OPEN ACCESS

International Journal of Applied Sciences and Biotechnology

A Rapid Publishing Journal

ISSN 2091-2609

Indexing and Abstracting

CrossRef, Google Scholar, Global Impact Factor, Genamics, Index Copernicus, Directory of Open Access Journals, WorldCat, Electronic Journals Library (EZB), Universitätsbibliothek Leipzig, Hamburg University, UTS (University of Technology, Sydney): Library, International Society of Universal Research in Sciences (EyeSource), Journal Seeker, WZB, Socolar, BioRes, Indian Science, Jadoun Science, Jour-Informatics, Journal Directory, JournalTOCs, Academic Journals Database, Journal Quality Evaluation Report, PDOAJ, Science Central, Journal Impact Factor, NewJour, Open Science Directory, Directory of Research Journals Indexing, Open Access Library, International Impact Factor Services, SciSeek, Cabell's Directories, Scientific Indexing Services, CiteFactor, UniSA Library, InfoBase Index, Infomine, Getinfo, Open Academic Journals Index, HINARI, etc.

CODEN (Chemical Abstract Services, USA): IJASKD

Vol-3(2) June, 2015

Available online at:

http://www.ijasbt.org

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http://www.nepjol.info/index.php/IJASBT/index

SEM-Biotech Publishing

Impact factor*: **1.422**
Scientific Journal Impact factor#: **3.419**
Index Copernicus Value: **6.02**

*Impact factor is issued by Universal Impact Factor. Kindly note that this is not the IF of Journal Citation Report (JCR).
#Impact factor is issued by SJIF INNO SPACE.

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R. Shashikumar et al. (2015) Int J Appl Sci Biotechnol, Vol 3(2): 202-209
DOI: 10.3126/ijasbt.v3i2.12536

ISSN (Online): 2091-2609
DOI Prefix: 10.3126/ijasbt

International Journal of Applied Sciences and Biotechnology (IJASBT)

Research Article

HIGH FREQUENCY PLANT REGENERATION OF *MUSA PARADISIACA* CV. KARIBALE MONTHAN

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Abstract

High frequency plant regeneration protocol has been standardized from banana cultivar *Musa paradisiaca* cv. Karibale Monthan, an endemic cultivar of Malnad region of Karnataka. The fruits are used as glomerular protective to solve kidney problems. To minimize the microbial contamination and to promote healthy growth, explants were treated with 70 % absolute alcohol for 6 min, 0.1 % Mercuric chloride for 10 min and 0.2 % for 10 min, 1 % Sodium hypochlorite for 15 min, 0.1 % Cefotaxime for 5 min and 0.05 % Gentamicin for 5 min. The high frequency shoot initiation (93.33 %) was recorded at 5 mg/l BAP. The synergetic effect of BAP (4 to 6 mg/l), TDZ (0.1 to 1.2 mg/l) and coconut water (0.1 to 0.9 ml/l) induced multiple shoot buds and it was optimized at the concentration of 5 mg/l BAP, 0.5 mg/l TDZ and 0.5 ml/l coconut water with 15.90 ± 1.66 frequency of shoots per propagule. Supplementation of 1.0 mg/l IBA induced 5.33 ± 1.21 numbers of roots with a mean root length of 7.50 ± 1.87 roots. The 99% of plantlets with distinct roots and shoots were successfully acclimatized in the green house and transferred to the field to evaluate the agro-morphological variations. The weight of the bunch (kg), number of hands in a bunch, number of fingers in a hand, length of the finger (cm), girth of the finger (cm) and girth of the pseudostem (cm) exhibited by *in vitro* plants were higher than the *in vivo* plants.

Keywords: *Musa paradisiaca* cv. Karibale Monthan; High frequency regeneration; Glomerular protective.

Introduction

Edible bananas (*Musa* spp.) are the major staple food for rural and urban consumers in India and an important source of rural income (Simmonds, 1962). The plant breeding in edible *Musa* spp. from their vegetative parts is practiced from ancient times because almost all of the banana cultivars are triploid, seedless or seed sterile (Duangkongsan and Promtab, 2014). Banana propagation through conventional breeding method is not an ideal method because the suckers may carry bacteria, fungi, viruses, weevils and nematodes (Arias, 1992; Sagi *et al*., 1998). Micropropagation technique has played a key role in producing high quality and disease free planting materials (Rowe and Rosales, 1996; Vuylsteke *et al*., 1997). To date, many investigator successfully carried out tissue culture protocol on a wide range of banana cultivars using apical meristem (Mante and Tepper, 1983), shoot-tip (Kanchanapoom and Chanadang, 2001; Buah *et al*., 2010), floral explants (Cronauer and Krikorian, 1985; Cote *et al*., 1996; Ganapathi *et al*., 1999; Gomez *et al*., 2001), protoplast culture (Panis *et al*., 1993), embryos culture (Escalant and Teisson, 1989), organ formation (Jarret *et al*., 1985) and immature fruits as a explants (John Nelson Buah, *et al*., 2000; Msogoya *et al*., 2006). Many species and cultivars of banana protocols are standardized and commercialized for clonal multiplication from the apical meristem (Rout *et al*., 2000).

The *Musa paradisiaca* cv Karibale is also known as 'Mara bale' and 'Male bale' which grows to a height of 3.0 - 4.0 m and the fruit skin is thick, yellowish-green in color with slight bloom but becomes black or blotched very soon. The fruit has a peculiar flavor which is much esteemed in South India. Due to the low yielding capacity with 50-60 fruits in a bunch this culinary variety is restricted to back yards, areca gardens and coffee estates (Yegna Narayan Aiyer, 1954). The plantain is grown largely in the heavy rain fall regions of Kerala and Karnataka. It is one of the oldest cultivated plant and popular for its great medicinal value, traditionally the plant parts like fruits, stem juice, flower juice have been used for treatment such as epilepsy, leprosy, alopecia (female), anasarca, pain reliever, cooling effect on burns, cancer, cataplasm, diabetes, diarrhea, snake bite, malignant ulcers, scorpion sting, lizard bites, dysentery, dyspepsia, cruptions, fractures, gangrene, headache, hematuria, hemiplegia, hemoptysis, hemorrhage, hypertension, mange, marasmus, migraine, nausea, otalgia, psoriasis, ringworm, septicemia, shingles, smallpox, sore, strain, syphilis, tuberculosis, warts, wound and to promote functional efficiency of kidneys (Alexandra *et al*., 2001; Sampath kumar *et al*., 2012). The protocol for mass

multiplication of plantlets for this indigenous banana cultivar Karibale Monthan has not yet been standardized so far. In the present study, an attempt has been made to develop high frequency regeneration protocol and evaluation of morpho-agronomic characteristics in the farmyard.

Materials and Methods

Material collection and maintenance

The healthy four week old sword suckers from *Musa paradisiaca* cv. Karibale Monthan weighing about one kilogram uniformly sized were identified from elite plants of high yielding and disease free mother plants. These suckers were removed from the pseudostem 15cm above the base level and collected from banana farmyards of Malnad region (Shivamogga District), Karnataka. The disinfection of suckers was carried out thoroughly washed with tap water, soaked in a solution of 0.2% Bavistin and 0.1%Streptocyclin for overnight. One whorl of leaf sheath was removed and corm portion trimmed to a width of 1 inch x 1 inch and height about 2 inches, dipped in 0.001 M Teepol solution for an hour and finally rinsed three to four time using distilled water to remove residual Teepol. From such trimmed suckers shoot tip measuring about 2.0 to 3.0 cm containing several sheathing leaf bases and axillary bud along with underlying rhizome tissue were isolated.

Surface sterilization

The sucker cubes were transferred to the laminar airflow chamber. Tissue blocks containing shoot-tips and rhizomatous bases were surface sterilized for six min in 70% ethanol, 0.2% Mercuric Chloride solution for ten minutes and rinsed three times repeatedly with sterile distilled water for five minutes. One whorl of leaf sheath was removed, corm portion trimmed and treated with 70% alcohol for six minutes, 0.1 % mercuric chloride for ten minutes and rinsed three times repeatedly with sterile distilled water for five minutes. Further, a whorl of leaf sheath was removed, corm portion was trimmed, dipped in 1 % sodium hypochlorite for fifteen minutes and rinsed three times repeatedly with sterile distilled water for five minutes. Then the cut surface of the sucker tissue was further trimmed and dipped in antibiotic solution containing Cifotaxime 0.1% and Gentamicin 0.05 % for 5 min. Finally, after the antibiotic treatment the suckers were treated with Ascorbic acid (100mg/l) for 10 min to avoid blackening of tissues due to phenolic exudation.

Initiation of organogenesis

After surface sterilization, the explants were aseptically inoculated on MS (Murashige & Skoog, 1962) basal medium augmented with 160mg/l Adenine sulfate (ADS), 100mg/l Tyrosine, 0.8 % Agar agar and 3 % (W/V) sucrose. The growth regulators consisted of different concentrations and combinations of BAP (6 – Benzyl amino purine) at the range of 1.0 – 10.0 mg/l. The pH of the media was adjusted 5.8 and thirty replicates were used for each of the concentrations.

Growth condition

A clean culture incubation room with provision for temperature, light and humidity control is preferred. The cultures should be incubated in the basal MS media supplemented with plant growth regulators. These cultures were induced for four weeks at 25±2ºC, with 16 h photoperiod and 40% relative humidity in growth chamber, illuminated by cool, white fluorescent lamps (40 Watts). The light intensity thus obtained is in the order of 30 to 50µ $E.m^2/sec$. There upon the healthy, contamination free explants should be taken for next multiplication stage.

Mass multiplication of propagules

The regenerated shoots obtained from initiation cultures were vertically marked into smaller pieces (usually 2 to 4) and transferred to MS medium supplemented with 4 to 6 mg/l BAP, 0.1 to 1.2 mg/l Thidiazuron (TDZ) and 0.1 to 0.9 ml/l coconut water for the induction of multiple shoot buds. The cultures were incubated for 4 to 5 weeks. The shoot buds were organized from the transversely cross-initiated shoot buds. At each cycle, organized multiple propagules were transformed to new organogenic media supplemented with the same concentration of growth regulators. To minimize further blackening, the meristematic tissues were transferred to the similar fresh medium frequently every 10 to 12 days for about one month by removing the blackish tissues using sterilized scalpel during the initial phase. During subculture, the cultures were checked for bacterial and fungal contamination which may appear within 15 days of incubation. At each cycle, organized multiple propagules were transformed to new organogenic media supplemented with the same concentration of growth regulators. The clumps of shoot propagules developed on multiplication media were transformed to elongation media. The shoot elongation media composed of 5 mg/l BAP. At this stage the regenerants were maintained for 2 -3 weeks and transformed to rooting media.

Rhizogenesis

At the end of multiple shoot generation cycles, individual shootlets attained the height of 4-5 cm were carefully isolated from the shoot clump and aseptically transferred to rooting media composed of MS basal nutrients augmented with 0.5 – 1.5 mg/l IBA, 0.5 – 1.5 mg/l IAA, 0.5 – 1.5 mg/l NAA and 0.2% activated charcoal to induce root formation.

The observations were made on the development pattern of plantlets after four weeks of incubation and the data were recorded.

Hardening and evaluation of morphoagronomic characters of the regenerants

Well developed plants were carefully removed and gently washed under running tap water to remove adhering pieces of gelled medium. These were then dipped in 0.2% Bavistin for about 5 min and transferred into polybag filled with sterile potting mixture (soil : cowdung : sand in 1:2:1 ratio) and were maintained in the green house for about 45 days under natural light with relative humidity of 90-100% at ambient temperature of about 25ºC.These plants were acclimatized in green house for about two months and transferred to the field to evaluate the agro-morphological variations along with vegetatively propagated Karibale plants of the same age at the experimental farm. Cultivation practices such as fertilizer application and irrigation followed conventional protocols (Chattopadhyay *et al*., 2001). Morphological parameters such as height of the plants (in ft), number of leaves, length of the leaves (in cm), width of the leaves (in cm), length of the bunch (in cm), weight of the bunch, number of hands in a bunch, number of fingers in a hand, length of the finger (in cm), girth of the finger (in cm) and girth of the pseudo stem (in cm) were recorded. All the data were analyzed by using ez. ANOVA software (version – 0. 98).

Results and Discussion

The *in vitro* propagation of banana provides excellent advantages because of high multiplication rate, physiological uniformity, availability of disease free material all the year round, short harvest interval and faster growth in the early growing stages when compared to conventional plant materials (Vuylsteke, 1989; Daniells & Smith, 1991). Previously, we reported the standardization of protocol for mass multiplication of endemic banana cultivar *Musa paradisiaca* cv. Puttabale using shoot tip explants (Venkatesh *et al*., 2013).

To control the bacterial and fungal contamination in banana culture, a number of antibiotics are being used to establish disease free culture (Habiba *et al*., 2002). Hamill *et al*., (1993) reported 10 percent contamination control during propagation of *Musa* cv. Williams using double infection with NaOCl. In the present study, aseptic culture of cultivar Karibale showed 100% survivability by treating with 70% absolute alcohol (6 min), 0.1 and 0.2% Mercuric chloride (10 min), 1% Sodium hypochlorite (15 min), 0.1% Cefotaxime (5 min) and 0.05% Gentamicin (5 min) as shown in **Table 1**. The surface disinfestations are very crucial in the culture explants of Karibale. Omitting of any of the above treatment and extension of period also affect the survival rate of the cultured explants.

After one week of primary inoculation tissue blackening, darkening of culture medium and explant growth inhibition was observed due to phenolic exudation. Strosse *et al*., (2004) reported that the cause of blackening and growth inhibition of the explant was due to the oxidation of polyphenols. Antioxidants reduce browning of tissues by inhibiting the oxidation of liable substrates and were successfully used in many monocotyledonous species (George, 1996). Therefore, the initial explants were treated with an antioxidant, ascorbic acid (100 mg/l) for 10min before inoculation and supplementation of 50mg/l ascorbic acid to the MS medium for culture establishment markedly reduced the phenolic exudation. The results were in agreement with the report of Mante & Tepper (1983), who has control the problem of oxidation of phenolic compounds using different antioxidants including citric acid, ascorbic acid, L-cystein and L-tyrosine in the medium.

Table 1: Standardization of different Sterilants treatment period for the surface sterilization of the explants

Sterilants used							Antioxidants treatment	No. of explants	Rate of contamination (%)	% of explant survival after 15 days
Trials	**70% Alcohol treatment (Min)**	**Mercuric chloride (Min)**		**1 % Sodium hypochlorite (Min)**	**0.1% Cefotaxime (Min)**	**0.05% Gentamicine (Min)**	**Ascorbic acid (100mg/l) (Min)**			
		0.1 %	**0.2 %**							
I	3	-	-	-	-	-	-	10	90	5
II	2	2	2	3	1	1	2	10	70	30
III	3	4	4	6	2	2	4	10	60	45
IV	4	6	6	9	3	3	6	10	50	50
V	5	8	8	12	4	4	8	10	30	70
VI	**6**	**10**	**10**	**15**	**5**	**5**	**10**	**10**	**-**	**100**
VII	7	12	12	18	6	6	12	10	-	100*
VIII	8	14	14	20	7	7	14	10	-	100**
IX	9	16	16	22	8	8	16	10	-	100***

"-" indicates no contamination
"*" Indicates explant death due to tissue killing

Table 2: Effect of various concentrations of BAP, TDZ and Coconut water on shoot bud organogenesis from *Musa paradisiaca*. Cv Karibale monthan

Plant growth regulators mg/l			Frequency of shoot initiation per explant %	Number of multiple shoot bud per propagule Mean ± S.D	Mean length Shoot per propagule (cm) Mean ± S.D
BA mg/l	TDZ mg/l	Coconut water ml/l			
1	-	-	16.66	-	-
2	-	-	23.33	-	-
3	-	-	36.66	-	-
4	-	-	46.66	-	-
5	-	-	**93.33**	-	-
6	-	-	53.33	-	-
7	-	-	43.33	-	-
8	-	-	26.66	-	-
9	-	-	23.33	-	-
10	-	-	06.66	-	-
4	0.1	-	-	3.50±1.08	-
4	0.3	-	-	4.20±1.03	-
4	0.5	-	-	6.50±1.35	-
4	0.7	-	-	4.50±0.53	-
4	0.9	-	-	3.30±1.16	-
4	1.2	-	-	2.80±0.63	-
5	0.1	-	-	5.10±0.99	-
5	0.3	-	-	6.30±1.16	-
5	**0.5**	-	-	**11.20±1.55**	-
5	0.7	-	-	6.60±2.01	-
5	0.9	-	-	4.80±1.32	-
5	1.2	-	-	3.50±1.35	-
6	0.1	-	-	3.80±1.03	-
6	0.3	-	-	9.70±1.57	-
6	0.5	-	-	7.10±1.20	-
6	0.7	-	-	6.00±1.25	-
6	0.9	-	-	3.40±0.84	-
6	1.2	-	-	3.00±0.67	-
4	-	0.1	-	2.80±1.32	-
4	-	0.3	-	2.80±1.48	-
4	-	0.5	-	4.60±1.78	-
4	-	0.7	-	3.40±1.17	-
4	-	0.9	-	3.00±1.25	-
5	-	0.1	-	4.20±1.03	-
5	-	0.3	-	4.10±1.37	-
5	-	**0.5**	-	**7.20±1.32**	-
5	-	0.7	-	3.30±1.16	-
5	-	0.9	-	3.50±1.08	-
6	-	0.1	-	2.90±1.20	-
6	-	0.3	-	3.40±1.43	-
6	-	0.5	-	6.10±1.37	-
6	-	0.7	-	5.10±1.37	-
6	-	0.9	-	3.90±0.99	-
5	0.3	0.3	-	8.70±1.77	-
5	**0.5**	**0.5**	-	**15.90±1.66**	-
5	0.7	0.7	-	10.50±1.43	-
2	-	-	-	-	1.33±0.52
3	-	-	-	-	2.33±0.52
4	-	-	-	-	4.17±0.98
5	-	-	-	-	**8.83±0.75**
6	-	-	-	-	4.33±1.21
7	-	-	-	-	3.83±0.75
8	-	-	-	-	3.33±1.21

The value of combination consisted of mean ± S.D. of 10 replicates.
The F-value is significantly different when $p < 0.05$.

The knowledge of endogenous hormonal levels in explant helps to minimize the number of attempts required to design the best suitable hormonal combination for multiple shoot culture induction (Mercier *et al*., 2003). The endogenous plant hormones are involved in the regulation of cell growth and development (White and Rivin, 2000) and their concentration varies from cell to cell and from tissue to tissue (Gokani *et al*., 1998). The reports also suggests that cell growth regulation may control by balance or ratio between hormones (White *et al*., 2000). The uptake of exogenous hormones from the medium represented an essential requirement, causing an increase of endogenous hormonal levels, which appear to be involved in the shoot organogenesis process (Mercier *et al*., 2003).

In the present study, shoot tip initiation growth was achieved on the media consists of MS basal nutrients supplemented with growth hormones at the range of 1.0-10.0 mg/l BAP (Table 2) resulted initiation of shoot tip was noticed after 15 days of culture. The initiation of shoot

tip growth was optimized at the concentration of 5 mg/l BAP with a frequency of 93.33% and the shoot tip explants were survived without phenolic oxidation. They remained green and initiation of shoot bud was noticed within 10 days of incubation (Fig. 1A). The lowest response (6.66%) was noticed at the concentration of 10 mg/l BAP. The results of the present study indicate that the 5mg/l BAP was the optimal level for efficient for shoot proliferation in *Musa paradisiaca* cv. Karibale Monthan.

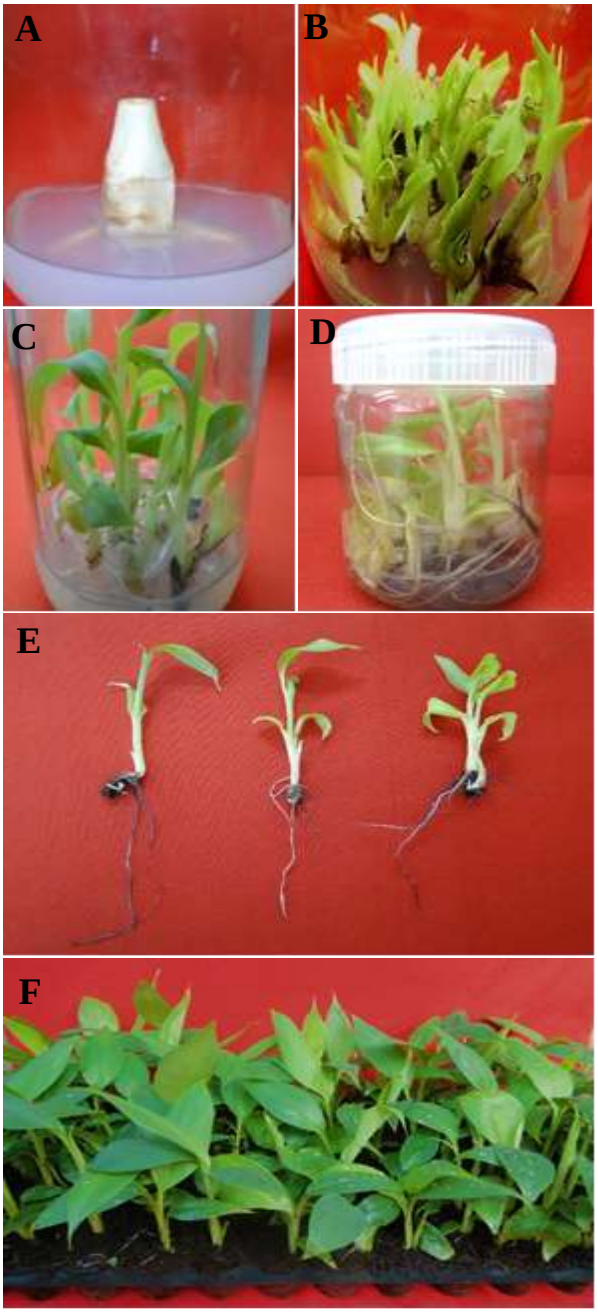

Fig.1: A. Inoculated banana apical disc on MS + 5mg/l BAP
B. Sprouting of multiple shoots from the exercised shoot meristem on MS + 0.5mg/l BAP+0.5mg/l TDZ+0.5ml/l Coconut water.
C. Elongation of multiple shoots on MS +BAP 5mg/l
D. Rhizogenesis from the base of the shoots on MS + IBA 100mg/l+200mg activated charcoal
E. Complete plantlet with distinct shoot and root formation
F. Primary hardening of *in vitro* regenerants in cocopeat

Subculturing of shoots was carried out on the MS medium supplemented with combinations of cytokinins to induce multiple shoots. After 2-3 weeks of subculture, axillary buds showed threefold increase in multiplication rate with well-defined tiny shoots. At the interval of 15 days resulted the increase in the rate of multiplication of shoots. To induce high frequency of multiplication rate, vertically cross-excised propagules were subcultured on the MS medium fortified with 4 to 6 mg/l BAP, 0.1 to 1.2 mg/l TDZ and coconut water 0.1 to 0.9 ml/l. The results showed that the multiple shoot formation occurred in all the combinations of growth regulators tested, and there were significant differences in the number and quality of shoots formed under each treatment. A frequency of 15.90±1.66 multiple shoots per explants (Fig. 1B) noticed at the concentration of 5 mg/l BAP with TDZ 0.5mg/l and coconut water 0.5ml/l. In contrast, 5 mg/l of BAP and 0.5mg/l of TDZ induces the 11.20±1.55 shoots per explants whereas 5mg/l BAP and 0.5 ml/l coconut water induced 7.20±1.32 multiple shoots/ per explant respectively.

The results revealed that, the combination of coconut water or TDZ alone with BAP did not enhance axillary shoot production when compared with combination of BAP, TDZ and coconut water. 5mg/l BAP, 0.5mg/l TDZ and 0.5ml/l CW was considered as optimal concentration for high frequency shoot multiplication. The results indicated that nutritional compositions present in the coconut water along with cytokinin activity (Kuraishi and Okumura, 1961; Richter *et al*., 2005) was found to be beneficial for inducing more number of shoots and to maintain culture quality for longer period when augmented to the media along with BAP. Similarly, Sanada mondal *et al*., (2012) reported that MS basal media supplemented with BAP along with coconut water increased the frequency of shoot regeneration and shoot length for the cultivar Dwarf Cavendish. Our results also showed that, TDZ along with BAP found to be more effective in small concentration to increase the multiplication rate by producing more and better quality shoots. But increase in TDZ concentration (upto 1.5 mg/l TDZ) decreased the caulogenic potency of the propagules. Arinaitwe *et al*., (2000) also reported the similar suppressive effect of TDZ in shoot proliferation at higher concentrations for the banana cultivars, 'Kibuzi' and 'Bwara'. In banana genotype cultures many investigators observed TDZ as an effective cytokine for inducing shoot bud proliferation (Thomas and Katterman, 1986; Huetteman and Preece, 1993).The multiple shoots were transferred to the shoot elongation medium augmented with 2-8mg/l BAP and the proliferation was highest at the concentration of 5mg/l BAP with a mean length of shoots 8.83±0.75 cm/propagule (Fig. 1C).

Table 3: Effects of different concentrations of IBA, IAA and NAA on root induction in *in vitro* regenerated shoots.

Auxin	Conc.(mg/ml)	Activated Charcoal mg/l	% of Shoot rooted	No of roots/Shoot	Root length(cm)
	0.5	100	23.33	2.50±0.55	4.50±1.05
IBA	**1.0**	200	**86.66**	**5.33±1.21**	**7.50±1.87**
	1.5	300.	33.33	3.00±0.63	4.83±1.17
	0.5	100	30.00	1.83±0.75	2.67±0.52
IAA	1.0	200	33.33	3.83±0.75	4.33±0.82
	1.5	300.	26.66	2.50±0.55	3.67±0.82
	0.5	100	23.33	1.17±0.41	2.17±0.75
NAA	1.0	200	36.66	2.50±0.55	3.83±1.33
	1.5	300.	20.00	1.50±0.55	4.67±1.21

Data were collected after 5 weeks of culture. Average values of 10 replicates and std. deviation (±).

Table 4: Morpho-agronomic characteristics of sucker derived *in vitro* regenerants of banana in the farmyard**.**

Planting material	Height of the plants (in ft)	No. of leaves	Length of the leaves (in cm)	Width of the leaves (in cm)	Length of the bunch (in cm)	Weight of the bunch (kg)	No. of hands in a bunch	No. of fingers in a hand	Length of the finger (in cm)	Girth of the finger (in cm)	Girth of the pseudo stem (in cm)
In vitro	**13.80±2.17**	**13.40±1.14**	**344.52±3.85**	**53.00±1.58**	**56.20±2.17**	**14.60±1.14**	**11.40±1.14**	**19.20±1.30**	**15.28±0.30**	**4.36±0.34**	**49.80±1.48**
In vivo	**12.84±1.85**	**12.20±1.92**	**339.32±2.46**	**44.20±1.48**	**55.20±3.03**	**13.40±0.89**	**9.80±1.30**	**18.60±1.67**	**14.60±0.43**	**4.19±0.12**	**44.40±1.14**

The value of each concentration consisted of mean ± S.D of 5 replicates.

Well-developed shoots were transferred to rooting medium with various concentrations of auxins such IBA, IAA and NAA. Among the concentrations tested, MS+1.0 mg/l IBA+200 mg activated charcoal 86.66% of shoots rooted with an average root of 5.33±1.21 per plants and a length of 7.50±1.87 roots per explant (Table 3). Plantlets with distinct roots and shoots were transferred to the plastic pots (Fig. 1D & 1E). After primary and secondary hardening, the 92 % of plantlets were survived in green house (Fig. 1F) and the well grown disease free plants were finally transferred to the field.

The success of micropropagation is achieved when the *in vitro* raised plants shows superior yield and growth characteristics than the sucker derived plants at field condition. Hence, morphoagronomical characters of *in vitro* raised plants were evaluated at standing crop stage in comparison with the *in vivo* plants (Fig. 2). The results of the study revealed that during vegetative growth, the height of the tissue cultured plants is higher (13.80±2.17) when compared to the sucker derived plants (12.84±1.85). Tissue cultured plants showed significantly higher values in the leaf growth such as the leaf length, leaf width and number of leaves when compared to the *in vivo* plants at standing crop stage. Alvarez (1997) reported that the high yield of the banana plant depends on the more number of leaves at harvest time. Similarly, more number of functional leaves (13.40±1.14) with significant increase in leaf length (344.52±3.85) and leaf width (53.00±1.58) were observed in *in vitro* plant. The yield of *in vitro* plants were compared with the *in vivo* plants which showed increase in bunch weight (14.60±1.14), number of hands (11.40±1.14), number of fingers in a hand (19.20±1.30) and length of the finger (15.28±0.30). The *in vitro* raised plants showed increased pseudostem girth (49.80±1.48) in field condition. The results were summarized in Table 4.

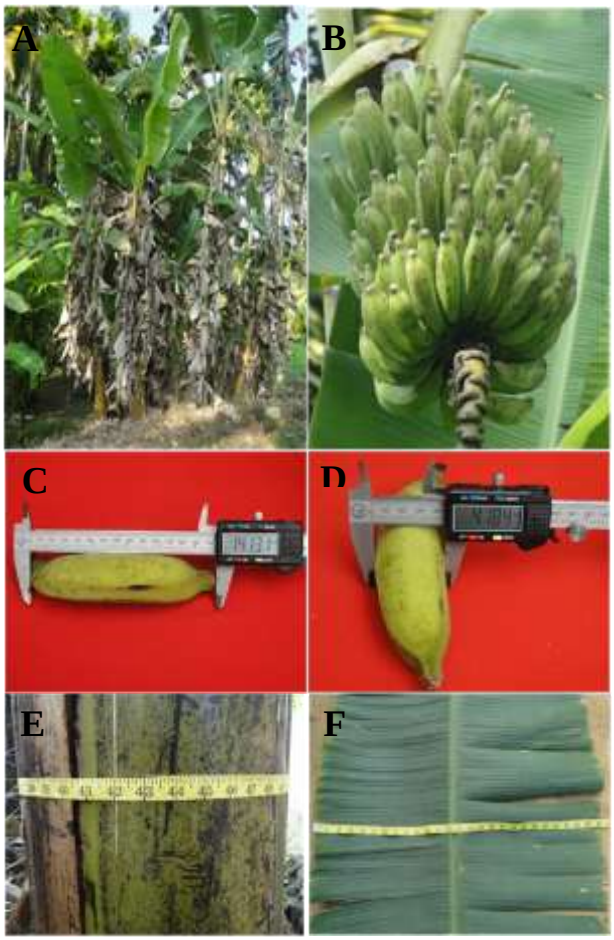

Fig. 2: (A) Sucker derived *in vitro* regenerant Karibale plant at standing crop stage. (B) Yield of the Sucker derived *in vitro* plant at field condition. (C), (D), (E) and (F) Evaluation of yield and growth parameters of *in vitro* regenerants.

The superior growth of the tissue-cultured plants observed could be accredited with well grown root and shoot systems in *in vitro* raised plants which helps them to uptake the nutrients and assimilation immediately after planting. The nutrient supplied during regeneration of *in vitro* plants may show carry over effect upon which they could depend while, the suckers lack root system due to paring in conventional method of planting which takes few weeks to recover and they have to depend on the stored food in the corm for initial growth (Robinson *et al*., 1993; Dzomeku *et al*., 2012). Many investigators reported the superior growth and yield of the *in vitro* regenerants compared to *in vivo* plants (Robinson, *et al*., 1993; John Nelson Buah, *et al*., 2000; Msogoya, 2006; Shaileas Vasane, *et al*., 2010).

Conclusion

Musa paradisiaca cv. Karibale Monthan known for its glomerular protection activity and there is an increasing demand for the fruits of this cultivar. Standardization of high frequency regeneration protocol of this endemic banana cultivar resulted higher rate of multiplication with 99% survival of plantlets in the green house. The subsequent evaluation of field performance of *in vitro* plants showed superior agronomical characters than the *in vivo* plants. To conclude, the result of this investigation, is applicable for mass multiplication and production of superior planting material which contributes significantly in crop performance.

Acknowledgements

The authors are grateful to the Registrar Kuvempu University and the Chairman, Department of Biotechnology and Bioinformatics, Kuvempu University for accessing the laboratory assistance and providing necessary facilities.

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