Turk J Agric For
28 (2004) 355-361

In Vitro Propagation of Some New Banana Types (*Musa* spp.)

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Received: 24.02.2004

Abstract: Three newly selected superior banana types were used to study the effects of different cytokinins and auxins on shoot multiplication and rooting. Benzylaminopurine [(BAP) (5, 10, 20 and 30 µM)] and thidiazuron [(TDZ) (0.4, 1, 2 and 3 µM)] were tested alone and with 1 µM indoleacetic acid (IAA) for the propagation stage. We compared basal Murashige and Skoog (MS) medium, active charcoal (5 g l^{-1}), indole-3-butyric acid (IBA) and naphthaleneacetic acid (NAA) for rooting. Shoot proliferation and elongation were significantly greater with TDZ than with BAP all 3 types. Furthermore, each cytokinin with IAA increased shoot proliferation and elongation more than their use alone. BAP below 20 µM or TDZ below 1 µM did not increase shoot proliferation, and BAP over 20 µM and TDZ over 2 µM suppressed shoot elongation. Charcoal alone was better for rooting than auxin treatments or MS medium alone. In conclusion, supplementation of 2 µM TDZ, and 1 µM IAA or 20 µM BAP and 1 µM IAA on MS medium, followed 5 g l^{-1} charcoal at the rooting stage were the best combinations for the in vitro propagation of banana types.

Key Words: Banana, *Musa* spp., tissue culture, thidiazuron, active charcoal

Bazı Yeni Muz Tiplerinin (*Musa* spp.) Doku Kültürü ile Çoğaltılması

Özet: Bu çalışmada deneme materyali olarak, yeni selekte edilmiş 3 superior muz tipi kullanılmış ve muz tiplerinin çoğaltılması ve köklendirilmesi üzerine farklı sitokinin ve oksin tiplerinin etkileri araştırılmıştır. Bu amaçla, çoğaltma aşamasında değişik benzylaminopurine [(BAP) (5, 10, 20 and 30 µM)] ve thidiazuron [(TDZ) (0.4, 1, 2 and 3 µM)] konsantrasyonlarının bağımsız ve 1 µM indoleacetic asit (IAA) ile olan kombinasyonları test edilmiştir. Ayrıca köklenme üzerine büyüme düzenleyici içermeyen temel Murashige Skoog (MS) ortamı, aktif kömür, indole-3-butyric asit (IBA) ve naphthaleneacetic asit (NAA)'in etkileri araştırılmıştır. Her üç tipte de sürgünlerin çoğalması ve uzaması, TDZ içeren ortamda BAP içeren ortamdan daha yüksek saptanmıştır. Ayrıca sitokininlerin IAA ile olan kombinasyonları, bağımsız kullanımlarına göre sürgün sayısını ve uzamasını arttırmıştır. BAP'ın 20 µM'ın ve TDZ'nin 1 µM'ın altında kullanımı sürgün çoğalmasını arttırmamış, BAP'nin 20 µM'ın ve TDZ'nin 2 µM'ın üzerinde kullanımı ise sürgün uzamasını engellemiştir. Aktif kömürün bağımsız kullanımı, köklenme açısından MS ortamı ve diğer oksin uygulamalarından daha başarılı bulunmuştur. Sonuç olarak, muz tiplerinin in vitro çoğaltılmasında MS besi ortamına 2 µM TDZ ve 1 µM IAA ya da 20 µM BAP ve 1 µM IAA , köklenme aşamasında ise MS besi ortamına 5 g l^{-1} aktif kömür ilavesi en uygun kombinasyonlar olarak belirlenmiştir.

Anahtar Sözcükler: Muz, *Musa* spp., doku kültürü, thidiazuron, aktif kömür

Introduction

Bananas and plantains are propagated vegetatively because almost all cultivated banana cultivars are triploid, seedless, or seed sterile. The materials used for conventional propagation include corms, large and small suckers, and sword suckers (Cronauer and Krikorian, 1984; Arias, 1992). However, conventional planting materials are not the ideal propagule, because they carry weevils, fungal pathogens, nematodes, and viruses (Arias 1992; Sagi et al., 1998) and also suffer from slow multiplication, bulkiness, and poor phytosanitary quality (Vuylsteke, 1989). Therefore, since 1985, shoot tip culture has been increasingly used in some countries (Israel, the Canary Islands, Taiwan and South Africa) as an alternative to conventional plant material (Robinson, 1996). In vitro propagation of bananas provides excellent advantages over traditional propagation, including a high multiplication rate, physiological uniformity, the availability of disease-free material all the year round, rapid dissemination of new plant materials throughout the world, uniformity of shoots, short harvest interval in comparison with conventional plants, and faster growth in the early growing stages compared to conventional materials (Vuylsteke, 1989; Daniells and Smith, 1991;

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Arias, 1992). Tissue culture also plays a vital role in the distribution of germplasm, conservation, safe exchange of internal planting material and rapid propagation of newly selected hybrid cultivars.

Apart from the influence of genotypes, shoot proliferation rate and elongation are affected by cytokinin types and their concentration. Adenine-based cytokinins are used in several *Musa* spp. for in-vitro propagation. N^6-benzylaminopurine (BAP) is the most commonly preferred cytokinin (Cronauer and Krikorian, 1984; Vuylsteke, 1989). The others are isopentyladenine (2-ip) (Dore Swamy et al., 1983), zeatin (Vuylsteke and De Langhe, 1985) and kinetin (Cronauer and Krikorian, 1984). The concentration of exogenous cytokinin appears to be the main factor affecting multiplication. For example, Wong (1986) stated that when 11.1 µM BAP is supplemented in the medium, each of the explants produces an average of 2.4 shoots, while increasing the BAP concentration to 22.2 µM and 44.4 µM results in 2.6 and 4.3 shoots per explant respectively. However, the optimum recommended BAP concentration is 20 µM for banana micropropagation (Vuylsteke, 1989). There are reports on the use of diphenyl urea derivatives in various cell-culture systems including both callus cultures and micropropagation of many woody-plant species (Sarwar et al., 1998; Victor et al., 1999; Ainsley et al., 2001; Joshi et al., 2003; Kadota and Niimi, 2003). However, the use of diphenyl urea derivatives (thidiazuron) in *Musa* shoot-tip culture is very rare. Diphenyl urea derivatives are used for propagating cultivars 'Kibuzi' (AAA), 'Bwara' (AAA) and 'Ndiziwemiti' (ABB) (Arinaitwe et al., 2000). These researchers stated that cultivars responded significantly better in their shoot proliferation responses to TDZ than BAP and that TDZ was more economical than adenine-based cytokinins.

The auxins (NAA, IAA or IBA) are most frequently used to induce root initiation in the banana (Vuylsteke, 1989). Rooting is also achieved on basal medium without any growth regulators (Cronauer and Krikorian, 1984; Jarret et al., 1985). The influence of active charcoal on rooting is reported in the literature. Cronauer and Krikorian (1984) reported that when NAA, IAA or IBA was added to the medium in the presence of 0.025% (w/v) active charcoal, no difference in rooting was observed.

Banana growers generally use conventional planting materials in Turkey. For this purpose, sword suckers are used for new plant establishment and reestablishment of old plantations. There are not enough government or private banana companies producing banana planting materials. Growers obtain their banana planting material from their orchards or from neighboring orchards. 'Dwarf Cavendish' is the only cultivar grown in Turkey. The first study on selection breeding of bananas in Turkey was carried out at the Department of Horticulture, Faculty of Agriculture, Akdeniz University in Antalya. Some banana types were selected. These selected types are well adapted to the cool subtropics and produce higher yield and quality than 'Dwarf Cavendish' (Gubbuk et al., 2004). Therefore, rapid propagation is needed to distribute the plants to growers. The optimization of multiplication and rooting of these new types will aid banana cultivation not only in Turkey but also in other tropical and subtropical regions.

In this study, the possibilities of rapid clonal propagation of newly selected banana types were investigated. For this purpose, the influence of BAP and TDZ concentrations alone and with 1 µM IAA on shoot proliferation and shoot elongation was investigated. The influence of MS medium, charcoal, 1 µM IBA and 1 µM NAA on rooting were also investigated.

Materials and Methods

Three newly selected banana types ('Alanya 5', Anamur 10' and 'Bozyazı 14') were used as experimental material. These types were selected from the 'Dwarf Cavendish' cultivar grown under open-field and greenhouse conditions in Turkey. 'Alanya 5', 'Anamur 10' and 'Bozyazı 14' were selected in Alanya, Anamur and Bozyazı, respectively. These off-types displayed higher levels of variability for morphological characters affecting yield than the control 'Dwarf Cavendish.' The main advantages of these types are that they are well adapted to the cool subtropics and are higher yielding and have better fruit quality than 'Dwarf Cavendish' (Gubbuk et al., 2004). Shoot tip explants of 'Alanya 5', 'Anamur 10' and 'Bozyazı 14' were excised from young suckers grown in the greenhouse. Explants were surface disinfected by washing with ethanol (95%) for 1 min. and then in a 10% hypochlorite solution for 30 min. Afterwards, the explants were rinsed 3 times with sterile distilled water and kept under aseptic conditions. Shoot tips (3-4 mm) were isolated aseptically and incubated on Murashige and Skoog (MS) (1962) medium. The multiplication medium

contained sucrose (30 g l^{-1}), agar (8 g l^{-1}) and BAP (0, 5, 10, 20 and 30 µM) or TDZ (0, 0.4, 1, 2 and 3 µM) alone and with 1 µM IAA. For rooting, MS with 1 µM IBA and 1 µM NAA and active charcoal (5 g l^{-1}) were used. The pH of the medium was adjusted to 5.7. Cultures were incubated at 26 ± 2 °C under a 16 h photoperiod. Light intensity was 39 µmol $s^{-1}m^{-2}$. Established cultures were routinely transferred every 3 weeks by subdividing shoot clusters with a scalpel. The explants were sub-cultured 3 times and after each sub-culture, the shoots per explant were counted and shoot lengths were measured. In the propagation stage, shoots per explant (determined by counting all shoots/explant) and average shoot length (determined by measuring 3 randomly chosen shoots) were recorded. In the rooting stage, plant height (determined by measuring the area between the starting point of the pseudostem and the point the first leaf emerged), root numbers (determined by counting all roots/plant), average root length (determined by measuring 5 randomly chosen roots) and stem diameter (determined by measuring the distance above 1 cm of pseudostem) were examined. Individual plantlets were transplanted into a sterile potting mixture. Twenty explants per treatment were arranged in a completely randomized design with 3 replicates. The results were analyzed in a completely randomized design using analysis of variance (ANOVA). Means were separated using the LSD multiple range test at 0.05 levels.

Results

The results of proliferation and shoot length response to different equimolar concentrations of BAP and TDZ alone and with 1 µM IAA are presented in Tables 1 and 2. The shoot proliferation and elongation response to TDZ were stronger than to BAP in all banana types. The control group (no plant growth regulators) of all banana types had the lowest shoot number and shoot length. It was observed that BAP concentrations below 20 µM did not improve shoot multiplication or elongation. However, increasing BAP above 20 µM reduced shoot elongation in all banana types (Table 1). BAP increased shoot proliferation in 'Alanya 5' from 2.37 to 5.18 at 5 µM and 20 µM before falling to 4.32 at 30 µM BAP. Similar results were observed in the other banana types. Furthermore, the same behavior was observed with the joint effect of BAP and IAA. BAP with 1 µM IAA increased shoot elongation compared to BAP alone (Table 1). While the most shoots were produced with 2 µM TDZ and 1 µM IAA, the longest shoots were observed with 1 µM TDZ and 1 µM IAA in all genotypes (Table 2). The joint effects of TDZ and 1 µM IAA were greater on shoot elongation than TDZ alone just as for BAP. The average shoots per explant varied between 0.83 and 7.51 and the average shoot length varied between 1.53 and 11.86 mm.

In the rooting study, active charcoal in MS medium gave the best results for all the examined features except for stem diameter (Table 3). However, plant height, roots per plant, average root length and stem diameter were lower under control (MS) treatment than under charcoal or auxin treatments. Plant height varied between 4.36 and 5.86 cm, depending on the treatment (Table 3). The greatest number of roots was in 'Anamur 10' types on active charcoal, with 12.76 roots per plant, followed by 'Alanya 5' (Table 3). The longest average root length was observed for all banana types with active charcoal (Table 3). On the other hand, the highest stem diameter was recorded for all banana types with 1 µM NAA, followed by active charcoal (Table 3). The highest stem diameter (6.31 mm) was recorded in 'Bozyazı 14' with 1 µM NAA.

Discussion

The experimental results indicated that the types of cytokinin and their concentration significantly influenced shoot multiplication and elongation. Moderate concentrations of cytokinins increased the shoot proliferation rate, but very high concentrations decreased multiplication and especially depressed shoot elongation. BAP below 20 µM did not improve shoot multiplication or elongation, and above 20 µM reduced shoot length. Supplementation of 20 µM BAP on MS medium produced the best multiplication and elongation in all 3 bananas. Furthermore, the joint effect of BAP and 1 µM IAA increased shoot elongation compared to BAP alone. The optimum BAP concentration does not vary significantly among researchers. For example, BAP at 22.2 µM was optimal in studies by Cronauer and Krikorian (1984) and Jarret et al. (1985) and at 20 µM in a study by Vuyslteke (1989). Wong (1986) stated that 44.4 µM BAP reduced shoot multiplication. Arinaitwe et al. (2000) stated that shoot proliferation was cultivar dependent. They reported that increasing BAP above 16.8 µM did not significantly increase shoot proliferation in 'Kibuzi' or

Table 1. Shoot proliferation and elongation responses to different BAP treatments alone and with 1 µM IAA in 'Alanya 5, 'Anamur 10' and 'Bozyazı 14' banana types.

Types	Treatments (µM)	No. of shoots (shoots/explant)	Shoot length (mm)
Alanya 5	Control (MS)	0.83 g*	1.33 g
	5 BAP	2.37 f	1.98 f
	10 BAP	4.50 c	3.83 e
	20 BAP	5.18 b	6.68 b
	30 BAP	4.32 e	5.36 c
	5 BAP + 1 IAA	2.36 f	2.05 f
	10 BAP + 1 IAA	4.52 c	4.00 d
	20 BAP + 1 IAA	5.26 a	9.03 a
	30 BAP + 1 IAA	4.40 d	6.66 b
	$LSD_{0.05}$	0.041	0.122
Anamur 10	Control (MS)	0.85 e	1.57 h
	5 BAP	2.41 d	3.64 g
	10 BAP	4.54 bc	9.58 c
	20 BAP	5.30 a	8.88 d
	30 BAP	4.34 c	7.64 f
	5 BAP + 1 IAA	2.45 d	3.68 g
	10 BAP + 1 IAA	4.59 b	11.86 a
	20 BAP + 1 IAA	5.38 a	10.03 b
	30 BAP + 1 IAA	4.58 b	8.73 e
	$LSD_{0.05}$	0.212	0.094
Bozyazı 14	Control (MS)	0.80 g	1.60 f
	5 BAP	2.41 f	3.68 e
	10 BAP	4.57 cd	7.25 d
	20 BAP	5.43 b	8.95 b
	30 BAP	4.45 e	7.68 c
	5 BAP + 1 IAA	2.44 f	3.74 e
	10 BAP + 1 IAA	4.58 c	7.95 c
	20 BAP + 1 IAA	5.51 a	9.51 a
	30 BAP + 1 IAA	4.51 de	7.80 c
	$LSD_{0.05}$	0.061	0.279

*Means followed by the same letters within columns and types are not significantly different according to $LSD_{0.05}$.

'Ndiziwemiti' cultivars. However, the cultivar 'Bwara' showed significant increases in shoot proliferation rates with increasing BAP concentrations from 5.0 to 8.0 shoots with an increase from 16.8 and 28.8 µM. Our results showed that TDZ can be used at a much lower concentration than BAP. A similar result was obtained by Arinaitwe et al. (2000). TDZ produced only modest shoot multiplication and elongation at concentrations below 1 µM. Shoot proliferation progressively increased with increasing TDZ concentration up to 1 µM for all genotypes. There are few reports of the use of TDZ in *Musa* spp. The first study was carried out on 'Kibuzi' (AAA), 'Ndiziwemiti' (ABB) and 'Bwara' (AAA) cultivars by Arinaitwe et al. (2000). They stated that TDZ shows higher cytokinin activity than BAP, zeatin, 2-ip or kinetin. Furthermore, the optimum TDZ concentration varied significantly by cultivar. For example, shoot multiplication in 'Ndiziwemiti' progressively increased with increasing TDZ concentrations; however, 'Bwara' and 'Kibuzi' decreased with increasing concentrations.

Rooting can be stimulated when individual shoots are

Table 2. Shoot proliferation and elongation responses to different TDZ treatments alone and with 1 µM IAA in 'Alanya 5, 'Anamur 10' and 'Bozyazı 14' banana types.

Types	Treatments (µM)	No. of shoots (shoots/explant)	Shoot length (mm)
Alanya 5	Control (MS)	0.85 h*	1.53 i
	0.4 TDZ	2.39 g	3.58 h
	1 TDZ	5.90 e	9.48 b
	2 TDZ	7.29 b	8.77 d
	3 TDZ	6.23 d	7.58 f
	0.4 TDZ + 1 IAA	2.44 f	3.79 g
	1 TDZ + 1 IAA	5.93 e	11.29 a
	2 TDZ + 1 IAA	7.35 a	9.18 c
	3 TDZ + 1 IAA	6.32 c	8.06 e
	$LSD_{0.05}$	0.049	0.132
Anamur 10	Control (MS)	0.86 h	1.57 h
	0.4 TDZ	2.44 g	3.64 g
	1 TDZ	5.93 f	9.58 c
	2 TDZ	7.33 b	8.88 d
	3 TDZ	6.30 d	7.64 f
	0.4 TDZ + 1 IAA	2.49 g	3.68 g
	1 TDZ + 1 IAA	6.01 e	11.86 a
	2 TDZ + 1 IAA	7.51 a	10.03 b
	3 TDZ + 1 IAA	6.38 c	8.73 e
	$LSD_{0.05}$	0.060	0.094
Bozyazı 14	Control (MS)	0.83 g	1.57 i
	0.4 TDZ	2.46 f	3.64 h
	1 TDZ	6.03 e	9.60 b
	2 TDZ	7.43 a	8.93 d
	3 TDZ	6.33 c	7.71 f
	0.4 TDZ +1 IAA	2.50 f	3.80 g
	1 TDZ +1 IAA	6.16 d	11.28 a
	2 TDZ + 1 IAA	7.48 a	9.15 c
	3 TDZ + 1 IAA	6.47 b	8.11 e
	$LSD_{0.05}$	0.081	0.065

*Means followed by the same letters within columns and types are not significantly different according to $LSD_{0.05}$.

transferred to basal medium alone (Cronauer and Krikorian 1984; Jarret et al., 1985). However, auxins may induce further root initiation (Vuylsteke, 1989). Vuylsteke (1989) found that NAA (1 µM) was more effective than IAA. The optimum IBA concentration was found to be 1 µM by Vuylsteke and De Langhe (1985). Our results show that when active charcoal was added to MS medium, it was not necessary to include IBA or NAA for rooting. Using active charcoal alone for rooting will reduce the cost of producing plantlets for the field. Cronauer and Krikorian (1984) reported no differences in the root-inducing effects of NAA, IAA or IBA in presence of 0.025% (w/v) activated charcoal. Hwang et al. (1984) recommended 0.1-0.25% activated charcoal.

In conclusion, TDZ was more effective in producing more and better quality shoots in in-vitro banana propagation than BAP. Shoot proliferation was significantly greater on medium with TDZ than on BAP for each of the 3 banana types. Explants on medium with 2 µM TDZ and 1 µM IAA produced 7-8 new shoots per explant for the 3 types, while 20 µM BAP with 1 µM IAA produced 5-6 shoots per explant. In the rooting stage

Table 3. Plant height, root numbers, root length and stem diameter responses to different treatments (Control (MS), active charcoal (AC; 5 g l^{-1}), 1 µM IBA, and 1 µM NAA) in 'Alanya 5', 'Anamur 10' and 'Bozyazı 14' banana types.

Types	Treatments	Plant height (cm)	No. of roots (roots/plant)	Root length (cm)	Stem diameter (mm)
Alanya 5	Control (MS)	4.43 d*	6.68 d	2.32 d	4.16 d
	AC (5 g l^{-1})	5.83 a	12.70 a	8.78 a	4.49 b
	1 µM IBA	5.53 b	10.82 b	3.48 b	4.35 c
	1 µM NAA	4.75 c	9.58 c	3.03 c	4.68 a
	$LSD_{0.05}$	0.069	0.038	0.096	0.052
Anamur 10	Control (MS)	4.47 d	6.76 d	2.36 d	4.23 d
	AC (5 g l^{-1})	5.86 a	12.76 a	8.84 a	4.54 b
	1 µM IBA	5.58 b	10.86 b	3.55 b	4.39 c
	1 µM NAA	4.76 c	9.64 c	3.10 c	4.74 a
	$LSD_{0.05}$	0.043	0.038	0.067	0.055
Bozyazı 14	Control (MS)	4.36 d	6.76 d	2.47 d	4.23 d
	AC (5 g l^{-1})	5.40 a	11.28 a	10.15 a	6.14 b
	1 µM IBA	5.23 b	10.83 b	3.66 b	4.41 c
	1 µM NAA	4.66 c	9.41 c	3.15 c	6.31 a
	$LSD_{0.05}$	0.043	0.078	0.094	0.064

*Means followed by the same letters within columns and types are not significantly different according to $LSD_{0.05}$.

charcoal alone was significantly better for plant elongation, roots per plant and root length than the other treatments. Overall we recommend the multiplication of banana explants on MS medium with 2 µM TDZ and in combination with 1 µM IAA or 20 µM BAP and 1 µM IAA followed by rooting with charcoal alone.

Acknowledgments

This work was supported by The Scientific and Technical Research Council of Turkey (TUBITAK). The authors thank Mr. Yılmaz Koçer from the Foreign Languages Department for proofreading the material and Dr. Barbara M. Reed of Agricultural Research Service, United States Department of Horticulture (USDA-*ARS)* for critically reviewing the manuscript.

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