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In Vitro Micro Propagation of Banana Variety (Butuzua-AAA-) Using Shoot Tip Culture

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Abstract

Banana is an attractive giant monocotyledonous plant family Musaceae. Conventionally, bananas propagate vegetatively through suckers with several draw bakes. However, the use of tissue culture planting material is an effective method to produce a mass of uniform bunches free from pests and diseases. The objective of this study was to optimize an in vitro protocol for micro-propagation of an economically important variety of bananas i.e. Butuzua (AAA) using shoot tip explants. Well-developed suckers were used as a source of explants. The study revealed that the percentage of initiation was best in Murashige and Skoog (MS) medium supplemented with 1.5 mg/l 6-Benzyl Amino-Purine (BAP) giving a result of 88.89% for the Butuzua variety. Hormonal combinations, of Murashige and Skoog's medium, supplemented with 4.0 mg/l of BAP and 0.5 mg/l of Naphthalene acetic acid (NAA) revealed that the highest mean shoot number 4.8 ± 0.7a and maximum shoot length (5.4±0.87) was recorded on MS media combination with 5.0 mg/l BAP + 0.5mg/l NAA. The mean root numbers (8.66) and the mean root length (7.5) were achieved in half-strength MS media with 1.0 mg/l of Indol Butyric Acid (IBA). The highest survival rate of the plantlet-92%- was recorded in coco peat substrate in the hardening stage in the greenhouse. Hence, it is important to use this in vitro micro propagation method as the best road map to large-scale propagation trials of banana plants.

Keywords: Banana; Butuzua (AAA); In vitro propagation; MS medium; Plant Growth regulators explante

Introduction

Bananas are attractive perennial giant monocotyledonous plants belonging to the genus Musa, order Zingiberales family Musaceae. They are extraordinarily significant to human societies and widely grown fruit crops in the tropical and subtropical regions of the world (Darvari et al., 2010; Rahman et al., 2013). Banana is a large, herbaceous plant (3.5 – 9) mater in height with a pseudo stem composed of tightly packed leaves arranged in sheaths (Jones, 2000).Majority of banana cultivars are triploid varieties (2n = 3x = 33 chromosomes) (Valmayor et al., 2002). These triploid genotypes are completely sterile and develop their fruits vegetatively (Bushra et al., 2012) [1].

Today, banana is distributed widely in Africa from East Africa, Central Africa to West Africa. It is a staple food and a good source of income for several African countries especially East Africa including Ethiopia and Central Africa (Viljoen, 2010). Banana was cultivated in Ethiopia for several years; the major banana growing regions being the Southern Nations and nationalities, Oromia and Amhara regions (MOA, 2011) [2].

Banana production is significantly contributing to food security as well as livelihood opportunities in Ethiopia (Alemu, 2017). However; many biotic and abiotic factors are responsible for the low yield of bananas in Ethiopia (Seifu, 2003). Propagation of bananas through tissue culture is a reliable solution to this problem. Micro propagation is preferred over the conventional method of propagation in banana owing to its faster multiplication rate continued throughout the year irrespective of seasonal variation (Rahman et al., 2004). It gives uniformity in planting materials, production of disease-free planting materials, higher bunch weight, more fingers and less variability in fruit size and shape (Lalrinsanga and Vanlaldiki 2013) [3].

Murashige and Skoog (1962) medium supplemented with different Growth regulators such as auxin, cytokinin, gibberellin, and abscisic acid has been used for the in vitro regeneration of various plants (Ali et al., 2015; Momena et al., 2014). Cytokinins for shoot formation and growth of buds, auxins for root formation mostly important in in-vitro culture media (North et al., 2012). The effectiveness of BAP over other cytokinins in inducing multiplication of shoot tip cultures has been reported in different banana cultivars (Buah et al., 2010; Farahani et al., 2008). Auxins such as Naphthalene acetic acid (NAA) have been reported to promote plant rooting in vitro (Hussein, 2012). In the past few years protocols have been standardized for in vitro propagation of a wide range of Musa species and cultivars (Sathiamoorthy. et al., 1998). However, no in vitro propagation protocol for the economically important banana cultivars known as Butuzua (AAA) and hence this study was initiated to develop a suitable in vitro micro propagation protocol for mass multiplication of this cultivar by using shoot tip cultures [4].

Materials and Methods

This experiment was carried out at Tigray Biotechnology Center formerly known as Mekelle plant tissue culture laboratory Pvt. Ltd. Co. Mekelle is the capital city of Tigray region, located in the Northern high lands of Ethiopia, 780 km north of Addis Ababa and 200km southeast of the historic city of Aksum [5].

Experimental design

In this study, MS medium (Murashige and Skoog, 1961) with BAP

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in the concentration of 0.0, 0.5, 1 and 1.5 was used for shoot initiation, (MS+BAP combination with NAA) (0.0, 1.0, 2.0, 3.0, 4.0, 5.0 BAP combined with 0.5 NAA) also used for shoot multiplication as well as rooting (half MS with 0.0, 0.5, 1.0, 1.2, 2.0, 2, 5 IBA). The experimental design employed was Completely Randomized Design (CRD) in three replications each.

Mother plant selection

The corms of the banana variety Butuzua, at six months of age were collected from the agricultural research center of Melkassa, Ethiopia. The mother plants were cleaned by washing with tap water, dipping in 50°C hot water for 10 minutes, and treating it with fungicidal and bactericidal chemicals of 0.25g/L redomil kocide and byliton. They were ex vitro cultivated under controlled environment to minimize contamination and enhance success in the in vitro culture. Corms were cultivated using coco peat in green house for two month by regular watering to develop a new disease free sucker (shoot tip) explants [6] (Figure 1).

Explants preparation and surface sterilization

The superfluous corm tissue, roots, and leaf sheaths were trimmed and removed from the pseudo stem. The shoot tips embedded inside the pseudo stems (2.0 – 3.0 cm) were used as an explant. These explants were taken to the laboratory for surface sterilization. Briefly, the explants were washed thoroughly under running tap water for 10 minutes to remove all unwanted particles. Explants were immersed in 0.25% redomil, byliton, and kocide solution mixed with Tween 20 for 20 minutes [7]. The explant was removed from the chemical and washed three times each with double distilled water for five minutes to remove the chemical. After this, the explants were treated with 10% (v/v) sodium hypochlorite solution for 15 minutes and they were washed three times with sterile distilled water.

Further sterilization was carried out in a laminar air flow chamber by using 0.1%HgCl2. The explant was immersed in 0.1 HgCl2 for 10 minutes and rinsed three times with sterile distilled water for five minutes in a laminar air flow cabinet before inoculation [8] (Figure 2).

Culture media preparation

An MS medium (Murashige and Skoog 1962) was used throughout the micro propagation study. The stock solutions of macronutrients, micronutrients, and vitamins, and other organic supplements were prepared separately. An appropriate amount of each nutrient was weighed and dissolved in double-distilled water in such a way that one nutrient was added after the first one is completely dissolved [9]. The plant growth regulators used for this study were prepared separately

Figure 1: Planting sterile mother plant in cocopeat.

Figure 2: Explant preparation from the developed sucker.

and a combination of cytokines such as 6-Benzyl Amino-Purine (BAP) for shoot induction and BAP and NAA for shoot multiplication and the auxin hormone, Indole Butric Acid for root growth induction and 30 g/l sugar was added as a carbon source and the pH was adjusted by using 1 N NaOH and 1N HCl. Agar was added at a concentration of 5% before autoclaving as a solidifying agent and 1.5 g/l of activated charcoal was used to prevent phenolic oxidation [10]. The volume was made up by adding double-distilled water. The media was autoclaved at 121°C 15 lbs/square inch pressure for 15 minutes and then it was allowed to cool at room temperature and stored in culture rooms until further use. The media was gently stirred till complete dissolution and the culture media was poured into labeled 40 ml plastic bottles and stored at a temperature of 40C for 3-7days for further use [11].

Culture conditions

The cultures were maintained at 16 hours light and 8hour dark photoperiod, the temperature was kept at 26 ± ˚C light with an intensity of 2000-2500 lux.

Data analysis

The data for the number of percentage survival of explants during culture initiation, average number of shoots, mean of shoot length, average number of roots, and mean of root length and percentage survival of plantlets during acclimatization were recorded during the experiment. All data were subjected to analysis of variance (ANOVA) to quantify the differences between treatments. Treatment means were separated using the least significant differences (LSD) at a probability level of $p \leq 0.05$. ANOVA was calculated using SPSS version 20 [12].

Results and Discussion

Effect of BAP on shoot initiation

The results showed that the highest number of responses (88.88% and 70.37%) was recorded for the variety of Butuzua with the treatment of 1.5 mg/l BAP and 1.0mg/l BAP respectively while the lowest numbers of responses were recorded (11.11%) from MS media free from growth regulators or control in these varieties of bananas [13].

The current result obtained from the variety Butuzua was similar to Ali et al. (2011) who reported that maximum shoot formation (88 %) was obtained on MS medium enriched with1.5 mg/l of BAP. Govindaraju et al. (2012) also reported that shoot proliferation of 80% was achieved on MS medium containing 2.0 mg/l Benzyl Amino Purine [14] (Table 1).

Number of shots per explant

Table 1: Effect of different levels (mg/l) of BAP in response to shoot initiation for banana varieties.

Concentration	% of shoot initiation
BAP	Butuzua
0	11.11
0.5	66.67
1	70.37
1.5	88.89

Among the different hormonal concentrations of BAP and NAA in the variety of Butuzua MS. However, there was no statistically significant difference between the two treatments of 5.0mg/l BAP + 0.5mg/l NAA and 4.0 mg/l BAP + 0.5 mg/l NAA (4.8±0.7) in the studied variety. On the other hand, the minimum average shoot numbers was recorded (1±0.0and 1.±1.0) for the control of this banana variety [15].

The result is in agreement with earlier reports of different authors on different varieties of banana (Khatun et al.; 2017; Miilion et al., 2015 and Gabriel et al., 2013). A similar result was reported by Miilion et al. (2015) as an interaction of 5.0 mg/l BAP + 0.5 mg/l NAA where the highest shoot number (2.6 shoots at 30 days of inoculation) was recorded for the banana variety of Grand Nain. Similarly, Khatun et al., (2017) have reported that the highest number of shoots (3.0 and 3.4) were recorded in MS medium supplemented with 5.0 mg/l BAP and 4.0 mg/l of BAP shoots per explant on the banana variety of Sabri. Similar to the current investigation lowest number of shoots per explant (1.0) was recorded in the treatment-free from growth hormone (control) on the variety of Sabri.

Gabriel et al. (2013) reported that there was a growing trend in the number of shoots with increasing levels of BAP in the culture medium from 1-4 mg/l. However, these authors reported that higher concentration of BAP (5mg/l and above) decreased the effects on the number of shoots produced. This implies that an optimum BAP level can be reached up to 4mg/l and any further increase is not beneficial for shoot production [16].

Shoot length

In this study, the maximum shoot length (5.4±0.87) was recorded on MS media supplemented with 5.0 mg/l BAP + 0.5mg/l NAA for the variety of Butuzua .The second most optimal response on shoot length was obtained (4.1 ± 0.3) on the MS media containing 4 mg/l BAP + 0.5mg/l NAA . On the other hand, the minimum shoot lengths and less response for shoot elongations were observed (0.83 ± 0.7) in the control group of MS medium free from plant growth regulators (BAP and NAA) in the banana variety of Butuzua [17-20].

A similar result was reported by Miilion et al., (2015) who found that maximum shoot length (5.8 cm) on MS medium combined with 5.0 mg/l BAP + 2.0 mg/l NAA concentration on the variety of Grand Nain.

The minimum response to shoot length and elongation reported by Adane, (2015) was similar with the finding of the current study [21,22] (Table 2).

Effects of the different concentration of IBA on root induction

Several roots per plantlet:

Half strength MS medium supplemented with 1.0 mg/l IBA showed the highest response for root number of butuzua variety of bananas. The maximum number of roots (8.6 ± 0.52) were found at a concentration of 1.0 mg/l IBA. The least mean number of roots was recorded in the case

Table 2: Effects of different concentration and combination of BAP and NAA on shoot number and shoot elongation of banana varieties.

BAP combined	NAA	Butuzua	
		Shoot number	length
0.0	0.0	1.0 ± 0.0^{d}	0.8 ± 0.7^{d}
1.0	0.5	2.3 ± 0.3^{c}	$2.6 \pm 0.2b^{c}$
2.0	0.5	3.1 ± 0.2^{c}	3.5 ± 0.5^{b}
3.0	0.5	4.0 ± 0.4^{b}	3.5 ± 0.4^{b}
4.0	0.5	4.8 ± 0.7^{a}	4.1 ± 0.3^{b}
5.0	0.5	5.4 ± 0.4^{a}	5.4 ± 0.8^{a}

Shoot length/shoot (mean ± S.D) Mean within a column followed by the same superscript letters are not significantly different at (P≤0.05).

Table 3: Effect of different concentration of IBA on root number of banana varieties.

IBA (mg/l)	Butuzua
0	2.0 ± 1.0^{e}
0.5	6.5 ± 0.5^{b}
1	8.6 ± 0.5^{a}
1.5	5.6 ± 0.5^{cbd}
2	5.2 ± 0.5^{cd}
2.5	4.5 ± 0.5^{cd}

Means and standard division with the same letter (s) down the column are not significantly different at 95% confidence interval (P≤0.05).

of a control group of half-strength MS medium without hormones. The number of roots increased with increasing concentration of IBA up to 2mg/l and declined beyond 5 mg/l (Rahman et al., 2002). In addition to that Ahmed et al. (2014) recorded the highest root number (6.66) on ½ MS media with 1.0 mg/l IBA on a banana variety of grand Nine. Shashi kumar et al. (2015) also explained MS + 1.0 mg/l IBA + 200 mg activated charcoal was the best concentration for rooting of banana which gave 5.33±1.21 average root per plant [23] (Table 3).

Root length

The present study obtained highest mean root lengths (7.5± 0.6) with a 1.0 mg/l concentration of IBA, which was statistically similar to 0.5mg/l IBA. This was followed by treatment with the concentration of 1.5 mg/l IBA which produced (5.6±0.5) mean root length per explant. The shortest length of root per explant (1.8±0.76) was also produced on MS medium free from growth regulator [24].

Al-Amin, et al (2009) also recorded the shortest length of root 2.0 cm from control in a banana variety of cv. BARI Banana-I. As regards the maximum length of root the present result is analogous with Ahmed et al., (2014) the longest root (7.80) was recorded on ½ MS media with 1.00 mg/l IBA and activated charcoal. These findings are in line with Shashi kumaret al., (2015) who reported, MS+1.0 mg/l IBA+200 mg activated charcoal was the best concentration for rooting of banana which gave 7.50±1.87 average length of roots per explant [25] (Table 4).

For the acclimatization and hardening process, the plantlets were taken out from the culture tubes and the root portion was washed with slightly boiled tap water to remove the attached medium. Well-rooted plantlets about 5–7 cm height of the banana variety were acclimatized in the greenhouse on a sterile plastic potting which contains coco peat alone and vermin compost. They were covered with a plastic film to retain moisture and gradually acclimatized to the outdoor conditions. The results of the current study indicated that coco peat alone was the best medium for hardening of in vitro derived plantlets of bananas.

Table 4: Effect of different level of IBA on root length of banana varieties.

IBA	Butuzua
0	1.67±0.50
0.5	7.10±0.65[a]
1	7.50±0.64[a]
1.5	5.60±0.50[b]
2	4.40±0.50[c]
2.5	4.20±0.50[d]
Means with the same letter (s) down the column are not significantly different at P≤0.05	

Table 5: The survival rate of acclimatized plantlets in a glasshouse using coco peat.

Varieties	Substrate	Total number of plantlet	Survival of plantlet	Survival (%)
Butuzua	Coco peat	50	46	92
	Vermicompost	50	39	78

Figure 3: Acclimatization and hardening of banana plantlets.

The maximum percentage of survival rate (90.0%) was recorded from coco peat on the two varieties of banana (Butuzua) after four weeks. In the culture that contained compost, less response (78%) was found and this could be true due to the nature of the coco peat medium which has spongy-like characteristics with higher water and air holding capacity that help the plantlet to have a good root system. The present result is in line with Patel et al. (2015) who found that maximum survival rate in four banana varieties namely, Grand Naine (87.5%), Mahalaxmi (85.6%), Shrimanti (81.9%), and Basarai (83.7%) in coco peat during hardening in poly house condition. Whereas, the culture containing vermin compost was unsatisfactory in plantlet responses in greenhouse conditions. Ali et al. (2011) also registered a successful hardening response of plantlet in the pot containing Peat moss [26,27] (Table 5 and Figure 3).

Conclusion

The present study has developed a simple and reproducible in vitro micro propagation protocol of banana tissue culture using shoot tip explants. For shoot initiation and shoot multiplication, the concentration of cytokine and auxins had significant effects in this variety. Among the treatments used in the study 5.0 mg/l BAP + 0.5 mg/l NAA showed the best response for shoot number (5.4). Similarly, MS medium containing 5.0 mg/l BAP + 0.5 mg/l NAA gave maximum shoot length (5.4cm). Whereas the highest leave numbers (3.8) were recorded on MS medium supplemented with 5.0 mg/l BAP + 0.5 mg/l NAA in the studied banana variety. After four weeks interval, well-grown shoots were transferred to root induction medium. Highly significant differences (p≤0.05) were observed on the treatments at 1.0 mg/l IBA and also provoke a greater number of root formation and differentiation. The rooted plantlets were successfully acclimatized in greenhouse conditions with a survival rate of 92 % in the coco peat medium of the banana variety, Butuzua. Hence, it is important to use this developed method in vitro micro propagation for large scale propagation trials of banana plants. It can also serve as a convenient method for the production of disease-free homogenized banana cultivars to generate a large number of plantlets in a short period of time and to establish a continuous production system. It can also help the enhancement of the economic benefit of farmers and industrialists.

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