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Effects of plant growth promoting bacterial isolates from Kavango on the vegetative growth of *Sorghum bicolor*

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Sorghum is an important cereal that is processed into a variety of foods and leisure beverages across the African continent. Low sorghum yields experienced in agriculture is a result of major production constraints such as soil nutrient deficiency and plant disease. It is important that the methods for crop production are of a sustainable nature as the chemical fertilizers in current use are detrimental to the natural environment. The aim of this study was to determine the effects of plant growth promoting (PGP) bacteria on growth of *Sorghum bicolor*. PGP bacteria isolated from the rhizosphere of *Pennisetum glaucum* (*Pseudomonas stutzeri* ACM2-32, *Kosakonia cloacae* FCM2-50, *Bacillus subtilis* ASM1-59 and *Bacillus amyloliquefaciens* LSM1-61) and *S. bicolor* (*Stenotrophomonas maltophilia* LCS2-11) plants in Kavango (Namibia), were used as peat-based inoculants to evaluate their effects on the growth of *S. bicolor*. The combination treatment T_9 (*B. amyloliquefaciens* LSM1-61: *K. cloacae* FCM2-50: *P. stutzeri* ACM2-32) significantly ($p = 0.032$) enhanced the biomass of *S. bicolor* as compared to the water control. Single inoculants consisting of *S. maltophilia* LCS2-11, *K. cloacae* FCM2-50 and *B. amyloliquefaciens* LSM1-61 and combination inoculants T_7, T_8 and T_9, enhanced *S. bicolor* root biomass as much as a commercial fertilizer control. These results indicate that the plant growth promoting bacteria induced a beneficial effect on growth of sorghum seedlings. The future work involves testing these promising inoculants on growth of these sorghum plants to maturity stage to determine effects on seed yield over three seasons in multi-location trials.

Key words: Rhizosphere bacteria, peat based inoculants, plant growth promoting bacteria, *Sorghum bicolor*.

INTRODUCTION

Africa's population might rise by 58% in 2030 (DESA, UN, 2013), consequently the demand for agricultural crops might increase significantly by then. The utilization of sustainable methods for enhancing harvest yield are gaining preference over the environmentally damaging agrochemicals which are currently being used. Solving agricultural concerns identified with the application of ecologically unfavourable fertilizers and the control of

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Table 1. Source and plant growth promoting trait profiles of bacterial isolates.

Bacterial isolate	Site	Plant rhizosphere	PO_3^{-4} solub.	N_2 fixation	IAA	Anti-fungal
S. maltophilia LCS2-11*	Lukas 2	Sorghum	-	+	+	-
P. stutzeri ACM2-32	ATR	Pearl millet	+	-	+	-
E. cloacae FCM2-50	Field E	Pearl millet	-	+	++	-
B. subtilis ASM1-59	ATR	Pearl millet	-	-	++	+
B. amyloliquefaciens LSM1-61	Lukas 2	Pearl millet	-	-	++	+

*Siderophore producing isolate; ATR coordinates - 17°53′49.75″ S 20°09′07.07″ E; Lukas 2 coordinates - 17°53′43.80″ S 20°14′05.26″ E; Field E coordinates - 17°54′04.40″ S 20°14′14.34″ E.

plant diseases can be fulfilled by using plant root associated bacteria (Akhtar and Siddiqui, 2011; Glick, 2012). Various groups of bacteria found in the volume of soil affected by the presence of plant roots (Uren, 2007), also known as the rhizosphere, have been shown to be beneficial for the growth, yield and crop quality of plants (Orhan et al., 2006). These bacteria are able to colonize the rhizosphere and in some instances enter the roots of plants eventually inducing a beneficial effect on the host plant (Kloepper et al., 1980). Mechanisms by the bacteria aimed at inducing plant growth promotion include production of antibiotics against pathogenic microorganisms, production of antifungal compounds, production of plant hormones, increasing the availability of soluble phosphorus, increasing iron availability to plants, nitrogen fixation and regulation of ethylene concentration (Lucy et al., 2004).

The sorghum plant has the capacity to grow in moderately poor semi-arid and sub-tropical conditions of Africa (Taylor, 2003). Sorghum (*Sorghum bicolor* (L.) Moench) is an important crop throughout Western, Eastern and Southern Africa, that is used mainly for food and beverage production. Sorghum meal is used for making porridge and as an added substance to lower consistency and increase supplement and caloric thickness in porridge produced from other grains (Smith and Frederiksen, 2000; Ohiokpehai, 2003). In beer production, sorghum malt is used in the saccharification of the starchy substrate prior to fermentation (Smith and Frederiksen, 2000). Despite the genetic potential, generally low sorghum grain yields are experienced as a consequence of major constraints such as nutrient deficiency, soil water deficiency and plant diseases (Wortmann et al., 2009).

Commercial inorganic fertilizers may offer a short term solution for crop production but the financial component and long term soil fertility concerns labels them as an unfavourable option (Namibia Resource Consultants and Vigne and Associates Consultants, n.d.). For those countries that do not manufacture fertilizers, as opposed to need to import them, bacterial inoculants can be used to produce local cereals and agricultural products at a reasonably less expensive cost. The utilization of rhizosphere related microorganisms offers an appealing option to agrochemicals considering the fact that their plant growth and crop yield enhancing capacities have been shown over the recent decades (Saharan and Nehra, 2011). This study was carried out to assess the effects of treating *S. bicolor* seed with peat based plant growth-promoting rhizobacterial suspensions.

MATERIALS AND METHODS

Bacterial isolates

Native bacterial isolates (*Stenotrophomonas maltophilia* LCS2-11 and *Pseudomonas stutzeri* ACM2-32, *Enterobacter cloacae* FCM2-50, *Bacillus subtilis* ASM1-59 and *Bacillus amyloliquefaciens* LSM1-61) exhibiting increasing nutrient availability, plant hormone production and anti-fungal capabilities were obtained from The Department of Biological Sciences, University of Namibia. The bacteria were isolated from the rhizospheres of pearl millet and sorghum plants that were grown in the fields of subsistence farmers along the Kavango River (Table 1).

Preparation of treatments

Bacterial isolates were grown in VM–ethanol broth at 28 ±2°C for 3 days. The bacterial cell concentration was adjusted to OD_{660} = 0.9 in 50 ml VM–ethanol broth volume, washed with sterile distilled water and resuspended in 50 ml of 0.85% NaCl. This procedure was repeated for some bacterial isolates depending on the number of treatments and number of replicates. Starke Ayres® palm peat was prepared according to the manufacturer's instructions and dried overnight at 60°C. Approximately 50 g dry palm peat was placed into separate aluminium foil containers and sterilized via autoclaving. The palm peat was then aseptically transferred into Ziploc® plastic bags, moistened with 5 ml sterile distilled water per bag and kept at 4°C.

The application of phosphate solublizers alone or in combination with nitrogen fixers is beneficial for the growth of cereal (Zaidi and Khan, 2005). Therefore, combination treatments were made up of one phosphate solubilizing isolate (*P. stutzeri* ACM2-32), a N_2–fixer and an isolate with antifungal capability. The inoculum treatments were prepared according to Rose et al. (2011) with slight modifications. Treatments (Table 2) consisted of 50 g palm peat and 20 ml of bacteria solution, that is, 3 ml bacteria-0.85% NaCl suspension + 17 ml sterile distilled water for single bacterial treatments and 3 ml bacteria-0.85% NaCl suspension (×3 different isolates) + 11 ml sterile distilled water for combination bacterial treatments, whereas 3 ml 0.85% NaCl + 17 ml sterile distilled water was the control. After transferring the bacteria solutions to the palm peat enclosed in Ziploc® bags, the treatments were incubated for 3 days at 30°C before applying to soil. The non-inoculum control treatments were a commercial fertilizer, Hygrotech Terra Nova

Table 2. Comparisons of treatments for root masses, plant masses and root : shoot ratios.

Treatment	Root mass (g)	Plant mass (g)	Root : shoot ratio
T_1	0.09 ± 0.01^{c}	0.31 ± 0.04^{b}	0.39
T_2	0.05 ± 0.01^{bc}	0.22 ± 0.06^{b}	0.31
T_3	0.09 ± 0.03^{c}	0.29 ± 0.02^{b}	0.42
T_4	0.07 ± 0.05^{bc}	0.17 ± 0.16^{b}	0.86
T_5	0.09 ± 0.02^{c}	0.39 ± 0.18^{b}	0.33
T_6	0.07 ± 0.01^{bc}	0.23 ± 0.11^{b}	0.50
T_7	0.08 ± 0.00^{c}	0.34 ± 0.08^{b}	0.33
T_8	0.08 ± 0.04^{c}	0.34 ± 0.30^{b}	0.39
T_9	0.10 ± 0.03^{c}	0.45 ± 0.04^{ab}	0.28
T_{10}	0.14 ± 0.07^{a}	0.83 ± 0.16^{ac}	0.20
T_{11}	0.07 ± 0.09^{bc}	0.18 ± 0.23^{b}	0.58
T_{12}	0.19 ± 0.05^{a}	0.39 ± 0.08^{b}	0.95

Data is presented as mean ±SD for root and plant masses and as decimal form for root mass : shoot mass. [a] = mean difference between treatment and peat + water is significant at the 0.05 level. [b] = mean difference between treatment and fertilizer is significant at the 0.05 level. [c] = mean difference between treatment and no peat is significant at the 0.05 level. T_1 = LCS2-11 (*Stenotrophomonas maltophilia*); T_2 = ACM2-32 (*Pseudomonas stutzeri*); T_3 = FCM2-50 (*Enterobacter cloacae*); T_4 = ASM1-59 (*Bacillus subtilis*); T_5 = LSM1-61(*Bacillus amyloliquefaciens*); T_6 = ASM1-59: LCS2-11: ACM2-32; T_7 = ASM1-59: FCM2-50: ACM2-32; T_8 = LSM1-61: LCS2-11: ACM2-32; T_9 = LSM1-61: FCM2-50: ACM2-32; T_{10} = Fertilizer; T_{11} = peat + water; T_{12} = no peat.

applied at 200 kg/hectare and a treatment with no peat.

Application of treatments and planting sorghum seeds

Plant pots (15 cm diameter x 12 cm depth) containing 1.6 kg of unprocessed arenosol type soil collected from a field (17°53′57.90″ S; 20°14′04.39″ E) were used in this study. Using a sterile trowel, treatments were transferred from the Ziploc® bags and mixed with soil in the plant pots. *S. bicolor* seeds bought from Rundu Open Market were surface sterilized by soaking in 70% ethanol for 5 min, then in 1.5% sodium hypochlorite for 1 min and rinsed three times in sterile distilled water. The seeds were dried for 2 h in sterile conditions and planted into the pots containing treatments. There were two replicates for each treatment with one seed planted per pot. After 25 days, the dry mass was determined by drying plants in an oven (50°C) until the weight remained constant; the length and mass of shoots and roots were recorded.

Specifics for greenhouse pot experiments

The pot experiments were carried out at the University of Namibia Main campus' (Windhoek) greenhouse facility for 25 days. The plant pots were arranged in a randomized block manner with two blocks. The plants were watered every day with an average atmospheric pressure of 1006.923 hPa, an average maximum temperature of 34.1°C and an average 13 h 26 m 58 s daylight length per day for the duration of the pot experiments.

Statistical analysis

SPSS statistics (SPSS, version 22.0.0.0, 2013) was used to analyse the data. Analysis of variance (ANOVA)\Kruskal-Wallis one-way analysis of variance procedure was performed followed by post hoc Fisher's least significant difference (LSD). All analyses were tested at 5% level of significance.

RESULTS AND DISCUSSION

Three of the single inoculant treatments (T_1, T_3 and T_5) and three combination treatments (T_7, T_8 and T_9) had comparatively similar growth effects on sorghum root mass as the fertilizer treatment. Treatment T_9 was able to enhance sorghum plant growth significantly as compared to the water control. Apart from inoculants T_2 and T_4, the remaining peat based bacterial suspensions evoked a valuable impact on the development of *S. bicolor*.

PGP and biocontrol bacteria inoculation effects on sorghum

Three of the single inoculant treatments (T_1, T_3 and T_5) and three combination treatments (T_7, T_8 and T_9) had comparatively similar growth effects on sorghum root mass as the chemical fertilizer treatment. Treatment T_9 was able to enhance sorghum plant growth significantly as compared to the water control. Though two of the single inoculants T_2 and T_4 did not bring about any improved growth on the plants, it was determinable that the peat based bacterial suspensions elicited a beneficial

effect on the growth of *S. bicolor*.

The results showed that single bacterial suspension treatments consisting of *K. cloacae* FCM2-50 (p = 0.089), *S. maltophilia* LCS2-11 (p = 0.089) and *B. amyloliquefaciens* LSM1-61 (p = 0.122) enhanced root growth of *S. bicolor*. The combination bacterial treatments T_7 (*B. subtilis* ASM1-59: *K. cloacae* FCM2-50: *P. stutzeri* ACM2-32) and T_8 (*B. amyloliquefaciens* LSM1-61: *S. maltophilia* LCS2-11: *P. stutzeri* ACM2-32) also produced enhanced root growth on *S. bicolor*. Treatment T_9 (*B. amyloliquefaciens* LSM1-61: *K. cloacae* FCM2-50: *P. stutzeri* ACM2-32) enhanced both *S. bicolor* root growth (p = 0.196) and whole plant biomass (p = 0.032). Unsurprisingly, the difference in mean root dry mass between the fertilizer and the water control was statistically significant (p = 0.044). *K. cloacae* FCM2-50, *B. subtilis* ASM1-59 and *B. amyloliquefaciens* LSM1-61 are described as high producers of IAA, thus enabling root growth stimulation. Additionally, *Kosakonia* spp. are known to promote seedling root elongation via ACC deaminase activity (Li et al., 2000).

The water control and the no peat treatments were significantly different (p = 0.003) with regard to sorghum plant dry mass. Sorghum plants that grew in the no peat control treatment often had greater and at times statistically significant than most of the inoculant treatments. However, the average root-shoot ratio of 0.95 (0.58 for water control) for sorghum plants in the no peat treatment suggests that nitrogen availability was lower in the no peat treatment as compared to the inoculation treatments.

N_2-fixing bacteria play a critical role in the accumulation of plant biomass by providing an environment where the plant acquires nitrogen for assimilation (Pilbeam, 2010). The root-shoot ratio of the plant is also determined by nitrogen availability. The average root : shoot ratio of T_4 (0.86) and the no peat treatment (0.95) were greater than that of the water control treatment (T_{11} = 0.58). The rest of the treatments had a smaller average root : shoot ratio as compared to the water control. A nitrogen deficiency often causes the growth of an increased root fraction so that the root system is allowed to increase nutrient acquisition (Pilbeam, 2010). As compared to the water control, the lower root-shoot ratios in plants treated with inoculations suggests that there was more nitrogen available as a result of the bacterial treatments.

We can conclude from our data that bacterial treatments were able to enhance sorghum growth, comparable to that of the commercial fertilizer in terms of root biomass. *K. cloacae* FCM2-50: *B. amyloliquefaciens* LSM1-61: *P. stutzeri* ACM2-32 enhanced sorghum plant biomass. Enhancement of sorghum growth in terms of root biomass comparable to the level of commercial fertilizer was accomplished by single inoculants of *S. maltophilia* LCS2-11, *K. cloacae* FCM2-50, and *B. amyloliquefaciens* LSM1-61. Similarly, combination inoculants of *B. amyloliquefaciens* LSM1-61: *K. cloacae* FCM2-50: *P. stutzeri* ACM2-32, *B. amyloliquefaciens* LSM1-61: *S. maltophilia* LCS2-11: *P. stutzeri* ACM2-32 and *B. subtilis* ASM1-59: *K. cloacae* FCM2-50: *P. stutzeri* ACM2-32 promoted sorghum vegetative root growth.

Conclusion

From this study, it is concluded that PGP bacteria inoculants improve the growth of sorghum seedlings to level comparable to chemical fertilizers. These findings show the possibility of using bacterial inoculants as an inexpensive, effective and environmentally friendly alternative for increased agricultural crop productivity. An added advantage is that these PGP bacteria are ecologically adapted to the soils of this agro ecological zone as they were originally isolated from there. The eventual goal is to prove that the inoculants facilitate and improve plant growth and increase grain seed yield in sorghum. By developing inoculants consisting of native PGPR and bio-control bacteria, we improve our potential to alleviate challenges of heavily depending on importing fertilizers in countries that do not have chemical fertilizer manufacturing companies like Namibia or where subsistence farmers do not afford the price of the chemical fertilisers. The advancement of field trials at multiple locations is a necessary step towards assuring the accomplishments of effective bacterial inoculants. These inoculants offer a cheap preferential option to support current and future sorghum based industries.

Conflict of interests

The authors did not declare any conflict of interest.

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