EFFECT OF THIDIAZURON ON *IN VITRO* PROLIFERATION CAPACITIES OF SOME BANANA (*Musa* spp.) CULTIVARS WITH WEAK MULTIPLICATION POTENTIAL

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Abstract

The multiplication and the distribution of plant material is one of the objectives of plant tissue culture laboratory of African Research Centre on Bananas and Plantains (ARCBAP). Some banana (*Musa* spp.) cultivars including Topala, Fougamou, Gros-Michel shows low proliferation when cultured in vitro. Other cultivars like Dwarf-Kalapua, Pelipita and Kalapua 2 take long time (two months) to be differentiated in *in vitro* multiplication. All these cultivars have interested agronomic characteristics and have partial or total resistance to Black Leaf Streak Disease (BLSD). In the aim of increasing proliferation rate, shoot tips of those cultivars were cultured on Murashige and Skoog nutrient salts supplemented with different concentrations of thidiazuron (0.05-2 µM) in comparison with the standard medium containing 2 mg/l of BAP. Growth parameters (length and diameter of the stem) and proliferation parameters (number of shoots, leaves and roots formed) were measured six weeks after introduction of explants on the medium during introduction and proliferation phases. Results showed that proliferation was better whichever the cultivar with low thidiazuron concentration (0.05 to 0.8 µM). The plant growth in treatments did not show any significant difference as compared to the control, however, plant development was ameliorated in all cultivars at thidiazuron concentration of 0.05-0.4 µM. Positive correlations were observed between shoot proliferation and leaf or root formation in Fougamou, Gros-Michel and Pelipita cultivars, except for cultivar Topala.

Key words: *Musa* spp, *in vitro* culture, thidiazuron, proliferation rate

Düşük Çoğalma Potansiyeline Sahip Bazı Muz (*Musa* spp.) Çeşitlerinin *In-Vitro*'da Çoğalma Kapasiteleri Üzerine Thidiazuron'un Etkisi

Özet

Afrika Muz ve Muz Türleri Araştırma Merkezi Bitki Doku Kültürü Loboratuvarının amaçlarından birisi; bitki materyalinin çoğaltımı ve dağıtımıdır. Topala, Fougamou ve Gros-Michel gibi çeşitler *In-Vitro'da* düşük çoğalma kapasitelerine sahip olup, Dwarf-Kalapua, Pelipita and Kalapua 2 gibi çeşitlerde ise farklılaşma uzun bir zaman (2 ay) almaktadır. Bu çeşitler, üstün tarımsal özelliklere sahip, Black Leaf Streak Disease (BLSD) hastalığına ise tam veya kısmen dayanıklıdırlar. Bu çalışma adı geçen çeşitlerin *In-Vitro* çoğalma oranları üzerine thidiazuron (TDZ)'un etkisinin belirlenmesi amacıyla gerçekleştirilmiştir. Bu amaçla çeşitlere ait meristemler farklı konsantrasyonlarda TDZ (0,05-2 µM) içeren Murashige ve Skoog besi ortamlarında kültüre alınmış ve sonuçlar 0,2 mg/l BAP içeren standart besin ortamı ile karşılaştırılmıştır. Meristemlerin besi ortamlarına yerleştirilmesinden 6 hafta sonra ve kültür süresince büyüme (sürgün boyu ve gövde çapları) ve çoğalma parametrelerine (sürgün sayısı, yaprak sayısı ve kök oluşumu) ait ölçümler yapılmıştır. Sonuçlar, tüm çeşitlerde TDZ'un düşük konsantrasyonlarında (0,05 – 0,8 µM) organ oluşumunun daha iyi olduğunu göstermiştir. Büyüme ölçütleri açısından uygulamalar arasında istatistiksel anlamda fark olmamasına karşın, tüm çeşitlerde 0,05-0,4 µM TDZ konsantrasyonlarının büyüme değerlerini artırdığı gözlenmiştir. Ayrıca Topala çeşidi dışındaki Fougamou, Gros-Michel, Dwarf-Kalapua, Pelipita ve Kalapua 2 çeşitlerinde sürgün oluşumu ile yaprak veya kök oluşumu arasında pozitif korelasyonlar belirlenmiştir.

Anahtar Kelimeler: *Musa* spp., *In-Vitro* Kültür, Thidiazuron, Organ Oluşumu.

1. Introduction

The micropropagation of banana allows production of disease-free plants and increases the rate of multiplication compare to mother plant in the field. In vitro proliferation is under the control of cytokinin concentrations and the optimum concentration depends on the variety (Vuylsteke, 1985; Vuylsteke et al., 1998). Observations made in CARBAP laboratory reveal that some cultivars of banana: Topala,

Fougamou, and Gros Michel have a low rate of proliferation. Others like Dwarf-kalapua, Pelipita and Kalapua 2 take more than two months before been differentiated for in vitro multiplication. This weak proliferation in *In-Vitro* culture is also observed in vivo by few suckers produced by mother plants in the field. These limiting factors considerably reduce the possibilities of in vitro plant production of these cultivars which have interesting agronomic and culinary characteristics (higher mean weight of bunch (17 to 25 kg), reduced height of pseudo term (not more than 3 m), acceptability by consumers under different culinary forms (chips, fries, boiled pulp etc.) behaviours of partial or total resistance to black leaf streak desease (Noupadja et al., (2001). New molecules with cytokinin effect exist and act at low concentration and their activity is sometime 100 times more efficient than the ordinary cytokinins. The diphenyl urea derived *N*-phenyl-*N'*-1,2,3-thiadiazol-5-ylurea (thidiazuron, TDZ) is characterized by its efficient effect at low concentration on some plant species (Arif et Kathamlan, 1992). It had been used for in vitro stimulation of proliferation of banana (Arimative et al., 2000) and for plant regeneration in callus of triploid banana (Scrangsam and Kanchanapoom, 2003). On the standard medium (Murashige and Skoog basal medium supplemented with 2mg.l^{-1} benzylaminopurine (BAP), Topala, Fougamou, Gros-Michel, Dwarf-Kalapua, Pelipita and Kalapua 2 showed a low proliferation rate.

This paper reports the influences of different concentrations of diphenyl urea derived *N*-phenyl-*N'*-1,2,3-thiadiazol-5-ylurea (thidiazuron, TDZ) on shoot proliferation rates in these six cultivars of banana.

2. Materials and methods

2.1. Plant materials

Shoot apices of six cultivars included Gros Michel (AAA), Topala (AAB), Fougamou, Dwarf-Kalapua, Pelipita and Kalapua 2 (ABB) were excised from in vitro propagated culture of the six banana (*Musa* spp) cultivars.

2.2. Methods

The basal medium used in the experiments was that of Murashige and Skoog (1962) supplemented with 2 ml/liter Morel vitamin (1950), 40 g.l^{-1} sucrose, 2-4 g.l^{-1} phytagel. The pH was adjusted to 5.8 with either 1 N NaOH or 1 N HCl prior to autoclaving for 20 min at 120°C and 1.05 kg/cm^2. The concentrations of thidiazuron (TDZ) were later diluted to 0.05, 0.1, 0.2, 0.4, 0.8, 1, 1.5 and 2µM. The basal medium containing 2mg.l^{-1} benzylaminopurine (BAP) was used as control. Cultures were incubated at 25 ± 1°C under 12h photoperiod of 20 µmol $m^{-2}.s^{-1}$ provided by cool white fluorescence lamps. All plant materials were cultured in 250 ml screw-topped jars containing 50 ml of medium. Shoot-tips were isolated aseptically and inoculated onto differently modified semi-solid nutrient media.

Six weeks after inoculation, the shoot-tips had formed shoot clusters. Screw-topped jars were aseptically opened and the parameters: height and diameter of shoots, number of shoots, number of leaves and number of roots were evaluated. Shoot clusters were sub-divided and individual shoot-tips, of comparable sizes, transferred onto fresh media.

The trial was laid out following a completely randomized bloc design with two factors: cultivar and concentration. There were 5 cultivars and 9 concentrations in two replications of 30 shoots observed per cultivar and concentration, with a total of 2700 observations. Data were expressed as means of two replicates. An ANOVA was performed separately on each variable using Analysis System (SAS) computer program and means were compared with using LSD tests ($LSD_{0,05}$).

3. Results

3.1. Proliferation

The results of analysis of ANOVA of

shoot proliferation response among the five selected cultivars are represented in Table 1.

Their proliferation responses were highly influenced by the TDZ, TDZ concentration and the cultivar. Shoot proliferation was cultivar dependent compared to 2mg/l of BAP. The optimum proliferation mean (44.0) was obtained with 0.1 µM TDZ while 0.2µM was needed for optimum means (28.0 and 21.5) respectively for Topala, Dwarf-Kalapua 2 and Pelipita. The higher proliferation mean (18.75) was obtained with 0.4 µM TDZ for Gros-Michel wile 0.8 µM was needed to obtain the optimum proliferation mean (18.5) for Fougamou. The control medium containing 2mg/l of BAP of all banana cultivars had the lowest shoot number. It was observed that the optimum TDZ concentration varied from one genomic group to other and within the same group: 0.05 – 0.2 µM for Topala (AAB), 0.8 µM for Fougamou (ABB), 0.05-0.4 µM for Gros-Michel (AAA), 0.1-0.4 µM for Dwarf-Kalapua (ABB) and 0.05-1 µM for Pelipita (ABB). Above 1µM of TDZ, the proliferation rate failed for cultivars or genomic groups.

3.2. Growth and development

The results showed (Table 2) that the TDZ concentrations from 0.05 to 1 µM did not present significant differences compared to the control. The same results were observed concerning the diameter of pseudo-stem (Table 3). Concerning the mean number of leaves formed (Table 4), except for Topala where the TDZ concentration (2 µM) inhibited the leaf formation; the other cultivars did not present significant difference at all TDZ concentrations compared to the control (Table 4). Concerning the mean number of roots per plant, the concentrations 0.05-0.1 µM were optimum for the cultivars Fougamou, Gros-Michel and Pelipita while it varied from 0.05 to 0.4 µM for Topala (Table 5).

The growth and the development study of the explants did not show in general significant variation at ($p < 0.05$) in the experimental conditions between the control and the TDZ concentrations. The TDZ have small effect on growth and development of the plants.

4. Discussion

The different results obtained on shoot proliferation showed that TDZ increased multiplication rate. The optimum concentration varied among cultivars and

Table 1. Effects of TDZ concentrations on proliferation rates (%) of different banana cultivars on MS medium.

Cultivar	BAP ($2mg.L^{-1}$)	Concentration of thidiazuron (µM)							
		0.05	0.1	0.2	0.4	0.8	1.0	1.5	2.0
Topala	20.0 cd[z]	34.0 ab	44.0 a	28.8 bc	23.5 d	12.8 de	11.0 de	6.8 e	2.3 e
Fougamou	5.5 bc	1.5 c	8.0 bc	7.8 bc	9.5 b	18.5 a	10.3 b	9.5 b	5.0 bc
Gros-Michel	9.5 d	14.0 bc	14.3 bc	17.0 ab	18.8 a	8.0 d	9.0 d	11.5 cd	9.0 d
Dwarf-Kalapua 2	2.5 dc	5.0 bc	5.3 abc	8.0 a	5.8 ab	3.0 bcd	1.8 d	3.0 bcd	2.0 d
Pélipita	7.0 d	18.0 ab	17.0 ab	21.5 a	14.8 bc	13.5 bc	13.3 bc	10.0 cd	7.0 d

[z]: Means followed by the same letters within each cultivar are not significantly different according to $LSD_{0,05}$.

Table 2. Effects of TDZ concentrations on the shoot heights (cm) of different banana cultivars on MS medium.

Cultivar	BAP ($2mg.L^{-1}$)	Concentration of thidiazuron (µM)							
		0.05	0.1	0.2	0.4	0.8	1.0	1.5	2.0
Topala	2.5 ab[z]	3.0 a	3.5 a	2.5 ab	2.0 ab	2.0 ab	2.0 ab	1.5 b	1.5 b
Fougamou	2.8 ab	2.5 ab	2.5 ab	2.5 ab	2.0 ab	2.0 ab	2.0 ab	2.0 ab	2.0 ab
Gros-Michel	3.0 a	3.0 a	3.0 a	2.5 a	2.3 a	2.5 a	2.5 a	2.5 a	2.0 a
Dwarf-Kalapua 2	2.8 ab	2.5 ab	3.0 ab	3.3 ab	2.0 ab	2.0 ab	2.0 ab	2.0 ab	1.8 ab
Pélipita	2.5 ab	3.0 a	3.4 a	3.5 a	2.5 ab	2.0 ab	2.0 ab	1.8 ab	1.5 b

[z]: Means followed by the same letters within each cultivar are not significantly different according to $LSD_{0,05}$.

Table 3. Effects of TDZ concentrations on the shoot diameters (mm) of different banana cultivars on MS medium.

Cultivar	BAP (2mg.L^{-1})	Concentration of thidiazuron (µM)							
		0.05	0.1	0.2	0.4	0.8	1.0	1.5	2.0
Topala	2.0 a[z]	2.5 a	2.0 a	1.5 a	1.5 a	1.0 a	1.0 a	1.0 a	1.0 a
Fougamou	1.5 a	1.5 a	1.5 a	1.8 a	1.8 a	2.0 a	1.0 a	1.0 a	1.0 a
Gros-Michel	2.5 a	2.0 ab	2.0 ab	2.0 ab	2.0 ab	1.8 a	1.5 ab	1.5 ab	1.5 ab
Dwarf-Kalapua 2	1.5 a	1.5 a	1.5 a	1.5 a	1.8 a	1.3 a	1.0 a	1.0 a	1.0 a
Pélipita	1.5 a	1.5 a	1.8 a	1.8 a	1.5 a	1.5 a	1.0 a	1.0 a	1.0 a

[z]: Means followed by the same letters within each cultivar are not significantly different according to $LSD_{0,05}$.

Table 4. Effects of TDZ concentrations on number of leaves formed by shoots of different banana cultivars on MS medium.

Cultivar	BAP (2mg.L^{-1})	Concentration of thidiazuron (µM)							
		0.05	0.1	0.2	0.4	0.8	1.0	1.5	2.0
Topala	1.0 ab[z]	2.3 a	3.33a	1.3 ab	2.0 ab	3.0 ab	3.3a	2.7 ab	0.0 b
Fougamou	15.0 ab	17.0 ab	22.0 a	18.0 a	18.0 a	9.3 bc	10.0 bc	4.7 c	4.7 c
Gros-Michel	32.3 ab	42.0 ab	27.0 ab	18.3 ab	43.0 a	16.7 b	42.0 ab	27.7 b	29.0 ab
Dwarf-Kalapua 2	11.7 a	10.7 ab	11.7 a	10.3 ab	6.7 abc	4.6 abc	6.3 abc	1.7 c	3.3 bc
Pélipita	13.7 abc	13.3 ab	19.0 ab	18.0 ab	19.7 a	5.0 c	8.0 abc	6.7 bc	4.7 c

[z]: Means followed by the same letters within each cultivar are not significantly different according to $LSD_{0,05}$.

Table 5. Effects of TDZ concentrations on number of roots formed by shoots of different banana cultivars on MS medium.

Cultivar	BAP (2mg.L^{-1})	Concentration of thidiazuron (µM)							
		0.05	0.1	0.2	0.4	0.8	1.0	1.5	2.0
Topala	3.0 a[z]	1.3a	0.3 a	1.0 a	1.3 a	0.0 a	0.0 a	0.0 a	0.0 a
Fougamou	5.7 bc	42.7 ab	60.0 a	37.3 b	27.3 a	7.3 de	9.0 cde	2.7 e	2.7 e
Gros-Michel	20.7 c	99.0 a	75.3 ab	50.7 abc	50.0 abc	26.3 bc	48.3 abc	37.3 bc	25.3 bc
Dwarf-Kalapua 2	12.7 a	9.0 ab	5.3 bc	1.3 c	1.0 c	1.3 c	1.0 c	1.3 c	1.3 c
Pélipita	25.0 ab	38.7 a	32.3 a	25.7 ab	5.7 bc	1.0 c	0.7 c	0.3 c	0.3 c

[z]: Means followed by the same letters within each cultivar are not significantly different according to $LSD_{0,05}$.

within the genomic group. The same result had been obtained by Arinaitwe (2000) when studied the effects of cytokinins and TDZ on proliferation rate of Kibusi (AAA), Bwara (AAA) and Ndiziwemiti (ABB) banana cultivars. The differences observed on proliferation rate were explained by the different uptake rate reported in different genomes (Blakesley, 1991), variable translocation rates to meristematic regions and metabolic processes, in which the cytokinin may be degraded or conjugated with sugars or amino acids to form biologically inert compounds as reported by Tran Thanh Van and Trinh (1990) and Kaminck (1992). This high cytokinin effect of TDZ has been reported by several workers (Thomas and Katterman, 1986; Fellman et al., 1987; Mok et al., 1987; Arinaitwe et al., 2000; Gübbük and Pekmezci, 2004). In all cultivars studied, high proliferation rate was obtained with low concentration of TDZ (0.1- 0.8 µM). This result was contrary to those obtained by Arinaitwe et al. (2000); Gübbük and Pekmezci, (2004) who used more than 1 µM of TDZ to obtain high proliferation rates. This could be explained by the difference of the cultivars used. High proliferation rates were reported at very low TDZ concentration in woody plants species, which have low proliferation on BAP supplemented medium (Arif and Kathamian, 1992; Huetteman and Preece, 1993). This behaviour is believed to be due to the ability of TDZ to increase the biosynthesis of endogenous adenine-type cytokinins (Huetteman and Preece, 1993), thus making

TDZ an effective cytokinin for stimulation of shoot bud proliferation in recalcitrant banana genotypes. Our results corroborate with this observation. Arinaitwe et al. (2000) reported that TDZ was effective against recalcitrant and increased proliferation rates in non-recalcitrant banana cultivars.

The experimental results obtained on shoots growth and development were not significant ($P< 0.05$) between the control, at different TDZ concentrations and within genomic groups. These results are similar to those reported by Vuylsteke and Delanghe (1985); but contrary to the observations made by Gübbük and Pekmezci (2004), who obtained significant shoot elongation and root formation after supplementing 1 µM IAA in basal medium containing different TDZ concentrations. The cultivars used in this experiment rooted easily in MS basal medium without auxin.

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