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Evaluation of Gelling Agent Alternatives during *in Vitro* Multiplication of *Yucca elephantipes* L.

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ABSTRACT

In vitro hardening materials like Agar and Phytigel are increasing in price, therefore cheaper gelling agents were needed. In this study, potato starch (*Solanum tuberosum*) and corn starch (*Zea mays* L.) at different rates (40, 50, 60, and 70 g/l) each alone or combined with agar at 2 or 4 g/l as well as 8 g agar/l as control were tested during *in vitro* multiplication of yucca (*Yucca elephantipes* L.). However, the first experiment, shoot proliferation percentage was 100% with all treatments (potato starch + agar) under study, media status was solid with any combination between potato and agar except that of potato alone was semi solid status. On the other hand, MS medium supplemented with potato alone at 70 g/l produced the most leaves per shoot and shoot length, while MS medium supplemented with agar at 4 g/l + potato at 40 g/l produced the greatest shoots per explant, with significant differences from the other treatments. For the second experiment, shoot proliferation percentage was 100% as well as media status was solid with all treatments (corn starch + agar) under study. MS medium supplemented with corn alone at 70 g/l provided the highest leaf number per shoot and shoot length, while MS medium supplemented with agar at 2 g/l + corn at 40 g/l produced the most shoots per explant with significant differences from the other treatments. Consequently, the present work suggests a low-cost alternative gelling agent that will reduce plant micro propagation production costs.

Keywords: *Yucca elephantipes*, *In vitro*, gelling agent, shoot proliferation.



INTRODUCTION

Yucca (*Yucca elephantipes* L.) plant belongs to Family *Asparagaceae* or *Agavoideae* (Rentsch, 2013). *Yucca* is a public plant used for indoor ornamentation and some of yucca species had medicinal usage (Cheeke *et al.*, 2006 and Patel, 2012). Also, it is an evergreen shrub single-stemmed and medium-sized. Plant leaves are sword-shaped, stiff, striped, spine-tipped, 35-55 cm long, 5 cm wide, long pointed, and mostly in rosettes at the ends of plant trunk (Abdul-Halem *et al.*, 2015).

One of the most important factors that affect the chemical and physical characteristics of the culture medium *in vitro*, is the type and concentration of gelling agent. Gelling agents make the medium firm and influence the diffusion characteristics of the medium. Consequently, solidifying agents can significantly impact the morphogenetic response, growth, and development of tissue cultured plant material (Das *et al.*, 2015 Mohamed *et al.*, 2021). Moreover, they can contribute to the occurrence of hyperhydricity (also known as vitrification) which is a common physiological disorder that causes shoots and leaves to become brittle, with a glassy appearance (Amer and Omar, 2019).

Agar is the most expensive substance in the media which contributes around 70% of costs (Mpatani and Vuai, 2019)

The layout of setback effective micropropagation protocols is a prerequisite in the adoption of the low cost micro propagation technology in growing countries. Agar has stayed the ultimate frequently utilized gelling agent for culture media used for both microbes and plants. The agar properties,

which create it a gelling agent of choice, are its high clarity, steadiness, its resistance to metabolism during culture and non-toxic nature (McLachlan, 1985; Henderson and Kinnersley, 1988). Prakash (1993) pointed out that a mixture of potato starch with agar in a ratio of (2:1) reduced the cost of gelling agent by 70-82%. Zimmerman *et al.* (1995) revealed that medium gelled with corn starch at 50 g/l + gelrite at 0.5 g/l was completely as efficacious as medium gelled with agar for apple and raspberry shoot proliferation. Also, Lalitha *et al.* (2014) indicated that corn flour at 22 g/l in combination with 3.5 g/l agar produced significantly higher and healthy micro-shoots of length. In addition, Ullah *et al.* (2015) suggested that the MS medium supplemented with the increasing rate of corn and potato (from 40 to 50 g/l) gelled with agar (2 g/l) progressively improved orchid growth performance.

Gelling agents used to solidify plant growth medium can contain many mineral nutrients reported to affect plant growth (Joshi, 2009). Generally, many studies have shown that the type of gelling agent used can influence tissues growth *in vitro* (Quiala *et al.*, 2014; Ramesh and Ramassamy, 2014, 2015; Al-Mayahi, 2015; Raina, 2017; Hegele *et al.*, 2021 Sánchez-Gutiérrez 2023), therefore the study on selecting commercial grades of gelling agents is very important. There are limited studies about gelling agent effect routinely used in micropropagation on palm plant growth and development (Al-Mayahi, 2021; Palanyandy *et al.*, 2020).

Several alternative gelling agents, such as corn starch, barley starch, white flour, laundry starch, semolina, potato starch, rice powder, sago, sweet potato, maize, rice, wheat,

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sorghum, cassava and opuntia pads have been proposed, (Ullah et al., 2015; Teixeira da Silva, 2015; Amlesom et al 2021 Biswas et al 2021 Sánchez-Gutiérrez, et al., 2023).

The present study was undertaken to look for additional easily and cheap available substitutes of agar to be utilized in tissue culture media to enhance *Yucca elephantipes* callus weight, number of leaves and shoots per cluster as well as shoot length during multiplication stage.

MATERIALS AND METHODS

The present study was carried out at the Plant Tissue Culture Laboratory of Horticulture Department, Faculty of Agriculture, Zagazig University, Egypt from January to September 2020. This study involved two experiments. The first one was to determine the effect of different rates (40, 50, 60 and 70 g/l) of potato starch either alone or combined with 2.0 or 4.0 g/l agar as well as 8.0 g/l agar as a control on shoot multiplication and growth of *Yucca elephantipes* L. during multiplication stage. The second one was devoted to assessing the influence of different rates (40, 50, 60 and 70 g/l) of corn starch either alone or combined with 2.0 or 4.0 g/l agar as well as 8.0 g/l agar as control on shoot multiplication and growth of *Yucca elephantipes* L. during multiplication stage.

Plant material source:

Explants shoot tips about 1.5- 2.0 cm in length of *Yucca elephantipes* were obtained from Plant Tissue Culture Laboratory, Faculty of Environ. Agric. Sci., Arish University, Egypt.

Culture media and growth conditions during the multiplication stage:

The culture medium was Murashige and Skoog (1962) basal medium. The medium was supplemented with 1 mg/l BA, 30 g/l sucrose. The pH was adjusted to be 5.8 before autoclaving at 120 °C for a duration of 20 min under a pressure of 1.2 kg/cm². Jars (60 × 120 mm) were used as culture vessels and each one was filled with 50 ml medium. The cultures were placed in an air-conditioned incubation room at a temperature of 25 ± 2 °C under 16 h/ day photoperiod which provided by cool white fluorescent lamps (light intensity of 2000 Lux). The explants at this stage were separated and transferred to MS medium supplemented with 1 mg/l benzyladenine (BA) with different rates of gelling agents source (potato or corn).

Recorded data

Growth traits:

After 6 weeks from culturing the following data were recorded: Shoot proliferation (%), media status, callus fresh and dry weights/cluster (g), callus fresh and dry weights/shoot (g), number of leaves/shoot, number of shoots/explant and shoot length (cm).

Chemical constituents:

After 6 weeks from culturing vegetative parts (shoots+ leaves) were used to determine some chemical constituents. Total carbohydrates, total nitrogen, total phosphorus and potassium percentages were estimated as outlined in A.O.A.C. (2012).

Statistical analysis:

The experimental design was completely randomized design. The collected data were subjected to statistical analysis according to Snedecor and Cochran (1980). Mean separation was done using the least significant different test

(LSD) at 5% level by using computer program of Statistix version 9 (Analytical software, 2008).

RESULTS AND DISCUSSION

Results:

Effect of potato starch as gelling agents on *Yucca elephantipes* L. shoot growth and multiplication during the multiplication stage:

As shown in Tables 1 and 2 and Plat. 1 supplementation of the medium with potato starch alone without agar addition produces semi-solid media status. While MS media supplemented with agar alone at 8 g/l or all combination treatments between agar and potato starch produced solid medium. Furthermore, all rates of potato as a gelling agent (40, 50, 60 and 70 g/l) alone or in combination with agar (at 2.0 or 4.0 g/l) recorded 100 % shoot proliferation. In addition, using 70 g/l potato starch individually as gelling agent produced the maximum growth values for all *Yucca -elephantipes* L. growth parameters i.e., fresh and dry weight of cluster and shoot (g) as well as the number of leaves/shoot and shoot length (cm), which gave, 5.91 and 0.45 (g) as well as 13.05 and 3.47 cm; respectively with increasing percentages estimated by 14.76% and 36.36% as well as 54.44% and 160.90 % for these traits, respectively compared with control mean values (8 g/l agar). Moreover, the combination between 4 g/l agar and 40 g/l potato starch as gelling agent significantly produced the maximum value regarding the number of shoots/explant (16.40) compared to the control and the other combinations under study.



Control (8g/l Agar)

70 g/l of Potato starch



2g/l Agar + 40 g/l Corn starch



Plat. 1. Using different gelling agents on *Yucca elephantipes* L. growth during the multiplication stage

Table 1. Influence of different potato starch (PS) rates alone or combined with 2 or 4 g/l of agar on shoot proliferation percentage, media status and fresh and dry weights of shoot per cluster of *Yucca elephantipes* as compared with 8 g/l agar

Gelling agent (g/l)	Shoot proliferation (%)	Medium status	Weight of cluster (g)		Weight of Shoot (g)	
			Fresh	Dry	Fresh	Dry
8 Agar (control)	100	Solid	5.15 ab	0.33 c	0.534b-f	0.034c-d
40 PS	100	Semi solid	4.70 b	0.42 a	0.338d-f	0.031d-f
50 PS	100	Semi solid	5.67 a	0.41 ab	0.722 bc	0.052b-f
60 PS	100	Semi solid	5.91 a	0.45 a	1.359 a	0.013 a
70 PS	100	Semi solid	2.12 f	0.18 de	0.855 b	0.073 b
2 Agar + 40 PS	100	Solid	2.10 f	0.19 de	0.156 g	0.014 f
2 Agar + 50 PS	100	Solid	2.94 de	0.18 de	0.601b-e	0.037c-f
2 Agar + 60 PS	100	Solid	3.87 c	0.34 bc	0.310e-g	0.027ef
2 Agar + 70 PS	100	Solid	1.26 g	0.14 e	0.537b-f	0.059bc
4 Agar + 40 PS	100	Solid	3.44 cd	0.23 d	0.213fg	0.014 f
4 Agar + 50 PS	100	Solid	1.62 fg	0.14 e	0.658b-d	0.055b-d
4 Agar + 60 PS	100	Solid	2.16 ef	0.17 de	0.450c-g	0.036c-f
4 Agar + 70 PS	100	Solid	1.38 fg	0.12 e	0.340d-g	0.029d-f

Table 2. Influence of different potato starch (PS) rates alone or combined with 2 or 4 g/l of agar on the number of leaves and number of shoots per cluster and shoot length (cm) of *Yucca elephantipes* L. as compared with control (8 g/l agar)

Gelling agent (g/l)	No. of shoots/explant	Shoot length (cm)	No. of leaves/shoot
8 Agar (control)	11.70 cd	1.33 c	8.45 de
40 PS	14.80 ab	1.76 bc	8.88 c-e
50 PS	9.20 de	1.84 bc	9.66 b-e
60 PS	5.60 fg	1.63 bc	9.23 b-e
70 PS	3.50 gh	3.47 a	13.05 a
2 Agar + 40 PS	14.20 abc	1.37 c	8.96 c-e
2 Agar + 50 PS	12.70 bc	1.74 bc	9.24 b-e
2 Agar + 60 PS	7.40 ef	2.25 b	10.01 b-d
2 Agar + 70 PS	2.90 h	2.05 b	10.69 b
4 Agar + 40 PS	16.40 a	1.70 bc	8.39 e
4 Agar + 50 PS	4.20 gh	1.72 bc	9.87 b-e
4 Agar + 60 PS	5.40 fgh	2.10 b	10.15 bc
4 Agar + 70 PS	4.20 gh	1.79 bc	9.64 b-e

Effect of potato starch as gelling agents on *Yucca elephantipes* L. chemical constituents during the multiplication stage:

Results recorded in Table 3 indicate that the combination between 4 g/l agar and 60 g/l potato starch as gelling agent significantly produced the maximum values regarding total carbohydrates percentage (21.16%) and total phosphorus percentage (0.713 %) compared to the control and the other combinations, in most cases, under study. Also, the combination between 2 g/l and 40 g/l potato starch as gelling agent significantly produced the maximum value regarding total nitrogen percentage (3.09 %) compared to the control and the other combinations under study. Moreover, the combination between of 4 agar and 40 or 70 g/l potato starch as gelling agents significantly produced the maximum values regarding potassium percentage (2.10 and 2.08 %) compared to the control and the other combinations under study.

Effect of corn starch as gelling agents on *Yucca elephantipes* L. vegetative growth during multiplication stage:

Data presented in Tables 4 and 5 reveals that all rates of corn starch (g/l) as a gelling agent (40, 50, 60 and 70 g/l) alone or in combination with agar (at 2.0 or 4.0 g/l) recorded 100 % shoot proliferation as well as these treatments also attributed to obtaining a solid media status. Furthermore, MS media supplemented with agar alone at 8 g/l or all combination treatment between 2 g/l agar and 40 g/l corn starch gave the highest values in fresh and dry weights of callus /cluster without significant differences between them in the second

season. Using corn starch at 70 g/l alone or plus 4 g/l agar as gelling agent gave the highest values concerning the number of leaves/ yucca shoots (12.95 and 12.49 leaves) during the multiplication stage with no significant differences between them. Also, the combination between 4 g/l agar and 40 g/l corn starch as gelling agent significantly recorded the maximum value in the number of shoots/explant (18.90 shoots) compared to the control and the other combinations under study. Similarly, using 60 g/l corn starch plus 2 g/l agar and 70 g/l corn starch alone as gelling agent produced the maximum values in shoot length of yucca elephantipes L., which gave, 2.67 and 2.41 (cm), respectively with increasing percentages estimated by 100.75 % and 81.20% for these traits, respectively compared with control mean value (8 g/l agar).

Table 3. Influence of different potato starch (PS) rates alone or combined with 2 or 4 g/l of agar on total carbohydrates, total nitrogen, total phosphorus and potassium percentages of *Yucca elephantipes* L. as compared with control (8 g/l agar)

Gelling Agent (g/l)	Total carbohydrates %	Total nitrogen %	Total phosphorus %	Potassium %
8 Agar (control)	18.34 i	2.62 i	0.675 e	1.80 fg
40 PS	18.39 hi	2.78 f	0.686 d	1.68 i
50 PS	19.06 g	2.98 c	0.710 ab	1.65 i
60 PS	19.36f	2.59 j	0.699 c	1.73 h
70 PS	19.77 e	3.03 b	0.665 f	1.83 f
2 Agar + 40 PS	20.11 d	3.09 a	0.702 c	1.92 d
2 Agar + 50 PS	18.04 j	2.47 k	0.678 e	1.78 g
2 Agar + 60 PS	18.45 h	2.45 k	0.712 ab	1.88 e
2 Agar + 70 PS	18.99 g	2.69 h	0.699 c	1.90 de
4 Agar + 40 PS	20.22c	2.75 g	0.708 b	2.10 a
4 Agar + 50 PS	20.03 d	2.88 e	0.702 c	1.98 c
4 Agar + 60 PS	21.16 a	2.86 e	0.713 a	2.01 b
4 Agar + 70 PS	21.02 b	2.93 d	0.683 d	2.08 a

Effect of corn starch as gelling agents on *Yucca elephantipes* L. chemical constituents during the multiplication stage:

Results listed in Table 6 pointed out that using 70 g/l corn starch individually as gelling agent produced the maximum chemical constituents values for total carbohydrates, total nitrogen, total phosphorus and potassium percentages which gave, 20.73, 2.86, 0.722 and 2.05 (%); respectively. In addition, the combination between 4 g/l agar and 50 g/l corn starch as gelling agent significantly produced

the maximum value regard total carbohydrates percentage (20.24) compared to control and the other combinations under study. Also, the combination between 2 g/l or 4 agar and 60 or 40 g/l corn starch as gelling agent significantly produced the maximum values regard total nitrogen percentage (2.83 and 2.83 %) compared to control and the other combinations

under study. Moreover, the combination between 2 g/l or 4 agar and 70 or 50 g/l corn starch as gelling agent significantly produced the maximum values regard potassium percentage (2.04 and 2.03 %) compared to control and the other combinations under study.

Table 4. Influence of different corn starch (CS) rates alone or combined with 2 or 4 g/l of agar on shoot proliferation percentage, media status and fresh and dry weights of shoot per cluster of *Yucca elephantipes* L. as compared with 8 g/l agar

Gelling agent (g/l)	Shoot proliferation (%)	Media status	Weight / cluster (g)		Weight of Shoot (g)	
			Fresh	Dry	Fresh	Dry
8 Agar (control)	100	Solid	5.15 a	0.33 a	0.534ab	0.034bc
40 CS	100	Solid	2.11 c-f	0.17 b-d	0.214cd	0.018c
50 CS	100	Solid	2.21 c-e	0.18 bc	0.199cd	0.016c
60 CS	100	Solid	1.90 c-g	0.19 b	0.188d	0.019bc
70 CS	100	Solid	1.88 d-g	0.17 b-d	0.626a	0.056a
2 Agar + 40 CS	100	Solid	3.35 b	0.28 a	0.202cd	0.017c
2 Agar + 50 CS	100	Solid	2.38 cd	0.19 b	0.279cd	0.022bc
2 Agar + 60 CS	100	Solid	1.28 g	0.12 cd	0.422a-c	0.039ab
2 Agar + 70 CS	100	Solid	1.37 fg	0.11 d	0.347b-d	0.028bc
4 Agar + 40 CS	100	Solid	1.52 e-g	0.15 b-d	0.191d	0.019c
4 Agar + 50 CS	100	Solid	2.68 bc	0.21 b	0.405a-d	0.033c
4 Agar + 60 CS	100	Solid	2.57 b-d	0.19 b	0.209cd	0.015bc
4 Agar + 70 CS	100	Solid	1.90 c-g	0.15 b-d	0.257cd	0.021bc

Table 5. Influence of different corn starch (CS) rates alone or combined with 2 or 4 g/l of agar on the number of leaves and number of shoots per cluster and shoot length (cm) of *Yucca elephantipes* L. as compared with control (8 g/l agar)

Gelling agent (g/l)	No. of shoots/explant	Shoot length (cm)	No. of leaves/shoot
8 Agar (control)	11.70 bc	1.33 d	8.45 ef
40 CS	11.20 bc	1.42 d	9.20 d-f
50 CS	11.80 bc	1.63 cd	10.45 b-d
60 CS	10.80 bc	1.44 d	10.48 b-d
70 CS	5.00 e	2.41 ab	12.95 a
2 Agar + 40 CS	18.90 a	1.55 cd	9.40 c-f
2 Agar + 50 CS	10.80 bc	1.55 cd	11.36 a-c
2 Agar + 60 CS	5.60 de	2.67 a	11.63 ab
2 Agar + 70 CS	6.00 de	1.98 bc	11.12 a-d
4 Agar + 40 CS	10.30 bc	1.57 cd	8.30 f
4 Agar + 50 CS	8.10 cde	1.57 cd	9.54 c-f
4 Agar + 60 CS	13.00 b	1.41 d	10.39 b-e
4 Agar + 70 CS	9.00 cd	1.72 cd	12.49 a

Table 6. Influence of different corn starch (CS) rates alone or combined with 2 or 4 g/l of agar on total carbohydrates, total nitrogen, total phosphorus and potassium percentages of *Yucca elephantipes* L. as compared with control (8 g/l agar)

Gelling agent (g/l)	Total carbohydrates %	Total nitrogen %	Total phosphorus %	Potassium %
8 Agar (control)	18.34 g	2.62 d	0.675 e	1.80 f
40 CS	18.33 g	2.34 g	0.608 i	1.78 f
50 CS	18.32 g	2.49 e	0.568 j	1.78 f
60 CS	19.21 ef	2.74 b	0.624 h	1.81 ef
70 CS	20.73 a	2.86 a	0.722 a	2.05 a
2 Agar + 40 CS	19.71 b-e	2.50 e	0.647 g	1.95 bc
2 Agar + 50 CS	18.80 fg	2.65 cd	0.678 e	1.93 c
2 Agar + 60 CS	19.88 bc	2.83 a	0.676 e	1.84 e
2 Agar + 70 CS	19.05 f	2.38 f	0.668 f	2.04 a
4 Agar + 40 CS	19.84 b-d	2.83 a	0.678 e	1.98 b
4 Agar + 50 CS	20.24 ab	2.73 b	0.678 e	2.03 a
4 Agar + 60 CS	19.61 c-e	2.66 c	0.704 b	1.89 d
4 Agar + 70 CS	19.33 d-f	2.71 b	0.689 c	1.69 g

Discussion:

The present work studied the influence of of potato starch as gelling agents on *Yucca elephantipes* L. shoot

growth and multiplication during multiplication stage (Tables 1 and 2). Likewise, Mohamed *et al.* (2010) on *Solanum tuberosum*, pointed out that the combination of agar and potato starch could offer a firm support for plant tissues and could be successfully utilized for micropropagation. Also, these results are in harmony with those reported by Ullah *et al.* (2015) on *Dendrobium sonia*. They demonstrated that Murashige and Skoog (MS) was the best medium when supplemented with agar at 2 g/l plus potato at 50 or 60 g/l as gelling agent treatment. Moreover Amlesom *et al.* (2021) on potato micropropagation found that MS with Potato LG gave the highest plant height values as compared to the control (Agar). In addition, Mohamed *et al.* (2021) found that when gelrite was used at a concentration of 2.0 or 2.5 g/L or phytigel was used at a concentration of 2.5 or 3.0 g/L, the best callus formation was seen. Also, it was seen that rice Sakha104 cultivar was better at making calluses than Giza178, no matter what kind of hardening agent was used or how much of it was used.

A lot of researchers have tried to find other gelling agents that could replace agar from tissue culture in part or in whole. Few workers have used starches (rice, corn, barley, wheat, etc. starches), gums (locust bean gum, guar gum, gum kartia, etc.), isabgol, etc., and said that by utilizing these gelling agents, the cost of the product from tissue culture operations can be cut while growth or yield can be improved (Biswas *et al.*, 2021).

The decrease on shoots number per explant which obtained from media contained 70 g/l corn starch could be due to the lowering on plant water potential below onset value for cell expansion which is a precondition for growth and shoots formation (Owens and Wozniak, 1991). Furthermore, Toaima *et al.* (2016) cleared that gelrite at the suggested concentrations of 0.2–0.25% was not enough for micropropagation of *G. paniculata*, or this type of gelling agent was not right for this species. When 8 and 10 g/l of agar were used, the amount of hyperhydricity was 13.4% and 8%, respectively. Compared to Agar Type 900, Gelrite® with a concentration of 3.5 g/l was chosen as an effective gelling

agent for turning polyembryoids into plantlets because 100% of the polyembryoids survived and 53.3% of the plantlets (multiple shoots with roots) were fully formed (Palanyandy *et al.*, 2020).

In the same time, Daud *et al.* (2011) on *Celosia* revealed that the combination of alternative gelling agent (40 g/l corn flour) with agar or gelling agent alone successfully regenerated shoots from stem segment explants. Also, Ullah *et al.* (2013) on orchid, indicated that the MS medium supplemented with the raising rate of corn from 50 to 60 g/l gelled with 2g/l agar progressively enhanced their growth performance than that of 8 g/l agar only. In general, Amlesom *et al.* (2021) pointed out that using corn and potato starches at 50 g/l as solidifying agent instead of agar can be efficient and cost effective for single node potato micropropagation. Also, Ibrahim *et al.* (2022) showed that agar alone and its mixtures with gillan gum at 2–6 g/l were the most effective gelling agents throughout the multiplication stage of *Paulownia* hybrid (*Paulownia elongata* × *Paulownia fortunei*).

CONCLUSION

The present study offers a new possibility of utilizing low cost alternative gelling agents (potato or corn starches) which will decrease the production costs considerably plant micro propagation techniques as presented in multiplication stage of *Yucca elephantipes* L. explants.

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تقييم بدائل عامل التصلب للإكثار الدقيق داخل الأنابيب لنبات اليوكا

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المخلص

نظرا للزيادة الحادة في أسعار مواد التصلب مثل الأجار والفيثاجيل لزراعة الأنسجة النباتية، كان من الضروري البحث عن البديل لعامل التصلب. اختبر عاملين من عوامل التصلب وهما نشا البطاطس ونشا الذرة بمعدلات مختلفة (40 و 50 و 60 و 70 جم / لتر) منفردة أو بالتداخل مع الأجار بمعدل 2 أو 4 جم / لتر بجانب معاملة الكنترول 8 جم أجار/لتر لمرحلة التضاعف لنبات اليوكا. في التجربة الأولى، كانت نسبة تكوين الأفرخ 100٪ مع جميع المعاملات بين نشا بطاطس + أجار، وكانت حالة الوسط صلبة مع أي توليفة بين البطاطس والأجار باستثناء البطاطس منفردة كانت حالة البيئة شبيه متصلبة. تم الحصول على أكبر عدد من الأوراق/فرخ وطول الفرخ في بيئة موراشيغ وسكوج المضاف إليها 70 جم/لتر دقيق البطاطس منفرداً، بينما تم الحصول على أكبر عدد من الأفرخ/منفصل نباتي في بيئة موراشيغ وسكوج المضاف إليها الأجار بمعدل 4 جم/لتر + 40 جم/لتر نشا البطاطس مع وجود اختلافات معنوية مع المعاملات الأخرى. بالنسبة للتجربة الثانية، كانت نسبة تكوين الأفرخ 100٪ وكذلك حالة الوسط صلبة مع جميع المعاملات (نشا الذرة + أجار) قيد الدراسة. تم الحصول على أعلى عدد أوراق/فرخ وطول الفرخ في بيئة موراشيغ وسكوج + 70 جم/لتر نشا الذرة بفرده، بينما تحقق أعلى عدد من الأفرخ/منفصل نباتي في بيئة موراشيغ وسكوج + أجار بمعدل 2 جم/لتر + 40 جم/لتر نشا الذرة مع وجود اختلافات معنوية مع المعاملات الأخرى، في معظم الحالات. تقدم الدراسة الحالية إمكانية جديدة لاستخدام عامل التصلب البديل منخفض التكلفة والذي سيقبل من تكاليف الإنتاج إلى حد كبير في تقنيات الإكثار الدقيق.