Research Article

Effect of different phytohormones on micropropagation of banana (*Musa sp.*) cultivars and their assessment through RAPD

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Abstract

A research was conducted to study the multiplication rates of banana shoot tips derived from different suckers under *in vitro* conditions during successive sub-culture of FHIA-23, KM5 and Basari and assessment of rapid amplified polymorphic DNA (RAPD) techniques of banana cultivars through molecular marker. Shoot tips of banana varieties were cultured on MS medium supplemented with different concentrations of Benzaldehyde alkaline phosphate (BAP). MS ½ with Indole butyric acid (IBA) 1 mg L⁻¹ + 5.0 g, 10 g, 20 g, 30 g and 40 g sugar L⁻¹ for root length, number of root and root weight were used for root induction. The results revealed that the best shoot initiation was observed in Basari using MS medium containing BAP 4 mg L⁻¹. Maximum shoot length was observed in KM5 under the concentration of BAP 3.0 mg L⁻¹. Maximum numbers of shoots and shoot weight were observed in KM5 followed by FHIA-23 and Basari variety. Maximum root length was observed in KM5 followed by FHIA-23 under the concentration of sugar 30 g L⁻¹ + IBA 1.0 mg L⁻¹. Maximum numbers of roots were observed in KM5, followed by FHIA-23 and Basari variety under concentration of 30 g L⁻¹ + IBA 1.0 mg L⁻¹. It was concluded that the KM5 variety was superior, followed by FHIA-23 and Basari. The concentration of 3.0 to 4.0 mg L⁻¹ of BAP were found better for shooting and 1.0 mg L⁻¹ of IBA + 30 and 40 g L⁻¹ sugars for root induction of banana micropropagation.

Keywords: Banana; BAP; DNA quantification; IBA; Micropropagation; Phytohormones; RAP

Introduction

Banana (*Musa sp.*) is an important fruit crop cultivated in Pakistan. The total cultivated area in Pakistan is about 348.0 thousand hectares with 154.8 thousand tons production annually. [1]. Composition of edible portion of the fruit is 74.8% water, 1.2% proteins, 0.2% fats, 0.84% ash, 0.6% fiber, 19.2% sugar as invert, 445 calories per pound and 0.39% malic acid [2]. It is rich in carbohydrates, calcium, phosphorus, and potassium. Blood pressure, ulcer and heat burn are also improved and controlled using banana [3]. Cavendish variety locally known as Basari, is particularly cultivated in Sindh, which in result holds about 98% of the plantation. Banana is of major importance to food security which as well provides a valued source of income. Banana tissue culture propagation has gained attention because of their ability to provide uniform genetically and disease-free planting materials. Rate of multiplication is a key factor by which the efficiency of the micro-propagation system is being affected. Israeli *et al.* [4]; Mendes *et al.* [5], observed and reported that multiplication rate of banana depends on the genotype, whereas cultures originated from same banana genotype cultured *in vitro*, have been observed with variable behaviour.

Banana's low yield and production in Pakistan is caused due to some biotic and abiotic reasons where the virus is included as one of the most important problems. Traditionally clonal propagation method seems to be incapable to meet the growing demand for disease-free materials of banana. Virus disease has greatly reduced the productivity of vegetatively propagated banana [6]. Banana production is being reduced by some internal and intracellular pathogens, where micropropagation under in-vitro is a possible way for the development of pathogen free plants [7]. Novak [8] have demonstrated banana tissue culture in liquid media through somatic embryogenesis. Whereas Muhammad *et al.* [9] also found that banana multiplication

rate depends on the genotype. An experiment was conducted for the standardization of *in vitro* culture technique for mass propagation of *Musa acuminata*, shoot tips were used as an explant and cultured on Murashige and Skoog (MS) medium. It was observed that highest results were obtained from MS medium supplemented with BAP + IAA at the concentration of 3.0 mg L⁻¹ and 0.5 mg L⁻¹ respectively [10]. Micro-propagation is the practice in which aseptic conditions are adapted using modern plant tissue culture methods to produce a large number of progeny plants by rapidly multiplying stock plant material. Propagation of banana through *in vitro* techniques using different explants has been observed by researchers [11]. *In vitro* propagation is the best alternative to overcome such limitations to produce disease free and sufficient amount of planting material [12]. Excessive supplementation of phytohormones increases the growth rate of plant biomass *in vivo* or *in vitro* to control plant growth and senescence [13, 14]. Currently, RAPD and simple sequence repeats (SSR) markers are effectively being applied for the detection of genetic similarities or dissimilarities in micropropagated material in a variety of plants [15, 16], which is also very simple, fast, cost-effective, highly discriminative and reliable [17].

The present research was conducted to study the multiplication rates of banana shoot tips derived from different suckers under *in vitro* conditions during successive sub-culture of FHIA-23, KM5 and Basari and assessment of RAPD techniques of banana cultivars through molecular marker. The study was carried out to meet the objectives to produce maximum micropropagulas with different phytohormones, extract DNA and assessment of banana clones through RAPD.

Materials and methods

The experiment was carried out at tissue culture laboratory, Plant Breeding and Genetics Division, Nuclear Institute of

Agriculture, Tandojam. The exotic clones of FHIA-23, KM5 and local Basari were used. Explants source were used from the top portion of the plant and six months age of sucker. Suckers of banana (*Musa spp.*) as explants material were selected from, experimental farm of Nuclear Institute of Agriculture (NIA), Tandojam and progressive farmers field near Tandojam locations. Meristematic stem tips were excised, ethanol (70%) was used for washing surface disinfected from microorganisms for one minute then were shacked in 10% commercial bleach for 20 minutes adding few drops of Tween-20. The experimental design laid out was Randomized Complete Block Design (RCBD) replicated thrice. Parameters including number of shoots, shoot length (cm), shoot weight (g), number of roots, root length (cm) and root weight (g) were determined.

Treatments

Shoot induction

Factor (A) = Banana cultivars (03)

V₁ = FHIA-23, V₂ = KM-5 and V₃ = Basari

Factor (B) = BAP concentrations (mg L⁻¹)

Concentrations = 0.0 mg L⁻¹, 0.5 mg L⁻¹, 1.0 mg L⁻¹, 2.0 mg L⁻¹, 3.0 mg L⁻¹, 4.0 mg L⁻¹, 5.0 mg L⁻¹ and 6 mg L⁻¹.

Root induction

Sugar concentrations (g + IBA mg L⁻¹)

Concentration = 5 + 1.0 mg L⁻¹, 10 + 1.0 mg L⁻¹, 20 + 1.0 mg L⁻¹, 30 + 1.0 mg L⁻¹ and 40 + 1.0 mg L⁻¹.

The initial media for explants were cultured on MS medium containing 10 µm BAP + 5 µm IAA along with Thiamine 2 mg L⁻¹ and Myo-inositol 100 gm L⁻¹. Multiplication mediums consisted MS salts with 0.0 mg L⁻¹, 0.5 mg L⁻¹, 1.0 mg L⁻¹, 2.0 mg L⁻¹, 3.0 mg L⁻¹, 4.0 mg L⁻¹, 5.0 mg L⁻¹ and 6.0 mg L⁻¹ of BAP. After four weeks the growth started. Sub culturing was done every fifth week and black tissues were eliminated prior to sub culturing. Number of shoots and roots were counted and weighed, which were observed after every subculture. After five subcultures, the shoots were shifted to rooting medium. Rooting medium MS½

containing 1.0 mg L⁻¹ IBA with five concentrations of sugars (0 mg L⁻¹ as control, 10 mg L⁻¹, 20 mg L⁻¹, 30 mg L⁻¹ and 40 mg L⁻¹). All cultures were supplemented with different sucrose and were maintained at 25±2°C with 16/8 h photoperiod (2000 lux light intensity).

Media preparation

To prepare 1liter media, initially 700 ml of distilled water was taken in 1liter beaker. Each chemical component was weighted accurately according to the required concentration and added in the beaker containing distilled water, which was then kept on magnet stirrer for complete mixing. After the process, the media was poured into bottles covered with caps. The medium was autoclaved at 121 °C, 15 psi for 20 minutes. After sterilization bottles were labelled with the name of the media.

Sterilization of the explants and culture

After removing the unnecessary portion of leaves, remaining distilled water was used for washing first, and then was sterilized with 70% ethanol for a minute and 10% sodium hypochlorite solution for 20 minutes. Explant material was rewashed with double distilled water two-three times after sterilization to remove any mark of disinfectant. Only the innermost leaves, which are supposed to be the infection free, were cut into 2-3 mm apical meristem. This apical meristem was cultured aseptically into the bottles of the media

Micropropagation

Four stages were adopted for micro-propagation for banana.

Stage 1: Plants were selected and maintained in a controlled environment. Explants were prepared, such as shoot tip or meristem and were transferred to a suitable culture medium.

Stage 2: In the second stage, the explants were induced to form multiple shoots, generally each explant produces five to six shoots within 4 to 5 weeks.

Stage 3: Multiple shoots produced in stage II were induced to produce rooting in a fresh nutrient medium. Rooted shoots were highly fragile and sensitive to moisture.

Stage 4: Hardening is a process by which rooted plantlets were prepared for soil conditions. The plants were acclimatized to face the field conditions. When plants were acclimatized over a period of time they were transferred to the field.

Molecular marker study in banana

Plant material

Three different banana cultivars were analyzed among them two were from exotic material (FHIA-23 (Exotic, AAAA), KM5 (Exotic, AAA), Basari (Local, AA), provided by Nuclear Institute of Agriculture (NIA), Tandojam. Following primers were utilized in this study; A-03 (AGGGGTCTTG), A-04 (AATCGGGCTG), A-17 (GACCGCTTGT).

Isolation of DNA

Young and fresh leaves from each banana were used for DNA extraction using MTAB method. Two hundred milligrams of fresh leaves were grounded in liquid nitrogen. The ground sample was placed in a 15 ml tube, 3 ml of the cell lysis solution was added and the tube was incubated at 65°C for one hour. Fifteen micro-liters of RNase solution were then added to the cell lysate and incubated at 37 °C. Protein precipitation solution after 30 minutes was added to a tube which was then vortexed for 20 seconds and then placed on ice for 30 minutes. Mixture was centrifuged at 2000 x g for 10 minutes. The supernatant containing DNA was poured into separate 15 ml centrifuge tube adding five millimeters of isopropanol, which was then precipitated by centrifuging at 2000 x g. The pellet was washed with 70% ethanol and the DNA samples were then hydrated with TE buffer. DNA was quantified on BIOMATE 3 (spectrophotometer).

Results and discussion

Days to initiation

The results indicated that cultivars, BAP concentrations and their interactions were highly significant at $P > 0.05$ probability level (Table 1). The results in (Table 2, Figure 1) shown for cultivars revealed that early days to initiation observed in KM5

was 16.06, FHIA-23 (17.58) and Basari variety (20.22). The results for BAP concentrations indicated that minimum early days to initiation (12.20) were observed under the concentration of BAP 4 mg L⁻¹, followed by concentration BAP 3 mg L⁻¹ (14.06) and control (24.76). The results indicated for their interactions that early days to initiation were observed in KM5 (10.16), followed by FHIA-23 (12.20) under the concentration of BAP 4 mg L⁻¹. The maximum days to initiation (27.46) were observed in Basari variety under control.

It was noted that early days to initiation were appeared when MS + 3.0 and 4.0 mg L⁻¹ BAP treatments were applied. The present findings are supported by Goshu *et al.* [18], who also reported that MS + 5 mg L⁻¹ BAP showed early days to initiation at BAP 4.0 and 3.0 mg L⁻¹, respectively.

Shoot length (cm)

The results indicated that cultivars, BAP concentrations and their interactions were highly significant at ($P > 0.05$) probability level (Table 1). The results for shoot length are shown in (Table 3, Figure 1) indicating that maximum shoot length (7.88 cm) was noticed in KM5 followed by FHIA-23 (7.34 cm), and minimum was noticed in Basari (6.60 cm) variety. The results for BAP concentrations indicated that maximum shoot length (9.89 cm) was in the concentration of BAP 4.0 mg L⁻¹ followed by concentration of BAP 3.0 mg L⁻¹ (9.77 cm), and minimum (3.43 cm) was observed in control. The results for their interactions showed that KM5 produced maximum shoot length (10.73 cm) under the concentration of BAP 3.0 mg L⁻¹, followed by concentration of BAP 4.0 mg L⁻¹ (10.64 cm) and minimum shoot length (2.98 cm) was observed in Basari variety under control. On overall, it was found that increasing the concentration of MS + BAP 4.0 mg L⁻¹ appeared to be better for shoot length. While further increase in concentrations of BAP did not prove to be feasible because shoot length was reduced when BAP concentration was increased.

The results are in line with Rahaman *et al.* [19]; Arinaitwe *et al.* [20], they also

observed a hard ball like structure being developed during shoot multiplication.

Table 1. Mean squares for various traits of Banana cultivars revealed by analysis of variance (ANOVA)

Sources of variation	Replication	cultivars	BAP	Cultivars *BAP	Error
	D.F=2	D.F=2	D.F=7	D.F=14	46
Days to initiation	2.060	106.282**	172.017**	2.544**	0.031
Shoot length	0.0042	9.8499**	45.7017**	0.2021**	0.0004
No. of shoot	0.0016	9.5886**	60.7688**	0.5791	0.0008
Shoot weight	0.0015	15.0733**	34.9071**	0.2814	0.0006
Sources of variation	Replication	cultivars	Sugar+IBA	Cultivars *Sugar+IBA	Error
	D.F=2	D.F=2	D.F=7	D.F=14	46
Root length	0.0001	1.4583**	14.9341**	0.0704**	0.0005
No. of root	0.0016	10.0636**	12.6966**	0.7394**	0.0004
Root weight	0.0009	5.4587**	16.7549**	0.1354**	0.0006

**= Significant at $P < 0.01$

Table 2. Effect of different concentrations of 6-benzylaminopurine (BAP) on days to initiation of banana cultivars

MS + BAP concentrations (mg L ⁻¹)	Number of explants	Cultivars			Mean
		FHIA-23	KM5	Basari	
0.0	10	24.43 b	22.40 d	27.46 a	24.76 a
0.5	10	14.43 l	13.33 m	20.30 f	16.02 f
1.0	10	16.43 j	14.30 l	18.23 h	16.32 e
2.0	10	17.33 i	15.30 k	17.43 i	16.68 d
3.0	10	13.26 m	12.40 n	16.53 j	14.06 g
4.0	10	12.20 n	10.16 o	14.23 l	12.20 h
5.0	10	20.23 f	19.26 g	23.23 c	20.91 c
6.0	10	22.33 d	21.36 e	24.36 b	22.68 b
Mean		17.58 b	16.06 c	20.22 a

Figure 1. Shoots of banana cultivar FHIA-23, KM5 and Basari as affected by different concentrations of 6-benzylaminopurine (BAP)

Number of shoots

The results for number of shoots in banana obtained from micropropagation were proliferated under various concentrations of

MS + BAP, their interactions were highly significant at $P > 0.05$ probability level (Table 1). Maximum number of shoots (6.41) were observed in KM5, followed by

(5.33) in FHIA-23 where minimum with 5.32 were observed in Basari (Table 4, Figure 1). The results under various concentrations of BAP indicated that maximum number of shoots (9.58) were observed under the concentration of BAP 4.0 mg L⁻¹, followed by the concentrations BAP 3.0 mg L⁻¹ (8.61) and control (2.49), respectively. The results indicated for their interactions were maximum (10.86) under the concentration of BAP 4.0 mg L⁻¹, followed by (9.26) in KM5 under the concentration of BAP 3.0 mg L⁻¹ and minimum number of shoots (2.13) were observed in Basari variety under control. Current results are supported by Etienne and Berthouly [21], where they observed the highest number of shoots per explants were with 5.0 mg L⁻¹ of BAP, the variation might possibly due to the different concentrations of BAP (cytokinin) being used Muhammad *et al.* [22].

Shoot weight (g)

The results for shoot weight of banana obtained from micropropagation under various concentrations of MS + BAP were significant at ($P > 0.05$) probability level (Table 1). The results in (Table 5, Figure 1) shows that maximum shoot weight with 7.52 g was observed in KM5, followed by (6.59 g) in FHIA-23 and minimum (5.32 g) in Basari variety. The results under various concentrations of BAP indicated maximum shoot weight (9.16 g) under the concentration of BAP 4.0 mg L⁻¹, followed by (8.57 g) under the concentration of BAP 3.0 mg L⁻¹ and minimum (3.49 g) was observed in control. The results for their interactions showed that maximum shoot weight (10.26 g) was observed in KM5, followed by FHIA-23 (9.34 g) under the

concentration of BAP 4.0 mg L⁻¹ and minimum shoot weight (2.95 g) was recorded in Basari variety under control. The overall result showed that MS + BAP concentrations of 3.0 and 4.0 mg L⁻¹ proved to be excellent for shoot weight in KM5 variety. Similar results have also been reported by Kadota and Niimi [23], who reported that MS medium supplemented with 25 µM BAP treatments had prolonged the leaves in banana.

Root length (cm)

Results for differences in the length of root between the various concentrations of sugar (0 + 05 mg L⁻¹, 1.0 + 10 mg L⁻¹, 1.0 + 20 mg L⁻¹, 1.0 + 30 mg L⁻¹ and 1.0 + 40 mg L⁻¹) + IBA were highly significant ($P > 0.05$) at probability level (Table 1). Maximum root length with 5.49 cm was observed in KM5, followed by FHIA-23 (5.26 cm) and minimum (4.87 cm) was observed in Basari variety (Table 6, Figure 2). For sugar concentrations + IBA, the results indicated that maximum with 6.41 cm was observed under the concentration of sugar 30 g L⁻¹ IBA 1.0, followed by 6.14 cm under the concentration of sugar 40 g L⁻¹ + IBA 1.0 whereas minimum (3.25 cm) was observed in control. For their interactions, maximum root length (6.84 cm) was observed in KM5, followed by (6.44 cm) in FHIA-23 under the concentration of sugar 30 g L⁻¹ + IBA 1.0 and minimum root length (3.04 cm) in Basari variety under control. The overall average showed that best root length was recorded under MS^{1/2} + sugar 30 g L⁻¹ + IBA 1.0. The results are supported by Molla *et al.* [24] where they observed 2.60-5.67 cm root length in 0.5 mg L⁻¹ IBA. Habiba *et al.* [25] also got more or less same observation during the experiments.

Table 3. Effect of different concentrations of 6-benzylaminopurine (BAP) on shoot length (cm) of banana cultivars

MS + BAP concentrations (mg L ⁻¹)	Number of explants	Cultivars			Mean
		FHIA-23	KM5	Basari	
0.0	10	3.34 t	3.98 s	2.98 u	3.43 h
0.5	10	5.25 q	5.98 p	4.86 r	5.36 g
1.0	10	7.43 l	7.86 k	6.45 m	7.25 e

2.0	10	8.48 i	9.18 e	7.87 k	8.51 c
3.0	10	9.53 d	10.73 a	9.06 f	9.77 b
4.0	10	10.16 c	10.64 b	8.87 g	9.89 a
5.0	10	8.15 j	8.58 h	7.46 l	8.06 d
6.0	10	6.35 n	6.06 o	5.26 q	5.89 f
Mean		7.34 b	7.88 a	6.60 c	

Table 4. Effect of different concentrations of 6-benzylaminopurine (BAP) on number of shoots of banana cultivars

MS + BAP concentrations (mg L ⁻¹)	Number of explants	Cultivars			Mean
		FHIA-23	KM5	Basari	
0.0	10	2.27 u	3.07 t	2.13 v	2.49 h
0.5	10	3.37 q	3.85 p	3.27 r	3.49 f
1.0	10	4.26 o	5.66 k	5.16 m	5.02 e
2.0	10	6.35 i	8.47 f	7.46 h	7.42 c
3.0	10	8.53 e	9.26 b	8.06 g	8.61 b
4.0	10	9.04 c	10.86 a	8.85 d	9.58 a
5.0	10	5.35 l	6.28 j	4.45 n	5.36 d
6.0	10	3.37 q	3.86 p	3.17 s	3.47 g
Mean		5.33 b	6.41 a	5.32 b	

Table 5. Effect of different concentrations of 6-benzylaminopurine (BAP) on shoot weight (g) of banana cultivars

MS + BAP concentrations (mg L ⁻¹)	Number of explants	Cultivars			Mean
		FHIA-23	KM5	Basari	
0.0	10	3.55 u	3.96 t	2.95 v	3.49 h
0.5	10	4.23 s	5.06 r	3.96 t	4.42 g
1.0	10	6.66 m	7.55 j	6.24 o	6.82 e
2.0	10	7.26 k	8.64 d	6.86 l	7.58 c
3.0	10	8.41 f	9.25 c	8.05 g	8.57 b
4.0	10	9.34 b	10.26 a	7.87 h	9.16 a
5.0	10	7.66 i	8.56 e	6.35 n	7.52 d
6.0	10	5.65 p	6.84 l	5.24 q	5.91 f
Mean		6.59 b	7.52 a	5.94 c	

Table 6. Effect of different concentrations of sugar g L⁻¹ + Indole-3-butyric acid (IBA) on root length (cm) of banana cultivars

MS ^{1/2} + Sugar concentrations g L ⁻¹ + IBA mg L ⁻¹	Number of explants	Cultivars			Mean
		FHIA-23	KM5	Basari	
5 + 1.0	10	3.25 m	3.47 l	3.04 n	3.25 e
10 + 1.0	10	4.74 j	4.94 i	4.23 k	4.64 d
20 + 1.0	10	5.75 g	5.96 f	5.12 h	5.61 c
30 + 1.0	10	6.44 b	6.84 a	5.95 f	6.41 a
40 + 1.0	10	6.15 d	6.25 c	6.03 e	6.14 b
Mean		5.26 b	5.49 a	4.87 c

Figure 2. Roots of banana cultivar FHIA-23 KM5 and Basari as affected by different concentrations of Sugar + indole-3-butyric acid (IBA)

Number of roots

The results of number of roots in banana obtained from micropropagation were proliferated for further study under various concentrations of Sugar (0 + 05, 1.0 + 10, 1.0 + 20, 1.0 + 30 and 1.0 + 40 g) + IBA, their interactions were highly significant ($P > 0.05$) at probability level (Table 1). Table 7 represents the results, where maximum number of roots (8.21) were observed in KM5, followed by (7.12) in FHIA-23 and minimum (6.60) in Basari variety (Fig.2). The results under various concentrations of Sugar + IBA indicated that maximum number of roots (8.58) were observed under the concentration of sugar 30 g L⁻¹ + IBA 1.0, followed by (7.91) under the concentration of sugar 10 g L⁻¹ + IBA 1.0 and minimum with 7.72 under the concentration of sugar 40 g L⁻¹ + IBA 1.0. The results for their interactions indicated maximum number of roots (9.84) were observed under the concentration of sugar 30 g L⁻¹ + IBA 1.0, followed by (8.83) in KM5 under the concentration of IBA 1.0 + sugar 10 g L⁻¹ and minimum number of roots (5.26) were observed in Basari variety under control. The overall average showed that best root length was recorded under MS^{1/2} + sugar 30 g L⁻¹ + IBA 1.0. The present results are similar to the findings of Gubbuk and Pekmezci [26]; Rahman *et al.* [19].

Root weight (g)

The results indicated that root weight of banana obtained from micropropagation under various concentrations of Sugar (0 + 05, 1.0 + 10, 1.0 + 20, 1.0 + 30 and 1.0 + 40 g) + IBA were significantly at ($P > 0.05$) probability level in (Table 1). The results in (Table 8, Figure 2) displays that maximum root weight with 7.07 g which was observed in KM5, followed by 6.53 g in FHIA-23 and minimum with 5.86 g in Basari variety. The results showed that maximum root weight with 7.75 g was observed under the concentration of sugar 30 g L⁻¹ + IBA 1.0, followed by (7.43 g) under the concentration of sugar 20 g L⁻¹ + IBA 1.0 and minimum root weight (4.58 g) in control. The results for their interactions showed that maximum root weight with 8.55 g was observed in KM5 under the concentration of sugar 30 g L⁻¹ + IBA 1.0 and minimum root weight with 4.06 g in Basari variety under control. Rahman *et al.* [27] in their study indicated that highest root weight was achieved under sugar 30 g L⁻¹ + 1.0 mg L⁻¹ IBA. This may be due to the genotype of their cultivar and the relatively low concentration of IBA used. In our study, it was evident that sugar 30 g L⁻¹ + 1.0 mg L⁻¹ IBA can increase the average root weight. This may have a positive influence on root weight Al-Amin *et al.* [28].

DNA quantification of banana by spectrophotometer

Extracted DNA was quantified by biomate spectrophotometer at 260/280. DNA absorbs ultraviolet light, with an absorption peak at 260 nm wavelength. A sample is exposed to the ultraviolet light spectrophotometer and a photo-detector measures the light that passes through the sample. The more absorbed by the sample, the higher the nucleic acid concentration in the sample. The protein concentration was determined at the 280 nm. The 260/280

ratio reflects the purity of DNA. Usually the ratio between 1.2-1.8 is considered to be the best for PCR amplification. The results of the Banana DNA extraction used in experiment are as follows:

High molecular weight DNA was extracted from banana clones through MATAB method. Extracted DNA of 7 banana clones were quantified by spectrophotometer at 260/280nm. The highest DNA ratio (1.03) was observed in Basari-3 and highest DNA concentration (2724 ng/ul) was in the FHIA-23 (Table 9).

Table 7. Effect of different concentrations of sugar g L⁻¹ + Indole-3-butyric acid (IBA) on number of roots of banana cultivars

MS ¹ / ₂ + Sugar concentrations g L ⁻¹ + IBA mg L ⁻¹	Number of explants	Cultivars			Mean
		FHIA-23	KM5	Basari	
5 + 1.0	10	4.54 n	6.67 k	5.26 m	5.49 e
10 + 1.0	10	7.75 f	8.83 b	7.14 i	7.91 b
20 + 1.0	10	6.84 j	7.64 g	6.14 l	6.87 d
30 + 1.0	10	8.64 c	9.84 a	7.25 h	8.58 a
40 + 1.0	10	7.85 e	8.07 d	7.24 h	7.72 c
Mean		7.12 b	8.21 a	6.60 c	

Table 8. Effect of different concentrations of sugar g L⁻¹ + Indole-3-butyric acid (IBA) on root weight (g) of banana cultivars

MS ¹ / ₂ + Sugar concentrations g L ⁻¹ + IBA mg L ⁻¹	Number of explants	Cultivars			Mean
		FHIA-23	KM5	Basari	
5 + 1.0	10	4.64 m	5.04 l	4.06 n	4.58 e
10 + 1.0	10	5.66 k	5.94 j	5.02 l	5.54 d
20 + 1.0	10	7.27 f	8.06 b	6.96 h	7.43 b
30 + 1.0	10	7.65 d	8.55 a	7.05 g	7.75 a
40 + 1.0	10	7.44 e	7.75 c	6.24 i	7.14 c
Mean		6.53 b	7.07 a	5.86 c	

Table 9. DNA Quantification of Banana by Spectrophotometer

Samples/Cultivars	DNA Ratio	DNA mg/ml	Protein mg/ml
Basari-1	0.86	51.94	2576
FHIA-23	0.99	62.21	2045
FHIA-23	0.85	51.92	2724
KM5-1	0.87	54.05	2593
Basari-2	1.001	51.91	1533
Basari-3	1.03	25.89	678.9
KM5-2	0.885	56.02	25.08

Conclusion

It was concluded that the days to initiation, number of shoots, shoot length and shoot

weight were better under the concentration of 4.0 mg L⁻¹, followed by 3.0 mg L⁻¹. The results indicated that the root length,

number of roots and roots weight were better under the concentration of 30 g sugar L⁻¹ + IBA 1.0 mg L⁻¹. The BAP under the concentrations of 4.0 to 3.0 and 30 g sugar L⁻¹ and IBA 1.0 mg L⁻¹ should be applied to achieve beneficial results in tissue culture technique.

Authors' contributions

Conceived and designed the experiments: AA Keerio & GS Mangrio, Performed the experiments: AA Keerio & AA Keerio, Analyzed the data: MI Keerio, NS Soomro, GS Nizamani, SA Soomro, A Khatri, FD Soomro, AA Sheikh & Hakim, Contributed reagents/ materials/ analysis tools: MI Keerio, NS Soomro, GS Nizamani, A Khatri, FD Soomro, AA Sheikh & Hakim, Wrote the paper: AA Keerio, GS Mangrio & SA Soomro.

References

1. GOP (2012). Economic Survey of Pakistan. 2011-2012. Ministry of Food, Agriculture and Livestock, Government of Pakistan, Statistics Division (Economic Wing), Islamabad, pp 21-22.
2. FAO, 2012. Food and Agriculture Organization Production Year Book. Food Outlook. pp 10-11.
3. USDA 2012. [www//nutrition/usda/bananas?portionid=58486&portionamount=500](http://www.nutrition/usda/bananas?portionid=58486&portionamount=500).
4. Israeli BA, Lichtenstein R, Lantz P, Mcgranaghan R, Allen A., Guzman JRA, Softley DD & Maciak B (2001). The Detroit Community - Academic Urban Research Center: Development, Implementation and Evaluation. *J Public Heal Manag Pract* 7(5): 1-19.
5. Mendes BMJ, Mendes FJ, Neto AT, Demetrio CGB & Puske OR (1996). Efficacy of banana plantlet production by micropropagation. *Pesqui Agropecu Bras* 31(12): 863-867.
6. Lepoivre P (2000). Banana in vitro regeneration: Virus eradication. Laboratory of Plant Pathology, University of Gembloux, Belgium, pp 22-24.
7. Ikram-ul-Haq, Gill NP, Ali S, Khaskheli NS & Ghaffar A (2017). Influences of phytohormones in plant nutrient cultures of in-vitro multiplying banana plantlets alters the cellular biocontents. *Int J Biosci* 11(5): 301-308.
8. Novak FJ (1989). Musa (Bananas and Plantains). In: Hammerschlag, F.A. and Litz., R.E. (eds), Biotechnology of Perennial Fruit Crops. CAB International, University Press, Cambridge. UK pp 124-129.
9. Muhammad A, Hussain I, Naqvi SMS & Rashid H (2005). Banana plantlet production through tissue culture. *Pak J Bot* 36(3): 617-620.
10. Anbazhagan M, Balachandran B & Arumugam K (2014). In vitro propagation of Musa acuminata (Banana). *Inter J Res Plant Sci* 4(1): 26-29.
11. Venkatachalam L, Thimmaraju R, Sreedhar RV & Bhagyalakshmi N (2006). Direct shoot and cormlet regeneration from leaf explants of 'Silk' banana (AAB). *Inter Vitro Cell Dev Biol Plant* 42(3): 262-269.
12. Belete G (2017). Review on In vitro propagation of sugarcane to advance the value of tissue culture. *Agri Res & Tech* 5(4): 1-7.
13. Bielach A, Hrtan M & Tognetti VB (2017). Plants under stress: Involvement of auxin and cytokinin. *Inter J Mol Sci* 18: 1-29.
14. Voesenek LACJ, Pierik R & Sasidharan R (2015). Plant Life without Ethylene. *Trend Plant Sci* 20(12): 783-786.
15. Ramage CM, Borda AM, Hamill SD & Smith MK (2004). A simplified PCR test for early detection of dwarf off-types in micropropagated Cavendish banana (Musa sp. AAA). *Scientia Horti* 103(1): 145-151.
16. Modgil M, Mahajan K, Chakrabarti SK, Sharma DR & Sobti RC (2005). Molecular analysis of genetic stability in micropropagated apple rootstock

- MM106. *Scientia Horti* 104(2): 151-160.
17. Pradeep Reddy M, Sarla N & Siddiq EA (2002). Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. *Euphytica* 128(1): 9-17.
 18. Goshu G, Singh H, Petersson KJ & Lundeheim N (2014). Heritability and correlation among first lactation traits in Holstein Friesian cows at Holeta Bull Dam Station, Ethiopia. *Inter J Anim Prod* 5(3): 47-53
 19. Rahman MZ, Nasiruddin KM, Amin MA & Islam MN (2004). In vitro response and shoot multiplication of banana with BAP and NAA. *Asian J of Plant Sci* 3:406-409.
 20. Arinaitwe G, Rubaihayo PR & Magambo MJS (2000). Proliferation rate effects of cytokinins on banana (*Musa* sp.) cultivars. *Scientia Hort* 86(1): 13-21.
 21. Etienne H & Berthouly M (2002). Temporary immersion systems in plant propagation. *Plant Cell Tissue Organ Cult* 69(3): 215-231.
 22. Muhammad A, Hussain I, Saqlan naqvi SM & Rashid H (2004). Banana plantlet production through tissue culture. *Pak J Bot* 36(3): 617-620.
 23. Kadota M & Niimi Y (2003). Effects of cytokinin types and their concentrations on shoot proliferation and hyperhydricity on In vitro pear cultivar shoots. *Pl Cell Tissue Org Cult* 72(3): 261-265.
 24. Molla MMH, Dilafroza Khanam M, Khatun MM, Al-Amin M & Malek MA (2004). Invitro rooting and Exvitro plantlet establishment of BARI Banana-I (*Musa* sp.) as influenced by different concentrations of IBA (Indole 3-butyric acid). *Asian J Plant Sci* 3: 196-199
 25. Habiba U, Reza S, Saha ML, Khan MR & Hadiuzzaman S (2002). Endogenous bacterial contamination during in vitro culture of table banana: Identification and prevention. *Plant tissue Cult* 12(2): 117-124.
 26. Gubbuk H & Pekmezci M (2004). In vitro propagation of some new banana types (*Musa* sp.). *Turk J Agric For* 28(5): 355-361.
 27. Rahman M, Khatun A, Rahman MS & Rashid MA (2013). Antioxidant, antimicrobial and cytotoxic activities of *Vitis trifolia* Linn. *J Dhaka Int Univ* 1(1): 181-184.
 28. Al-Amin MD, Karim MR, Amin MR, Rahman S & Mamun ANM (2009). In vitro propagation of banana. *Bangladesh J Agri Res* 34(4): 269-278.