Journal of Scientific and Innovative Research 2021; 10(1): 16-22
Available online at: www.jsirjournal.com

Research Article

ISSN 2320-4818
JSIR 2021; 1(1): 16-22

Received: 22-02-2021
Accepted: 19-03-2021

YinYin Mon
Plant Tissue Culture Laboratory, Biotechnology Research Department, Myanmar

Kay Thi Oo
Plant Tissue Culture Laboratory, Biotechnology Research Department, Myanmar

Kyaw Kyaw Linn
Plant Tissue Culture Laboratory, Biotechnology Research Department, Myanmar

Zin Mar Lynn
Plant Tissue Culture Laboratory, Biotechnology Research Department, Myanmar

Nyo Zin Hlaing
Plant Tissue Culture Laboratory, Biotechnology Research Department, Myanmar

Nay Chi Ko Ko
Plant Tissue Culture Laboratory, Biotechnology Research Department, Myanmar

Correspondence:
YinYin Mon
Plant Tissue Culture Laboratory, Biotechnology Research Department, Myanmar
Email:
yinyinmon88.ym@gmail.com

Evaluation of Plant Growth Parameters by *In Vitro* and *Ex Vitro* Inoculation of Micropropagated Banana Plantlets with Rhizospheric and Endophytic Bacterial Inoculum

Yin Yin Mon[*], *Kay Thi Oo, Kyaw Kyaw Linn, Zin Mar Lynn, Nyo Zin Hlaing, Nay Chi Ko Ko*

Abstract

Banana propagation is dependent on propagation by tissue culture for industrial purposes. Due to lack of native endophytes in tissue culture plantlets, reintroduction of beneficial microorganisms to tissue culture plantlets become popular as a useful foundation for improving the level of establishment, increasing plant growth parameters, protecting the plantlets against pest and diseases and overall performance for field plantation. In the present study, rhizospheric and endophytic four bacterial species (Pseudomonas fluorescens, Bacillus putida, Bacillus pumilus, Bacillus amyloliquefaciens were isolated from different parts of healthy banana plants such as root, leaf and pseudostem. Banana tissue culture plantlets were cultured for root formation stage on MS medium and used as host for artificial inoculation. Then, bacterial inoculation was carried out with tissue culture plantlets both in vitro and ex vitro conditions. All banana plantlets used for this experiment were successfully acclimatized and survived in the greenhouse. The experiment consisted of three treatments: (1) bacterial inoculum with in vitro rooted plantlets for 2 weeks (2) bacterial inoculum with ex vitro (hardening) plantlets (3) control with sterilized distilled water. Among these treatments, in vitro inoculation affected significantly increased plant height, number of leaves and plant girth for all data collection times with the highest increment indices: (24.0, 7.0, and 7.3). Ex vitro inoculation showed the second highest increment indices: (22.0, 6.9 and 6.3) for plant height, number of leaves and plant girth, respectively. The lowest number of plant growth parameters were observed in control treatment for all four data collection time (19.0, 6.2, and 6.0). According to the results, application of mixtures of bacterial inoculum was effective for enhanced plant growth parameters of tissue culture banana plantlets under greenhouse condition. In addition, two weeks artificial inoculation during in vitro plantlets stage was found the most suitable method for application of microbes with tissue culture banana plantlets which can promote the microbial rhizosphere of banana prior to field plantation.

Keywords: in vitro inoculation, ex vitro inoculation, banana, tissue culture, rhizospheric and endophytic bacteria.

INTRODUCTION

Banana and plantain *(Musa spp.)* are world-wide economically important fruit crops. Dessert banana takes second position in world's fruit trade after citrus, whereas plantains or cooking bananas are major staple food in many parts of the tropics and subtropics [1]. Bananas are widely cultivated in 130 countries for food security and economy of millions of people. The total cultivation area covered approximately 10.3 million ha in the tropical and subtropical regions of the world [2]. Commercial cultivation of banana depend mainly on the large amount of organic inputs to soil such as nitrogen, potassium, phosphorus, calcium and magnesium for increased production [3]. Moreover, banana production is threatened by several important fungal, bacterial, and viral pathogens such as the burrowing nematode *Radopholus similis*, banana weevil *Cosmopolites sordidus*, *Mycosphaerella* leaf spots, Fusarium wilt fungal disease, banana bract mosaic virus, banana bunchy top virus, banana streak virus and banana dieback diseases.

Several control strategies have been applied to overcome these problems using crop rotation, flood fallowing, chemical fumigation and the use of organic inputs to soil. However, these applications cannot control the disease effectively [4, 5]. Under these conditions, use of microorganisms as biological control agent became popular as an alternative control management of these problems. Biocontrol microorganisms

can protect and promote plant growth by colonizing and multiplying in both rhizosphere and plant system [6, 7]. The growth of banana plants is closely related to the bio- diversity in the rhizosphere. Under constant supply of normal nutrition, the roots of the banana plants are well developed. The exudates from the root hairs or root tip promote the growth of rhizosphere organisms [8], which in turn enhance the biodiversity of soil. Endophytes are beneficial microorganisms that colonize the plant through the root system and survive showing no symptom within the plant. Biofertilizers based on rhizobacteria played an important role in maintaining soil fertility and also enhance absorption of both water and mineral nutrients by the plant [9, 10].

Biohardening is a process that manipulates the selected microbes in the plant roots by root feeding or soil drenching [11, 12]. Endophytic bacteria can induce the plant growth by secreting useful secondary metabolites, growth hormones in host plants were observed in studies on banana and other crops [13, 14]. Bioformulations of mixtures of endophytic *Bacillus pumilus* and *B. subtilis* isolated from banana cv. Grand Naine and rhizobacterial isolate *Pseudomonas fluorescens* (Pf1) were found to be effective in increasing the growth and physiological parameters such as pseudostem girth and height, number of leaves, phyllochron, and leaf area in biohardened plants under greenhouse study. Artificial inoculation of banana tissue culture plantlets with endophytes from native healthy banana plants in plantation resulted in wilt disease reduction and growth promotion in the greenhouse [15]. Artificial inoculation of banana tissue culture plantlets with indigenous endophytes showed that the re-introduction promoted in pseudostem height, pseudostem diameter and leaf area [16]. Kavino et al. (2010) found that plant growth promoting rhizobacteria PGPR-treated tissue culture banana showed increased plant growth and gave high vigour plantlets [17]. Smith et al. (2003) reported that the introduction of *Pseudomonas* strains 84 and 4B to banana roots of tissue-cultured plants at de-flasking stage improved plant growth and reduced infection of *Foc* in rhizomes under greenhouse conditions [18]. *Trichoderma* also enhanced the growth of plants and increased the root volume compared to untreated tissue culture control plants.

Biological control using endophytic and rhizospheric microorganisms can be especially useful for tissue culture banana plants. Inoculation of these plantlets with such beneficial microbes can enhance growth and pest and disease resistance under field condition. Therefore, bioinoculation should be considered as one of the most efficient biological strategies for improving the quality of micropropagated seedlings, field plant development, disease control and increased productivity [19-21].

The aim of this study was to evaluate the effect of two bacterial application methods and to characterize the most effective method for application of rhizospheric and endophytic bacterial inoculumn in promoting growth of tissue culture banana.

MATERIALS AND METHODS

Micropropagation of banana

Tissue culture Cavendish banana were produced through shoot tip culture method. The suckers of elite banana were peeled off and cut into 3 cm×4 cm (basal diameter×length). They were surface sterilized in 70% ethanol followed by three rinses in sterile distilled water. The suckers were trimmed to a length of 1.0–2.0mm in the laminar flow cabinet to obtain the meristem tissue, leaving a meristematic dome with one or two leaf initials. Thus prepared explants were cultured on Murashige and Skoog (MS) medium supplemented with 6-benzylamino purine (BAP) from 2.0 mg/L. The cultures were incubated at 26 ± 2 °C with a 16 h photoperiod. The cultures were routinely sub-cultured into new media at monthly intervals for shoot proliferation. Three months-old shoot primordial clumps were dissected into individual plants and transferred to a rooting medium containing citric acid for root formation, which does not promote further shoot proliferation. The rooted plantlets were utilized for artificial inoculation.

Bio-Control Agents

To investigate *in vitro* co-cultured tissue culture banana plantlets for plant growth parameters, combination of rhizospheric and endophytic four bacterial species (*Pseudomonas fluorescens, Bacillus putida, Bacillus pumilus, Bacillus amyloliquefaciens*) was conducted. These bacterial strains were isolated from different parts of healthy banana plants such as root, leaf and pseudostem. The experiment consisted of the following three treatments: (1) bacterial inoculum with *in vitro* rooted plantlets for 2 weeks (2) bacterial inoculum with *ex vitro* (primary hardening) plantlets (3) control with sterilized distilled water.

Inoculum

Pseudomonas fluorescens, Bacillus pudita, Bacillus amyloliquefaciens and *Bacillus pumilus* were maintained on NA and King's B (KB) medium at -20°C. Bacterial cells were harvested by centri -fugation at 4500 ×g for 15 min, washed and re-suspended with 10 mM NaH_2PO_4 containing 0.8% NaCl at pH 6.5 (phosphate buffered saline or PBS). Inoculum density was adjusted using a spectrophotometer (600 nm) to approximately 8.0–9.2 × 10^8 cfu/ml.

Inoculation of bacterial inoculum in tissue culture banana plantlets

Banana tissue culture plantlets were cultured for root formation stage on MS medium and used as host for artificial inoculation. Rhizospheric and endophytic bacterial inoculum was poured using a sterilized syringe into the banana tissue culture bottles, then holding bottles and gently shaking bacterial solution to make sure that this solution to contact all roots of plantlets well. Then, inoculated plantlets in the bottles were maintained for two weeks at growth room. After two weeks, the plantlets in the bottles were transferred to the greenhouse for acclimatization. After removing the agar from roots under water, plants were shifted to plastic bags having mixture of sterilized sand and soil in 1:1 ratio. These bags were covered with plastic bags to maintain the humidity.

In the same time, 20 plantlets inoculated with control sterilized distilled water were acclimatized the following steps descried above. Among them, 10 plantlets were selected for bacterial inoculation. Bacterial inoculum was placed in the centre of sterilized sand and soil mixture in each of the plastic bags. Then, covered the bacterial inoculum with sterilized sand and soil mixture again. Rooted banana plantlets were placed on top of the mixture and finally a further amount of mixture were added to cover the plant roots. The remaining 10 plantlets were acclimatized according to the normal hardening procedure described above and they were used as the control treatment in the greenhouse.

When these plantlets reached to 5-6 leaves and healthy root system during hardening stage, they were used in data collection for plant growth parameters. The pot culture experiments laid to find out the effect of biohardening of tissue culture banana plants with rhizospheric and endophytic bacteria. The greenhouse experiment consists of 3 treatments and each treatment comprises ten plants.

Morphological characters

Observations on morphological characters such as plant height (cm), pseudostem girth (cm), number of leaves were made at the nursery stage after bacterial inoculation. Plant height (distance from the base of the plant to the point of the youngest leaf emergence), girth of the pseudostem (measured at 3cm above the soil) and number of healthy functional leaves (leaves were considered healthy when > 75% of the leaf area was green as opposed to yellow, brown or dry), were measured every week. There were 3 treatments for plant growth parameters: (1) control); (2) *ex vitro* inoculation (3) *in vitro* inoculation. The data were collected at two weeks interval during 2 months in the greenhouse. There were 4 times of data collection for plant growth parameters.

DATA ANALYSIS

The data were statistically analyzed using the software Statistix 8.0 version. Means of plant growth parameters were analyzed using analysis

of variance (ANOVA) and treatment means were compared by LSD at 0.05 and 0.01 significant probability levels.

RESULTS AND DISCUSSIONS

Acclimatization of banana plantlets

After 2 weeks bacterial inoculation into *in vitro* culture bottles, these rooted banana plantlets bottles were transferred into greenhouse for acclimatization. Another rooted banana plantlets bottles were transferred for acclimatization stage. These plantlets were used for control and for *ex vitro* bacterial inoculation. There were 10 plantlets for each treatment. All banana plantlets (control plantlets, *in vitro* inoculated plantlets and *ex vitro* inoculated plantlets) were successfully acclimatized and survived in the greenhouse (Figures 4- 8).

Plant height (cm)

Plant height per plantlet as affected by different treatments is described in Table 1 and 4. In the analysis of variance and mean squares of plant height (cm) for four times of data collection, it was observed that plant height was highly significant among treatments for four times of data collection (Figure1).

The greatest number of plant height (13.7, 16.0, 22.3, 24.0) was observed from *in vitro* inoculation for all 4 weeks data collection which was highly significant among other treatments. The lowest plant height (8.2, 11.0, 14.9, 19.0) was observed in control plantlets which was statistically different among other treatments. In comparison with control treatment, *ex vitro* inoculation showed significant difference plant height at 4th week. But, *in vitro* inoculation gave plant height significantly different than *ex vitro* inoculation for four times.

According to the results, bacterial inoculum treated plantlets gave the greater plant height compared with control plantlets. Lian et al. (2009) observed that addition of a crude endophytic inoculum to tissue culture banana plantlets resulted in an increased plant growth parameters (height, girth, leaf area) as well as substantial reduction in the infection and severity of Fusarium wilt disease [15]. Smith et al. (2003) reported that the introduction of *Pseudomonas* strains to banana roots of tissue-cultured plants at de-flasking stage improved plant growth and reduced infection of *Foc* in rhizomes under greenhouse condition [18]. Thangvelu et al. 2015 found that rhizospheric bacterial isolates *B. cereus*, *P. putida* and *Bacillus* sp. also significantly increased the plant growth parameters, including plant height (92% increase), girth (up to +80%), number of leaves (up to +52%) and number of roots (up to +101%) compared to the *Foc* alone-inoculated control plants [22]. In our results, plants treated with bacterial inoculum grew relatively taller than control plantlets and plant height was gradually increased at 1st, 2^{nd,} 3rd week and 4th week. Therefore, the application of bacterial inoculation possess plant growth promotion characters in pot trials.

Number of leaves

Number of leaves per plantlet as affected by different treatments is described in Table 2 and 4. In the analysis of variance and mean squares of number of leaves for four times of data collection, we observed that the number of leaves were significant at 3rdth week and 4th week respectively. The number of leaves was significance at 3rd week and 4th week but there was no significant differences between treatments at 1st week and second week (Table 2, Figure 2).

The highest number of leaves (4.7, 5.8, 6.9, 7.0) was observed in *in vitro* inoculation treatment among three treatments. The lowest number of leaves (4.0, 5.0, 5.8, 6.2) was observed in control treatment for all data collection times (Table 4). The number of leaves in *ex vitro* inoculation treatment were slightly increased for 4 weeks data collection time. There was significant difference between control and *in vitro* inoculation at 2nd, 3rd and 4th week. There was also significant difference between control and *ex vitro* inoculation at 3rd and 4th week respectively. But no significant leaf number was observed between *in vitro* and *ex vitro* inoculation. These results indicated that the number of leaves was influenced by the different treatments. Plants inoculated with bacterial inoculum resulted more leaves than control treatment. Suthar et al. (2012) observed that micropropagated plantlets of *Boswelliaserrata Roxb* were biotized using an endophytic fungus *Piriformospora indica*. In his result, plantlets inoculated with *P indica* attained maximum height of 6.5cm along with increased number of leaves (5.8) per plantlet as observed after 1 month of their growth in the greenhouse as compared to control plantlets where an average of 5.6 cm height with 4.6 leaves per plantlet [23]. Kidane and Laing (2010) observed that treatments involving combinations of nonpathogenic F. oxysporum, T. harzianum Eco-T®,silicon and mulch had significantly higher number of leaves, stem height and girth size than single applications of the treatments [24]. Reintroduction of mixture of naturally-occurring uncultivated endophytes (dominated by γ-Proteobacteria) isolated from native healthy banana plant into tissue culture banana plantlets led to 67% suppression rate of wilt disease at the fifth month after pathogen infection on plantlets in the greenhouse [16]. In addition to disease suppression, growth of host plantlets was also promoted with the inoculation of these endophytes both in pathogen- infected and healthy control plants. These results were in accordance with number of leaves in this experiment. Plants treated with bacterial inoculum had relatively more leaves compared to control plants. This indicated that this bacterial inoculation to tissue culture banana plantlets caused an increased in the number of leaves in greenhouse experiment.

Plant girth (cm)

Plant girth per plantlet as affected by different treatments is described in Table 3and 4. In the analysis of variance and mean squares of plant girth (cm) for 4 times of data collection, there was significant differences among treatments for all 4 times (Figure 3).

The highest number of plant girth was observed in *in vitro* inoculated plantlets at all 4 weeks data collection and plant girth was slightly increased during 4 times of data collection. The lowest plant girth was observed in control plantlets. *In vitro* inoculation resulted significant difference in plant girth compared with *ex vitro* inoculation at four times. Although *ex vitro* inoculation did not showed significant difference with control, *ex vitro* inoculated plantlets had greater plant girth than control at all data collection times. This may be due to the pseudo-stem diameter of micropropagated banana plantlets was influenced by the bacterial inoculation. Smith et al. (2003) observed that free of non-pathogenic micro-organisms in the tissue culture plantlets may be overcome by reintroduction of microorganisms or their mixtures which could improve the level of establishment, protect the plantlets against pest and diseases and overall performance [18]. Our results were in accordance with Fernandes et al. (2013) in which endophytic bacteria *Klebsiella pneumoniae* isolated from roots of bananas cultivars Tropical and Galil 18 gave the highest pseudostem height, number of leaves and pseudostem diameter of micropropagated 'Prata Anã' banana plantlets [25]. For the data analysis of plant girth, bacterial inoculation of *in vitro* and *ex vitro* banana plantlets gave higher number of plant girth than the untreated control plantlets under greenhouse experiment.

Endophytes are beneficial bacteria which live inside the plants. Studies on banana and other crops explained that endophytic bacteria can induce the plant growth by secreting useful secondary metabolites, growth hormones in host plants. In addition, endophytes may provide resistance by inducing systemic resistance in a host against a wide range of pathogens. The application of microbial inoculation in tissue culture plantlets resulted the possible outcomes which was free from pathogenic

infections in *in vitro* co-cultures and on hardening stages [26]. *In vitro* co-cultures of tissue culture plantlets with microbial consortia showed enhanced growth characters like pseudostem height and girth number of leaves, leaf area and phyllochron

also improved significantly in the nursery stage of tissue culture banana [17, 21, 27]. When micropropagated banana plants variety 'Gran Enano' were inoculated with mycorrhiza and rhizobacteria (monoculture and combined), plant growth parameters as total fresh weight, shoot dry weight, stem length and leaf area were significantly higher than in non-

inoculated banana plantlets [28]. Similarly, according to the results, combinations of *Pseudomonas* and *Bacillus* strains applied as *in vitro* co-cultures and *ex vitro* hardening stages significantly enhanced the physiological characters of tissue culture banana plantlets. In our experiment, bacterial inoculation was carried out both *in vitro* and *ex vitro* banana plantlets and resulted increased plant growth parameters in both treatments. But *in vitro* inoculation gave the best plant growth parameters. It indicates that these inoculated bacteria can colonize and multiply within the *in vitro* banana plantlets bottles and then enhances the plant growth parameters under greenhouse condition. *Ex vitro* inoculation also results increased plant growth although *in vitro* inoculation gave the highest growth among other treatments. The results of the present study suggest that inoculation with combination of rhizospheric and endophytic four bacterial species (*Pseudomonas fluorescens, Bacillus putida, Bacillus pumilus, Bacillus amyloliquefaciens*) was very effective in enhancing plant vigour under greenhouse condition.

Table 1: Analysis of Variance and Mean squares of Plant Height (cm), where; ** high significant at 0.01 probability level, *: significant at 0.05 probability level, ns: no significant

S.O.V	d.f.	1st week	2nd week	3rd week	4th week
Replications	9	6.8481	10.0148	0.81852	1.03367
Treatments	2	78.6333**	76.3000**	4.43333**	4.07233**
Error	18	3.8926	5.3370	0.84074	0.62233

Table 2: Analysis of Variance and Mean squares of Number of Leaves where; ** high significant at 0.01 probability level, *: significant at 0.05 probability level, ns: no significant

S.O.V	d.f.	1st week	2nd week	3rd week	4th week
Replications	9	0.22593	0.67037	0.75556	1.21852
Treatments	2	1.43333^{ns}	1.63333^{ns}	3.43333**	1.90000*
Error	18	0.69259	0.55926	0.54444	0.52963

Table 3: Analysis of Variance and Mean squares of Plant Girth (cm), where; ** high significant at 0.01 probability level, *: significant at 0.05 probability level, ns: no significant

S.O.V	d.f.	1st week	2nd week	3rd week	4th week
Replications	9	0.44815	0.90741	0.81852	1.03367
Treatments	2	3.60000**	4.23333**	4.43333*	4.07233**
Error	18	0.30370	0.64074	0.84074	0.62233

Table 4: Mean performance of Plant Growth Parameters; Number of leaves, Plant height (cm), Plant Girth (cm) on different treatments. Means with the same letter are not significantly different from each other at P = 0.05 according to LSD test.

Plant Growth Parameters	**Data Collection Times**	**Treatments**				
		Control	***Ex vitro* Inoculation**	***In vitro* Inoculation**	**Pr>F**	**CV%**
No of Leaves	1st week	4.0A	4.6A	4.7A	0.16	18.77
	2nd week	5.0B	5.5AB	5.8A	0.0797	13.76
	3rd week	5.8B	6.7A	6.9A	0.0084	11.41
	4th week	6.2B	6.9A	7.0A	0.0488	10.86
Plant Height (cm)	1st week	8.2B	10.0B	13.7A	0.000	18.55
	2nd week	11.0B	12.0B	16.0A	0.0002	17.37
	3rd week	14.9B	17.5B	22.3A	0.0003	17.70
	4th week	19.0C	22.0B	24.0A	0.0009	12.79
Plant Girth (cm)	1st week	3.5C	4.1B	4.7A	0.005	13.44
	2nd week	4.2B	4.8AB	5.5A	0.0071	16.56
	3rd week	5.0B	5.4B	6.3A	0.0158	16.47
	4th week	6.02B	6.34B	7.25A	0.0073	12.07

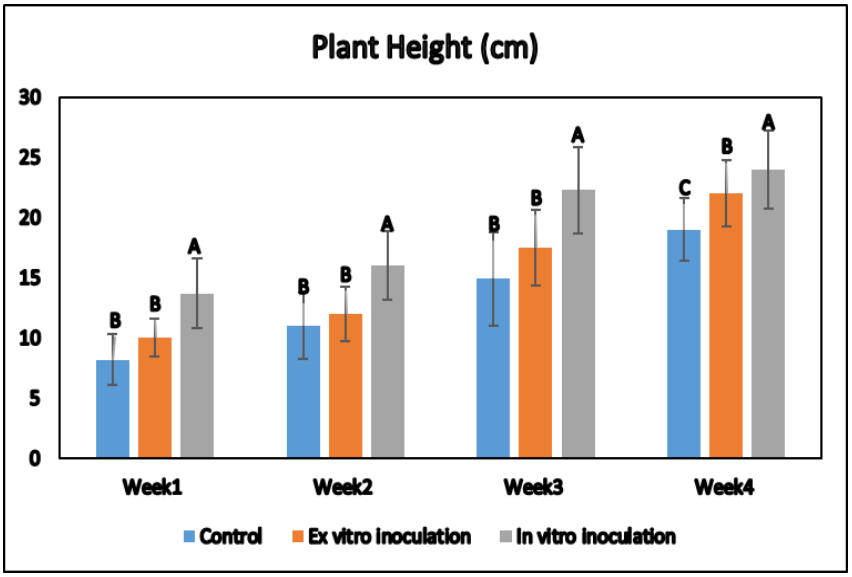

Figure 1: Plant height (cm) at 1st, 2nd, 3rd and 4th weeks in pot trials during 3 months bacterial inoculation. For each week, columns with different letters represent plant height that differ significantly from each other (P<0.05). Error bars: Standard deviation of the mean

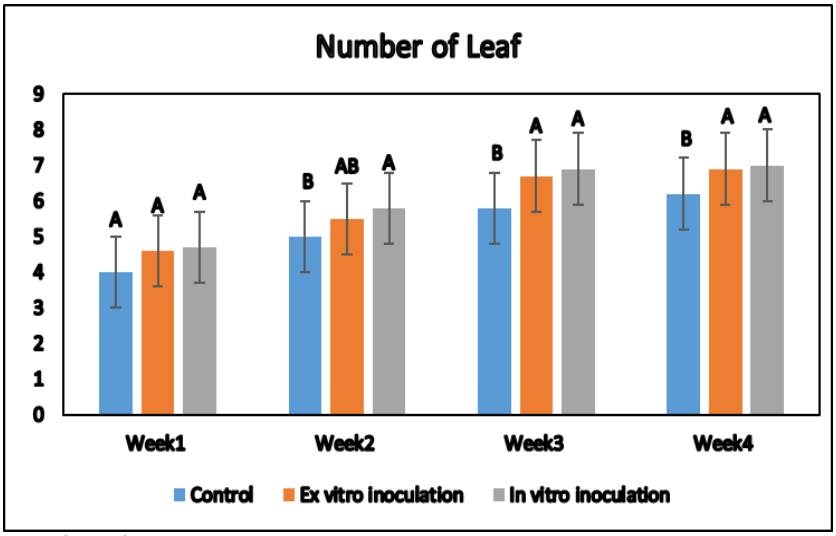

Figure 2: Number of leaves at 1st, 2nd, 3rd and 4th weeks in pot trials during 3 months bacterial inoculation. For each week, columns with different letters represent leaf numbers that differ significantly from each other (P<0.05). Error bars: Standard deviation of the mean

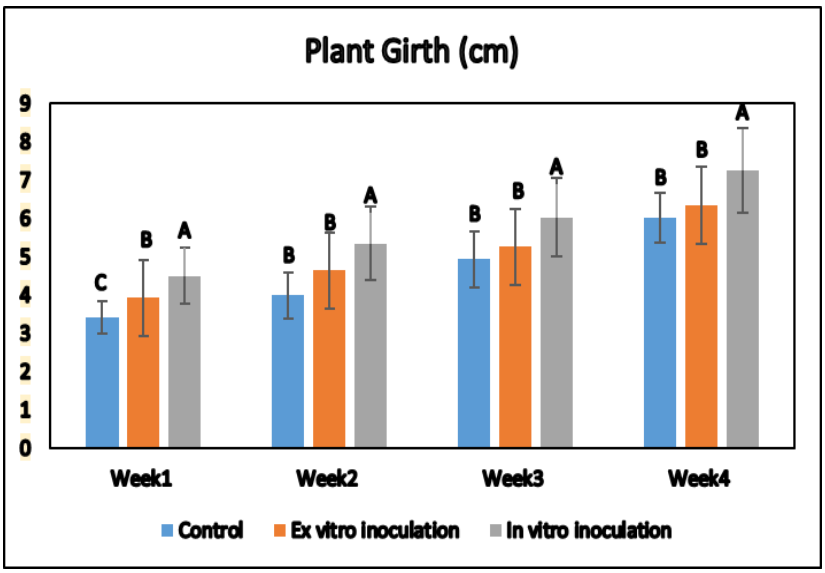

Figure 3: Plant girth (cm) at 1st, 2nd, 3rd and 4th weeks in pot trials during 3 months bacterial inoculation. For each week, columns with different letters represent plant girth that differ significantly from each other (P<0.05). Error bars: Standard deviation of the mean

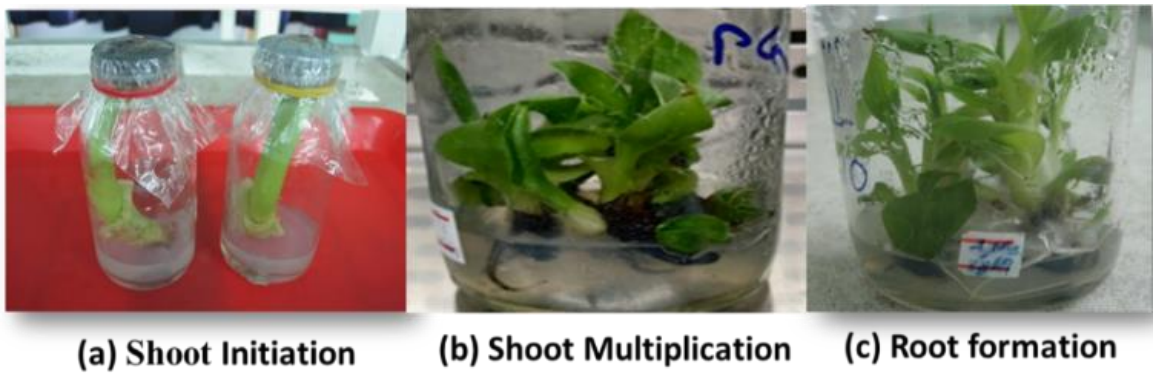

Figure 4: Micropropagation of Cavendish banana through shoot tip culture method.

Figure 5: *In vitro* and *ex vitro* bacterial inoculated plantlets were successfully acclimatized under greenhouse condition.

Figure 6: Growth of control banana plantlets at 2nd, 3rd & 4th week under greenhouse condition.

Figure 7: Growth of *ex vitro* inoculated banana plantlets at 2nd, 3rd & 4th week under greenhouse condition.

Figure 8: Growth of *in vitro* inoculated banana plantlets at 2nd, 3rd & 4th week under greenhouse condition

CONCLUSION

This study confirmed the application of mixtures of bacterial inoculum was effective for enhanced plant growth parameters in tissue culture banana plantlets. These findings indicate that two weeks artificial inoculation during *in vitro* plantlets stage was found the most suitable method for application of microbes with tissue culture banana plantlets before field plantation. Further studies must be applied to evaluate multiplication and viability of these bacterial inoculum, plant growth parameters and yield under field condition.

Acknowledgment

I am very grateful to our director, Dr. Aye Aye Khai, Director and Head, Biotechnology Research Department, Ministry of Education, Kyaukse Township, Mandalay Division, Myanmar, for her permission and encouragement throughout this study. I wish to express my thanks to our research team from Plant Tissue Culture Laboratory, Biotechnology Research Department for their kind help during this research.

REFERENCES

1. Banerjee N, Sharma AK. *In vitro* response as a reflection of genomic diversity in long-term cultures of *Musa.* Theoretical and Applied Genetics 1998; 76:733-736.
2. Lava Kumar P, Selvarajan R, Iskra Caruana ML, Chabannes M, Hanna R. Control of plant virus diseases vegetatively-propagated crops, by G. Loebenstein and N. I. Katis. New-York: Academic Press (Advances in Virus Research), 2015, 229-269.
3. Mia BA, Shamsuddin ZH, Wahab Z, Marziah M. Effect of plant growth promoting rhizobacterial (PGPR) inoculation on growth and nitrogen incorporation of tissue-cultured Musa plantlets under nitrogen-free hydroponics condition. Australian Journal of Crop Science 2010; 4(2):85-90.
4. Herbert JA, Marx D. Short-term control of Panama disease in South Africa. Phytophylactica 1990; 22:339-340.
5. Moore NY, Bentley S, Pegg KG, Jones DR. Fusarium wilt of banana. Musa disease fact sheet. INBAP 1995; 5:1
6. Weller DM, Raaijmakers JM, McSpadden Gardener BB, Thomashow LS. Microbial populations responsible for specific soil suppressiveness to plant pathogens. Annual Review of Phytopathology 2002; 40:309-48.
7. Fravel D, Olivain C, Alabouvette C. *Fusarium oxysporum* and its biocontrol. New Phytologist 2003; 157:493-502.
8. Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM. The role of root exudates in rhizosphere interactions with plants and other organisms. Ann. Rev. Plant Biol 2006;57:233-266.
9. Akhtar A, Hisamuddin M, Robab A, Sharf R. Plant growth promoting Rhizobacteria: an overview. J. Nat. Prod. Plant Resour. 2012;2(1):19-31.
10. Posada LF, Ramirez M, Ochoa-Gomez N, Cuellar-Gaviria TZ, Argel-Roldan LE, Ramirez CA, Villegas-Escobar. Bioprospecting of aerobic endospore forming bacteria with biotechnological potential for growth promotion of banana plants. Sci. Hortic 2016;212:81-90.
11. Harish S, Kavino M, Kumar N, Saravanakumar D, Soorianathasundaram K, Samiyappan R. Biohardening with plant growth promoting rhizosphere and endophytic bacteria induces systemic resistance against banana bunchy top virus. Applied Soil Ecology 2008;39(2):187-200.
12. Kumar A, Munder A, Aravind R, Eapen SJ, Tummler B, Raajmakers JM. Friend or foe; genetic and functional characterization of plant endophytic Pseudomonas aeruginosa. Environmental Microbiology 2013;15(3):764-779.
13. Beneduzi A, Ambrosini A, Luciane MPP. Plant growth-promoting rhizobacteria (PGPR): Their potential as antagonists and biocontrol agents. Genetics and Molecular Biology 2012;35:1044-1051.
14. Van Loon LC. Plant responses to plant growth-promoting bacteria. European Journal of Plant Pathology 2007;119:243-254.
15. Lian J, Wang Z, Cao L, Tan H, Inderbitzin P, Jiang Z et al. Artificial inoculation of banana tissue culture plantlets with indigenous endophytes originally derived from native banana plants. Biological Control 2009; 51:427-434.
16. Jie L, Zifeng W, Lixiang C, Hongming T, Patrik I et al. Artificial inoculation of banana tissue culture plantlets with indigenous endophytes originally derived from native banana plants. Biol. Control 2009;51:427-434.
17. Kavino M, Harish S, Kumar N, Saravanakumar D, Samiyappan R. Effect of chitinolytic PGPR on growth: yield and physiological attributes of banana (Musa spp.) under field conditions. Appl. Soil Ecol. 2010;45:71-77.
18. Smith L, Keef DO, Smith M, Hamill S. The benefits of applying rhizobacteria to tissue cultured bananas. Banana Topics Newsletter, 2003; 33:1-4.
19. Tsavkelova EA, Klimova SY, Cherdyntseva TA, Nestrusov AI, Weber OB, Baldani JI et al. Pesquisa Agropecuária Brasileira. Brasília 2000;35(11):2227-85.
20. Mia MAB, Shamsuddin ZH, Wahab Z, Mahmood M. High-yielding and quality banana production through plant growth-promoting rhizobacterial inoculation. Fruits 2005;60(3):179-185.
21. Kavino M, Kumar N, Damodaran T, Harish S, Saravanakumar D. Biochemical markers as a useful tool for the early identification of *Fusarium oxysporum* f.sp. *cubense* race1 resistance banana clones. Archives of Phytopathology and Plant Protection 2007;42:1069-1078.
22. Thangavelu RM, Gopi M. Field suppression of Fusarium wilt disease in banana by the combined application of native endophytic and rhizospheric bacterial isolates possessing multiple functions. Phytopathologia Mediterranea. 2015;54(2):241-252.
23. Suthar KR, Purohit SD. Biopriming of Micropropagated Boswellia serrata Roxb. Plantlets – Role of endophytic root fungus Piriformospora indica. Indian Journal of Biotechnology 2012;11:304-308.
24. Kidane EG, Laing MD. Integrated Control of Fusarium Wilt of Banana (Musa spp.). In: Dubois T (Eds), Proc. IC on Banana & Plantain in Africa, Acta Hort. 2010; 879:315-321. ISHS,
25. Fernandes T, Nietsche S, Costa MR, Aparecida A. Potential use of endophytic bacteria to promote the plant growth of micropropagated banana cultivar Prata Anã. African Journal of Biotechnology 2013; 12(31):4915-4919.
26. Nowak J, Pruski K. Priming tissue cultured propagules. p.69-81. In: Low Cost Options for Tissue Culture Technology in Developing Countries. Proc. Technical meeting organized by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, held in Vienna 2002.
27. Kavino M, Manoranjitham SK, Balamohan TM, Kumar N, Karthiba L, Samiyappan RJ. Enhancement of Growth and Panama Wilt Resistance in Banana by In Vitro Co-Culturing of Banana Plantlets with PGPR and Endophytes." Acta horticulturae 2014;1024, 37.
28. Rodriguez-Romero AS, Guerra MSP, Jaizme-Vega MDC. Effect of arbuscular mycorrhizal fungi and rhizobacteria on banana growth and nutrition. Agron. Sustain. Dev. 2005;25(3):395-399.