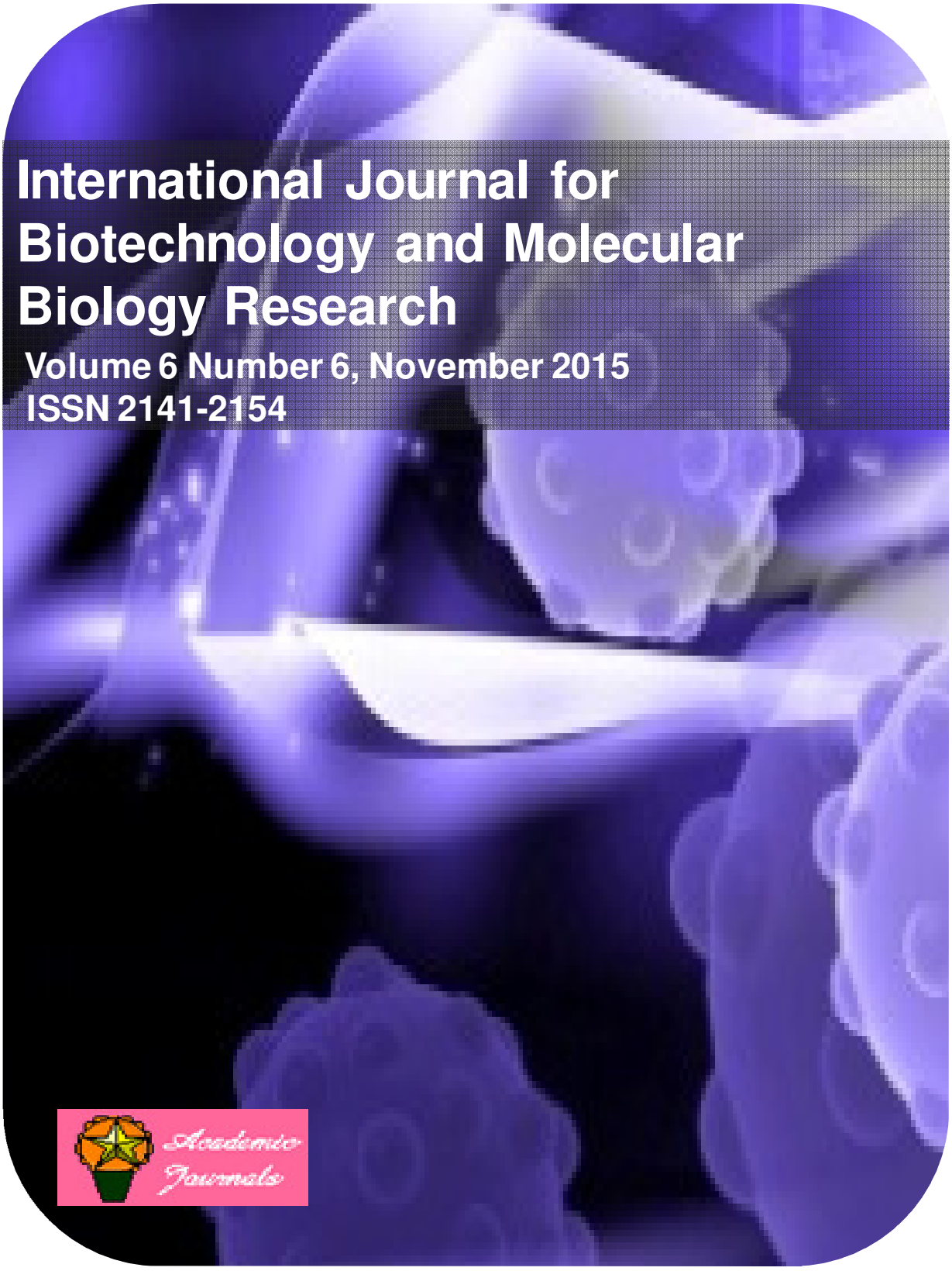

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Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

Pelczar JR, Harley JP, Klein DA (1993). Microbiology: Concepts and Applications. McGraw-Hill Inc., New York, pp. 591-603.

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Full Length Research Paper

In vitro propagation of two elite cooking banana cultivars- FHIA 17 and INJAGI

Jane Kahia[1]*, Felicien Ndaruhutse[2], Bancy Waweru[3], Ntirugulirwa Bonaventure[3], Amini Mutaganda[3], Peter Yao Sallah[2], Njenga Peter Kariuki[4] and Theodore Asiimwe[3]

[1]World Agroforestry Centre (ICRAF), Cote d' Ivoire Country Program Cocody Mermoz,Abidjan, 08 BP 2823, Côte d'Ivoire.
[2]Department of Crop Production and Horticulture, Faculty of Agriculture, National University of Rwanda, P. O. Box117, Huye, Rwanda.
[3]Rwanda Agriculture Board (RAB), P. O. Box 5016, Kigali, Rwanda.
[4]Jomo Kenyatta University of Agriculture and Technology, P. O. Box 62000, Nairobi, Kenya.

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Explants of two elite cooking banana cultivars, FHIA17 and INJAGI were collected from healthy source of stock plants growing in the field. Sterilization was evaluated using different concentrations (20, 30, 40, 50 and 60%) of a commercial bleach (JIK) for 25 min. Effects of cytokinins benzyl amino purine (BAP), 2-isopentenyl adenine (2iP) and kinetin each evaluated at 5, 10, 20, 24 and 40 µM and thidiazuron (TDZ) at 0.1, 0.5, 1 and 1.5 µM, on microshoot induction were investigated. Effects of auxins viz. napthaleneacetic acid (NAA), indole-3-acetic acid (IAA), 2,4-dichlorophenoxyacetic acid (2,4-D) and indole-3-butyric acid (IBA each evaluated at 5, 10, 20 and 40 µM) on rooting were tested as well. The highest (91%) numbers of clean explants were obtained when the explants were subjected to sterilization in 40% Jik for 25 min. The FHIA 17 explants cultured on MS media supplemented with 40 µM kinetin produced the highest (3.00±0.35) mean number of microshoots. On the other hand, INJAGI explants cultured on MS media supplemented with 0.1 µM TDZ yielded the highest mean number (1.84±0.24) of microshoots and the highest mean length (0.99±0.13). Rooting was achieved in all media supplemented with the auxins evaluated except on 2,4-D. These results have an important significance on the application of tissue culture in propagation of cooking bananas in Rwanda, which is highly desired to support the government policy of replacing old unproductive bananas with elite high yielding varieties.

Key words: FHIA17, INJAGI, banana, microshoot, cultivar.

INTRODUCTION

Banana (*Musa* spp.) belongs to the Musaceae family. They are number four on the list of staple crops in the world and one of the biggest profit yielders in supermarkets, making them critical for economic and global food

*Corresponding author. E-mail: J.Kahia@cgiar.org.

security (Frison and Sharrock, 1999). It is the main staple food and a key component of food security in the Great Lakes region of Eastern Africa (CIALCA, 2007) especially in parts of Rwanda, Tanzania and Uganda, where it makes the highest contribution to household income (Smale and Tushemereirwe, 2007). The cultivation of bananas in Rwanda and in many parts of the world is constrained by pests and diseases, especially of viral origin (Okech et al., 2004). The viral diseases are caused by pathogens that are difficult to control and are transferred by vegetative propagation, often resulting in significant loss of crop and sometimes producing no or poor quality product. Conventionally, bananas are propagated by suckers, which are collected from an existing field or from a multiplication plot planted only for the production of suckers. This mode of propagation has its own limitations such as presence of diseased mother plants and supply is season dependent (Hanumantharaya et al., 2009). On the contrary, the numbers of suckers harvested are few as one mother corm can only produce ten suckers per year or less depending upon variety and clone (Bohra et al., 2013). There is therefore urgent need to look for feasible alternatives of propagating bananas in Rwanda. Tissue culture or *in vitro* propagation offers such alternative and large numbers of plants can be regenerated using only a few explants (Dadjo et al., 2014). *In vitro* propagation of bananas has advantages over traditional propagation methods, which include large scale production of disease-free planting materials all year round, physiological uniformity and the availability of disease-free material (Daniells and Smith, 1991; Abeyaratne and Lathiff, 2002; Waman et al., 2014). Furthermore, micro-propagated bananas plants, in general, give higher yields and have shorter production periods as compared to conventional propagated bananas (CIAT, 2006). Moreover, tissue cultured banana are the preferred planting material in commercial plantations all over the world (Clifford et al., 2011). Currently, tissue cultured banana planting materials are not widely available to farmers in Rwanda, Tanzania and Uganda due to insufficient dissemination channels in this region. This is attributed to lack of understanding of the benefits of this technology and lack of agronomic data demonstrating the benefits of tissue cultured bananas (Bjorn et al., 2006).

Although a number of studies have been carried out on the use of cytokinins on micropropagation of bananas, the results have not been consistent. Murashige and Skoog (1962) media (MS) supplemented with adenine-based cytokinins especially BAP is commonly used in several *Musa* spp. (Madhulatha et al., 2004; Venkatachalam et al., 2007; Bairu et al., 2008; Jafari et al., 2011). Other studies have reported the use of isopentyladenine (2-ip) (Dore et al., 2008), zeatin (Vuylsteke and Langhe, 1985) and kinetin (Cronaeur and Krikorian, 1984). However, the use of diphenyl urea derivatives (such as TDZ) is rare, except for a few reports (Youmbi et al., 2006; Shirani et al., 2009; Bohra et al., 2014).

Therefore, considering the above facts, the current study was undertaken with the following objectives: i) To study the effect of adenine and phenylurea types of cytokinins and their concentrations on microshoot proliferation of two elite banana cultivars INJAGI and FHIA17; ii) To determine the effect of auxins (type and their concentrations) on *in vitro* root development.

MATERIALS AND METHODS

Suckers of approximately one foot long of two cooking banana cultivars FHIA17 and INJAGI were collected from healthy, true-to type source plants grown in the banana field gene bank at Rubona Research Station, Rwanda. They were taken to the plant tissue culture laboratory at Rwanda Agriculture Board (RAB) located in Rubona, Southern, Rwanda (Altitude: 1630 m asl, 2°29'07''S, 29°47'49''E). Thereafter, they were thoroughly washed with running tap water. Suckers showing coloration were discarded to reduce the possibility of contamination. The remaining suckers were trimmed with a knife to approximately 5 cm^3 of tissue enclosing the meristem and were then transferred to sterile environment inside a lamina flow hood. Pre sterilization was carried out by using 70% ethanol solution for one minute and then rinsing two times with sterile distilled water. The explants were subjected to further sterilization using varying concentrations (20, 30, 40, 50 and 60%) of a commercial bleach (JIK® (Reckitt and Benckiser (Kenya) Ltd) solutions containing 2-3 drops of Tween 20 (wetting agent) for 25 min. The explants were then rinsed in four changes of sterile distilled water to remove the traces of the sterilizing agent. The sterilized explants were handled with sterile forceps and reduced further to a final size of 3-4 mm in length. Care was taken to ensure that tissue enclosing the meristem was not completely severed. The explants were then cultured on the media under evaluation.

Media preparation

Induction of microshoots was carried out by using full strength MS basal medium supplemented with 30 mg/l cysteine, 100 mg/l inositol and 3% sucrose. To this medium, BAP, 2iP, kinetin each evaluated at 5, 10, 20 and 40 µM and TDZ at 0.1, 0.5, 1 and 1.5 µM, were added in separate experiments. Half strength MS medium supplemented with NAA, IAA, 2,4-D and IBA were evaluated for rooting each at 5, 10, 20 and 40 µM. The rooting medium also contained inositol (100 mg/l) and 2% sucrose. Media without any growth regulator was used as the control. The pH was adjusted to 5.8 using 1 M NaOH or 0.1 M HCl before agar (7%) was added. The media was then heated to dissolve the agar and dispensed in 40 ml aliquots into culture vessels before autoclaving at 1.06 kg cm^{-2} and 121°C for 15 min.

Culturing and incubation

The culturing of plant materials was carried out under sterile environment in the laminar airflow cabinet. The explants were dissected using sterile blade and forceps and inoculated into a glass vessel containing 40 ml of the sterile media under evaluation. Fifteen culture vessels were used per treatment and these were sealed with parafilm before incubating in a growth room maintained at 25 ± 2°C under the cool white fluorescent lights and 16 h photoperiod.

Table 1. Effect of different JIK® concentrations on elimination of surface contamination from cooking banana explants.

Concentration of JIK® (v/v)	Duration of immersion in JIK® (minutes)	Initial number of explants	Type of contamination	Contaminated explants	Clean explants	Clean explants (%)
20	25	12	Fungus Bacteria	2 4	6	50
30	25	12	Fungus Bacteria	2 2	8	67
40	25	12	Fungus Bacteria	0 1	11	91
50	25	12	Fungus Bacteria	0 0	Damaged	Damaged

Acclimatization

The *in vitro* regenerated plantlets were removed from the culture vessels and the roots gently cleaned with running tap water to remove the adhering agar. The plants were then taken to the green house, where they were treated with 2% fungicide (Redomil, Syngenta, USA) for 20 min. A weaning pot (16.5 by 13.5 cm) was filled with sterile potting mixture containing top soil, sand and manure mixed in the ratio of 3:2:1(w/w). The pot was placed in a basin containing tap water to allow the potting mixture to take up water until the top became moist. The pot was then removed from the basin and the plantlets carefully planted using sharp wooden sticks. After two weeks, the holes of the weaning pot were half way opened and eventually fully opened after one month.

Experimental design, data collection and analysis

The experiments were laid out in factorial completely randomized design (CRD) with variety as first factor, type of cytokinin/auxin as second and concentration as the third factor with fifteen explants per treatment. The trial was repeated three times. The contamination percentage was calculated using the formula shown below. Twenty microshoots per treatments were used for the rooting experiment and the number of roots were counted, while their length was measured in cm. The collected parameters were analyzed using SAS software (SAS Institute, 2001). Two-way ANOVA was used to demonstrate factor effects and their interactions using the roc GLM procedures. Treatments means compared using least significant difference test (LSD) at 5% level of significance.

The contamination percentage was calculated using the following formula:

$$\text{Contamination (\%)} = \frac{\text{Number of cultures contaminated}}{\text{Total number of cultures inoculated}} \times 100$$

RESULTS

Results of the effect of different JIK® concentrations on percent clean explants fourteen days after culturing are presented in Table 1. There was no contamination observed, when explants were sterilized in 60% JIK (data not shown). However, the explants were all damaged by the sterilant. The highest number of clean explants (91%) was obtained when the explants were treated with 40% JIK for 25 min.

There was significant difference between the two cultivars (FHIA 17 and Injagi) in terms of number of shoot, root and their lengths (P<.0001) thus data were analyzed separately. Type of cytokinin/auxin significantly affected the proliferation and elongation of microshoot and root. Similarly, significant (P<.0001) interactions between the type of cytokinin/auxin and concentration were observed (Tables 4 and 5).

Of the four cytokinins evaluated, TDZ was the best cytokinin for microshoot regeneration in cultivar INJAGI, while Kinetin showed the best response for regeneration of FHIA 17 microshoots (Table 2). IAA was observed to be the best auxin for induction and elongation of roots in both banana cultivars evaluated (Table 3).

For shoot proliferation and elongation, 40 µM kinetin yielded highest number of shoots in INJAGI, whilst 1. 0 µM TDZ yielded the longest shoot for FHIA 17 (Table 4). However, for Injagi, 0.1 µM TDZ led to more and longer shoots as compared to other concentrations evaluated. As for root proliferation and elongation, 40 µM IAA yielded the highest number of root and length as compared to the other auxins for FHIA whilst 10 µM IAA produced the highest mean number of roots in INJAGI (Table 5).

Among all the TDZ concentrations evaluated for FHIA 17, the media supplemented with 1.0 µM TDZ produced the highest (1.84 ± 0.27) mean number of shoots per explant and the highest (0.92 ± 0.11cm) mean length. For this cultivar, increasing the concentration of TDZ from 0.5 to 1.0 µM resulted in the doubling the number of microshoots per explant and increased the microshoot length by almost three times. However, increasing the concentration of TDZ from 1.0 to 1.5 µM resulted in a slight decrease in the number of microshoots per explant and mean length. For the cultivar INJAGI, the media

Table 2. Effects of different cytokinins on *in vitro* shoots regeneration and elongation in FHIA 17 and INJAGI banana cultivars.

Cytokinins	FHIA 17		INJAGI	
	Number of microshoots	Length of shoots (cm)	Number of microshoots	Length of shoots (cm)
TDZ	1.28 ± 0.62[a]	0.55 ± 0.04[a]	1.39 ± 0.62[a]	0.83 ± 0.62[a]
KIN	1.65 ± 0.42[b]	0.46 ± 0.04[ba]	0.88 ± 0.42[b]	0.53 ± 0.42[b]
BAP	1.47 ± 0.55[c]	0.35 ± 0.04[c]	1.09 ± 0.55[b]	0.42 ± 0.55[b]
2iP	1.29 ± 0.68[d]	0.40 ± 0.05[bc]	0.98 ± 0.68[b]	0.43 ± 0.68[b]
P value	0.1324	0.003	0.0062	<.0001
LSD	0.3587	0.1071	0.2963	0.1238

Values represent means ± SE. Means within a column followed by different letters are significantly different at *P* = 0.05. LSD: least significant difference (LSD).

Table 3. Effects of different auxins on *in vitro* roots regeneration and elongation in FHIA 17 and INJAGI banana cultivars.

Auxins	FHIA 17		INJAGI	
	Number of roots	Length (cm) of roots	Number of roots	Length of roots (cm)
IBA	3.71 ± 0.18[b]	2.92 ± 0.12[b]	3.07 ± 0.15[c]	3.17 ± 0.11[b]
NAA	2.07 ± 0.15[c]	1.50 ± 0.11[c]	3.47 ± 0.18[b]	3.16 ± 0.12[b]
IAA	4.37 ± 0.16[a]	4.28 ± 0.12[a]	4.53 ± 0.13[a]	4.35 ± 0.11[a]
2,4-D	0.00 ±0[d]	0.00 ±0[d]	0.00 ±0[d]	0.00 ±0[c]
P value	<.0001	<.0001	<.0001	<.0001
LSD	0.4049	0.2968	0.3828	0.2783

Values represent means ± SE. Means within a column followed by different letters are significantly different at *P* = 0.05.

supplemented with 0.1 µM TDZ produced the highest (1.84 ± 0.24) mean number of microshoots per explant and the highest mean length (0.99 ± 0.13 cm).

Among all the BAP concentrations evaluated for FHIA 17, the media supplemented with 5 µM BAP produced the highest mean number (2.20 ± 0.24) of microshoots per explant and the highest mean length (0.48 ± 0.06 cm). Increasing the concentration of BAP in the media from 10 to 20 µM decreased the number of microshoots per explant and their lengths significantly in FHIA 17 cultivar. For INJAGI, the media supplemented with 20 µM BAP produced the highest (1.66 ± 0.22) number of microshoots per explant.

Among all the kinetin concentrations evaluated for FHIA 17 and INJAGI, the media supplemented with 40 µM kinetin gave the highest mean number of microshoots per explant (3.00 ± 0.35 and 1.36 ± 0.29), respectively. This concentration also produced microshoots with the highest mean length. Increasing the concentration of kinetin from 10 to 40 µM for FHIA 17 increased the mean number of microshoots per explants by almost five times.

For FHIA 17, there were no significant difference in mean number of microshoots per explant produced at all the 2iP concentrations evaluated although 20 µM 2-iP produced the highest (1.49 ± 0.29) mean number of microshoots. However, for INJAGI, the media supplemented with 5 µM 2iP produced the highest (1.34 ± 0.32) mean number of microshoots per explant although it was not significantly different from 10 and 20 µM.

Among all the IBA concentrations evaluated for FHIA 17 and INJAGI, the media supplemented with 5 µM IBA produced the highest mean number of roots per explant (4.57 ± 0.36) and the highest mean lengths (3.57 ± 0.34 cm), respectively. Increasing the IBA concentration from 5 to 40 µM decreased the number of roots per explant and their lengths in both cultivars.

Among all the NAA concentrations evaluated for FHIA 17 and INJAGI, the media supplemented with 5 µM NAA produced the highest mean number of roots per explant and the highest mean lengths. Increasing the concentration of NAA in the media from 5 to 40 µM decreased the number of roots per explant and their lengths in both cultivars.

Among all the IAA concentrations evaluated for FHIA 17, the media supplemented with 40 µM IAA gave the highest (5.19 ± 0.32) mean number of roots per explant and the highest (4.68 ± 0.27 cm) mean length. However, for INJAGI, the media supplemented with 10 µM IAA

Table 4. Effect of the type of cytokinins and concentration on *in vitro* microshoots proliferation and elongation from FHIA 17 and INJAGI banana explants.

Cytokinins	Concentration	FHIA 17		INJAGI	
		Number of shoot	Length of shoot (cm)	Number of shoot	Length of shoot(cm)
TDZ	0.1	1.16±0.17^{gef}	0.46±0.05^{cd}	1.84±0.24^{a}	0.99±0.13^{a}
TDZ	0.5	0.836±0.11^{gfh}	0.28±0.03^{de}	1.06±0.20^{edfhcg}	0.78±0.10^{ba}
TDZ	1.0	1.84±0.27^{cbd}	0.92±0.11^{a}	1.23±0.19^{ebdfc}	0.76±0.08^{bac}
TDZ	1.5	1.29±0.21^{efd}	0.55±0.09^{cb}	1.44±0.24^{bac}	0.81±0.08^{ba}
KIN	5	1.35±0.19^{efd}	0.45±0.06^{cd}	0.62±0.10^{h}	0.45±0.06^{edf}
KIN	10	0.66±0.13^{gh}	0.28±0.06^{de}	0.78±0.09^{efhg}	0.40±0.04^{edf}
KIN	20	1.61±0.19^{cebd}	0.43±0.05^{cd}	0.79±0.16^{edfhg}	0.43±0.06^{edf}
KIN	40	3.00±0.35^{a}	0.70±0.07^{b}	1.36±0.29^{bdac}	0.84±0.10^{a}
BAP	5	2.20±0.24^{b}	0.48±0.06^{cd}	0.88±0.10^{edfhg}	0.36±0.05^{edf}
BAP	10	2.09±0.23^{cb}	0.42±0.04^{cd}	1.12±0.23^{ebdfhcg}	0.59±0.08^{bdc}
BAP	20	1.270.10^{gefd}	0.320.05 ^{de}	1.66±0.22^{ba}	0.53±0.07^{edc}
BAP	40	0.33±0.12^{h}	0.22±0.08^{e}	0.73±0.18^{fhg}	0.23±0.08^{f}
2ip	5	1.10±0.21^{gef}	0.39±0.06^{cde}	1.34±0.32^{ebdca}	0.45±0.06^{edf}
2ip	10	1.27±0.25^{efd}	0.42±0.06^{cd}	1.194±0.19^{ebdfcg}	0.35±0.05^{ef}
2ip	20	1.49±0.29^{ced}	0.41±0.06^{cde}	0.774±0.12^{fhg}	0.46±0.10^{edf}
2ip	40	1.31±0.22^{efd}	0.42±0.07^{cd}	0.65±0.14^{hg}	0.49±0.08^{ed}
LSD		0.6124	0.1984	0.5672	0.2371
P value		<.0001	<.0001	<.0001	<.0001

Values represent means ± SE. Means within a column followed by different letters are significantly different at *P* = 0.05.

Table 5. Effect of growth regulators type and concentration on *in vitro* microshoot and roots proliferation and elongation from FHIA 17 and INJAGI banana explants.

Auxin	Concentration	FHIA 17		INJAGI	
		Number of root	Length of root (cm)	Number of root	Length of root (cm)
IBA	5	4.57±0.36^{ba}	4.13±0.24^{bc}	3.57±0.34^{de}	4.03±0.15^{b}
IBA	10	3.98±0.33^{bc}	3.00±0.16^{d}	3.17±0.27^{gef}	3.37±0.14^{c}
IBA	20	3.44±0.35^{dce}	2.55±0.15^{ed}	2.67±0.30^{gh}	2.69±0.16^{d}
IBA	40	2.87±0.30^{fe}	2.03±0.16 ^{f}	2.86±0.31^{gf}	2.60±0.23^{ed}
NAA	5	0.91±0.13^{g}	0.65±0.08^{g}	4.64±0.41^{bc}	4.11±0.28^{b}
NAA	10	2.14±0.28^{f}	1.97±0.21^{f}	3.55±0.29^{def}	3.15±0.11^{c}
NAA	20	3.02±0.32^{de}	2.28±0.19^{ef}	2.15±0.20^{h}	2.17±0.16^{e}
NAA	40	2.23±0.27^{f}	1.13±0.16^{g}	3.55±0.26^{def}	3.23±0.17^{c}
IAA	5	3.66±0.23^{dc}	3.93±0.15^{c}	3.91±0.14^{d}	4.34±0.14^{b}
IAA	10	4.57±0.34^{ba}	3.97±0.20^{c}	5.36±0.25^{a}	3.94±0.18^{b}
IAA	20	4.08±0.28^{bc}	4.56±0.30^{ba}	4.00±0.23^{dc}	5.02±0.31^{a}
IAA	40	5.19±0.32^{a}	4.68±0.27^{a}	4.86±0.27^{ba}	4.11±0.12^{b}
2,4-D	5	0.00±0^{h}	0.00±0^{h}	0.00±0^{i}	0.00±0 ^{f}
2,4-D	10	0.00±0^{h}	0.00±0^{h}	0.00±0^{i}	0.00±0 ^{f}
2,4-D	20	0.00±0^{h}	0.00±0^{h}	0.00±0^{i}	0.00±0^{f}
2,4-D	40	0.00±0 ^{h}	0.00±0^{h}	0.00±0^{i}	0.00±0 ^{f}
LSD		0.7335	0.4887	0.6842	0.4611
P value		<.0001	<.0001	<.0001	<.0001

Values represent means ± SE. Means within a column followed by different letters are significantly different at *P* = 0.05.

produced the highest (5.36 ± 0.25) number of roots per explant. Microshoots cultured on MS media supplemented with 2, 4-D at all the concentrations evaluated did not induce roots. The microshoots turned brown and died.

Plate 1. Microshoot induction, elongation and rooting.

Plate 2. Weaning of plantlets in the green house.

The regenerated plantlets were successfully acclimatised in the greenhouse (Plate 2). The steps for regenerating plantlets developed in the current study involved culturing the meristem tip culture on MS media supplemented with cytokinins, induction of multiple shoots, elongation and rooting of microshoots and acclimation of plantlets in the greenhouse (Plates 1 and 2).

DISCUSSION

Surfaces of plant carry a wide range of microbial contaminants. To avoid this source of infection, the explant tissues must be thoroughly surface sterilized before inoculating it on the nutrient medium. The success in tissue culture depends on the effectiveness of the sterilization methods used on the explants prior to culture initiation (Yildiz and Celal, 2002). Among the sterilants that are normally used, sodium hypochlorite has been found to be highly effective against all kinds of microbes. The highest (91%) numbers of clean explants were obtained when the explants were subjected to sterilization in 40% Jik for 25 min. The results of the current study concur with those of Reza et al. (2013) who reported high percent (99%) clean explants when a commercial bleach (chlorox) was used on some *Musa* species.

Plant growth regulators play a vital role in the regeneration of plantlets through different *in vitro* culture techniques, as they influence different plant processes comprising mostly of growth, differentiation and development (Hobbie, 1998). Adenine-based cytokinins are commonly used in several banana (*Musa)* species for *in vitro* propagation and N6-benzylaminopurine (BAP) is the most commonly preferred cytokinin, (Vuylsteke, 1989; Madhulatha et al., 2004; Venkatachalam et al., 2007; Bairu et al., 2008; Jafari et al., 2011). The others are isopentyladenine (2-ip) (Dore et al., 2008), zeatin (Vuylsteke and Langhe, 1985) and kinetin (Arteca, 1996; Madhulatha et al., 2004). However, the use of diphenyl urea derivatives (such as TDZ) in *Musa* mersitem-tip culture is rare. INJAGI banana explants cultured on 20 µM BAP produced the highest number of microshoots. These results are in agreement with those of Vuylsteke (1989) who reported that the optimum recommended BAP concentration is 20 µM for banana micropropagation. BAP was found to be optimal at 22.2 µM in the studies by other researchers

(Cronaeur and Krikorian, 1984; Vuylsteke, 1989; Vuylsteke and Langhe, 1985; Venkatachalam et al., 2007; Jarret et al., 1985; Bairu et al., 2008). However, during the current study, for the cultivar FHIA 17, the optimum concentration of BAP was found to be 5 µM. The results of the present study indicate that optimal concentrations of BAP depend on the genotype. Increasing the concentration of BAP in the media to 40 µM decreased the number of micro-shoots per explant and their mean length significantly in both varieties. These results are in agreement with Venkatachalam et al. (2007) who reported that a reduction in the number as well as length of shoot occurred with exposure to high levels of BAP alone (44.44 µM) in banana cv. Nanjanagudu Rasabale (AAB). These workers alluded to the fact that rate of multiplication in banana is positively related with concentration of BAP and at high concentration it has an inhibitory effect.

There are few reports on the use of TDZ in *Musa* spp. The first study was carried out on ÔKibuziÕ (AAA), ÔNdiziwemitiÕ (ABB) and ÔBwaraÕ (AAA) cultivars by Arinaitwe et al. (2000). The optimum TDZ concentration varied significantly by cultivar. For example, shoot multiplication in ÔNdiziwemitiÕ progressively increased with increasing TDZ concentrations; however, ÔBwaraÕ and ÔKibuziÕ decreased with increasing concentrations. Similar results were observed during the current study, whereby for INJAGI, it was observed that the number of microshoots decreased with increasing concentration of TDZ. On the other hand, for FHIA 17, increasing the concentration of TDZ up to 1.0 µM significantly increased the number of microshoots. Hamide and Mustafa (2004) while working on some new banana cultivars reported that TDZ at low concentration shows higher cytokinin activity than BAP, 2ip or kinetin. During the present study, the lowest TDZ concentration (0.1 µM) gave the highest number of microshoots in cultivar INJAGI. However, for FHIA 17, the best response was achieved when the meristem tips were cultured in a media supplemented with 40 µM Kinetin. This could be explained by the fact that *in vitro* bud differentiation and development in banana was cultivar dependant. Variation in the activity of different cytokinins can be explained by differences in the uptake rates reported in different genomes (Blakesly, 1991), varied translocation rates to meristematic regions, and metabolic processes in which cytokinin may be degraded or conjugated with sugars or amino acids to form biologically inert compounds as reported by Kaminek (1992). Recently, Bohra et al. (2014) reported that TDZ was most efficient to improve total propagation rate in isolated bud clumps in an *in vitro* recalcitrant mixed diploid banana variety Elakki Bale.

Synthetic growth regulating chemicals that have been found most reliable in stimulating adventitious root production are the auxins, that is, IAA, NAA, IBA and 2,4-D (Arteca, 1996). Auxins, (IBA, IAA, and NAA at concentration of 0.2, 1.0 and 0.5 mg/l respectively) are most frequently used to induce root initiation in the banana (Vuylsteke, 1989; Arteca, 1996). During the current study, the root induction and elongation response to IAA were better than for all other auxins evaluated. These findings are contrary to those of Vuylsteke (1989) who reported that NAA was more effective than IAA in banana tissue culture. Rooting was also achieved on basal medium without any growth regulator. The results obtained from the rooting experiments in the current study showed that microshoots cultured on 2,4-D supplemented media did not produce roots at all the concentrations tested. The explants became brown and died within two weeks. There is no previous work reported on use of 2, 4-D to induce roots in banana microshoots. This could be because 2, 4-D is known to induce callus but interestingly in this study, there was no callus formation at all in the concentrations evaluated. The plantlets regenerated in the present study survived when they were transferred to the soil for acclimation. This is contrary to the research by Arinaitwe et al. (2000) who reported that, plantlets regenerated in the presence of TDZ or kinetin did not survive upon transfer to the soil.

A genotype dependent protocol for the micropropagation of two elite banana cultivars in Rwanda was developed. This protocol will provide the basis for the mass production of studied cultivars and will go a long way in supporting the Rwandan government policy of replacing old unproductive bananas with elite high yielding cultivars.

Conflict of Interests

The authors have not declared any conflict of interest.

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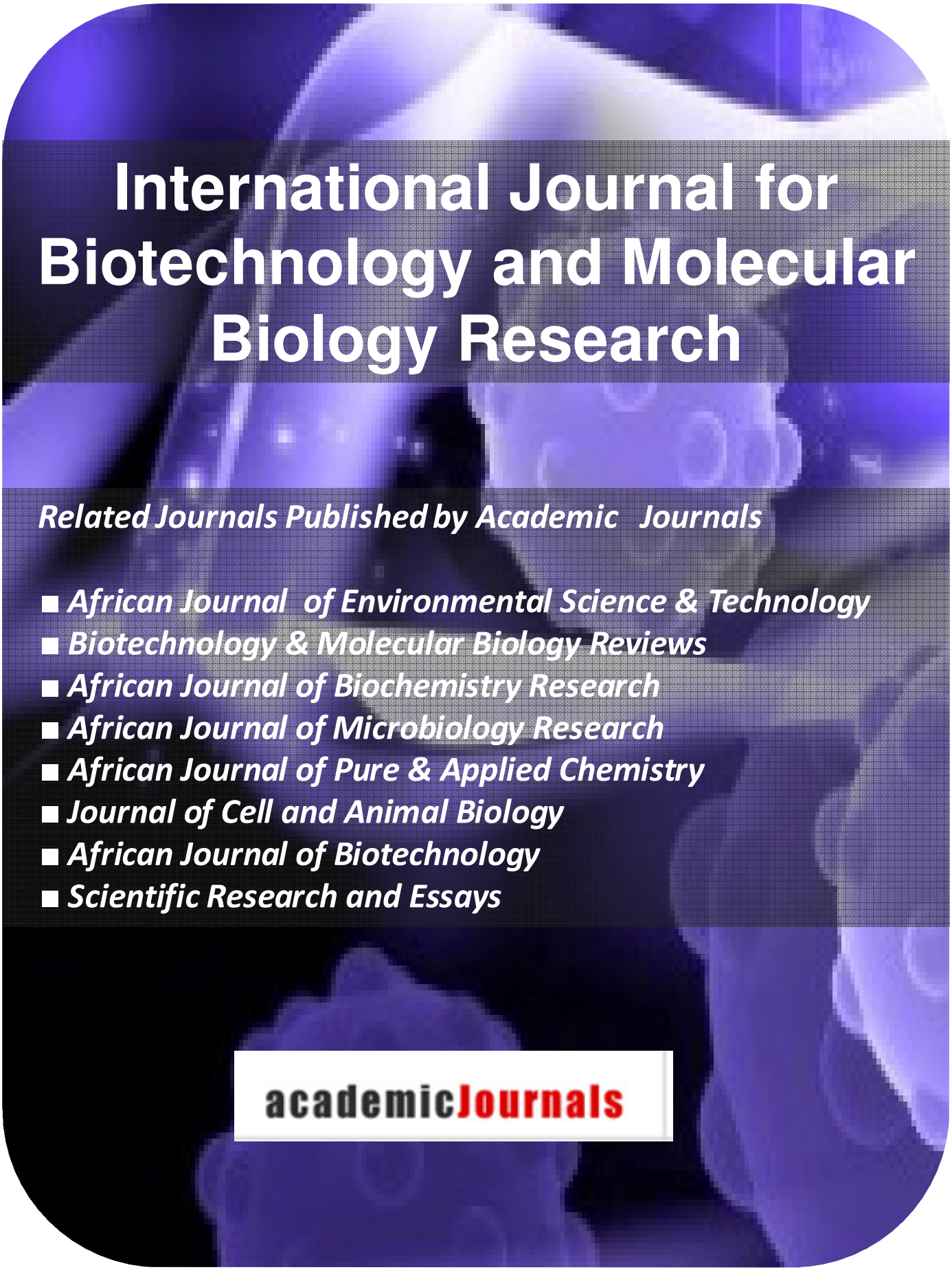

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