PROPAGATION AND ARTIFICIAL SEEDS PRODUCTION FROM SINGLE NODE EXPLANTS OF *MACLURA* THAT CULTURED *IN VITRO*

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ABSTRACT:
In this study, an ideal protocol for the *in vitro* propagation of Maclura plants was established. The effect of using sodium alginate at different concentrations on the production of artificial seeds from single nodes was investigated. The results showed that the Maclura plant can be successfully propagated in vitro by culturing single nodes on Woody Plant Medium (WPM) with 0.6mg /L GA3, resulting in a percentage response of up to 100%. The highest average number and length of shoots was achieved at this concentration with 5 shoots/explant and 1.31 cm, respectively. At the multiplication stage, the treatment with 0.5mg/L BA + 0.1mg/L NAA) was superior, resulting in the highest average number of shoots of 5.50 shoots/explant. At the rooting stage, the use of IBA at 3mg/L was best and resulted in the highest average number of roots (5.60 roots/shoot). The survival rate of 100% was achieved when soil and pitmoss were used in a 1:1 ratio after the seedlings had been transferred to the field for two months. The results of the artificial seed experiment showed that the interaction between 2% sodium alginate + 15 g/L calcium chloride achieved the highest percentage of survival, 80% after one month of cold storage at 4c◦.

Key word: tissue culture, micropropagation, Maclura, single nodes, artificial seeds

اكثار وأنتاج البذور الصناعية من العقد المفردة للماكلورا خارج الجسم الحي

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المستخلص:

في هذه الدراسة تم التوصل الى بروتوكول مثالي لإكثار نبات الماكلورا خارج الجسم الحي , وتم دراسة تأثير استعمال الصوديوم الجنيت بمستويات مختلفة لانتاج البذور الصناعية من العقد المفردة . اذ بينت النتائج ان نبات الماكلورا من الممكن اكثاره بنجاح خارج الجسم الحي، وذلك بزراعة العقد المفردة في الوسط الغذائي WPM والمجهز o.6 ملغم /لتر GA3 والذي اعطى نسبة استجابة بلغت 100% واعلى معدل لعدد الافرع واطوالها اذ بلغت 5 فرع.جزء نباتي$^{-}$ و1.31 سم على التوالي. أما في مرحلة التضاعف الخضري فقد تفوقت معاملة 0.5 ملغم . لتر$^{-1}$ BA + 0.1 ملغم . لتر$^{-1}$ من NAA , اذ اعطت اعلى معدل لعدد الافرع بلغ 5.50 فرع.جزء نباتي$^{-1}$. وبالنسبة لمرحلة التجذير فقد تفوق الاوكسين IBA (عند التركيز 3 ملغم . لتر$^{-1}$ اذ اعطى اعلى معدل لعدد الجذور بلغ 5.60 جذر . فرع $^{-1}$، وبلغت نسبة نجاح عملية الاقلمة 100 % عند استعمال التوليفة المكونة من الزميج والبتموس وبنسب 1:1 و بعد شهرين من نقل النبيتات الى ظروف الحقل، اظهرت نتائج تجربة انتاج البذور الصناعية على تفوق معاملة التداخل بين التركيزين 2 % الصوديوم الجنيت + 15 غم /لتر كلوريد الكالسيوم أعلى نسبة بقاء بلغت 80% بعد شهر من الحفظ بالتبريد على درجة حرارة 4 مئوي.

الكلمات المفتاحية: الزراعة النسيجية، الاكثار الدقيق، الماكلورا، العقد المفردة، البذور الصناعية.

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INTRODUCTION

The Maclura tree, whose scientific name is *Maclura Tinctoria* L., is a deciduous dioecious tree that usually grows to a height of 15 to 30 meters (4). Its round leaves and dense, spreading crown are its characteristic features. The Maclura tree is one of about 1,500 species in the Moraceae family, which comprises 63 different genera (14). This tree is found in many parts of the world, including tropical regions from North America to South America and in many parts of Asia, including China, Japan, Korea, Taiwan, India and Thailand (15). The economic value of the maclura tree goes far beyond fruit production. It also has other useful economic properties, such as preventing soil erosion, maintaining soil fertility and producing biofuels similar to petroleum-based fuels (23). Despite their fantastic reputation, maclura fruits are best enjoyed raw or made into liquids due to their high seed content and sweet, juicy flesh (10). The wood of this tree, which can range in color from yellow to reddish-brown, is known for its sturdiness and longevity. According to (18), this dye can be isolated and then used to dye textiles and clothing. This wood is particularly valuable because of its high mechanical strength and resistance to bacteria and disease (7). According to (11), it is of great importance for the construction and furniture industries. Note that the fruit, bark, wood and leaves of this tree are rich in biologically active chemicals such as flavonoids and phenols. The numerous therapeutic properties of these substances include antioxidant, anti-inflammatory, antibacterial and anti-cancer activities (19).

One of the greatest obstacles to the widespread cultivation of the maclura tree is that it reproduces by seed. According to (10), the germination rate of Maclura trees is only 30%. Another disorder that prevents propagation is self-incompatibility (9; 20). The Maclura tree, and in particular the Maclura tinctoria tree, has received surprisingly little attention from researchers, although it and its variants are very important. This requires additional treatment that could lead to the production of counterfeit seed. For this purpose, alginate can be applied to the endogenous embryos, buds or calluses before preservation. Over several epochs to provide the basis for later harvests (21). Maclura artificial seeds are produced using the same technology that has proven successful in the production of artificial seeds for apples (2; 5), pomegranates (16) and pears (17) from shoot tips, terminal shoots and side shoots. After conventional plant tissue culture techniques have failed, this approach is used to expand the plant population (12). In addition, Maclura can be treated by microvegetative propagation in case of poor propagation. According to (6), this method increases propagation rates while maintaining physiological properties in balance. With this in mind, this study investigated the components that make up an integrated breeding program for Maclura production, including:

1- The unique method of micropropagation of Maclura involves the use of individual nodules as explant nodles.

2- Production of spurious Maclura tree seeds from individual nodes using sodium alginate in varying quantities. The seeds are stored at 4 degrees Celsius for one to three months before being tested for transformation.

MATERIALS AND METHODS

This study was conducted in the Plant Tissue Culture Laboratory of the Ministry of Agriculture/Horticulture Department - Abu Ghraib from 6.6.2023 to 6.6.2024.

1- propagation of Maclura plants

a- plant Initiation Stage: The 1.5 cm long nodes were selected from young Maclura plants grown in the Botanical Garden in Zafaraniya, Ministry of Agriculture. Explants were then taken to the laboratory and left for a while under running tap water. Then were rinsed with water and liquid soap to remove any particles adhering to them, such as dust. The leaves were then removed from the nodes and cut into 1 cm long cuttings from a node. These cuttings were then placed in a cabinet with stratified airflow and surface sterilized for ten minutes with a NaOCl solution (a commercially available minor FAS solution, 6% concentration) at a concentration of 3% (2). Explants were then cultured in initiation media containing 1.5 mg/L BA and varying amounts of Gibberellic acid (GA3) in WPM culture medium, starting at 0 mg/L up to 0.6 mg/L (24; 27).

b- Vegetative multiplication stage: A total of 160 shoots that produced from initiation stage were grown in a woody plant medium (WPM) with different doses of benzyladenine (BA; 0, 0.5, 1 and 2 mg/L) and a-naphthaleneacetic acid (NAA; 0 , 0.1, 0.2 and 0.4 mg/L). Ten shoots were then used for each of the sixteen interactions on these shoots. The number of shoots and their length were measured after six weeks.

c- Rooting stage: Shoots were cultured on WPM medium containing 0, 1, 2 and 3 mg/L indolebutyric acid (IBA) after being selected based on the interactions that resulted in the most successful vegetative multiplication. The number of roots, root length and rooting percentage were calculated according to (10; 13).

d- The acclimatization stage: The ratio tested was one part sand to three parts peat moss and one part glass sand. The plants were then kept in a controlled environment in plastic containers in the growth chamber for eight weeks. The temperature was 27 ± 2 C, and the light intensity was 3000 lux per day. After eight weeks, the plants were moved to a greenhouse in order to follow their development and determine the survival rate after two months.=

2- Artificial seed production

a- Preparation and sterilization of Sodium Alginate (SA) solution*:* Prepare separate solutions of SA in concentrations of 1, 2 and 3%. Dissolve 10, 20 or 30 grams of the substance together with WPM (without growth regulators) in distilled water. Add the WPM until the solution has reached a volume of one liter. Bring the pH of the solution to 5.7+0.1 with constant stirring using a magnetic stirrer after adding 30 g/L sucrose. According to (1), sterilization in an autoclave leads to loss of gelatinization properties; therefore, Al-Jubori (3) states that chlorine dioxide was used for sterilization.

b- Preparation of calcium chloride solution $CaCl_2$: Prepare $CaCl_2$ solutions with concentrations of 10, 15 and 20 g/L by dissolving $CaCl_2$ solutions in distilled water in a volumetric flask, make up to one liter with additional distilled water and stir continuously with a magnetic stirrer. After twenty minutes in the autoclave, the solution was sterilized and stored in the culture chamber for future use.

c- Preparation of explant*:* Individual nodes were used in the experiment for plant propagation. The nodes were carefully pulled out with tweezers and a knife, and the leaves surrounding each node were also plucked off. Under the light microscope in the stratification booth, it was found that the length of single node was about 2.5-3 mm.

d- Encapsulation and artificial seed production:

1- All single nodes that prepared should be thoroughly mixed with SA in Becker using a magnetic stirrer.

2- Withdraw the explants with a pipette that has a hole with a diameter of two to four millimeters. This ensures that there is only one explant in the capsule. The mixture of explant and SA was then placed in calcium chloride and the capsules left to stand for fifteen minutes.

3- After the calcium chloride was extracted from the capsules, then capsules were sieved three times with sterile distilled water through small diameter sieves to remove any residual calcium chloride solution. The synthetic seeds were then placed in 5-milliliter plastic tubes containing WPM medium along with 1+0.2 mg/L BA and NAA. These tubes were then stored at a temperature of four degrees Celsius for one to three months.

4- When the storage period was over and ten days had elapsed after sowing the artificial seeds in WPM medium (without growth regulators), we divided the total number of germinated seeds by the total number of seeds and multiplied the result by 100 to obtain the percentage of germination. Thus, the germination percentage was calculated.

e- Incubation conditions*:* At a temperature of 24±2 C and a light intensity of one thousand lux, the plantlets were cultivated in the growth chamber for a total of sixteen hours of light and eight hours of darkness.

3-Statistical analysis*:* A completely randomized design with ten replicates was applied to examine the effects of treatments. Data were analyzed using Genstat software. Test of least significant differences (LSD) at 5% level of probability was used to compare the calculated averages of traits.

RESULTS AND DISCUSSION

Initiation Stage

1- The initiation of tissue cultures from the cultivation of single nodes: Single nodes grown in a culture medium containing 0.6 mg/L GA_3 showed the highest percentage of response, reaching 100%, as well as the highest average number of shoots and their length, reaching 5.00 shoots per expalnt and 1.31 cm, respectively, according to the results of the initiation experiment presented in Table 1 compared to other concentrations, this is a considerable increase. This may be due to the efficiency of GA_3 at a concentration of 0.6 mg, in addition to its important role in stimulating the initiaion and development of shoots in single nodes cultures.

Table 1. Effect of gibberellic acid concentrations on the percentage response of single nodes of Maclura after 30 days of cultivation on WPM medium

GA3 concentration	Response percentage	number of shoots	shoots lengths
0	0.00	0.00	0.00
0.1	0.00	0.00	0.00
0.3	60.00	2.60	1.28
0.6	100.00	5.00	1.31
LSD	0.95	0.76	0.0067

2-Vegetative multiplication stage

a- number of shoots: The number of shoots produced varied significantly between the BA concentrations added to the culture medium. Table 2 shows that the concentration of 2 mg/L was much better than the other BA concentrations, with a maximum number of shoots of 3.93. Moreover, the number of shoots decreased steadily with decreasing BA concentration, finally reaching a single shoot in the control group. The number of shoots is significantly influenced by the NAA concentration, as shown in Table 2. The highest number of shoots, 3.45, was observed at a concentration of 0.1 mg NAA, while the lowest number, 2.30, was observed at a concentration of 0.4 mg NAA. The data in Table 2 clearly show that the BA/NAA interaction has a significant effect on the average number of shoots. A notable difference between the cultures with BA and NAA is the number of shoots formed; the one with 0.5 mg BA and 0.1 NAA gave the highest number of 5.50 shoots, this phenomenon may be attributed to the reciprocal interaction between cytokinins and auxins in the cultivated ex plant. As is well established, these two regulators are indispensable for cell division and elongation in tissue culture. The efficacy of cytokinin in inducing proliferation is enhanced by the presence of auxin in the medium.

Table 2. Effect of BA and NAA on the average number of multiplying shoots after 30 days of cultivation on WPM medium

BA concentration	NAA concentration				BA Average
	0	0.1	0.2	0.4	
0	1.00	1.00	1.00	1.00	1.00
0.5	3.20	5.50	2.20	3.20	3.53
1	2.30	4.30	2.70	2.00	2.83
2	5.20	3.00	4.50	3.00	3.93
LSD		0.4276			0.2138
Average NAA	2.93	3.45	2.60	2.30	
LSD		0.2138			

b- shoots lengths (cm): The results show that the amount of BA has a significant influence on the shortening of the shoots. At all doses, shoots length decreased; however, the greatest reduction occurred at 2 mg/L BA when shoot length reached 1.40 cm. Table 3 shows that shoot length reached 3.12 cm in the cytokine-free control group, but this percentage is the lowest (Table 3). The length of the shoots is clearly influenced by NAA, as can be seen in Table 3. The maximum rate of shoot length, which reached 2.28 at a concentration of 0.4 mg, is an indication of this. It was also found that the average length of reproductive shoots was clearly influenced by the interaction between BA and NAA. The fact that all

interactions containing BA and NAA showed significantly shorter average lengths (mean = 3.92 compared to the culture medium without BA and with 0.4 mg NAA) demonstrates this, the reason for this phenomenon may be attributed to the addition of cytokinins to the nutrient medium, which leads to an increase in their concentration within the shoots. This, in turn, results in a reduction of the role of accumulated auxins responsible for cell elongation, consequently leading to a decrease in shoot lengths. Conversely, the superiority of auxins in terms of average shoot length may be due to the reduced number of formed shoots, thereby allowing them to better utilize the substances present in the medium. This is in addition to the role of auxins in cell elongation (8;12).

Table 3. The effect of BA and NAA on the average length of multiplying shoots after 30 days of cultivation on WPM medium

BA concentration	NAA concentration				BA Average
	0	0.1	0.2	0.4	
0	2.56	2.92	3.06	3.92	3.12
0.5	1.35	1.06	1.80	1.44	1.41
1	1.55	1.36	1.97	2.30	1.80
2	1.23	1.70	1.22	1.45	1.40
LSD		0.1225			0.0612
Average NAA	1.67	1.76	2.01	2.28	
LSD		0.0612			

c-Rooting: Table 4 shows that IBA has a considerable influence on the rooting percentage. This is due to the fact that the concentration of 1 mg resulted in a rooting percentage of 100% in contrast to the other IBA concentrations. The rooting percentage was highest at 3 mg IBA, which was significantly different from the other dosages, reaching 5.60. The average root length was 1.90 cm at a concentration of 1 mg IBA, while it was shorter at lower concentrations, The reason for this may be attributed to the efficacy of IBA in promoting root formation on shoots at a concentration of 3 mg/L. This is in addition to its relatively high stability, which is due to its resistance to the enzymes responsible for auxin degradation.

Table 4. Effect of IBA concentrations on rooting percentage, number of roots, and their lengths of Maclura shoots after 30 days of cultivation on WPM medium

IBA concentration	Rooting	Number of roots	Root lengths
	percentage		
0	0.00	0.00	0.00
1	100.00	2.60	1.90
2	80.00	4.40	1.70
3	50.00	5.60	1.40
LSD	0.95	0.64	0.20

3- Artificial seed production

a- Effect of SA and $CaCl_2$ concentrations on the percentage of germination of artificial seeds after one month of cryopreservation: The results can be seen in Table 5, which shows the effects of SA and CaCl2 and their interaction on the germination percentage of the adulterated seeds. The results show that the 2% SA concentration outperformed the other treatments, reaching the maximum germination percentage of 50%. However, at a 3 concentration, the germination percentage dropped to zero. Presentation of $CaCl_2$: The highest germination percentage of 36.67 percent was achieved at a concentration of 15 gm/L, which was obviously the best of the bunch.

Table 5. Effect of SA and CaCl2 concentrations on the percentage of germination of Maclura artificial seeds after storing them for a month at 4°C

SA concentration	$CaCl_2$ concentration			Average SA
	10	15	20	
1	20	30	50	33.33
2	60	80	10	50
3	0	0	0	0
LSD		1.481		0.855
Average CaCl2	26.67	36.67	20	
LSD		0.855		

The lowest germination percentage was 20% at a concentration of 20% g/L. Among the intervention therapies, the one with an SA content of 2 was the most effective. The maximum germination rate of 80% was achieved with 15 gm/L $CaCl_2$, which was different from the other treatments. However, there were no statistically significant changes in the germination rate for the intervention

therapy with 3% SA or one of the other $CaCl_2$ concentrations.

b- The effect of SA and CaCl2 concentrations on the percentage of germination of artificial seeds after three months of cryopreservation: As for the maximum germination percentage (13.33%), the results showed that SA concentrations of 1 and 2% were much better. However, at a concentration of 3%, the artificial seeds germinated at zero percent. When comparing the different $CaCl_2$ concentrations, the use of 15 g/.L was noticeably higher. The highest germination rate was 13.33% at the concentrations, while the lowest was 6.67% at the concentrations of 10 and 20% g/L. The strongest effects came from the concentrations. At concentrations of 2% SA and 15 g/L $CaCl_2$, the interaction treatment performed significantly better than the other treatments. In contrast, there was no discernible change in the germination rate after the interaction treatment with 3% SA in addition to the other CaCl2 concentrations. It had the best germination rate of all seeds tested at 30% (Table 6). Since these concentrations give a capsule with a good moisture content and a hardness comparable to that of the vegetable component during formation, the interaction treatment with SA concentrations of 2% with 15 g/L $CaCl_2$ is optimal. The reason for this is that the concentrations of the elements used in the capsule determine these parameters and that the composition of the capsule determines the gelatinization process. Encapsulation refers to the ion exchange that takes place between the sodium ions in sodium alginate SA and the calcium ions in calcium chloride $CaCl_2$.

Table 6. Effect of SA and $CaCl_2$ concentrations on the percentage of germination of Maclura artificial seeds after storing them for three months at a temperature of 4°C

SA concentration	$CaCl_2$ concentration			Average SA
	10	15	20	
1	10	10	20	13.33
2	10	30	0	13.33
3	0	0	0	0
LSD		1.352		0.781
Average CaCl2	6.67	13.33	6.67	
LSD		0.781		

When a divalent ion is replaced by a monovalent ion in alginate, a polymeric product is formed by ion exchange between the carboxyl group and the polysaccharide molecules. It should be mentioned that alginate is a polysaccharide, which means that there is one carboxyl group for each sugar molecule (22). Low SA and $CaCl_2$ concentrations, on the other hand, could cause the seeds to be too brittle for handling or transportation due to their lack of strength. In addition, the circulation of these compounds severely impairs seed germination (25). However, if these compounds are present in high enough concentrations, they can form seeds that are so hard that explants suffocate when trying to extract them (8).

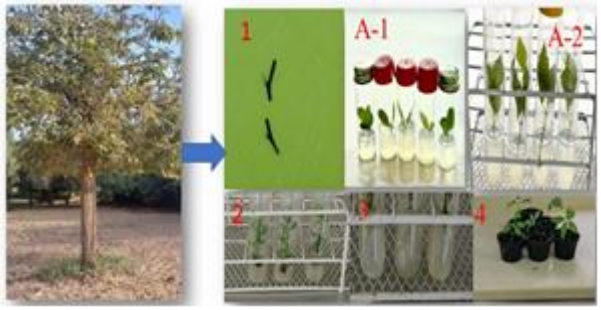

Figure 1.Micro*propagation of Maclura plants*

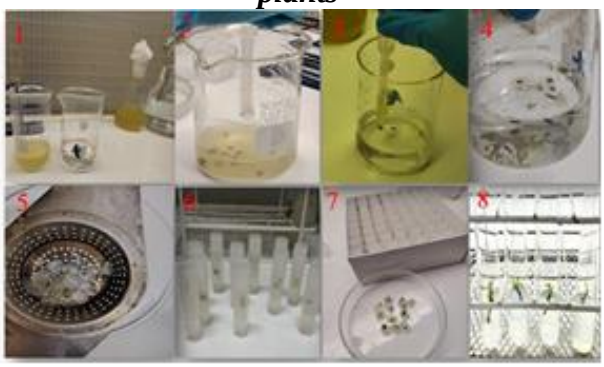

Figure 2. Steps for production Artificial seed

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