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BRIEF COMMUNICATION

Effect of bacteria isolated from composts and macrofauna on sorghum growth and mycorrhizal colonization

B. Hameeda · M. Srijana · O. P. Rupela · Gopal Reddy

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Summary Beneficial plant–microbe interactions in the rhizosphere are primary determinants of plant health and soil fertility. The effect of combined inoculation of plant growth-promoting bacteria, *Bacillus circulans* EB 35, *Serratia marcescens* EB 67 and *Pseudomonas* sp. CDB 35 and arbascular mycorrhizal fungi, *Glomus* spp. on sorghum growth and mycorrhizal colonization was investigated. Plant growth observations taken at 45 days after sowing (DAS) revealed that all the three strains applied along with arbascular mycorrhizae (AM) improved plant biomass from 17 to 20% and mycorrhizal colonization from 25 to 35%. Further studies at 90 DAS also showed improvement in plant growth parameters measured. It was apparent that all the three strains stimulated plant and root growth in combination with AM and infection of sorghum roots with mycorrhizae at 45 DAS was equal to or even greater than the AM + rock phosphate (RP) inoculation at 90 DAS. This shows the possible reduction of AM culturing period to 45 days compared to its 3-month culturing in the pot cultures.

Keywords Arbascular mycorrhizae · Bacteria · Composts · Plant growth promotion · Sorghum

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Introduction

Beneficial free-living soil bacteria that increase plant growth are generally referred to as plant growth-promoting bacteria (PGPB) and are found in association with the roots of various plants (Kloepper et al. 1991; Sajjad et al. 2001). An additional possibility is that the beneficial effects of some PGPB are due to their interactions with Arbascular mycorrhizal fungi (AMF) (Marschner and Baumann 2003; Artursson et al. 2006). AMF and bacteria can interact synergistically to stimulate plant growth through a range of mechanisms that include improved nutrient acquisition (Barea et al. 2002), inhibition of plant pathogenic fungi (Budi et al. 1999) and enhancement of root branching (Gamalero et al. 2004).

Arbascular mycorrhizae (AM) are an important component of the soil microflora, promoting nutrient uptake in plants in exchange for carbon compounds. They are ubiquitous in geographic distribution occurring with plants growing in arctic, temperate and tropical regions alike. These fungi belong to the genera *Endogone, Glomus, Entrophosphora, Gigaspora, Acaulospora, Scutellispora*, are obligate symbionts and are grown in association with living tissues (Al-Raddad 1995). The most widely used is pot culture, where the fungi are usually maintained in conjunction with suitable host plant roots (Ferguson and Woodhead 1982). The making of arbascular mycorrhizal inoculants is relatively expensive and involves extended culture periods of several months. Hence, the development of rapid and more efficient culture system remains an important challenge for commercialization. In the present study, PGPB isolated from composts and macrofauna promoting plant growth were applied

along with AM to evaluate growth of sorghum (sweet stalk) ICSV 93046 and colonization of mycorrhizae in roots under glasshouse conditions.

Materials and methods

Isolation and screening of bacteria

Two hundred and seven bacteria were isolated from composts (farm waste compost, rice straw compost and *Gliricidia* vermicompost) and macrofauna (earthworms, centipedes, slugs and snails) and screened for plant growth-promoting and antagonistic traits.

Twelve bacterial isolates with plant growth-promoting and antagonistic traits were identified by staining, morphology, cultural, growth and biochemical characters as per *Bergey's manual of determinative bacteriology* (Krieg and Holt 1984). Out of them three strains, *Bacillus circulans* EB 35, *Serratia marcescens* EB 67 and *Pseudomonas* sp. CDB 35 promoted growth of pearl millet under glasshouse conditions and were identified at Microbial Type of Culture Collection, Chandigarh, India for confirmation (Hameeda et al. 2006a). These three PGPB (EB 35, EB 67 and CDB 35) were applied along with *Glomus* spp. to evaluate plant growth and colonization of mycorrhizae in sorghum roots under glasshouse conditions.

Evaluation of bacteria and/or mycorrhizae under glasshouse conditions

For this study, unsterilized low-P soil from BR 1D field at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) was used as potting medium in 21-cm diameter plastic pots. Nitrogen was applied at 40 kg N ha^{-1} and phosphorus was applied as rock phosphate (RP) at 20 kg P ha^{-1} wherever specified. AMF, *Glomus* spp., was prepared as sand inoculum and applied at the rate of 1% and mixed evenly with the soil before filling up the pots. Sorghum seeds (ICSV 93046) were coated with a peat (Biocare Technology Pvt., Chatswood, NSW, Australia)-based inoculum of bacteria (10^8–10^9 c.f.u. g^{-1} peat) using 1% carboxymethylcellulose as adhesive and the cell count was 10^6–10^7 c.f.u. per seed before sowing.

The treatments included combination of mycorrhizae and bacteria with and without RP and uninoculated plants served as controls. Three seeds were sown and thinning was done to one per pot within a week after germination. The plants were watered with deionized water every alternate day. The temperature of the glasshouse ranged from 22 to 32°C (average 26°C). Harvesting of the plants was done at vegetative growth period [45 days after sowing (DAS)] and flowering period (90 DAS). Plant growth measurements such as shoot length, leaf area, plant dry weight and root volume (by water displacement method) were taken. Mycorrhizal colonization of roots in terms of percent infection was measured according to the method of Phillips and Hayman (1970).

Statistical analysis

The experiments were arranged in completely randomized block design with three replications in each treatment and repeated twice. The data were subjected to analysis of variance using the Genstat 6.1 statistical package (Lawes Agricultural Trust, Rothamsted, UK). Mean values in each treatment were compared using least significant differences (LSD) at 5% probability ($P = 0.05$).

Results

Compatibility of PGPB (*B. circulans* EB 35, *S. marcescens* EB 67 and *Pseudomonas* sp. CDB 35) with mycorrhizae (*Glomus* spp.) was studied on sorghum (ICSV 93046) in pots using unsterilized soil. Seed treatment with all three isolates showed significant increase in growth parameters at first harvest (45 DAS) when compared to the uninoculated control. The improvement in plant growth parameters ranged between 36 and 39% for shoot length, 10–14% for leaf area, 17–20% for dry weight and 27–70% for root volume when compared to AM + RP application. It was observed that mycorrhizal colonization of sorghum root was 46% with *B. circulans* EB 35, 48% with *Pseudomonas* sp. CDB 35 and 56% with *S. marcescens* EB 67 applied along with AM + RP (Table 1). However, when bacteria were applied alone, mycorrhizal colonization was less and was 17% with EB 35, 44% with EB 67 and 42% with CDB 35.

A further increment in most of the growth parameters studied was also noticed after second harvest (90 days). Increase in shoot length was 18–25%, leaf area 2–3%, dry weight 5–19%, root volume 71–129% by EB 35, EB 67 and CDB 35. Mycorrhizal root colonization showed an improvement at 90 DAS and the increase was 14% with EB 35, 11% with EB 67 and 9% with CDB 35 when compared to 45 DAS. However, when bacteria were applied along with AM and RP the increase was 4% (EB 35), 12% (CDB 35) and 18% (EB 67) (Table 2). Co-inoculation of all the three PGPB significantly increased most of the parameters

Table 1 Effect of dual inoculation of bacteria and AM on growth of sorghum ICSV (93046) (sweet stalk) and mycorrhizal association in roots (45 DAS)

Treatments	Shoot length (cm)	Leaf area (cm^2)	Dry weight (g)	Root volume (ml)	AM colonization (%)
Control	64	122	1.2	0.6	8
AM	84*	413*	5.2*	2.8*	15*
RP	78*	392*	4.7*	2.7*	14
AM + RP	64*	418*	5.4 *	3.0*	21*
B. circulans EB 35	84 (31)*	431 (3)*	5.8 (7)*	3.4 (13)*	17*
B. circulans EB 35 + AM RP	87 (36)*	460 (10)*	6.3 (17)**	3.8 (27)*	46**
S. marcescens EB 67	87 (36)*	456 (9)*	6.1 (13)*	4.2 (40)**	44**
S. marcescens EB 67 + AM RP	89 (39)**	478 (14)*	6.4 (18)**	5.1 (70)**	56**
Pseudomonas sp. CDB 35	83 (30)*	453 (8)*	6 (11)*	4.1 (37)**	42**
Pseudomonas sp. CDB 35 + AM RP	87 (36)*	461 (10)*	6.5 (20)*	4.6 (53)*	48*
Mean	83	406	5.3	3.2	29
LSD	3.5	93.1	1.03	1.0	6.7
CV (%)	11	13	11	19	14

LSD least significant difference, *CV* coefficient of variance

Values are means of three replications and done twice, data calculated per plant

AM arbascular mycorrhizae (*Glomus* spp.), N = 40 kg h^{-1} (applied twice, during sowing and 45 DAS for all the treatments except control); P = 20 kg h^{-1}, *RP* rock phosphate (wherever mentioned)

* Means are significantly different than control (uninoculated) at $P = 0.05$ when compared by LSD

** Means are significantly different than AM + RP treatment at $P = 0.05$ when compared by LSD. Values in parentheses are per cent increase over AM + RP treatment

analysed when compared to control. Mycorrhizal colonization study revealed that when bacteria were applied along with AM the percentage of colonization was significant within 45 DAS and was equal to that of AM + RP application that showed 41% after 90 DAS (Tables 1, 2).

Discussion

In this study, it was observed that dual inoculation of PGPB, *B. circulans*, *S. marcescens* EB 67, *Pseudomonas* sp. CDB 35 along with AM (*Glomus* spp.) showed significant increase in all growth parameters of sorghum.

Table 2 Effect of dual inoculation of bacteria and AM on growth of sorghum (ICSV 93046) (sweet stalk) and mycorrhizal association in sorghum roots (90 DAS)

Treatments	Shoot length (cm)	Leaf area (cm^2)	Dry weight (g)	Root volume (ml)	AM colonization (%)
Control	269	85	8	4.9	13
RP	403*	120	17*	7	23
AM	453*	115	17*	8.3	27*
AM RP	481*	124	18*	8.2	41*
B. circulans EB 35	516 (7)**	109	19 (4)*	11.7 (43)*	31*
B. circulans EB35 + AM RP	567 (18)**	122	19(5)*	14 (71)*	50*
S. marcescens EB 67	561 (17)**	111	20 (9)**	14.6 (78)*	55**
S. marcescens EB67 + AM RP	600 (25)**	128 (3)	22 (19)**	18.8 (129)*	74**
Pseudomonas sp. CDB 35	555 (15)*	112	19 (5)*	14 (71)*	53**
Pseudomonas sp. CDB35 + AM RP	573 (19)**	127 (2)	20 (11)**	15.2 (85)*	60**
Mean	115	490	18	11	41
LSD	16.2	57.6	2.3	4.8	11.6
CV (%)	8	7	8	25	17

Values are means of three replications and data calculated per plant after 90 DAS

AM arbascular mycorrhizae (*Glomus* spp.)

N = 40 kg h^{-1} (applied twice, during sowing and 45 DAS for all the treatments except control). P = 20 kg h^{-1}, *RP* rock phosphate (wherever mentioned)

* Means are significantly different than control (uninoculated) at $P = 0.05$ when compared by LSD

** Means are significantly different than AM + RP treatment at $P = 0.05$ when compared by LSD. Values in parentheses are per cent increase over AM + RP treatment

Dual inoculation of *Bacillus* spp. and *Glomus manihotis* significantly improved banana growth parameters (Rodriguez-Romero et al. 2005) and *Pseudomonas fluorescens* and *Glomus mosseae* BEG 12 increased mycorrhizal colonization of tomato root and improved plant growth (Gamalero et al. 2004). Recently it was reported that AM fungi and bacteria interact synergistically to stimulate plant growth through a range of mechanisms that include improved nutrient acquisition and inhibition of fungal plant pathogens (Artursson et al. 2006). In the present study, we have used low-P unsterilized soil to evaluate the activity of PGPB and AM in soil conditions. In soils with low P-bioavailability, free-living phosphate-solubilizing bacteria may release phosphate ions from sparing soluble inorganic and organic P compounds in soil and thereby contribute with an increased soil phosphate pool available for the extraradical AM fungal hyphae to pass on to the plant (Artursson et al. 2006).

The two strains used in this study, *S. marcescens* EB 67, *Pseudomonas* sp. CDB 35 are reported to have phosphate solubilizing ability and promote maize growth in glasshouse and field conditions (Hameeda et al. 2006b). Mostly P solubilization is due to organic acids that are products of fermentative and respiratory metabolism of carbohydrates and other carbonaceous substrates. AM fungi contribute to this pool by stimulating host plants to release root exudates by a mechanism or mechanisms not clearly understood at present (Habte 2006). However, most of the previous reports showed maximum plant growth and P uptake in sterilized soils where both AM and phosphate solubilizing microorganisms were inoculated (Kucey 1987). *S. marcescens* EB 67 inoculated along with AM + RP increased mycorrhizal colonization of sorghum. This suggests that the strain behaves as a mycorrhizal helper bacterium in sorghum. Previous reports have shown that 'Mycorrhizal helper bacteria' mainly of *Pseudomonas* spp., enhanced mycorrhizal formations significantly in plants (Garbaye and Bowen 1989; Duponnois and Plenchette 2003).

Per cent infection/colonization of mycorrhizae in plants due to application of bacteria along with AM within 45 days was same as that of AM inoculation alone at 90 days (Tables 1, 2). This suggests the possible reduction of AM culturing period by 45 days compared to its usual pot culture method that needs around 3 months. Previous reports (Bhowmik and Singh 2004) due to the inoculation of *Azospirillum* with *Glomus* spp. also showed significant improvement in AM colonization. PGPB are known to synthesize biologically active substances (plant growth hormones) that affect AM spore germination and hyphal elongation that in turn increase root biomass, which accelerates the AM root colonization (Azcon 1987). However, studies of Bending et al. (2002) showed that there is evident diversity among mycorrhiza-promoting gram-positive bacteria and *Bacillus* sp. doubled ectomycorrhizal colonization in roots, whereas *Pseudomonas*, *Serratia* and *Burkholderia* spp. inhibited the same. However, their studies revealed that growth promotion by bacteria in the *Pinus sylvestris-Suillus luteus* symbiosis did not result from enhanced mycorrhiza formation and it is possible that growth promotion resulted from shifts in the relative proportions of fungi forming ectomycorrhizae, leading the changes in the functional benefits to the plants from its associated fungus community.

Although there have been a substantial number of studies of interactions between AM fungi and bacteria, the underlying mechanisms of these associations are not well understood. More insight into these mechanisms will enable optimization of the effective use of AM fungi in combination with bacterial partners as a tool for increasing crop yields.

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