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THE EFFECT OF 6-BENZYLAMINOPURINE, THIDIAZURON AND THE TYPE OF EXPLANTS ON *in vitro* PROPAGATION OF *Yucca elephantipes* Regel

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Abstract. *Yucca elephantipes* is an important commercial ornamental pot plant, excellent for growing in flats, patios or winter gardens. Traditional vegetative propagation of the most decorative yuccas is complicated due to a very low rate of propagation, so *in vitro* culture is an alternative method for commercial propagation of these plants. The influence of BA (0.4, 2.2, 4.4, 11.1, 22.2 μM) and TDZ (0.5, 2.3, 4.5, 11.4, 22.7 μM) on shoot multiplication of *Yucca elephantipes* Regel on Murashige and Skoog (MS) medium was studied. Explants cultured on medium without growth substances were used as a control. The two types of explants used in the experiment: shoot tips and nodal segments of shoots, were obtained from aseptically grown shoot clusters. When comparing regeneration capability of 2 types of *Yucca elephantipes* explants, it was found that more newly formed shoots and roots were obtained from nodes. The highest formation of shoots was obtained from nodes on MS medium supplemented with 4.5 μM TDZ or 11.1 and 22.2 μM BA (6.5, 6.0, 5.8, respectively). The shoots regenerated from nodes showed best elongation. On the control medium and on the media with the lowest level of BA or TDZ, their average length was 31.0–37.8 mm. The growth regulator-free medium and the media with a low level of BA were the most effective in inducing roots.

Key words: Yucca, BA, TDZ, shoot tips, nodes

INTRODUCTION

Yucca elephantipes or Spineless Yucca (Agavaceae) is native to Mexico. Its shiny green leaves are pliable and lack the sharp spines on the tips that are so characteristic of most yuccas. With age the trunk becomes rough and thick, and when mature it develops a swollen base. Propagation of *Yucca* by cuttings and offsets produces only few plants so studies on possibility of *in vitro* propagations are performed [Pierik and Steegmans, 1983, Bentz et al. 1988, Atta-Alla and Van Staden 1997, Sakr et al. 1999].

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The present study was designed to compare two types of explants (shoot tips and nodes) and two cytokinins (BA, TDZ) in five concentrations and to choose optimal for proliferation.

MATERIALS AND METHODS

Shoot tips and axillary buds were taken from plants growing in greenhouse. They were disinfected in sodium hypochlorite containing 0.75% of active chlorine for 45 minutes and rinsed 3 times in sterilized water. The explants were cultivated on the basic Murashige and Skoog (MS) (1962) medium containing: mineral salts and thiamine – 0.4 $mg \cdot dm^{-3}$, pyridoxine – 0.5 $mg \cdot dm^{-3}$, nicotinic acid – 0.5 $mg \cdot dm^{-3}$, glycine – 2 $mg \cdot dm^{-3}$, myo-inositol – 100 $mg \cdot dm^{-3}$, sucrose – 30 $g \cdot dm^{-3}$ and Agar-Agar (Sigma) – 6.5 $g \cdot dm^{-3}$, and supplemented with benzyladenine (BA) 4.4 µM and indole-3-acetic acid (IAA) 0.6 µM. After several months of multiplication, shoot tips of 3 cm in length and nodal parts of shoots of 1 cm in length were dissected from the shoot clusters and used in the experiment. Two cytokinins: BA (0.4, 2.2, 4.4, 11.1, 22.2 µM) and thidiazuron (TDZ) (0.5, 2.3, 4.5, 11.4, 22.7 µM) were used to examine the production of new shoots. A control medium without cytokinin was included. The pH of the media was adjusted to 5.7 before autoclaving. There were four replications per treatment, each consisting of 5 explants/Erlenmeyer flask. The experiment was repeated twice. The cultures were maintained at 22°C and light intensity of 35 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and 16-h photoperiod.

The following characters were evaluated after 8 weeks: number of newly (axillary and base-adjoined) formed shoots and their maximal and average length, fresh weight of shoots/explant, number, length and fresh weight of roots. The results of the experiment were analyzed statistically using a standard statistical procedure with one factorial design and the Tukey test was used to estimate the differences between the means at a 5% level of significance.

RESULTS AND DISCUSSION

When comparing regeneration capability of 2 types of *Yucca elephantipes* explants, it was found that more newly formed shoots and roots were obtained from nodes (tab. 1, 3). Many previous investigations indicated that aseptic culture of yucca could be established using: shoot tips [Bentz et al. 1988, Atta-Alla and Van Staden 1997, Atta-Alla et al. 1997], lateral shoots [Arce-Montoya et al. 2007], the base of the seedling stem [Arce -Montoya et al. 2006], epicotyl from seedlings [Karpov 2004]. At the shoot multiplication stage, the following were used: shoot tips from *in vitro* shoot cultures [Atta-Alla and Van Staden 1997], single shoots trimmed to 2 cm in length [Bentz et al. 1988], stems with the apex after removing leaves and roots [Arce-Montoya et al. 2007], individual new shoots [Arce-Montoya et al. 2006], adventitious microshoots from epicotyl [Karpov 2004].

Table 1. Regeneration and growth of new shoots from shoot tips of *Yucca elephantipes*, depending on type and concentration of cytokinin, after 8 weeks of *in vitro* culture

Tabela 1. Regeneracja i wzrost pędów z wierzchołków pędów *Yucca elephantipes,* w zależności od rodzaju i stężenia cytokininy, po 8 tygodniach kultury *in vitro*

Cytokinin – Cytokinina		Number of shoots/ explant Liczba pędów/eksplantat	Maximal length of shoots Maksymalna długość pędów (mm)	Average length of shoots Średnia długość pędów (mm)	Fresh weight of shoots/explant Świeża masa pędów/eksplantat (mg)
Type Rodzaj	concentration stężenie (µM)				
Control Kontrola	0	1.2 d*	31.0 bcd	17.3 cde	228.7 d
BA	0.4	1.1 d	42.0 bc	23.1 bc	230.1 d
	2.2	1.2 d	78.0 a	33.4 a	601.5 b
	4.4	1.5 cd	76.0 a	32.2 ab	850.7 a
	11.1	1.9 bc	38.0 bcd	21.2 c	856.5 a
	22.2	1.5 cd	31.0 bcd	15.0 cde	476.2 bc
TDZ	0.5	1.9 bc	46.0 b	18.1 cd	248.5 d
	2.3	1.9 bc	33.0 bcd	9.6 de	554.1 b
	4.5	2.2 ab	30.0 bcd	9.5 de	292.3 d
	11.4	2.5 a	27.0 cd	9.7 de	266.2 d
	22.7	1.9 bc	20.0 d	7.7 e	317.2 cd
Mean Średnia		1.7	41.1	17.9	447.5

*Values in vertical columns followed by the same letter do not differ significantly at p = 0.05
Wartości w kolumnach oznaczone tą samą literą nie różnią się między sobą istotnie przy p = 0,05

Table 2. Regeneration and growth of roots from shoot tips of *Yucca elephantipes*, depending on type and concentration of cytokinin, after 8 weeks of *in vitro* culture

Tabela 2. Regeneracja i wzrost korzeni z wierzchołków pędów *Yucca elephantipes,* w zależności od rodzaju i stężenia cytokininy, po 8 tygodniach kultury *in vitro*

Cytokinin – Cytokinina		Shoots forming roots Pędy tworzące korzenie (%)	Number of roots/explant Liczba korzeni/ eksplantat	Maximal length of roots Maksymalna długość korzeni (mm)	Average length of roots Średnia długość korzeni (mm)	Fresh weight of roots/explant Świeża masa korzeni/ eksplantat (mg)
Type Rodzaj	concentration stężenie (µM)					
Control Kontrola	0	6.7	3.0 a*	86.0 a	46.3 b	68.1 b
BA	0.4	73.3	2.3 b	98.6 a	84.1 a	213.5 a
	2.2	6.7	1.0 c	50.0 b	50.0 b	48.6 b
	4.4	26.7	1.5 b	26.5 c	21.6 c	36.6 b
	11.1	0	0 d	-	-	-
	22.2	0	0 d	-	-	-
TDZ	0.5	0	0 d			
	2.3	0	0 d	-	-	-
	4.5	0	0 d	-	-	-
	11.4	0	0 d	-	-	-
	22.7	0	0 d	-	-	-
Mean Średnia		10.3	0.7	65.3	50.5	91.7

*Values in vertical columns followed by the same letter do not differ significantly at p = 0.05
Wartości w kolumnach oznaczone tą samą literą nie różnią się między sobą istotnie przy p = 0,05

Fig. 1. Shoot regeneration from shoot tips of *Yucca elephantipes* on MS medium supplemented with different concentration of BA, after 8 weeks of *in vitro* culture

Ryc. 1. Regeneracja pędów z wierzchołków pędów *Yucca elephantipes* na pożywce MS uzupełnionej BA w różnych stężeniach, po 8 tygodniach kultury *in vitro*

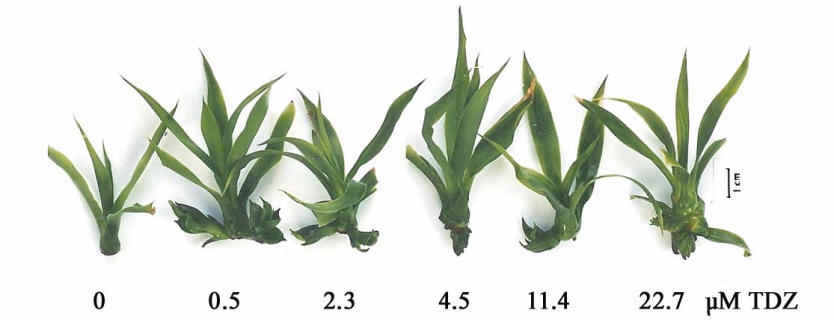

Fig. 2. Shoot regeneration from shoot tips of *Yucca elephantipes* on MS medium supplemented with different concentration of TDZ, after 8 weeks of *in vitro* culture

Ryc. 2. Regeneracja pędów z wierzchołków pędów *Yucca elephantipes* na pożywce MS uzupełnionej TDZ w różnych stężeniach, po 8 tygodniach kultury *in vitro*

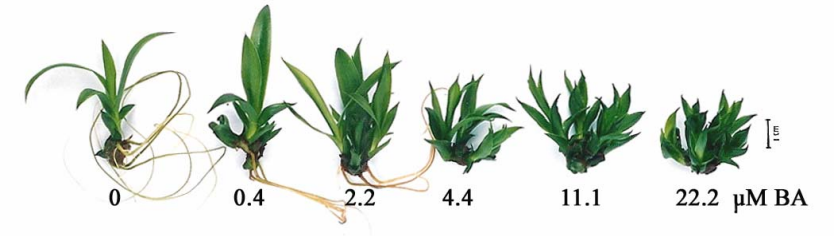

Fig. 3. Shoot regeneration from nodes of *Yucca elephantipes* on MS medium supplemented with different concentration of BA, after 8 weeks of *in vitro* culture

Ryc. 3. Regeneracja pędów z węzłów *Yucca elephantipes* na pożywce MS uzupełnionej BA w różnych stężeniach, po 8 tygodniach kultury *in vitro*

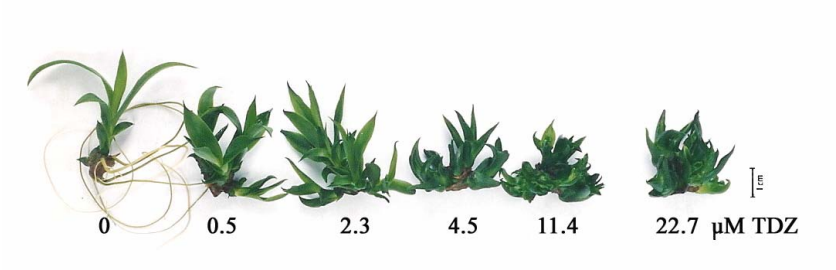

Fig. 4. Shoot regeneration from nodes of *Yucca elephantipes* on MS medium supplemented with different concentration of TDZ, after 8 weeks of *in vitro* culture

Ryc. 4. Regeneracja pędów z węzłów *Yucca elephantipes* na pożywce MS uzupełnionej TDZ w różnych stężeniach, po 8 tygodniach kultury *in vitro*

The analysis of shoot regeneration from shoot tips of *Yucca elephantipes* demonstrated significant differences in number of shoots, depending on the type and concentration of cytokinins. TDZ at an amount of 11.4 and 4.5 µM had the most beneficial influence on the regenerative abilities of shoot tips cultured on the growth media (2.5 and 2.2, respectively). On the control medium and on the media containing 0.4–4.4 µM BA, many fewer shoots were obtained (1.1–1.5). In analysing the number of shoots regenerated from the nodal segments of shoots, it was found that the highest number of shoots was obtained at a concentration of 4.5 TDZ or 11.1 and 22.2 µM BA (6.5, 6.0, 5.8, respectively). In the present study, the highest concentration of TDZ caused production of stunted shoots that could not be separated (fig. 4). Significantly more transferable shoots were produced on media with BA. Similarly, Richwine et al. [1996] observed stunted axillary shoots of *Hesparaloe parviflora* when a high BA concentration was used.

In the micropropagation of numerous plants of the Agavaceae family, benzyladenine was found to be more effective than other cytokinins. Bentz et al. [1988] reported that the highest total number of *Yucca glauca* shoots was produced at 32 µM BA, while the highest number of large shoots was at 10 and 32 µM BA. BA was the best cytokinin for induction of multiple shoots in *Y. filamentosa* var. Variegata [Atta-Alla et al. 1997]. Karpov [2004] obtained 7-8 adventitious shoots from epicotyl on medium containing BA 6.7 µM and NAA 0.3–0.5 µM. *Y. valida* was multiplied on medium supplemented with 5 µM BA and 1 µM IAA [Arce-Montoya et al. 2006] or 10 µM BA and 5 µM IAA [Arce-Montoya et al. 2007]. For *Hesperaloe parviflora* plants of the Agavaceae family, total axillary shoot multiplication was the greatest on medium with 6 µM BA [Richwine et al. 1996], but in the case of *Beaucarnea gracilis* and *B. recurvata* Osorio-Rosales and Mata-Rosas [2005] found that the highest formation of shoots from longitudinal sections of seedlings was on MS medium supplemented with 22.2 µM BA. The studies of Bettaieb et al. [2008] showed that multiplication of *Nolina recurvata* (6 axillary shoots/explant) is the best on medium with 4.45 µM BA and 0.5 µM IBA.

Atta-Alla and Van Staden [1997] obtained maximum shoot production of *Y. aloifolia* (6.6 shoots/explant) on MS medium supplemented with 4.5 µM TDZ and 1.1 µM NAA. In the studies on micropropagation of 5 genotypes of *Agave*, the highest shoot

Table 3. Regeneration and growth of new shoots from nodes of *Yucca elephantipes*, depending on type and concentration of cytokinin, after 8 weeks of *in vitro* culture

Tabela 3. Regeneracja i wzrost pędów z węzłów *Yucca elephantipes,* w zależności od rodzaju i stężenia cytokininy, po 8 tygodniach kultury *in vitro*

Cytokinin – Cytokinina		Number of shoots/explant Liczba pędów/eksplantat	Maximal length of shoots Maksymalna długość pędów (mm)	Average length of shoots Średnia długość pędów (mm)	Fresh weight of shoots/eksplantat Świeża masa pędów/eksplantat (mg)
Type Rodzaj	concentration stężenie (μM)				
Control Kontrola	0	2.3 e*	92.0 a	37.8 a	384.4 e
BA	0.4	2.4 e	86.0 ab	32.8 ab	499.5 e
	2.2	4.1 d	56.0 cde	31.0 abc	884.4 d
	4.4	4.7 cd	49.0 ef	26.3 cd	980.6 cd
	11.1	6.0 ab	46.0 ef	24.0 de	1322.8 b
	22.2	5.8 ab	48.0 ef	22.5 de	1352.3 b
TDZ	0.5	5.3 bc	71.0 abc	31.0 abc	988.8 cd
	2.3	4.9 c	63.0 cd	27.5 bcd	996.3 cd
	4.5	6.5 a	34.0 fg	19.1 ef	1215.2 bc
	11.4	5.0 c	27.0 g	18.2 f	1456.7 b
	22.7	5.3 bc	27.0 g	17.8 f	1839.3 a
Mean Średnia		4.8	54.5	26.2	1083.7

*Values in vertical columns followed by the same letter do not differ significantly at p = 0.05
Wartości w kolumnach oznaczone tą samą literą nie różnią się między sobą istotnie przy p = 0,05

Table 4. Regeneration and growth of roots from nodes of *Yucca elephantipes*, depending on type and concentration of cytokinin, after 8 weeks of *in vitro* culture

Tabela 4. Regeneracja i wzrost korzeni z węzłów *Yucca elephantipes,* w zależności od rodzaju i stężenia cytokininy, po 8 tygodniach kultury *in vitro*

Cytokinin –Cytokinina		Shoots forming roots Pędy tworzące korzenie (%)	Number of roots/explant Liczba korzeni/ eksplantat	Maximal length of roots Maksymalna długość korzeni (mm)	Average length of roots Średnia długość korzeni (mm)	Fresh weight of roots/explant Świeża masa korzeni/ eksplantat (mg)
type rodzaj	concentration stężenie (μM)					
Control Kontrola	0	100.0	3.3 b*	183.0 a	130.3 a	138.4 b
BA	0.4	100.0	5.2 a	113.0 b	62.4 b	256.8 a
	2.2	94.4	2.6 bc	57.0 c	11.2 c	59.5 c
	4.4	38.9	1.4 cd	21.0 d	13.0 c	9.3 d
	11.1	0	0d	-	-	-
	22.2	0	0 d	-	-	-
TDZ	0.5	0	0 d	-	-	-
	2.3	0	0 d	-	-	-
	4.5	0	0 d	-	-	-
	11.4	0	0 d	-	-	-
	22.7	0	0 d	-	-	-
Mean Średnia		30.3	1.1	93.5	54.2	116.0

*Values in vertical columns followed by the same letter do not differ significantly at p = 0.05
Wartości w kolumnach oznaczone tą samą literą nie różnią się między sobą istotnie przy p = 0,05

production efficiencies for *A. cupreata* and *A. karvinskii* were obtained with 6.7 and 4.4 μM BA (10.5 and 6.1 shoots/explant, respectively). In *A. difformis* and *A. obscura*, the best responses were obtained with 0.9 μM TDZ, which yielded 8.5–11.0 shoots/explant [Rosales et al. 2008].

Cytokinins affected the growth of shoots of *Yucca elephantipes*. The shoots produced from nodes were longer than those obtained from shoot tips. The strongest elongation growth of shoots was noted on the control medium (maximum length 92 mm, average length 37.8 mm) and on the media with the lowest level of cytokinins, in the case of shoots from nodal segments (fig. 3, 4) . The shoots formed from shoot tips had the largest length on the medium containing BA 2.2 and 4.4 μM (maximum length 78 and 76 mm, average length 33.4 and 32.2 mm, respectively) (fig. 1, 2). The length of shoots was significantly reduced by a higher cytokinin concentration. Similar effects were obtained by Bentz et al. [1988] and Atta-Alla and Van Staden [1997].

The shoots obtained from shoot tips were characterized by lower fresh weight (mean 447.5 mg) in comparison to those regenerated from nodes (mean 1083.7 mg). When assessing the fresh weight of newly formed shoots from 2 types of explants, a significant influence of cytokinins was observed. On the medium containing the highest level of TDZ (22.7 μM), the shoots from nodes reached the highest value of fresh weight (1839.3 mg). It was because of symptoms of slight hyperhydricity. Arce-Montoya et al [2006] observed slight hyperhydricity and abnormal morphological development of *Yucca valida* on medium with 20 μM BA and 5 μM IAA. When the plants were taken back to medium supplemented with 5 μM BA and 1 μM IAA, such symptoms disappeared. Chukwujekwu et al. [2002] noted that BA (4.4–13.3 μM) induced hyperhydricity in tissue culture of *Aloe polyphylla*. Atta-Alla and Van Staden [1997] reported that treatments with TDZ produced excessive callus formation of *Yucca aloifolia.*

During the experiment, the formation of roots of *Yucca elephantipes* was observed. The nodal segments of shoots had a higher rooting ability. Optimal root formation was noted on the control medium and on the media containing a low level of BA (0.4 and 2.2 μM), where 100 and 94.4% of the nodes formed roots. *In vitro* rooting was obtained on plant growth regulator-free medium in other Agavaceae species [Bentz et al. 1988, Binth et al. 1990, Santacruz-Ruvalcaba et al. 1999, Osorio-Rosales and Mata-Rosas 2005, Rosales et al. 2008]. Sakr et al. [1999] observed good formation of *Yucca elephantipes* roots on medium with 2.2 μM BA and 9.9 μM IBA. For many Agavaceae species, medium containing IBA is recommended for rooting [Atta-Alla and Van Staden 1997, Ray et al. 2006]. In the present experiment, TDZ at all the studied concentrations and BA at 11.1 and 22.2 μM completely inhibited rhizogenesis. Optimal elongation growth of roots was noted from the nodes on the control medium (maximum length 183 mm, average length 130.3 mm). The highest number of roots (5.2) and root fresh weight (256.8) were obtained from the nodes on the medium containing 0.4 μM BA.

CONCLUSIONS

1. A comparison of regenerative capabilities of shoot tips and nodal segments of shoots showed that many more shoots and roots regenerated from nodes than from shoot tips.

2. The highest formation of shoots was obtained from the nodes on MS medium supplemented with 4.5 μM TDZ or 11.1 and 22.2 μM BA.

3. The highest TDZ concentration caused slight hyperhydricity and abnormal morphological development.

4. The best elongation of shoots was found on the control medium and on the media with the lowest level of BA or TDZ in the case of the shoots regenerated from nodes and on the media with 2.2 and 4.4 μM BA for the shoots obtained from shoot tips.

5. The growth regulator-free medium and the media with a low level of BA were the most effective in inducing roots.

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WPŁYW 6-BENZYLOAMINOPURYNY, TIDIAZURONU I RODZAJU EKSPLANTATU NA ROZMNAŻANIE *Yucca elephantipes* Regel *in vitro*

Streszczenie. Juka słoniostopa (*Yucca elephantipes*) jest cenną rośliną pojemnikową do mieszkań, patio, a także do ogrodów zimowych. Tradycyjna metoda rozmnażania wegetatywnego większości gatunków ozdobnych juki jest mało wydajna, stąd zastosowanie metody kultur *in vitro* stwarza duże możliwości zwiększenia współczynnika mnożenia przy produkcji tych roślin na skalę masową. Badano wpływ BA (0,4; 2,2; 4,4; 11,1; 22,2 µM) i TDZ (0,5; 2,3; 4,5; 11,4; 22,7 µM) na namnażanie pędów *Yucca elephantipes* Regel na pożywce Murashige'a i Skooga (MS). Eksplantaty rosnące na pożywce bez regulatorów wzrostu stanowiły kontrolę. Pobrano dwa rodzaje eksplantatów: wierzchołki pędów i odcinki węzłowe, które pochodziły z kultur sterylnych. Porównując zdolności regeneracyjne eksplantatów *Yucca elephantipes*, stwierdzono, że więcej nowych pędów i korzeni regenerowało z odcinków węzłowych. Najlepszą regenerację pędów uzyskano z odcinków węzłowych na pożywkach z dodatkiem 4,5 µM TDZ lub 11,1 i 22,2 µM BA (6,5; 6,0; 5,8, odpowiednio). Pędy uzyskane z węzłów wykazywały lepszy wzrost elongacyjny. Na pożywce kontrolnej oraz w obecności niższych stężeń BA lub TDZ ich średnia długość wynosiła 31,0-37,8 mm. Najsilniejszą indukcję korzeni obserwowano na pożywce kontrolnej oraz przy dodatku BA w niskim stężeniu.

Słowa kluczowe: Juka, BA, TDZ, wierzchołki pędów, węzły

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