Factors Affecting the Micro-propagation Steps of *Yucca desmetiana*

Ramez S. Thabet and Mohammed M.M. Abass
Dept. Ornamental Plants Res., Hort. Res. Inst., Agric. Res. Cent., Dokki, Giza, Egypt.

ABSTRACT

This experiment was carried out in Plant Tissue Culture Laboratory at El-Zohryia Botanical Garden, Hort. Res. Instit., Agric. Res. Center, Egypt during the period of 2022 to 2023.The objective of this study was to assess a protocol for micro-propagation of *Yucca desmetiana* plant. Terminal buds were sterilized with Clorox at (20, 25 or 30 %) or mercuric chloride (MC) solution ($Hgcl_2$) at (0.1, 0.2 or 0.3 %) for 15, 20 and 25 min for sterilizing explants. In establishment stage, various MS medium strengths (¼, ½, ¾ or full strength) were used. For multiplication stage various concentrations of BAP (0, 1, 3, 5, 7, 9 or 11 mg/l) were examined. At rooting stage, IBA or NAA at different concentrations (0, 1, 3, 5 or 7 mg/l) were tested. Also, different media types were noticed during acclimatization stage.

The obtained results referred to that sterilizing explants with Clorox 25 % for 25 min gave the best result of survival percentage (100 %), while the lowest contamination percentage were noticed when explant was exposed to MC at (0.1, 0.2 and 0.3 % for 15 min). The highest shoots in establishment stage were achieved with ¾ or full strength of MS medium. Moreover, 5 mg/l BAP gave the highest values in multiplication stage for the same parameters. Using NAA at 7 mg/l concentrations recorded the longest roots during rooting stage while IBA at 7 mg/l gave more roots. Media mixture of peat moss+ sand +perlite (1: 1: 1 v/v/v) at acclimatization stage.

Keywords: *Yucca desmetiana*-MS strength- BAP- Rooting- Media mixture

INTRODUCTION

Yucca desmetiana is an interesting 'soft leafed' yucca with new green foliage that turns to an attractive burgundy red as it ages. This species is well-liked for landscaping because it grows well as a specimen plant and only has one stem. There are about fifty plant species in the Agavaceae family that belong to the genus Yucca. Worldwide, traditional medicinal practices and landscape use have long been associated with species of the Yucca genus (Simmons-Boyce and Tinto, 2007and Patel, 2012). One of the primary sources of steroidal saponins is Yucca, hence various extracts are sold for use as surfactant additions in beverages, cosmetics, animal feed, and agricultural items (Jiménez et al., 2021).

Micro-propagation has undoubtedly contributed much to plant science and agriculture. With the use of this technique, a single mother stock can yield a huge number of genetically identical plants. Compared to conventional propagation techniques, tissue culture plant production yields disease- and virus-free plants, which is an advantage (Shibli et al., 1997, Oliveira et al., 2000 and Ibrahim, 2003).

Chemical sterilization with sodium hypochlorite (NaOCl), proposed initially by Pais et al. (2016) provided satisfactory results for Gerbera hybrida cv. Essandre, Hesami et al., (2017) on Ficus religiosa concern the lowest rate of contaminations well as Laksana et al. (2023) on Bucephalandra aquatic plant regarding a decrease of the growth of germ-free cells. In addition, Abou Dahab et al. (2005) indicated that the best treatment which can be recommended to obtain free of contamination Ruscus hypoglossum explants was 0.4% mercuric chloride.

Half strength media showed the maximum average shoot length and the greatest number of induced shoots and roots

per explant. On full medium, regeneration was correlated with the maximum number of leaves and the average root length of spearmint (Fadel et al., 2010). The increased concentration of MS medium resulted in mass gain of baruzeiro (Dipteryxalata Vog.) plants (Pinhal et al., 2017).

Three MS salt concentrations (full, 1/2 or 1/4 strength) were investigated. Ruscus hypoglossum shoots multiplied most when MS medium was used at full salt strength (Abou Dahab et al., 2005). In three consecutive subcultures, Fathy et al. (2018) investigated the effects of Benzyl adenine (BA) concentrations of 0.2, 0.4, and 0.6 mg/l on the multiplication of shootlets. When compared to other treatments, the multiplicity media with 0.6 mg/l of BA had the highest survival rate (100%) and the highest number of shootlets per transplant (3.4) following the initial subculture.

Moreover, Abass et al. (2016) revealed that peat moss and perlite at equal volume proportions seemed to be the suitable medium for Aglaonema commutatum plantlets acclimatization. Also, El-Shamy et al. (2022) pointed out that the best survival percentage of Begonia plantlets at acclimatization stage (90 %) was observed when plantlets cultured in a mixture of peat-moss and sand at 3/1(v/v).

This study primarily sought to ascertain how Yucca desmetiana explants responded to various sterilization compounds (NaOCl or $HgCl_2$) at the established stage, evaluate the effects of medium strength and BAP on multiplication stage, examine the effects of auxins (IBA or NAA) on explants height and rooting traits at the rooting stage, and examine the effects of peat moss, coco peat, sand and perlite on plantlets acclimatization.

MATERIALS AND METHODS

This experiment was carried out in Plant Tissue Culture Laboratory at El-Zohryia Botanical Garden, Hort. Res. Instit., Agric. Res. Center, Egypt during the period 2022 - 2023.

Explant source:

Mother plants of *Yucca desmetiana* L. were collected from El-Zohryia Botanical Garden region, Egypt. Terminal shoots at one-year age about 4 - 5 cm were cut from mother plants and the leaves were removed and the bases of leaves were preserved (so as not to damage the axillary buds or tissues). The lower leaves completely removed and the parts containing clear dust and microbes were eliminated until the white tissues were reached. Thus, the buds reached a length of about 1.5- 2 cm. The buds were transferred to the laminar air flow hood and initially disinfected with ethyl alcohol (70 %)for 5 min by spraying three times and left until dry.

Sterilization stage:

Yucca desmetiana explants were sterilized with sodium hypochlorite5% in Clorox at different concentrations (20, 25 or30 %) or mercuric chloride (0.1, 0.2 or 0.3 %) each for 15, 20 or 25 min. Explants were thoroughly rinsed three times with sterile distilled water after each previous step. Finally, explants were cut into shoot tip explants (1.5 - 2 cm length) and culture at 250 ml glass containers and then incubated. Free contaminated *Yucca desmetiana* plants and survival percentage were recorded after 3 weeks.

Establishment stage:

All explants free contamination and survival were cultured on different strength of MS (Murashige and Skoog, 1962) medium (¼, ½, ¾ or full strength) with 3 replicates for each treatment. The data were recorded as the number of shoots, number of leaves/cluster and fresh weight of cluster (g) after 4 weeks.

Multiplication stage:

The best treatment in establishment stage was ¾ or full strength MS medium. Thus, ¾ strength medium four times were examined with various concentrations of benzyl amino purine (0, 1, 3, 5, 7, 9 or11mg/l). for three subcultures and the clusters were transplanted every 6 weeks, for one subculture shoots number /explant, number of leaves/shoot and fresh weights of cluster (g) were recorded.

Rooting stage:

Similar shoots of *Yucca desmetiana* (about 2-3 cm length) obtained from multiplication experiments were cultured on ¾ MS Free growth regulators medium for 3 weeks before starting on the rooting experiment. Then the shoots transferred to medium containing ¾ MS and activated charcoal at 1 g/l supplemented with different concentrations (0, 1, 3, 5 or7 mg/l) of indole butyric acid (IBA) or naphthalene acetic acid (NAA). After 4 weeks rooting percentage, root sand leaves number/ shoot, plant height (cm) and root length (cm) were recorded.

Every experiment culture previously described was kept in a growth environment with a temperature of 25 ± 2º C and a photoperiod of 16 hours each day, all supplied by cool white fluorescent lights with 2000 Lux of light intensity.

Acclimatization stage:

Rooted shoots, measuring around 3–4 cm in length, were placed in black, 8 cm diameter plastic pots with peat moss and perlite (1:1, v/v), peat moss + sand (1:1, v/v), coco peat + sand (1:1, v/v), coco peat + sand + perlite (1:1:1, v/v/v) or peat moss+ sand + perlite (1:1:1, v/v/v) to help them get acclimated. Transparent polyethylene bags were used to cover the cultured pots. Holes were created in covered bags after a week. Every week, these holes grew larger. Plantlets were ready to be moved to the exterior of the greenhouse after four weeks. In this stage, plant height (cm), leaves and roots number/ plantlet, and root length (cm) were recorded. Also, chlorophyll a, b, a+ b and carotenoids contents (mg/100 g as fresh weight) were determined according to Mazumder and Majumder (2003).

Statistical analysis:

All collected data were analyzed with analysis of variance (ANOVA). This experiment was designed as completely randomized design (CRD) with 3 replicates for each treatment. Separation of means among treatments was determined using L.S.D test at 5%, according to Steel and Torrie (1980).

RESULTS AND DISCUSSION

Sterilization stage:

Data presented in **Table (1)** reveal that Clorox at 30 % significantly recorded the highest explant free contaminated (76.6 %) compared to the other sterilization compounds. The lowest values in this regard were noticed when explants depressed in mercuric chloride at 0.1 % compared to the other ones under study. Sterilized explants for 15 min gave the lowest free contaminated explants (43.3 %) compared to the longest durations (20 and 25 min). sterilizing explants with Clorox 25 % for 25 min gave the best result of survival percentage (100 %), while the lowest contamination percentage (30.0 and 40.0%) were noticed when explant was exposed to mercuric chloride 0.1, 0.2 or 0.3 %for 15 min, respectively. Clorox sterilization has biocide qualities because of its effects on lipid and fatty acid degradation and enzymatic inactivation caused by oxidative processes triggered by chlorine ions (Estrela et al., 2002; Emmanuel et al., 2004). In addition, Abou Dahab et al., (2005) demonstrated that increasing mercuric chloride concentrations increased percentage of free of contamination explants of *Ruscus*

hypoglossum. According to Antony et al. (2015) *Tectona grandis* treated with HgCl 0.1% for five minutes demonstrated improved bud break, and at 0.1 and 0.15 percent, there was less bacterial and fungal contamination. Also, the *Momordica cymbalaria* explants treated with 0.15% of mercuric chloride were more effective than other concentrations the explants (Madhale, 2016).

Table (1). Effect of sterilization compound concentrations (Clorox or mercuric chloride), duration and their interaction treatments on free contaminated and survival % *Yucca desmetiana* plants.

Treatments	Free contaminated (%)				Survival percentage (%)			
	15 minutes	20 minutes	25 minutes	Mean (A)	15 minutes	20 minutes	25 minutes	Mean (A)
NaOCl 20%	40.0	50.0	60.0	**50.0**	70.0	80.0	80.0	**76.7**
NaOCl 25%	50.0	60.0	100.0	**70.0**	80.0	90.0	100.0	**90.0**
NaOCl 30%	60.0	80.0	90.0	**76.6**	70.0	90.0	90.0	**83.3**
$HgCl_2$ 0.1 %	30.0	60.0	60.0	**50.0**	60.0	60.0	60.0	**80.0**
$HgCl_2$ 0.2 %	40.0	50.0	70.0	**53.3**	60.0	60.0	70.0	**63.3**
$HgCl_2$ 0.3 %	40.0	70.0	80.0	**63.3**	60.0	70.0	80.0	**70.0**
Mean (B)	**43.3**	**61.6**	**76.6**		**66.7**	**75.0**	**80.0**	
L.S.D. at 5 %	**A= 5.22**	**B= 2.37**	**A×B= 7.04**		**A= 8.78**	**B= 6.21**	**A×B= 15.21**	

Establishment stage:

As shown in **Table (2)** and **Fig. (1)** it is clear that using ¾ or full strength of MS medium significantly increased number of shoots, number of leaves and fresh weight of shoot compared to the lowest strength (¼ and ½ strength). In general, the lowest values in this connection were noticed when the strength of MS medium was ¼ strength compared to the other concentrations under study. According to El-Afry et al. (2017), inoculating explants on a medium with half the strength produced the greatest numbers of *Phytolacca dioica* shoots and leaves. The results of employing MS medium at maximum strength were the longest shoots.

Table (2). Effect of medium salts strength on shooting behavior of *Yucca desmetiana* plants.

Treatments	Number of shoots	Number of leaves/cluster	Fresh weight of cluster (g)
¼ MS	3.00	24.78	2.97
½ MS	3.88	36.72	4.69
¾ MS	5.44	48.11	6.44
full MS	5.44	49.56	6.63
L.S.D. at 5 %	**0.252**	**2.161**	**0.292**

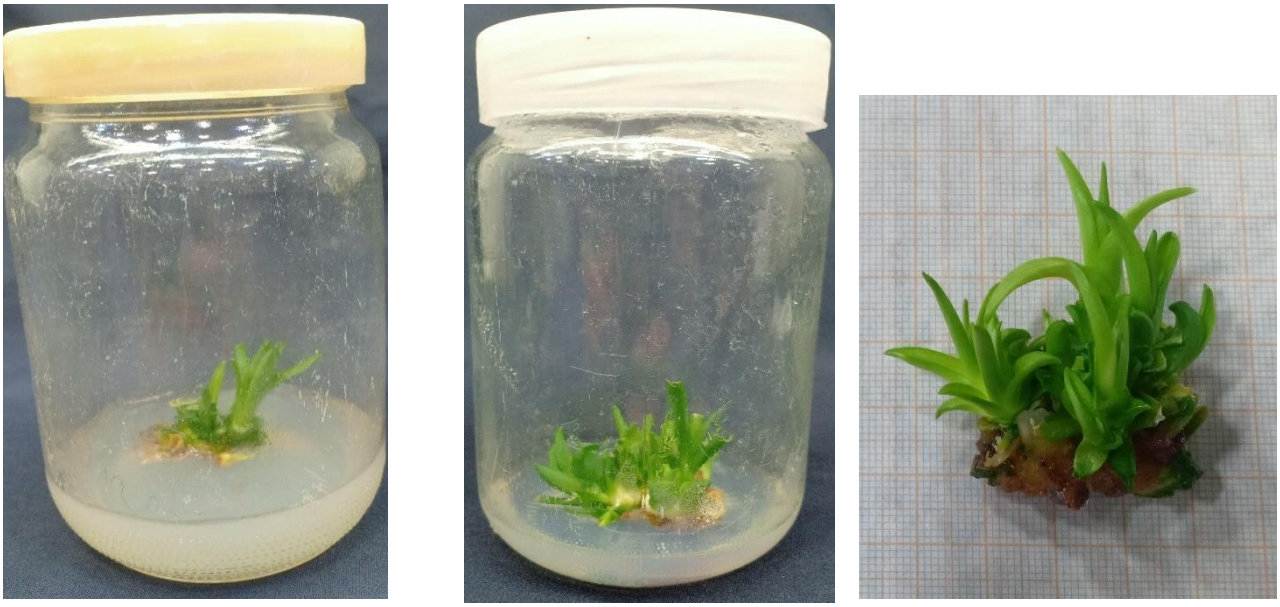
Multiplication stage:

From the previous stage the best strength of MS medium ¾ strength. This strength was used on multiplication stage with different concentrations of BAP. Data illustrated in Table 3 and Fig. 2 show that addition of BAP at concentration 5 mg/l to the ¾ strength of MS medium significantly increased the number of shoots, number of leaves, fresh weight of shoot. In this respect, Atawian et al. (2016) reported that at the first and second subcultures, 2.0 mg/l BAP exhibited the maximum shoot proliferation among the varied concentrations, with 56 and 42 shoots per explant, respectively. In both subcultures, the treatment of 0.25 mg/l BAP resulted in the production of the longest shoot of *Ananas comosus*. Moreover, adding BAP at 10 mg/l~~and~~ on MS medium at 3/4 strength, the greatest number of shoots and leaves were produced. The maximum shoot length and leaf count of *Phytolacca dioica* were obtained using MS medium at full strength in combination

with BAP at 3 or 10 mg/l (El-Afry et al. 2017).

Table (3). Effect of different concentrations of (BAP) on shooting behavior of *Yucca desmetiana* plants

Treatments	Number of shoots	Number of leaves/cluster	Fresh weight of cluster (g)
Control (free hormone)	3.22	13.56	5.04
1 mg/l BAP	3.56	14.89	4.99
3 mg/l BAP	6.78	32.89	6.99
5 mg/l BAP	16.56	78.00	8.29
7 mg/l BAP	12.00	52.44	7.09
9 mg/l BAP	6.45	31.67	6.78
11 mg/l BAP	3.55	15.56	5.66
L.S.D. at 5 %	**0.691**	**3.425**	**0.178**



Control (without BAP) **BAP at 5 mg/l** **BAP at 5 mg/l**

Fig.(1). Effect of (BAP) on multiplication of *Yucca desmetiana*

Rooting stage:

All shoots with length (2-3 cm) were cultured on MS medium with 3 mg/l AC and different type and concentrations of IBA or NAA. Data recorded in Tables 4as well as illustrate in Fig.3indicate that using any concentration of IBA or NAA significantly increased rooting percentage, number of roots/ plantlet and the longest root length compared to control. While, IBA or NAA gave no significant differences regard number of leaves. The highest values of rooting % and number of roots achieved with 7 mg/l of IBA compared to the other concentrations under study. In this connection, Abd El-Gawad et al. (2012) noticed that the greatest significant result for coffee plantlet development and root formation (length of plantlets and roots and number of leaves and roots/plantlet) was obtained at the rooting stage using half strength MS medium with IBA at 3 mg/l. Also, Atawia et al. (2016) showed that pineapple explants grown on MS media with 3 mg/l of IAA generated the longest roots, whereas 1.0 mg/l of IAA produced the greatest number of roots-10.67 roots per shootlet. In this concern, Markovic´ and Grbic´ (2020) reported that shoot cuttings of Dianthus giganteiformis sub sp. Kladovanus

with 1 to 4 nodes should be rooted on half- strength MS medium.

Table (4). Effect of type of auxins (IBA or NAA) with different concentrations on rooting behavior of *Yucca desmtiana* plants

IBA concentrations (mg/l)	Rooting (%)	Number of roots	Root length (cm)	Plantlet height (cm)	Leaf number
0 (Control)	33	1.33	4.57	7.38	9.11
1 IBA	100	4.00	6.31	7.42	10.67
3 IBA	100	8.78	7.27	7.35	10.91
5 IBA	100	12.89	7.19	7.11	10.44
7 IBA	100	15.67	7.88	7.05	10.45
1 NAA	100	4.29	7.33	8.25	9.00
3 NAA	100	5.38	8.13	8.56	9.45
5 NAA	100	8.78	8.54	8.59	9.44
7 NAA	100	11.72	9.84	8.32	10.22
L.S.D. at 5 %	**1.15**	**0.68**	**0.28**	**0.31**	**0.96**

Control **IBA at 7 mg/l** **NAA at 7 mg/l**

Fig. 2. Effect of auxins (IBA ~~and~~ or NAA) on rooting of *Yucca desmetiana*

Acclimatization stage:

Data tabulated in **Tables (5 and 6)** and illustrated in Fig. 4indicate that using coco peat + sand + perlite (1: 1: 1 v/v/v) media mixture at acclimatization stage recorded the highest values of number of root and root length average as well as chlorophyll a, b and a +b contents. In addition, utilizing and media mixture not significantly affected plant height, number of roots and root length average **(Table 5).** The lowest values in leaf pigments (chlorophyll a, b, a+ b and carotenoids) contents were observed when plantlets were grown in coco peat + sand mixture compared to the other media mixture types under study **(Table 6).** Furthermore, De Stefano et al. (2022) indicated that the combination of coconut coir and horticultural charcoal was more effective in acclimating *Epidendrum nocturnum* to its new environment than the combination of sphagnum moss, horticultural charcoal, and wood bark. This was demonstrated by higher values of plant height and number of leaves at 30, 90 and 120 days after transplantation. Moreover, Saleh et al. (2024) explained that the successive rooted *Capparis spinosa* explants

transferred on peat moss: vermiculite 2:1 which scored 41.66 % acclimatized plantlets.

Table (5). Effect of media mixture types on plant height (cm), number of leaves, rooting percentage, number of roots, and longest root length (cm) of *Yucca desmtiana* plants

Media types	Plant height (cm)	Number of leaves	Number of roots	Roots length (cm)
Peatmoss + perlite	11.33	5.67	2.00	12.23
Peatmoss + sand	11.33	5.33	2.67	10.37
Coco peat + sand	11.67	4.33	2.00	11.25
Coco peat + sand + perlite	11.83	5.33	2.67	11.27
Peatmoss + sand + perlite	12.17	5.67	2.33	10.40
L.S.D. at 5 %	**N.S.**	**1.26**	**N.S.**	**N.S.**

Table (6). Effect of media mixture types at acclimatization stage on chlorophyll a, b, a +b and carotenoids contents (mg/100 g as fresh weight) of *Yucca desmetiana*

Media types	Chlorophyll (a)	Chlorophyll (b)	Chlorophyll (a + b)	Carotenoids
Peatmoss + perlite	1.161	0.790	1.951	0.711
Peatmoss + sand	1.076	0.522	1.598	0.510
Coco peat + sand	1.006	0.512	1.518	0.414
Coco peat + sand + perlite	1.275	0.776	2.051	1.337
Peatmoss + sand + perlite	1.181	0.543	1.724	1.068
L.S.D. at 5 %	**0.181**	**0.162**	**0.299**	**0.253**

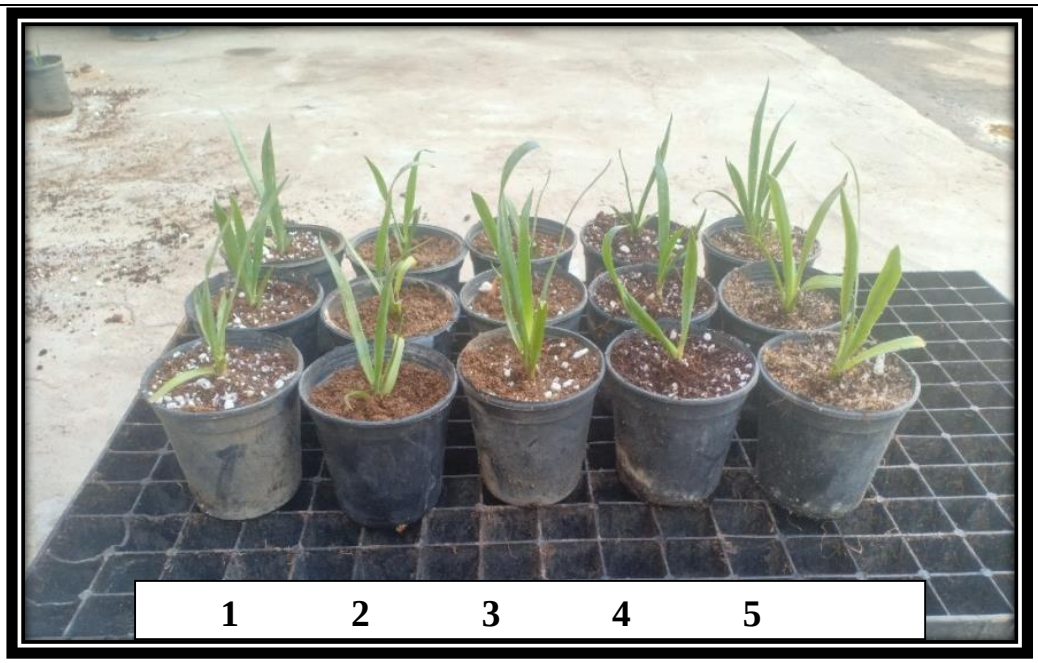


Fig. 3: Different media mixture types at acclimatization stage

1. Peatmoss + perlite. 2. Peatmoss + sand. 3. Coco peat + sand + perlite
4. Peatmoss + sand + perlite. 5. Coco peat + sand

Conclusion:

From above mentioned results it is clear that, utilizing surfactant NaOCl at 25 % for 25 min gave the best result of survival percentage on the surface sterilization. In multiplication stage, using ¾ or full with 5

mg/l BAP recorded the best result at rooting stage, adding 7 mg/l of NAA recorded the best rooting behavior. Coco peat + sand + perlite (1: 1: 1 v/v/v) media mixture at acclimatization stage recorded the highest growth parameters.

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الملخص العربى

العوامل المؤثرة على مراحل الإكثار الدقيق لنبات اليوكا ديسميتيانا

رامز صابر ثابت ، محمد محسن محمد عباس
قسم بحوث نباتات الزينه، معهد بحوث البساتين، مركزالبحوث الزراعيه، الجيزه، مصر

أجريت هذه التجربة بمعمل زراعة الأنسجة النباتية بحديقة الزهرية –معهد بحوث البساتين – مركز البحوث الزراعية بالدقي - مصر خلال الفترة من 2022 إلى 2023. كان الهدف من الدراسة الحالية هو تقييم بروتوكول محدد جيدًا للإكثار الدقيق لنبات يوكا ديسميتيانا. تم تعقيم البراعم الطرفية (طول 1.5 إلى 2.0 سم) بإستخدام كلوركس(20 و25 و30%) أو كلوريد الزئبق (0.1 و0.2 و0.3 %) لمدة 15 و20 و25 دقيقة على التوالي لتعقيم المنفصلات النباتية. في مرحلة التضاعف، تم فحص قوة البيئة المختلفة (¼، ½، ¾ والقوة الكاملة) ثم بعد ذلك تأثير تركيزات مختلفة من البنزيل أمينو بيورين (صفر، 1، 3، 5، 7، 9 و11 جزء / المليون) على عدد البراعم والأوراق والوزن الطازج للأفرع. في مرحلة التجذير، تم إختبارحمض الإندول بيوتريك أوحمض النفثالين أسيتك بتركيزات مختلفة (صفر، 1، 3، 5 و 7 جزء في المليون). كما أختبرت أنواع مختلفة من بيئات النمو خلال مرحلة الأقلمة.

أشارت النتائج المتحصل عليها إلى أن تعقيم المنفصلات النباتية بإستخدام كلوركس25% لمدة 25 دقيقة أعطى أفضل نتيجة لنسبة البقاء المئوية (100%)، في حين لوحظت أقل نسبة تلوث (30,0 و 40,0%) عند تعريض المنفصلات النباتية لكلوريد الزئبق 0,1، 0,2 و0,3 % لمدة 15 دقيقة، على التوالي. تم تحقيق أعلى عدد للبراعم والأوراق وأثقل الأوزان الطازجة للافرع في مرحلة التضاعف بإستخدام ¾ أو القوة الكاملة لبيئة النمومع وجود إختلافات معنوية مع قوى البيئة الأخرى قيد الدراسة. علاوة على ذلك، فإن إستخدام 5 جزء في المليون من بنزيل أمينو بيورين أعطى أعلى القيم في مرحلة التضاعف لنفس الصفات المدروسة. سجل إستخدام حمض الإندول بيوتريك أوحمض النفثالين أسيتك بتركيز 7 جزء في المليون أعلى نسبة مئوية للتجذير وعدد الجذور/نبتة وأطول طول للجذر خلال مرحلة تجذير. سجل خليط بيئات النمو بين الكوكوبيت + الرمل + البيرلايت (1: 1: 1 حجم/حجم/حجم) في مرحلة الأقلمة أعلى القيم في عدد الجذور ومتوسط طول الجذر وكذلك المحتويات من الكلوروفيل أ، ب، أ + ب.