Potential use of endophytic bacterial and fungi as bio fertilizer to promote plant growth in tissue culture banana

Ruth Murunde*, Irene Muriithi and Henry Wainwright
Real IPM Limited Company, kenya.

✉ Corresponding author*: ruth.murunde@realipm.com
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ABSTRACT

Banana is of special need to human society and are ranked as fourth most important food in the world after rice, maize. In Kenya, production of banana is constrained by among others declining soil fertility due to repeatedly application of fertilizers. Sustainable complementary response to declining soil fertility would be to increase the biological inputs of nutrients by exploitation of microorganisms. Endophytes which are mutualistic symbionts living symptomatically within plant tissues have been reported to have beneficial effects on plant growth, therefore the effect of three endophytic Bacterial isolate (Bacillus subtilis and Serratia nematodiphila), Fungal isolate (Trichoderma asperrellum), originating from Kenya were evaluated by inoculating on Grand Naine and William Hybrid banana cultivars under greenhouse condition at weaning stage and after two months for fifteen weeks. Plant responses to endophyte treatment was assessed on plant height, girth, number of functional leaves, fresh and dry roots weight. Improved growth of all parameters was observed for plants inoculated with endophytes when compared to the control. The study shows that the endophytes tested as growth promoters were found to have a significant effect in both cultivar plantlets. All treatments showed promising growth promoting properties. Isolate TR (Trichoderma asperrellum) induced the largest increases in plant height 129.2cm (Grand Naine) 114.0cm (William Hybrid) ) at 15th week, however all the treatment did not differ significantly in other growth parameter (Pseudo stem diameter, and total number of leaves ) at P= 0.05. The study shows that endophytes have potential to enhance growth of tissue-cultured banana plants.

Key Words: Musa spp. Serratia Nematodiphila, Trichoderma asperellum, Graid Naine and William Hybrid


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I. Introduction

Bananas are of special significance to human society being the fourth most important food in the world after maize, wheat and maize (Scot et al. 2006). Banana provides an essential food source for more than 400 million people throughout the developing countries of tropics and sub tropics (Frison and Sharrock, 2001). In Kenya, banana production area covers a about 63,290 ha with an estimated average yield of 19 tonnes per hectare as opposed to an average potential yield of 35-45 tonnes per hectare (FAOSTAT, 2011; HortiNews, 2013). Increased trade in local, regional and international markets has also made them an important cash crop, and in some cases, banana is a key source of income for rural populations (Frison and Sharrock, 2001). However, banana production in Kenya is constrained by among others, declining soil fertility (Vanlauwe and Giller, 2006; Okumu, 2008) and no fertilizer recommendation in place which is much required (Sultana et al. 2015). Soil fertility was reported to be a major problem in tissue cultured banana production in Central Kenya (Okumu, 2008), where yields depended more on soil fertility (67%) than either farm management (23%) or pests and diseases (10%). In vitro and ex-vitro establishment of tissue culture plants for growth and development is significant (Sultana et al. 2011; Siddique et al. 2006; Siddique et al. 2007; Siddique et al. 2007a). However, in this study, the suggested causes include insufficient application of livestock manure due to cost implications especially for the farmers without livestock, and limited use of inorganic fertilizers, which are expensive and therefore unaffordable for most banana farmers in Kenya. Farmyard manure and mulching are used to maintain or increase soil organic matter reserves in banana production. However, according to (Vanlauwe and Giller, 2006), organic inputs alone cannot sustain crop production in resource poor farming systems due to limitations in their quality and availability. A sustainable and alternative approach would be to exploit microorganisms as to increase the supply of nutrients, soil aggregate stability and increase soil fertility, which are largely untapped natural resources for plant growth promotion (Sultana et al. 2017; Siddique et al. 2017; Sultana et al. 2015; Thomas and Soly, 2009). Endophytes are increasingly gaining scientific and commercial interest because of their potential to improve plant quality and growth and their close association with internal tissues of host plant (Carroll, 1992; Schulz et al. 1999). Despite the fact that the interaction between entophytic bacteria and host plants has not been fully understood, it is well established that some of these interactions are beneficial to the plant (Rosenblueth and Martinez-Romero, 2006). Endophytic bacteria are reported to enhance plant growth in non-leguminous crops and improve their nutrition through nitrogen fixation, phosphate solubilisation production (Dobereiner and Baldani, 1998; Sturz et al. 2000; Sevilla et al. 2001; Hurek et al. 2002; Boddey et al. 2003; Iniguez et al. 2004; Ryan et al. 2008; Uribe et al. 2010). Besides bio-fertilization, endophytic bacteria (Bacillus spp) are also reported to promote plant growth and yield through production of Phytostimulators such as Phytohormones, the cofactor Pyrroquinoline quinone and the volatile acetoin; or by producing stress controllers like the enzyme 1-aminocyclopropane-1-carboxylate(ACC) deaminase, which facilitate plant growth and development by lowering plant ethylene levels; or indirectly through biological control of plant diseases or induced resistance response (Lugtenberg and Kamilova, 2009). In return, the plant protects endophytes and provides them with nutrients in form of photosynthates.

Application of Trichoderma spp has stimulated plant growth to crop seeds, seedlings and reduce the plant pathogen (Inbar et al. 1994; Rabeendran et al. 2000). Several authors have reported in crease in plant growth because of Trichoderma application on several crops and plant species, these include marigold, petunia and verbena (Ousley et al. 1994), sweet corn (Björkman et al. 1998), cabbage and lettuce (Rabeendran et al. 2000) and cucumber (Chang et al. 1986). Furthermore, several reports have also reported the use of Trichoderma spp. to control plant pathogens on a wide range of economically important crops (Lewis et al. 1996; Ahmed et al. 1999; Mathre et al. 1999). A favourable endophyte–host association in commercial cultivars would minimize the usage of agricultural inputs,
such as fertilizer and pesticides, thus saving costs and reducing pollutants to the environment. Therefore, there is need to isolate more microbial inoculants endophytes as alternative approach to fertilizer to improve nutrients uptake by plants to improve plant quality and growth of bananas. This study sought to determine the use of bacterial and fungi isolate as bio fertilizer to improve the plant growth of tissue culture bananas in Kenya.

II. Materials and Methods

The experiments were conducted in greenhouse at Real IPM Kenya; Banana plantlets were obtained from Jomo Kenyatta University of Agriculture and Technology commercial tissue culture laboratory. The Real IPM Company (K) Ltd that were previously isolated from Kenyan soils and identified by CABI microbial identification service, Egham UK, supplied the three isolates namely; *Bacillus subtilis*, *Trichoderma asperellum* and *Serratia nematodiphila* were isolates. For fungal isolates they were identified based on their morphological characteristic and subsequently identified by DNA sequencing (S.radu) whilst Bacterial isolates they were identified using BIOLOG Microlog Station system (Ver.4.2, modified from Magyarosy et al. 2002) that was repeated twice.

A split plot design was used to layout the experiment that was conducted in two phases namely weaning stage and potting stage, it comprised of two factors: endophyte and cultivar. The four endophytic isolates were three *Bacillus subtilis*, *Trichoderma asperellum*, *Bacillus subtilis + Trichoderma asperellum*, and *Serratia nematodiphila*. Two tissue culture banana cultivars William Hybrid (*Musa* spp. AAA) and Grand Naine (*Musa* spp. AAA) were used. The experiment was replicated three times with nine plants per replicate including control.

Inoculation of the plantlets was done at the hardening and subsequently at the potting phase. Hence plantlets (7-10 weeks) with 3-4 fully developed leaves in nutrients Agar (Murashige and skoog,1961) were removed from the culture tubes and washed toughly with tap water to remove the attached medium. The plants roots were inoculated with bacterial or fungal suspension by dipping the plant roots for one hour in the suspension, plants without bacterial or fungal were used as control. Plantlets, they were transferred to planting trays containing sterilized soil media that was sterilized at 80°C for 30 minutes. To minimize contamination each plantlet with different treatments was planted in a separate tray each replicated six times in each treatment and placed under greenhouse condition with 25°C ± 2°C, Misting was done regularly to minimize the humidity. After sixty days, the banana plants roots (8 to 10 cm height, 3 to 4 leaves) were dipped into bacterial or fungal suspension for one hour before planted individually into the potting pot, (2.5 liters) with a bottom plate, the planting media contained sand, soil and vermin-compost at ratio of 1:1:1.5 grams. Diammonium phosphates (DAP) fertilizer in each pot was also incorporated into the soil media since plant growth promoting microorganisms have previously been reported to often be effective under low-nutrient conditions and have little or no measurable effect on plant growth, when the plants are grown in nutrient-rich soil under optimal conditions and maintained in the greenhouse. Each plantlet was inoculated by soil drenching with 5ml/litre of Bacteria isolate *Bacillus subtilis* (5.37×10⁸ cfu/ml) *Serratia nematodiphila* (4.4×10⁷ cfu/ml) and fungal *Trichoderma asperellum* (5.86×10⁸ cfu/ml) endophytes on weekly bases for nine weeks. Watering were done when necessary.

Increase in four-growth parameters plant height, stem diameter, root fresh /dry weight and total number of leaves plant were recorded at fourteen-day intervals. Height was measured from the base of the corm up to the angle where the ‘leaf breaks’. The diameter of the stem diameter was measured using cross sections of the pseudo stem at distances half the height of the plant. Root mass was
quantified by weighing the total primary and secondary roots (air-dried) of each plant. All ‘opened’ leaves were calculated for total number of leaves.

Statistical Analysis: Data was subjected to Analysis of Variance with General Linear Model (GLM) procedures using Genstat software (Payne et al. 2011) and means separated by LSD Tukey at P≤0.05.

III. Results and Discussion

Interaction between banana cultivars and treatments was not significant (P>0.05) for any growth parameter therefore whenever variance homogeneity was assumed data for the two varieties were pooled.

Plant Growth Parameters:

Effect of endophyte isolate in plant height
There was a gradual increase of plant height after treating plants with endophytic isolate at weaning stage (6th weeks) and potting stage (15th Weeks) in both bananas varieties (Grand Naine and William Hybrid) (Figure 01A, 01B). There was no significant different in plant height at 15th week among the treatment with (P<0.015). When all the treatments were compared with the control, plants inoculated with endophytic isolates Trichoderma asperellum differed significantly in plant height at P<0.351 with Bacillus subtilis, Trichoderma +Bacillus subtilis and Serratia nematodhiphila in both varieties, (Figure 01A, 01B). Trichoderma asperellum led to plants grew taller than those treated with Bacillus subtilis, Trichoderma + Bacillus subtilis and Serratia nematodhiphila. The bacteria endophyte Bacillus subtilis demonstrated the potential as an efficient plant growth.

Effect of endophyte on Stem diameter
Inoculation of plants with endophytic isolates Bacillus subtilis, Trichoderma asperellum, Trichoderma + Bacillus subtilis and Serratia nematodhiphila led to an increase in plant stem diameter at 6th week to 15th week, however, when all the treatment they were compared to control plants they did not differ significantly at P<0.05 in Grand Naine and William hybrid varieties (Figure 02A, 02B). Trichoderma asperellum had the highest stem diameter although statistically the plants did not differ from other treatments at (P<0.002).

Effect of endophyte on the number of leaves
There was an increase in number of plants leaves after inoculating the plants with endophytic isolates in Grain Naine and William Hybrid varieties at weaning stage (6th week) and at potting stage (15th week) (Figure 03A, 03B). When all the treatments were compared to control, the increase number of functional leaves did not differ significantly(P<0.034) at 15th week, however, Trichoderma asperellum had more leaves compared to control plants in both varieties (Grand Naine, William hybrid) (Figure 03A,03B).

Effect of endophyte Plants fresh and dry shoot weight
There was no significant different in fresh plant in all the treatment when they were compared to control. When all the tested endophytic were compared to control, there was a significant increase in shoot weight (Figure 04B). There was a significant different between Serratia nematodiphila and Trichoderma asperellum and Trichoderma asperellum + Bacillus subtilis differed significantly in fresh plant weight with other treatments. In Grand Naine variety, results to had the highest fresh weight when plants were inoculated with Serratia nematodiphila (11.5g) Trichoderma asperellum (10.3g) and Trichoderma asperellum + Bacillus subtilis (10.2g) did not differ significantly in increase shoot fresh weight (P=0.041) (Figure 04A, 04B). Inoculating plants with isolate Serratia nematodiphila
resulted to a significant increase in dry shoot weight (g) in Grand Naine compared to William hybrid, with values 5.5g, 1.6g respectively (Figure 04B). Root fresh weight of plants inoculated with endophytic isolates Bacillus subtilis, Trichoderma asperellum, Trichoderma asperellum + Bacillus subtilis, Serratia nematodiphila was relatively higher than of the control, although not significant (P=0.0125) from each other (Figure 05A) in both banana varieties however Serratia nematodiphila resulted to have low fresh root weight (135.4g) as compared to other tested endophytic (Figure 05A). Root dry weight of plants treated with the endophytic isolates Serratia nematodiphila was relatively lower than of the control although not significant from Bacillus subtilis in William Hybrid (P=0.034), however, all the isolates caused an increase of dry plant weight in Grand Naine varieties although not significant from each other when were compared to control (Figure 05B), promoter however it did not differ significantly at P<0.005.

This study reports, the potential of indigenous endophytes species and their effect on growth of tissue culture bananas in Kenya. Most endophytes form either beneficial, detrimental association with their host plant and they have been reported to be beneficial to plant growth (Clay and Scharl, 2002). The present study examined the effect of endophytic on growth of tissue culture banana. The endophytes tested (Bacillus subtilis, Trichoderma asperellum and Serratia nematodiphila formed a beneficial association with banana plantlets at weaning stage (6th week) and at Potting stage (15th week). As the growth of the inoculated plantlets was slightly better than that of plantlets without endophytes (control). However there was differences in growth between endophytes inoculated plants and control in both bananas varieties, an increase in plant growth was observed for the plants inoculated with the three endophytic isolates, at 6th week, and 16th week after Inoculation, Trichoderma asperellum resulted to significantly higher plant height in Grand Naine variety as compared to William Hybrid at 15th week, similar results were reported by (Niere, 2001), who reported enhanced growth in 22 week old banana cultivars with F. oxysporum endophytic isolate. Other study that were done by (Ting et al. 2008), reported a positive effect of endophytes on increase in growth of banana who reported an increase in height, pseudo stem diameter, and number of leaves in plants inoculated with F.oxysporum endophytes. Bio-priming of vegetables seeds (Kales, Carrots and Onions) with Trichoderma spp. and Bacillus spp. that demonstrated a beneficial increase in percentage of seed germination and growth was also reported by (Ruth and Henry, 2018). Trichoderma gamsii has also been reported as endophytic in control of Fusarium wilt in banana. Reintroducing of naturally occurring endophytes to tissue culture bananas plantlets resulted in a substantial reduction in the infection and severity of Fusarium wilt diseases (67%) as well as increased plant growth parameters (height, girth, leaf area) (Lian et al. 2009).

Ting et al. (2008) also demonstrated that endophytes (Serratia and Fusarium oxysporm) isolated from wild bananas can promote the growth of banana plantlets and render tolerance towards Fusarium wilt. Earlier work by Zuraida et al. (2000) also showed that treatment of banana plantlets with Agrobacteria and Azospirillum resulted in 79% and 11% increase in soluble nitrogen, respectively, as compared to the control (Ngamau et al. 2012) also demonstrated capacity of endophytic bacteria associated with bananas in Kenya to fix free nitrogen having grown on nitrogen-source free medium and showed varied nitrogenase activity.

Bacillus spp. also has been widely been used for many years in extensive research in an attempt to increase plant growth and suppress the activities of soilborne plant pathogens (Turner and Backman, 1991; Holl and Chanway, 1992; Gutierrez Mañero et al. 1996; Kim et al. 1997a; Probanza et al. 1996) in an experiment held that two B. pumilus and one strain of B. licheniformis showed significantly (P < 0.05) increased growth of European alder [Alnus glutinosa (L.) Gaertn.]. They reported that the Bacillus strains used increased the aerial surface and length of European alder by 163 and 182
percent respectively compared to the untreated controls also reported that strains of *B. subtilis* and *B. pumilus* were able to increase germination speed and dry biomass of loblolly pine (*Pinus taeda L.*) and slash pine (*Pinus elliottii L.*) seedlings. According to (Shishido et al. 1995), two strains of *B. polymyxa* inoculated onto lodgepole pine seeds under greenhouse conditions increased seedling length, shoot and dry biomass by 18, 24 and 27 percent respectively, compared to inoculated control. Turner and Backman (1991) reported an increase of 17 percent in peanut yield after seeds were treated with *B. subtilis* and grown under field conditions.

![Figure 01A, 01B. Increase mean number (±) of plant height in Graid Naine and William Hybrid banana varieties inoculated with endopytic isolates (♦ = Control - Untreatment, ▲ = BS- Bacillus subtilis, ▲ = TR-Trichoderma asperellum, ×=TR+BS-Trichoderma asperellum +Bacillus subtilis, *SN-Serratia nematodiphila* respectively. When assessed at weaning stage (6th week) and at potting stage (15th week). Vertical lines indicate standard error.](image-url)

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Figure 02A, 02B. Mean number (±) of stem diameter(cm)(girth) Graid Naine and William Hybrid banana varieties inoculated with endopytic isolates ♦ =Control –Untreatment, ■ = BS- Bacillus subtilis, ▲ =TR-Trichoderma asperellum, ×=TR+BS-Trichoderma asperellum +Bacillus subtilis, *SN-Serratia nematodhiphila respectively. When assessed at weaning stage (6th week) and at potting stage (15th week). Vertical lines indicate standard error.

Figure 03A, 03B. Mean number (±) of plant leaves Graid Naine and William Hybrid banana varieties inoculated with endopytic isolates ♦ =Control –Untreatment, ■ = BS- Bacillus subtilis, ▲ =TR-Trichoderma asperellum, ×=TR+BS-Trichoderma asperellum +Bacillus subtilis, *SN-Serratia nematodhiphila respectively. When assessed at weaning stage (6th week) and at potting stage (15th weeks). Vertical lines indicate error bar.
Figure 04A, 04B. mean number of fresh and dry plants shoots weight of tissue culture bananas, 15th weeks (potting stage) after inoculation with endophytic isolates, □=Control – Untreatment, ■= BS- Bacillus subtilis, □= TR-Trichoderma asperellum, □= BS+TR-Trichoderma asperellum + Bacillus subtilis, □= SN-Serratia nematodhiphila respectively. As compared to positive control. □ =Grand Naine □ = William Hybrid. Values with the same letter in the same vertical bar and variety are not significantly different as determined by Tukey’s studentized range test.
Figure 05A, 05B. Mean number of fresh and dry plant roots weight of tissue culture bananas, 15th weeks (potting stage) after inoculation with endophytic isolates, ■=Control – Untreatment, ■= BS- Bacillus subtilis, ■=TR-Trichoderma asperellum ■=BS+TR-Trichoderma asperellum +Bacillus subtilis, ■=SN-Serratia nematodiphila respectively. As compared to positive control. ■=Grand Naine ■= William Hybrid. Values with the same letter in the same vertical bar and variety are not significantly different as determined by Tukey’s studentized range test.

IV. Conclusion

From the study, the results here demonstrate that endophytes isolated locally in Kenya can promote the growth of tissue culture bananas plantlets, however application of endophytes is strongly recommended of growth promotion at weaning stage and potting stage as shown in this study in order to allow the establishment of endophytes early. Similar Recommendation by Ting et al. (2008) of application of endophytes at the nursery stage on tissue cultured clones to allow establishment of microbes prior to transplanting to the field as it can promote plant growth. The potential of endophytic bacteria in sustainable agriculture is immense. However, their biology and ecology are far from being fully understood and require further studies.

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