

Effect of Media Type and BAP Concentrations on Micropropagation during Multiplication Stage on Ponytail Palm (*Beaucarnea recurvata* Lem.)

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Abstract: Ponytail palm (*Beaucarnea recurvata* Lem.; Family Asparagaceae) is one of the most important plants in the internal and external coordination. This work was carried out to study the effect of media type (MS, B5 and WPM) and Benzylaminopurine BAP at 0, 0.2, 0.4 and 0.6 mg/l during multiplication stage. The shoot tips were collected from *in vitro* seedlings cultured on MS medium without growth regulators. B5 medium supplemented with 0.4 mg/l (BAP) increased number of shoots (3.40 shoots/clump) and number of leaves (24) plant compared with other treatments. The B5 medium is preferable within mass production and featured commercial. The BAP Concentration 0.4 mg/l promotes shoots initiation and development with B5 medium more than MS and WPM.

Keywords: Ponytail palm, *Beaucarnea recurvata*, tissue culture, micropropagation, BAP, media type, MS, B5, WPM

INTRODUCTION

Beaucarnea (Asparagaceae) is a Mexican and Guatemalan genus that inhabits dry tropical areas. Most of the species are endangered under the Mexican legislation because they have a high horticultural demand and are threatened by habitat destruction. (Martínez *et al.*, 2014).

Ponytail palm grows in full sun or partial shade on a wide range of soils. Soil must have good drainage as plants tend to develop root rot on poorly-drained soils. Plants moved from indoors to permanent outside locations should be gradually exposed to the increase in light and temperature change. Ponytail palm is evergreen sharp tree grows very slowly up to 30 feet in height but rarely exceeds 10 feet (Samyn, 1997).

Plants which were cultured on a low concentration of BAP produced highest number of shoots (Vardja and Vardja, 2001). The effect of medium containing BAP on *Aloe barbadensis* more efficient and produced the greatest number of plantlets, the multiplication was greater with BA (Adelberg and Naylor-Adelberg, 2012).

The highest number of shoots of *Aloe vera* was obtained in medium containing BAP comparison with another cytokinins (Lavakumaran and Seran, 2014). *Agave Americana* planted on MS medium supplemented with BAP had the highest number of shoots per callus after 4 weeks from culturing (Lecona-Guzmán *et al.*, 2017). Shoots regenerated of *Agave angustifolia* from shoot tip on the media with 0.4 mg/l produced the best number of shoots. The analyses of variance showed that the concentrations of benzylaminopurine (BAP) had highly significant effects on leaf number, leaf length, number of shoots, fresh weight and total dry weight (Rios-Ramírez *et al.*, 2017).

The main objective of this investigation was to identify the effect of media type and BAP Concentrations on micropropagation during multiplication stage on Ponytail palm (*Beaucarnea recurvata* Lem.).

MATERIALS AND METHODS

This study was carried out in the plant tissue culture laboratory in the Department of Horticulture, Faculty of Agriculture, Suez Canal University, Ismailia during the period 2013 – 2015.

Preparation of Explants:

The seeds were collected from healthy fruiting mother plants, then washed with running water for 15 minutes, Soaked in sterile distilled water for 24 hours in the refrigerator, then sterilized with 70% ethanol for 1 minute, followed by 20% v/v Clorox (sodium hypochlorite 5.2%) with Tween 80 (2 drops/100 mL) for 10 minutes on Magnetic Stirrer (Osorio-Rosales and Mata-Rosas, 2005).

Culturing medium:

In the starting stage, Murashige and Skoog (1962) medium supplemented with vitamins 4.4 g/l, 30 g/l sucrose, and 6g/l Agar was used. The pH of the culture medium was adjusted at 5.7± 0.1 prior to addition of agar. The culture media were distributed into culture Jar 60 x 90 mm where immediately capped with polypropylene closure and autoclaved at 121°C at 15 lb/in² for 20 min and then incubated at 25°C ± 1°C for testing against contamination (Rani *et al.*, 2014).

Explant establishment:

Sterilized seeds were cultured on the MS medium as mentioned before in the starting stage. Culture Jar were incubated at 25± 2°C and 16/8 hours (day/night) light using white fluorescent tubes giving intensity of about 1500 Lux. After four weeks the germination rate was 90%, all survival explants were transferred into the media of multiplication stage.

Survived and established shoot tip explants were transferred and re-cultured different media, Murashige and Skoog (MS) (Murashige T. and Skoog F, 1962) Gamborg medium (B5) (Gamborg *et al.*, 1968) and Woody Plant Medium (WPM) (Lloyd. and Mccown., 1980) All media were supplemented with BAP (0, 0.2, 0.4, 0.6 mg/l) + 30 g/l sucrose + 6.0 g/l agar in medium.

At the end of multiplication stage after four weeks data recorded per cluster as: shoot number, Plant length (cm), Plant weight (g) and number of leaves.

Statistical analysis:

Experiment in this work was designed as factorial experiment in a complete randomized design. Data were computerized and subjected to statistical analysis using Costat “version 6400” statistical software. The differences between means of treatments were tested using LSD Test at 0.05 level (Snedecor and Cochran., 1980).

RESULTS AND DISCUSSION

a. Main effect of media type and BAP concentrations on the *Beaucarnea recurvata* Lem.:

Data presented in Table (1) and Figure (1) showed that difference between three types of media (MS, B5 and WPM) and showed that B5 medium was more effective than other media in shoot tip multiplication.

Result indicated that media type affected the shoots number. B5 medium was significantly increased the shoot number (3.25).

Data in Table (2) shows the interaction between media type (MS, B5, WPM) and BAP concentrations. The B5 medium with 0.4 mg/l BAP was more significantly than MS and WPM media on number of shoot (5.40 shoots/clump). The MS Medium with zero BAP was more effective than B5 and WPM on shoot length (13.00 cm). The MS and WPM with 0.6 mg/l BAP had same fresh weight (3.8 gm) and WPM with 0.6 mg/l BAP produced high number of leaves (30/plant).

The difference between media is due to the difference in the composition of each medium where B5 medium contain a greater proportion of potassium nitrate comparison with other media, it is containing monosodium phosphate and ammonium sulfate and is not found in other media according to (Enriquez-del Valle *et al.*, 2016).

Table (1): Main effect of media type and BAP concentrations on number of shoots, shoot length, fresh weight and number of leaves of *Beaucarnea recurvata* *in vitro* micropropagated during multiplication stage

Treatment		No. of shoots per clump	Shoot length (cm)	Fresh weight (g)	No. of leaves
Media	MS	2.40 b	10.20 a	3.00 a	20.00 a
	B5	3.25 a	7.55 b	2.21 b	20.55 a
	WPM	2.80 ab	7.60 b	2.23 b	20.45 a
	L.S.D. 0.05	0.415	0.567	0.176	0.720
BAP conc. mg/l	0.0	1.47 d	7.63 b	1.39 d	9.13 d
	0.2	2.27 c	9.20 a	2.30 c	17.00 c
	0.4	4.47 a	9.83 a	2.69 b	26.86 b
	0.6	3.07 b	7.13 b	3.53 a	28.33 a
	L.S.D. 0.05	0.479	0.655	0.204	0.832

Means of each column in each treatment have the same letter/s are not significantly different at 0.05 level of probability according to Duncan's multiple range test.



Table (2): Effect of interaction between media type and BAP concentrations on number of shoots, shoot length, fresh weight and number of leaves of *Beaucarnea recurvata* *in vitro* micropropagated during multiplication stage

Treatment Media	BAP conc. mg/l	No. of shoots per clump	Shoot length (cm)	Fresh weight (g)	No. of leaves
MS	0.0	1.00 f	13.00 a	1.50 de	11.20 h
	0.2	2.00 de	9.60 b	3.56 a	19.20 e
	0.4	4.20 b	12.20 a	3.10 b	24.00 d
	0.6	2.40 de	6.00 d	3.82 a	25.60 c
B5	0.0	1.60 ef	4.30 e	1.66 d	6.80 j
	0.2	2.60 cd	10.10 b	2.06 c	17.40 f
	0.4	5.40 a	8.00 c	2.18 c	28.60 ab
	0.6	3.40 bc	7.80 c	2.92 b	29.40 ab
WPM	0.0	1.80 def	5.60 d	1.02 f	9.40 i
	0.2	2.20 de	7.90 c	1.28 ef	14.40 g
	0.4	3.80 b	9.30 b	2.78 b	28.00 b
	0.6	3.40 bc	7.60 c	3.84 a	30.00 a
L.S.D.	0.05	0.829	1.134	0.352	1.440

Means of each column in each treatment have the same letter/s are not significantly different at 0.05 level of probability according to Duncan's multiple range test

CONCLUSION

Different composition of the media in the macro elements, micro elements and vitamins lead to different characteristics of vegetative growth of the plant also affects the number of plants produced.

For producing the greatest number of plantlets of *Beaucarnea recurvets* Lem. must use B5 medium supplemented with 0.4 mg/l BAP for four weeks.

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تأثير البيئات المختلفة مع تركيزات البنزويل أمينو بيورين أثناء مرحلة التضاعف علي نبات ذيل الحصان (القله)

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نخيل ذيل الحصان (القله) (*Beaucarnea recurvet* Lem) هي واحدة من أهم النباتات في التنسيق الداخلي والخارجي. أجريت هذه الدراسة بمعمل زراعة الأنسجة في قسم البساتين ، كلية الزراعة ، جامعة قناة السويس ، الإسماعيلية خلال الفترة ٢٠١٣ - ٢٠١٥ ، لدراسة تأثير استخدام البيئات المختلفة (MS، B5 و WPM) و BAP تركيزات ٠ ، ٠.٢ ، و ٠.٤ ملجم / لتر خلال مرحلة التضاعف وذلك في تجربة عاملية في تصميم كامل العشوائية. وكانت أفضل النتائج هي استخدام بيئة B5 مضاف إليها ٠.٤ ملجم / لتر بنزويل أمينو بيورين حيث زاد الأفرع المتضاعفة (٣.٤٠) وعدد الأوراق (٢٤ / نبات) بالمقارنة مع البيئات الأخرى.