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Response of micropropagated tissue culture banana *(Musa spp.)* to acclimatization using arbuscular mycorrhiza fungi

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ABSTRACT

The production of banana seedlings through tissue culture provides a reliable source of numerous, disease free, quality plantlets. However, the micropropagation process is faced with a number of challenges. In this study we are focusing on the improvement of survival and growth of the resultant plantlets during the acclimatization process. The viability of arbuscular mycorrhiza fungi (AMF) symbiont in the acclimatization process was assessed. Tissue culture bananas (Kienyeji, Kiganda and Ng'ombe local cultivars) were inoculated with single species AMF *Rhizophagus irregularis* and *Funneliformis mosseae* as well as commercial AMF Rhizatech. The survival rate was assessed and plant growth evaluated at a two week interval. Destructive harvesting was conducted followed by assessment of root, shoot and total biomass. The results demonstrated that Kiganda treated with *Rhisophagus irregularis* and *Funneliformis mossae* recorded better survival rates compared to treatments with NPK fertilizer and commercial AMF (Rhizatech). Kienyeji cultivar however had a better survival rate when treated with commercial AMF (Rhizatech). Ng'ombe cultivar was very resilient having 100 % survival rate when treated with indigenous AMF, commercial AMF (Rhizatech) and NPK. There were significant differences in the shoot dry weight, root dry weight and biomass in the three banana cultivars. Kiganda and Kienyeji cultivars had the highest biomass when treated with *Funneliformis mossae* of 0.52 g and 0.83 g respectively. The results in this study confirm that the use of AMF in the acclimatization process improves the overall seedling output of the micropropagation process during the nursery stage of production.

Key words: Mycorrhiza, acclimatization, micropropagation, banana seedlings

INTRODUCTION

Banana belongs to the *family Musaceae* and is a popular fruit worldwide. Most popular commercial propagation strategy is through *in-vitro* micropropagation (Sinha *et al.*, 2018). This ensures the production of clean seedlings within a short duration. The development of a successful protocol for *in-vitro* micropropagation of bananas requires that it is able to produce numerous viable, pest and disease free plantlets that are genetically uniform to the mother plant (Elyazid *et al.*, 2021).

The acclimatization phase, which occurs after the *in vitro* regeneration of bananas, is one of the most important steps in the physiological development and adaptation of banana plantlets. Despite the micro-propagated plantlets being disease free at this stage, they still lack the essential AMF that would enhance the plant growth and vigor (Gaidashova *et al.*, 2010), hence the need for inoculation. Successful AMF inoculation during acclimatization phase has been demonstrated (Declerck *et al.*, 2002; Ortas *et al.*, 2017) and it is suggested that the symbiosis between the banana root system and the AMF is useful leading to the rigorous development of micro-propagated banana plantlets. Jaizme-Vega *et al.* (1997) associates

the benefits of AMF-banana plant root symbiosis to increased photosynthetic rates, enhanced water and nutrient absorption and increased stress tolerance. Various strains of AMF have been reported to interact differently with specific banana cultivars hence the need to screen for specific AMF strains that work best with particular banana cultivars (Jefwa *et al.*, 2012). The current study aimed at determining the response of tissue culture micropropagated banana plantlets to acclimatization using arbuscular mycorrhiza fungi (AMF).

MATERIALS AND METHODS

Source of tissue culture banana plantlets

Tissue cultured banana plantlets of local cultivars Kienyeji, Kiganda and Ng'ombe were obtained through low-cost tissue culture micro-propagation at the plant tissue culture laboratory, , Kenyatta University. Initial culture establishment, shoot multiplication and rooting was carried out for a period of 120 days.

Acclimatization efficacy of Arbuscular Mycorrhiza Fungi (AMF)

The study assessed the effectiveness of Commercial AMF (Rhizatech), *Rhizophagus irregularis* and *Funneliformis mosseae* in establishment and development of banana plantlets produced by low cost tissue culture technology during the acclimatization phase. Spores were bulked and multiplied using sterile soil and sand (1:1) and Bermuda grass. The plantlets were transferred to planting pots containing sterile soil and vermiculite (ratio 1:1), Different treatments were administered including different AMF species applied at rate of 5 g per pot which contains approximately 100 spores (Table 1).

Experimental design

The experiment was laid out in a RCBD with five replications for all treatment and was maintained at field conditions with constant monitoring of Relative humidity and temperatures for a period of 70 days (Fig. 1). Three mycorrhizal innoculants, *F. mosseae* (BEG 12), *R. irregularis* (BEG 44) donated by the International Bank of the Glomeromycota INRA, France and commercial AMF (Rhizatech) (*G. mosseae*, *G. Inraradices*, *G. Etunicatum* and *G. aggregatum*) from Dudutech were used. The treatments comprised of non-inoculated, negative control (No treatment), positive control (NPK) or inoculation with *R. irregularis, F. mosseae or* Rhizatech. Observable features that were recorded during hardening stages included plant height, leaf number, total dry weight, shoot dry weight, root dry weight and survival rates.

Data Analysis

Banana growth parameters and response of micropropagated banana plantlets to AMF inoculation were analyzed using Analysis of Variance (ANOVA) with Statistical Analysis computer Software version 9.0. The data on response of micropropagated banana plantlets to AMF inoculation was analyzed using Analysis of variance (ANOVA). Means were separated using Tukey's Honest Significant Difference at $p < 0.05$.

RESULTS

I*n-vitro* low-cost micropropagated banana plantlets survival response to acclimatization using AMF

In vitro regenerated Kiganda banana cultivars plantlets treated with *R. irregularis* and *F. mossae* had better survival rates compared to those treated with NPK fertilizer and commercial AMF (Rhizatech). Kienyeji cultivar however had a better survival rate when treated with commercial AMF. Ng'ombe cultivar was very resilient having 100 % survival rate when treated with indigenous as well as commercial AMF (Rhizatech) and NPK (Fig. 2).

Effect of AMF treatment on growth of low cost micro-propagated Kiganda, Kienyeji and Ng'ombe banana cultivars

There was a significant difference in the number of leaves determined over a 10-week duration in the different treatments ($p \leq 0.05$). Kiganda cultivar had the highest mean number of leaves (7.00) after 10 weeks of treatment when inoculated with *R. irregularis* and the control had the lowest mean (2.30). Kenyeji had no significant difference ($P = 0.061$) in the number of leaves between the different treatments. Ng'ombe cultivar miropropagated plantlets had the highest mean number of leaves when they were inoculated with *F. mossae* (Table 2).

The plant height determined over 10-week duration in the different treatments varied with Kiganda cultivar having the highest increase in height with *R. irregularis* (6.15 cm) while the lowest height increase was recorded in the control (1.72 cm). Kienyeji had the highest increase in height when treated with *F. mossae* (4.13 cm) and lowest in the control at 1.23 cm (Table 3).

The different treatments showed significant difference in plant height recorded at two week intervals with the highest mean height recorded in Kiganda cultivar micropropagated plantlets treated with *R. irregularis* and lowest mean plant height in the control and those treated with NPK. Similar results were recorded in Kienyeji cultivar. However, in Ng'ombe cultivar treated with NPK had the highest mean plant height followed by those treated with commercial AMF (Rhizatech).

Effect of AMF treatment on biomass of micro-propagated Kiganda, Kienyeji and Ng'ombe banana cultivar plantlets

Micropropagated banana plantlets acclimatized using AMF and used to determine fresh and dry biomass are shown in Fig, 3. Biomass determined by drying the fresh micropropagated banana plantlets showed significant differences (P = 0.018) between Kiganda, Kienyeji and Ng'ombe cultivars (Table 4).

There was a significant effect of both banana cultivar (p =0.02) and treatment (p =0.035) on shoot dry weight. The interaction between cultivar and treatment was also statistically significant (p = 0.021). Specifically, Kienyeji cultivar had the highest biomass when treated with *F. mossae* compared to the rest of the treatments.

Based on the root dry weights results, there was a significant effect of both banana cultivar (p = 0.01) and treatment (p = 0.015). Additionally the interaction between the banana cultivar and treatment was significant (p = 0.019). The results demonstrated a significant effect of both banana cultivar (p < 0.001) and treatment (p = 0.018) on total biomass. The interaction between the banana cultivar (p < 0.001) and treatment total biomass was statistically significant (p = 0.021) (Table 4).

Shoot dry weight was greater compared to root dry weight for Kiganda cultivar in *R. irregularis,* commercial AMF (Rhizatech) and *F. mossae* treatments, however for Kienyeji cultivar treated with *R. irregularis*) and commercial AMF (Rhizatech) had a greater root total biomass compared to the shoot total biomass.

DISCUSSION

In banana farming, there has been increased demand for planting materials that cannot be sufficiently supplied through traditional vegetative propagation (Ferdous *et al.*, 2015; Muthee *et al.*, 2019). Micropropagation involves the production of plantlets in a controlled microenvironment resulting in numerous uniform disease-free plantlets. Therefore *in vitro* micropropagation is a significant tool in the improvement of banana production by producing sufficient plantlets within a short duration (Ferdous *et al.*, 2015). There are several limitations that are associated with micropropagation which include the expensive nature of the process, poor acclimatization of the resultant plantlets as well as the risk of changes in the genetic make-up of the resultant plantlets (Gupta *et al.*, 2020).

Due to the delicate nature of micropropagated banana plantlets, efficient acclimatization strategies are essential in improving survival rate and growth of the plantlets. There has been a significant improvement in growth and development of banana plantlets by the use of exotic AMF species (Jefwa *et al.*, 2012). The current study aimed at assessing the effect of indigenous AMF species in the survival rate and growth of low-cost micropropagated banana plantlets. Microorganisms that form symbiotic relationships with plants play an important role in sustainable agricultural practices because they improve the plant performance under biotic and abiotic stress conditions (Smith and Read, 1997). Therefore, the use of these microorganisms offers an environmentally beneficial alternative that reduces the use of chemicals in control of pests and diseases (Hajek & Eilenberg, 2018). Mutualistic root endophytic fungi tend to induce systemic resistance to pathogens and pests in the host plant. They increase tolerance of crops to biotic and abiotic factors (Sommermann *et al.*, 2018).

The plantlets that were treated with *R. irregularis* and *F. mossae* and commercial AMF bioinnoculant (Rhizatech) showed the highest survival rates. It has been reported that AMF improves water and nutrient uptake, growth as well as increase in plant tolerance to biotic and abiotic stressors (Chandrasekaran *et al.*, 2014). Additionally, it has also been reported that AMF has an involvement in host plant physiology thus play a role in influencing plant response to stressors (Plouznikoff *et al.*, 2016). Plantlets treated with the 2 AMF showed greater growth performance. Similarly, Jefwa *et al.* (2012) and Simó-González *et al.* (2019) reported an increase in plant height, in plant s treated with AMF cultures. However, *Musa* spp. has been associated with several AMF species and research has indicated that it is highly dependent on AMF (Srivastava *et al.,* 2014).

The highest increase in biomass was recorded in Ng'ombe cultivar treated with *R. irregularis* while for Kienyeji cultivar the highest increase in biomass was recorded in plantlets treated with *F.mossae*. Oye-Anda *et al.* (2020) reported that banana plants colonized by AMF had a notable increase in biomass and this has been attributed to the ability of AMF mycelium to enhance uptake of high amounts of nutrients. AMF provides plants with minerals and in turn receive products of photosynthesis such as sugars (Keymer *et al.*, 2017).

It has been established that AMF directly affects K uptake especially in soils with minimal available P and has been reported to reduce fertilizer requirement by 25 % (Berruti *et al.*, 2016). Dodd and Ruiz-Lozano (2012) reported that soil parameters have a direct effect on AMF activities in the soil. According to Bukovská *et al.* (2018),

use of manure improves the proliferation of hyphae thus improving mycorrhizal root growth and nutrient uptake by the bananas.

AMF has been reported to reduce occurrence as well as the severity of pathogens found above and belowground (Li *et al.*, 2013). Similarly, Koffi *et al.* (2013) reported that AMF played a significant role in the decrease of incidences of nematodes. William *et al.*, (2017) reported that in some cases, high nutrients may reduce AMF colonization but still lead to improved plant performance, while in others, the opposite effect may occur.

CONCLUSION

The results of the study showed that the use of AMF in the nursery stage of micropropagated banana seedlings resulted in increased growth of the plants as well as biomass accumulation. This offers a viable strategy to ensure the survival and vigor of the seedlings. However, the specificity of this symbiotic interaction requires further screening to select suitable host and treatment combination to ensure optimal conditions for the host plant.

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Conflict of Interest

The authors declare that they have no conflict of interest.

TABLES

Table 1: Treatments used to determine AMF efficacy in improving plantlets survival rate and plant development

Treatment	Composition
Treatment 1	Soil + Vermicompost + *Rhizophagus irregularis*
Treatment 2	Soil + Vermicompost + Commercial AMF (Rhizatech)
Treatment 3	Soil + Vermicompost + *Funneliformis mosseae*
Treatment 4	No treatment (control)
Treatment 5	NPK fertilizer (17:17:17)

AMF, Arbuscular mycorrhizal Fungi; N, Nitrogen; P, Phosphorus; K, Potassium

Table 2: Mean number of leaves of AMF treated low cost tissue micro-propagated Kiganda, Kienyeji and Ng'ombe banana cultivars plantlets recorded in 10 week duration

		No. of leaves over a 10 week duration				
Cultivar	Treatment	Week 2 Mean± SE	Week 4 Mean± SE	Week 6 Mean± SE	Week 8 Mean± SE	Week 10 Mean± SE
Kiganda	*Rhisophagus irregularis*	3.50±0.29^{a}	4.20±0.48^{a}	5.30±0.58^{a}	5.80±0.25^{a}	7.00±0.41^{a}
	Rhizatech	3.50±0.50^{a}	4.00±0.75^{a}	5.00±0.65^{a}	3.50±1.32^{b}	5.25±1.80^{b}
	Funneliformis mossae	2.75±0.48^{a}	3.50±0.65^{a}	4.50±0.78^{a}	5.00±0.41^{a}	6.50±0.29^{ab}
	No treatment	2.25±0.25^{a}	2.50±1.18^{a}	3.50±1.15^{a}	4.30±1.18^{a}	2.30±1.31^{c}
	NPK	3.00±0.58^{a}	4.30±1.50^{a}	3.80±2.32^{a}	5.00±1.44^{a}	2.80±1.60^{c}
Kienyeji	*Rhisophagus irregularis*	3.00±0.41^{a}	2.00±0.71^{a}	2.30±0.75^{a}	3.00±1.08^{a}	3.00±1.00^{a}
	Rhizatech	3.00±0.41^{a}	3.00±0.71^{a}	3.50±0.29^{a}	3.50±0.29^{a}	4.00±0.00^{a}
	Funneliformis mossae	3.50±0.29^{a}	2.50±0.29^{a}	2.50±0.87^{a}	3.00±1.00^{a}	3.25±1.11^{a}
	No treatment	2.75±0.25^{a}	4.00±0.96^{a}	2.50±0.96^{a}	1.75±1.03^{a}	2.00±1.22^{a}
	NPK	3.50±0.29^{a}	1.80±1.03^{a}	2.00±1.15^{a}	2.00±1.22^{a}	2.00±1.22^{a}
Ng'ombe	*Rhisophagus irregularis*	3.00±0.00^{a}	4.25±0.25^{a}	4.25±0.25^{a}	4.50±0.29^{a}	4.50±0.29^{ab}
	Rhizatech	3.25±0.48^{a}	4.00±0.41^{a}	3.75±0.25^{a}	4.50±0.29^{a}	4.25±0.25^{ab}
	Funneliformis mossae	3.25±0.25^{a}	3.75±0.25^{a}	4.75±0.25^{a}	5.25±0.25^{a}	5.00±1.00^{a}
	No treatment	3.75±0.25^{a}	3.50±0.50^{a}	4.00±0.41^{a}	4.00±0.41^{a}	3.75±0.25^{b}
	NPK	3.50±0.29^{a}	3.75±0.25^{a}	3.75±0.25^{a}	4.25±0.25^{a}	4.00±0.00^{b}

Values are expressed as mean ± standard error of the mean. Means having the same letters within columns are not significantly different according to Tukey's HSD at 5% level. NPK, Nitrogen, Phosphorous and Potassium.

Table 3: Plant height of AMF treated low cost micro-propagated Kiganda, Kienyeji and Ng'ombe cultivars plantlets recorded in 10-week duration

Cultivar	Treatment	Plant height over 10 week duration				
		Week 2 Mean± SE	Week 4 Mean± SE	Week 6 Mean± SE	Week 8 Mean± SE	Week 10 Mean± SE
Kiganda	*Rhisophagus irregularis*	4.23±0.38[a]	5.50±0.25[a]	6.28±0.25[a]	9.48±0.83[a]	10.38±0.97[a]
	Rhizatech	5.25±0.56[a]	7.38±0.41[a]	6.78±1.01[a]	6.85±2.36[a]	7.95±2.65[a]
	Funneliformis mossae	6.30±1.11[a]	7.92±0.65[a]	7.10±1.63[a]	8.32±1.02[a]	11.13±1.26[a]
	No treatment	3.78±0.38[a]	4.65±1.65[a]	3.90±2.34[a]	4.67±2.72[a]	5.50±3.85[b]
	NPK	3.30±0.38[a]	4.72±0.65[a]	4.40±0.52[a]	5.00±1.33[a]	6.65±2.08[a]
Kienyeji	*Rhisophagus irregularis*	6.08±0.37[a]	6.75±1.98[a]	7.00±1.94[a]	7.40±2.01[a]	7.65±2.35[a]
	Rhizatech	6.23±0.39[a]	6.95±0.57[a]	8.00±0.59[a]	8.45±0.61[a]	8.88±0.59[a]
	Funneliformis mossae	6.00±0.35[ab]	7.17±1.74[a]	9.00±0.79[a]	9.50±2.23[a]	10.13±2.29[a]
	No treatment	4.67±0.24[b]	4.72±1.89[b]	5.50±1.30[b]	5.72±1.92[b]	5.90±1.97[b]
	NPK	5.20±0.16[b]	5.55±2.73[a]	8.05±2.38[a]	8.25±2.86[a]	9.50±2.88[a]
Ng'ombe	*Rhisophagus irregularis*	6.05±0.46[a]	7.50±0.79[ab]	9.00±0.31	9.30±0.36	9.53±0.41[a]
	Rhizatech	4.78±0.20[a]	5.93±0.37[b]	8.91±0.77	9.28±0.77	9.45±0.72[a]
	Funneliformis mossae	4.95±0.40[a]	7.42±0.32[ab]	8.70±0.14	8.98±0.16	8.35±0.26[a]
	No treatment	5.15±0.26[a]	7.15±0.14[ab]	8.40±0.26	8.70±0.28	8.83±0.28[a]
	NPK	5.73±0.39[a]	8.25±0.09[a]	9.38±0.19	9.65±0.19	9.80±0.21[a]

Values are expressed as mean ± standard error of the mean. Means having the same letters within columns are not significantly different according to Tukey's HSD at 5% level. NPK, Nitrogen, Phosphorous and Potassium

Table 4: Response and symbiotic efficiency of micro-propagated Kiganda, Kienyeji and Ng'ombe cultivar plantlets treated with AMF

Variable	Shoot dry weight (g)	Root dry weight (g)	Total Biomass (g)
Banana Cultivar			
Kiganda	0.21±0.07[b]	0.09±0.02[b]	0.30±0.01[b]
Kienyeji	0.38±01[a]	0.11±0.01[a]	0.49±0.03[ab]
Ng'ombe	0.44±0.47[a]	0.14±0.01[a]	0.58±0.02[a]
Treatment			
Rhisophagus irregularis	0.34±0.01[a]	0.12±0.03[a]	0.46±0.05[ab]
Rhizatech	0.31±0.01[a]	0.11±0.01[a]	0.32±0.01[b]
Funneliformis mossae	0.43±0.02[a]	0.15±0.01[a]	0.58±0.07[a]
No treatment	0.26±0.13[b]	0.05±0.11[b]	0.31±0.01[b]
NPK	0.35±0.05[a]	0.12±0.01[a]	0.47±0.01[ab]
P value			
Cultivar	0.02	0.01	<0.001
Treatment	0.035	0.015	0.018
Cultivar*Treatment	0.021	0.019	0.021

Values are expressed as mean ± standard error of the mean. Means having the same letters within columns are not significantly different according to Tukey's HSD at 5% level. NPK, Nitrogen, Phosphorous and Potassium.

FIGURES

Figure 1: Acclimatization of micropropagated banana plantlets using AMF. A, Primary acclimatization; B, Secondary acclimatization; C, Growth monitoring; D; Fully developed banana seedlings.

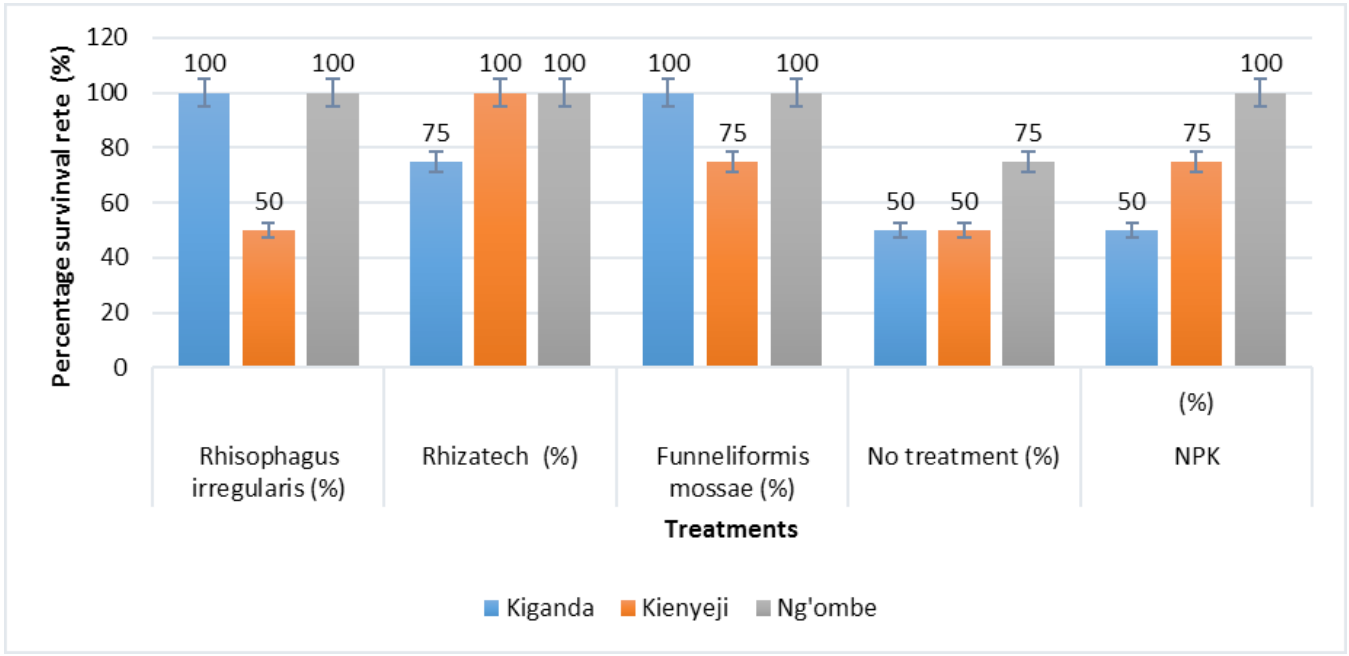

Figure 2: Survival rate of AMF treated low-cost micro-propagated Kiganda, Kienyeji and Ng'ombe banana cultivars plantlets. NPK, Nitrogen, Phosphorous and Potassium.

Figure 3: Micropropagated banana plantlets acclimatized using AMF. A, Fresh Kiganda cultivar plantlets; B, Dry Kiganda cultivar banana plantlet.

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