thoroughly washed, sterilized and filled in 3-L pots. Thirty-six pots were inoculated with commercial AMF products and manufacturer's recommendation were duplicated. To ensure enough propagules for plant root colonization, respective doses were supplied twice. Three control pots were inoculated with the soil at the rate of 100 g per pot and, three non-inoculated pots were added as a control treatment. Maize (*Zea mays* L.) (Hybrid 513) seeds from a Kenyana seed company (www.kenyaseedcompany.co.ke) were used as the test crop. Seeds were surface-sterilized using in 3.3% $Ca(ClO)_2$ for 5 min and five healthy seeds were sown in each pot before spraying approximately 1 cm of sterile sand over and placing in Sunbags (Sigma-Aldrich # B7026). Pots were arranged in a randomized block design over a greenhouse bench and sand moisture was kept at 80% of field capacity. After 1 wk of growth, plant pots were thinned to 2 and substrate moisture was increased to 90% of field capacity for the remaining 17-wk growth period. Modified Hewitt nutrient solution (Jaizme-Vega et al. 1991) was divided into five stocks of solution: Stock 1: KNO_3 (40.44 g L^{-1}), Stock 2: $CaNO_3 \cdot 4H_2O$ (47.23 g L^{-1}), Stock 3: $MgSO_4 \cdot 7H_2O$ (36.97 g L^{-1}) a: KH_2OPO_4 (0.027 g L^{-1}); b: KH_2OPO_4 (0.272 g L^{-1}); Stock 4: NaEDTA-Fe (4.21 g L^{-1}), Stock 5: $MnSO_4 \cdot 4H_2O$ (2.23 g L^{-1}); H_3BO_3 (3.09 g L^{-1}); $ZnSO_4 \cdot 2H_2O$ (0.268 g L^{-1}) and $CuSO_4 \cdot 5H_2O$ (0.375 kg L^{-1}) and applied weekly at the rate of 10 mL per plant to ensure nutrient availability for normal plant growth. Low P stock solution 3a for the first 2 wk and higher P stock solution

Table 1. List and characteristics of tested commercial AMF inoculants

Inoculants	AMF speciesnumber	No. AMF propagules	Other organisms	Added fertilizer N–P–K
1	NA	100 g^{-1}	–	–
2	NA	100 g^{-1}	–	–
3	4	39 g^{-1}	–	5–0–2, Kelp
4	4	30 cc^{-1}	–	3–1–2, Kelp
5	4	30 cc^{-1}	7 Ectomycorrhizae 2 *Trichoderma* 4 bacteria	4–1–2
6	10	44 g^{-1}	–	Humic acid, kelp, vitamins
7	4	13 cc^{-1}	–	5–0–2
8	9	13 cc^{-1}	11 Ectomycorrhizae 2 *Trichoderma* 15 bacteria	4–1–2 kelp
9	1	200 000 g^{-1}	–	–
10	4	50 cc^{-1}	–	–
11	1	NA	–	–
12	6	NA	–	–

b starting at 3 wk after planting were applied at the rate of 2 mL per plant.

Mycorrhizal Root Colonization and AMF Spore Assessment

At harvest, two random test plants were uprooted carefully from each pot treatment. Roots were washed and preserved in 70% ethanol. The root mycorrhizal colonization was assessed by staining with Trypan Blue (Phillips and Hayman 1970) and root colonization levels were assessed using the technique developed by McGonigle et al. (1990) targeting 50 root intersections. AMF spores were extracted from a 100-g sample of sand by wet-sieving and sucrose gradient (Dalpé and Hamel 2007). Sand was thoroughly mixed with 0.5 L of water and two drops of Tween 80 and, passed through superposed 38-, 150- and 500-µm sieves; sievings were distributed into 100-mL tubes containing 25 mL of water filled from the bottom with 50% sucrose solution (vol/vol) before centrifuging the mixture at 2500 $\times g$ for 4 min. Supernatants were recovered, washed through a 38-µm sieve to dilute the sucrose solution and condensed to approximately 20 mL volume. Spores were isolated manually from concentrated supernatants under a dissecting microscope (Olympus SZ-STS), transferred to microscopic slides with a micro-pipette and mounted in polyvinyl alcohol, lactic acid, glycerol (Omar et al. 1979) and polyvinyl alcohol, lactic acid, glycerol–Melzer's reagent (1:1). Identification of spores was performed under a light microscope (Nikon Eclipse 800) using original descriptions, synoptic keys and specialized websites (www.zor.zut.edu.pl/Glomeromycota; invam.caf.wvu.edu). Identified species recovered were compared with strains listed in the corresponding commercial inoculants.

Evaluation of Commercial AMF Inoculants into an Indigenous Soil

Experimental Design and Inoculation

This experiment was established in a randomized block design with three replicates in the same greenhouse. PVC tubes with a volume of 8 L (15.6-cm diameter) closed at the bottom with a double nylon 1-mm mesh were filled with the same soil described in the previous experiment. Inoculation of mycorrhizal products was performed as in the first experiment and a control treatment (not inoculated) was added. To ensure normal plant growth, nutritive solutions were supplied with KNO_3 (0.562 g kg^{-1} of dry soil), NH_4NO_3 (0.385 g kg^{-1}), $MgSO_4$ (0.133 g kg^{-1}) and $CaCl_2$ (0.287 g kg^{-1}) and $MgCl_2$ (0.290 g kg^{-1}) thoroughly mixed with soil before planting. Phosphorus was applied at 60 kg ha^{-1} of a sparingly soluble Mijungu phosphate rock (30% of P_2O_5) mixed separately for each tube. Plants were watered daily (100–150 mL) during the experiment by adding distilled water (6 wk). Twice per week, soil moisture content was adjusted to 90% of the field capacity (29.5%).

Table 2. Characteristics of the Kenyan soil used in the study

Class	Texture	pH (H_2O)	Olsen P (ppm)	ExNa (100 g)	ExCa (100 g)	ExMg (100 g)	ExK (100 g)	ExNa (100 g)	Organic C (%)	AMF spores number (100 g)
Humic Nitisol	clay	6.13	4.27	0.23	12.71	3.29	1.73	0.07	2.63	1200

Data Collection and Analysis

Plant shoot height was measured twice per week. Chlorophyll concentration percentages in the two youngest fully developed leaves were determined at 6 wk after planting using a chlorophyll meter (Model SPAD 502; Konica Minolta Sensitive, Inc; 101 Williams Drive Ramsey, NJ). At harvest, plants were cut 1 cm above the soil surface, soil debris was washed from the roots, and a fresh subsample was collected in 70% ethanol for mycorrhiza colonization assessment as in the first experiment. Shoots and roots were oven dried for 72 h at 65°C and dry biomasses was recorded.

For both experiments, analysis of variance on factors (biomass, chlorophyll content or root colonization) was carried out using General Linear Model procedures of SAS software (SAS Institute, Inc. 2006) with the LSD option for the test of means separation. Significance was evaluated at $P \leq 0.05$.

RESULTS

Effects of Commercial AMF Inoculants on Maize Root Colonization and AMF Spore Survey in Sand Culture

Among the 12 mycorrhizal inoculants tested, three (2, 10 and 12) significantly ($P < 0.001$) increased maize root colonization by mycorrhiza compared with the soil (19%). Seven inoculants (1, 3, 6, 7, 8, 9 and 11) induced root colonization at much lower levels (from 0.7 to 3%) than the soil inoculation, while two inoculants (4 and 5) did not colonize roots (0%) as in control (sand only) (Fig. 1).

Overall, 11 AMF species (9 *Glomus*, 1 *Paraglomus* and 1 *Gigaspora*) were declared to be present in the commercial inoculants studied (Table 3). A total of 13 AMF spore species were extracted and identified from the 18-wk-old sand trap cultures. Among them, five species were not listed in the commercial inoculants. *Gigaspora gigantea*, *Acaulospora scrobiculata*, *Glomus fasciculatum and G. pallidum* were extracted from a unique commercial inoculant trap culture, while *spores* of *A. tuberculata,* a species not listed in any inoculants, were found in abundance within seven of tested inoculants. Except for *G. clarum*, *G. irregulare, G. etunicatum, G. mosseae and Paraglomus brasilianum*, there were few matches between claimed and trapped species. In the non-inoculated pot treatment, no AMF spores were detected.

Mycorrhizal Potential of Commercial Mycorrhizal Inoculants in Soil

Effects of commercial mycorrhizal inoculation on maize plant growth and yield in the Kenyan soil are summarized in Table 4. After 21 and 24 d of growth, shoot heights of maize plants inoculated with inoculants 2 and 10 were found significantly higher ($P < 0.05$) than the control. However, at the end of the 6-wk growth period, plant heights, shoot and root dry weight, and shoot:root ratio were not significantly different from the control. Significant negative effects obtained with inoculant 12 also totally disappeared as well as negative effect on chlorophyll leaf content induced by inoculant 7. Inoculation of commercial mycorrhizal products did not allow significant total dry biomass increases of maize plant compared to control treatment. In all treatments (inoculated or not), a high level of P deficiency has been observed.

In terms of mycorrhizal potential, inoculants 2, 4, 7, inoculants 6, 9, 12 and inoculant 10 significantly increased root colonization percentage at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively, compared with soil alone (Fig. 2). The highest root colonization levels were obtained with inoculants 9 (46.83%), 10 (56.67%) and 12 (47.67%). The effects of inoculants 1, 3, 5, 8 and 11 were similar to the control treatment.

DISCUSSION

Root Colonization Potential of Commercial Inoculants in Sterile Sand Culture

Ten of the twelve mycorrhizal inoculants tested succeeded in colonizing maize roots in sterile sand pot cultures (Fig. 1). These results are different from those reported by Corkidi et al. (2004) and Tarbell and Koske (2007), who found that most commercial inoculants were unable to colonize roots. However, only three mycorrhizal inoculants 2 ($P < 0.05$), 10 ($P < 0.001$) and 12 ($P < 0.01$) colonized maize plant roots at higher rates than the Kenyan soil. Consequently, those inoculants might be promising candidates for greenhouse plant production. However, there is a need to test inoculants under field conditions to confirm their performance as suggested by Sorensen et al. (2008). The remaining AMF inoculants, which gave root colonization levels lower than the soil, are indeed inefficient under these specific soil and crop environment conditions. However, it is not known if they would perform well under other growth conditions and with other crops.

Inoculants AMF Spore Assessment in the Sand Trap Culture

Some AMF species advertised on the commercial inoculant labels were found sporulating in pot cultures, while some others were not detected. The 18-wk trap pot culture period might be not long enough to allow establishment and differentiation of all AMF species of an inoculant even though Khaliq et al. (2010) considered this period as sufficient to allow AMF propagules to revitalize, infect maize roots, and sporulate. In fact, AMF species such as *Acaulospora* and *Gigaspora* genera possess a low sporulation potential, mostly differentiating fewer than a dozen spores within a 6-mo growing period (Dalpé and Declerck 2002). Others, generally small-spore species, such as *G. irregulare*, can produce hundreds of spores within a 4-mo period in a unique in vitro root culture (St-Arnaud et al. 1996).

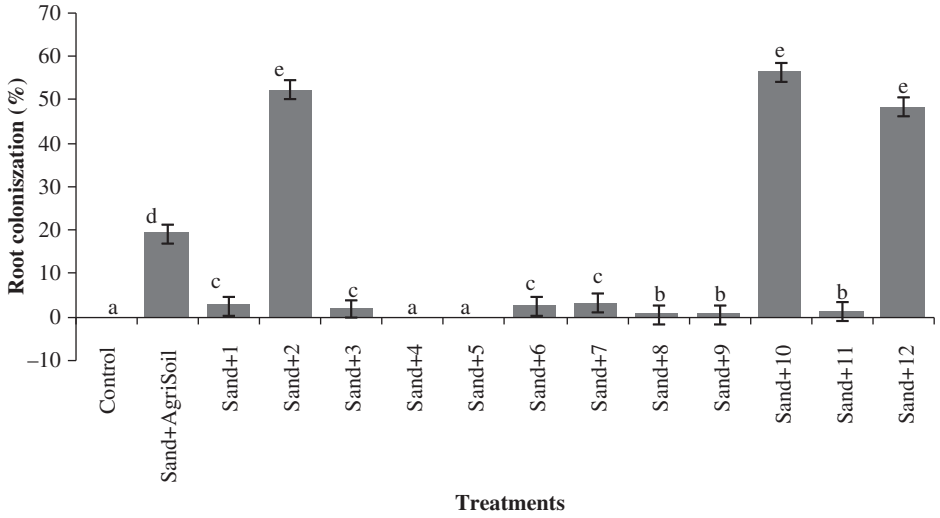

Fig. 1. Percent root colonization of maize plants grown in sterile sand inoculated with the 12 commercial inoculants, soil and without inoculation (Control). Bars represent the standard error of the difference (SED) for effects of product application. Values with different letter are significantly different at $P < 0.05$.

Based on these assumptions, the presence in some pot cultures of species that were neither advertised by inoculant producers nor found in the control pots remains unexplained. The use of Sunbags minimized cross-contamination between pots and should considerably reduce the risk of introducing outside AMF species. On the other hand, industrial production of mycorrhizal inoculants is mostly performed under greenhouse pot culture conditions, with quality control, propagule density and AMF species identity verified through subsample analyses (Gianinazzi and Vosátka 2004; Fortin et al. 2008). Producers use diversity of biological inoculants and of formulations going from a single ubiquity AMF species to a range of strains and species with or without added fertilizers hoping by those strategies to provide optimal inoculants suitable for a diversity of applications. The 12 commercial inoculants tested were in vivo mass produced in containers with the advantage of low-cost technology, but risky in term of potential contamination by outside AMF organisms.

For commercial use, high root colonization and sporulation potentials to ensure rapid propagation and maximise AMF strain viability and productivity over time are preferential selection criteria of AMF strains (Fortin et al. 2002). Meanwhile, inoculant producers opt to add non-microbial ingredients such as fertilizers, vitamins, and other substrates as stated by Schweinsberg-Mickan and Müller (2009) and confirmed in Table 1.

Table 3. Arbuscular mycorrhizal fungi species' composition of commercial inoculants and species detected from 18-wk-old trap cultures

Inoculants	Claimed species	Species detected
1	*G.* spp.[z]	*A. tuberculata*[z], *G. mosseae, G. constrictum, G. deserticola*
2	*G.* spp.	*G. deserticola, G. etunicatum, G. mosseae,*
3	*G. aggregatum, G. etunicatum, G. intraradices G. mosseae*	*A. tuberculata, G. etunicatum, G. mosseae,*
4	*G. aggregatum, G. etunicatum, G. intraradices, G. mosseae*	*G. mosseae*
5	*G. aggregatum, G. etunicatum, G. intraradices, G. mosseae*	*A. tuberculata, G. mosseae,*
6	*Gi. margarita, G. aggregatum, G. clarum, G. deserticola, G. etunicatum, G. intraradices, G. monosporum, G. mosseae, P. brasilianum*	*A. tuberculata, G. constrictum, G. etunicatum, G. irregulare***
7	*G. aggregatum, G. etunicatum, G. intraradices, G. mosseae*	*G. clarum, G. deserticola, P. brasilianum*
8	*Gi. margarita, G. aggregatum, G. clarum, G. deserticola, G. etunicatum, G. intraradices, G. monosporum, G. mosseae, P. brasilianum*	*A. scrobiculata, Gi.* sp., *G. fasciculatum,G. irregulare, G. pallidum*
9	*G. intraradices*	*Gi. margarita, Gi.* [illegible] *G. irregulare*
10	*G. claroideum, G. etunicatum, G. geosporum, G.intraradices, G. mosseae*	*A. tuberculata*
11	*G. intraradices*	*A. scrobiculata, G. fasciculatum, G. irregulare*
12	6 beneficial mycorrhiza from arid zones	

[z]A, Acaulospora; Gi, Gigaspora; G, Glomus; P, Paraglomus
[illegible]ously identified as *G. intraradices* (Sokolski et al. 2010).

Table 4. Effects of commercial arbuscular mycorrhizal fungi inoculants on growth parameters of 6-wk-old maize cultivated under the Kenyan soil

	Effects on plant growth parameters			
Treatments	Shoot height 21–24 DAP (cm)	Shoot height at 6 WAP (cm)	Chlorophyll (%)	Total dry biomass (g)
Control: Soil without inoculant	8.03 ± 2.35*A*	84.173 ± 1.732*A*	27.631 ± 2.148*A*	11.373 ± 2.138*A*
Soil + Inoculant 1	9.934 ± 0.347*A*	84.000 ± 2.437*A*	28.800 ± 1.828*A*	11.470 ± 3.999*A*
Soil + Inoculant 2	11.330 ± 1.351*B*	93.672 ± 1.377*A*	27.935 ± 6.356*A*	15.347 ± 2.777*A*
Soil + Inoculant 3	9.572 ± 2.351*A*	90.752 ± 2.566*A*	N/A	19.763 ± 2.861*A*
Soil + Inoculant 4	9.470 ± 1.742*A*	93 ± 4.122*A*	24.933 ± 0.115*A*	11.777 ± 1.268*A*
Soil + Inoculant 5	8.00 ± 3.542*A*	91.504 ± 4.351*A*	25.372 ± 1.392*A*	11.553 ± 1.830*A*
Soil + Inoculant 6	6.874 ± 1.562*A*	85.171 ± 3.743*A*	N/A	10.870 ± 0.789*A*
Soil + Inoculant 7	8.433 ± 3.437*A*	83 ± 2.643*A*	23.07 ± 3.963*B*	10.603 ± 0.270*A*
Soil + Inoculant 8	10.377 ± 3.452*A*	85.502 ± 2.548*A*	28.60 ± 1.034*A*	14.053 ± 3.690*A*
Soil + Inoculant 9	8.472 ± 4.333*A*	84.17 ± 3.571*A*	N/A	13.553 ± 2.968*A*
Soil + Inoculant 10	11.2 ± 4.658*B*	90 ± 5.372*A*	27.53 ± 4.659*A*	18.230 ± 6.765*A*
Soil + Inoculant 11	8.474 ± 3.534*A*	85.173 ± 2.873*A*	N/A	11.540 ± 4.833*A*
Soil + Inoculant 12	5.237 ± 2.373*C*	90.676 ± 1.734*A*	34.87 ± 1.721*C*	16.333 ± 3.346*A*

A–C Different letters across columns indicate significant ($P < 0.05$) difference compared with the control.

Inoculants 4, 5, 6 and 8, advertised to contain *Trichoderma*, bacteria and ectomycorrhizal fungi in combination with N, P and K fertilizers, gave mitigated results. When mixed inoculants are developed, each added organism (bacteria, PGPR or ectomycorrhizal fungi, etc.) is expected to have a synergistic effect with other inoculant components in order to optimize product efficacy. Potential inhibitory effects between abiotic growth promoters, microbial organisms and native soil microorganisms may also progressively take place. It is then imperative to evaluate commercial inoculants locally before large-scale use for crop production, especially when multi-species and ingredients are added to the inoculants.

Mycorrhizal Potential of Commercial Inoculants in Soil

The increased shoot heights obtained with inoculants 2 and 10 at 21 and 24 d after planting (Table 4) may indicate their capacity to rapidly establish functional symbiosis with maize plants compared with other tested inoculants. However, since native AMF isolates are sometimes more mutualistic than non-native ones (Lambert et al. 1980), significant growth effect from

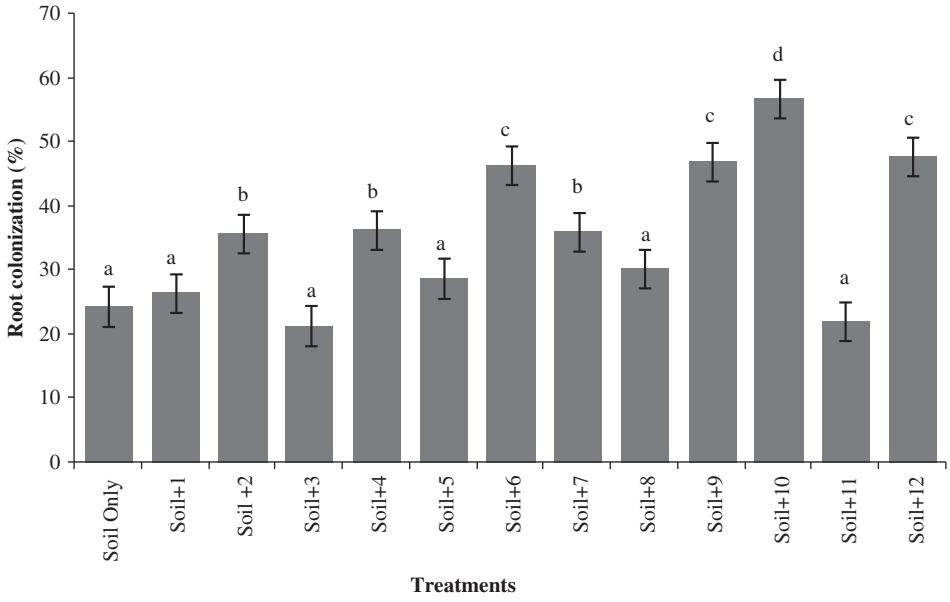

Fig. 2. Percent root colonization of maize plants grown in soil inoculated or not with commercial mycorrhizal inoculants. Bars represent the standard error of the difference (SED) for effects of products application. Values with different letter are significantly different at $P < 0.05$.

products 2 and 10 before the 6-wk growth period might be due to performing native AMF activity. Combination of exotic strains with native ones may allow significant increases of plant root colonization, but is not necessarily translated to higher plant yields, as appears with inoculants 4, 6, 9 and 12. As a result, seedling growth parameters measured at 6 wk were not different between treatments. Interestingly, products such as inoculant 2, 4, 6, 7, 9, 10 and 12 induced maize root colonization at a rate significantly higher than the indigenous soil. These findings are in line with the results of Corkidi et al. (2004) who stated that a 6-wk growth period is sufficient to allow mycorrhizal development and good AMF maize root colonization. On another hand, there was a slight increase of dry biomass observed with inoculants 2, 10 and 12 showing high root colonization rates in sterile sand pot (Fig. 1) and in the soil pot cultures (Fig. 2). These results suggest that these inoculants may have good potential and, as such, can be considered as performing mycorrhizal inoculants. For inoculants 6 and 9, the 6-wk growth period may have been long enough to allow good root colonization, but this is not reflected in plant biomass increase (Table 4). This result confirms that root colonization efficiency might be used as an indicator of inoculant viability, but not of plant growth efficiency, as is often expected in field studies (Bilalis and Karamanos 2010). Similarly, no direct correlation was established between inoculant mycorrhizal potential and its AMF propagule density or species diversity. One convincing example is inoculant 9, which was claimed to contain a unique AMF strain at a tremendously high propagule concentration (200 000 propagules g^{-1}), but colonized plant roots at a very low level in sterile sand with almost 50% more root colonization than when mixed with soil. The addition to soil of a large amount of spores from a unique AMF species may stimulate the growth a native AMF population. Even so, the root colonization levels observed did not increase plant biomass. Similar behavior was recorded with inoculant 6, which contained multiple AMF species in the range of 44 propagules per gram mixed with 11 ectomycorrhizal fungi plus kelp, humic acid and vitamins.

As demonstrated, the benefits expected from the use of commercial mycorrhizal inoculants in maize production, up to 6 wk of growth, could not be clearly determined from the inoculant formulation, labels or plant growth promotion advertisements made by producers. Results of the study indicated that a 6-wk growth period is not long enough to get significant increases of maize shoot dry biomasses from any of the commercial inoculants tested. For a complete evaluation of the inoculants' potential, it is important to pursue similar tests up to maize maturity in order to compare final crop yields. The effectiveness of commercial inoculants depends on environmental and soil conditions, such as mineral concentration, microbial composition and crop type. To ensure successful use of inoculants, preliminary trials must be carried out under local growth conditions with the soil and host-plants expected to be used in the field. On the other hand, good root colonization of commercial mycorrhizal inoculants does not translate to plant growth and yield, and may increase the necessity to investigate the functionality of a mycorrhizal symbiosis formed by a commercial inoculant. Molecular tools could also be used to elucidate the root occupancy capacity of exotic strains within indigenous soils.

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