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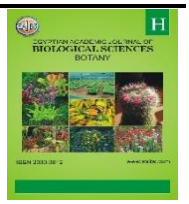

Propagation of Jojoba Plant *(Simmondsiachinensis (Link) Schnieder*) by Tissue Culture Under Different Growth Regulators Concentration

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ABSTRACT

Jojoba plants *(Simmondsia chinensis*) are excellent plant for the development marginal lands, Jojoba propagate by different vegetative methods including (stem cuttings, grafting, air-layering, root cutting and tissue) or direct seed propagation. Jojoba is cultivated for its valuable oil and bioactive components which are used in various industries such as pharmaceutical products, cosmetics, producing biodiesel fuel as well as biodegradable lubricants, jojoba is considered as a new solution of biofuel in the coming era. The purpose of this study is to evaluate different concentrations of growth regulators for optimal callus induction in Jojoba plant via enhanced axillary bud proliferation from nodes explant cultured on full strength MS medium, 30g/l sucrose, 4g/l gel rite augmented with various concentrations of plant growth regulators (T1:T16) with the combination between BA, NAA.The results indicated that certain treatments could enhance the initiation stage, leading to more shoots and greater shoot length, while others may not contribute significantly to the growth of the male jojoba plant. The best results in callus induction in 2.0 mg/l (2, 4 D) with different concentrations of BA at concentrations. The pH was adjusted to 5.7 of the MS basal medium. The percentage of callus induction, size and color were recorded after 35 days in culture. Under aseptic conditions, the callus induction response with combination of (BA,2,4D) in the MS basal medium, 2.0 mg/l the highest value (42.85%) in male of Jojoba but the lowest response appeared at the same concentration giving a value of (27.27%) in female (J14), the callus size in female (J15) was small (+++) and its color was Yellowish green but the interaction between BA, NAA (2.0 mg/l), showed a maximum response reached 83.3% in Male of Jojoba but, the lowest value (68.75%) in female (J15) but the callus size was large(++++) and its color was Yellowish green. Regarding callus induction stage, the best results were recorded when the explants were cultured and sub cultured on MS medium plus BA and NAA at 2.0 mg/l, each in turn, and higher callus induction in males suggests better propagation potential but it is not the economic way.

INTRODUCTION

Simmondsia chinensis (Link) Schn. (commonly known as jojoba) is classified as a nontraditional crop suitable for arid and semi-arid regions. This plant exhibits a natural resilience to saline soils and elevated temperature conditions (National Academy of Sciences (U.S.) Advisory Committee on Technology Innovation, 2002). The seeds of jojoba are notable for their lipid content, comprising 40–60% of their dry weight in the form of liquid wax. This wax and its derivatives present significant potential for diverse applications across cosmetics, pharmaceuticals, lubricants, extenders, and antifoaming agents (Mills *et al*., 1997; Benzioni *et al.*, 1999). There has been a growing interest in the agricultural cultivation of jojoba, with increasing knowledge gained each year regarding its cultivation requirements, planting densities, management practices, productivity, propagation methods, and genetic enhancement (National Academy of Sciences (U.S.) Advisory Committee on Technology Innovation, 2002; Mills *et al*., 1997).

Jojoba is a dioecious species that cannot commence sexual reproduction until it reaches flowering, typically occurs 2–4 years post-germination. The jojoba plant (Simmondsia chinensis (Link) C. Schneider), belonging to the Simmondsiaceae family, is primarily a woody, evergreen, perennial shrub that produces small seeds containing a waxy liquid akin to spermaceti (Kubitzki *et al.*, 2003; Van *et al.*, 2004). Recognized as a medicinal and oil-producing multipurpose species (Kumar *et al.*, 2012), jojoba's significance lies in its seeds, which store liquid wax. This wax is extensively utilized in lubricants, antibiotic production, anti-inflammatory treatments, hair care, and medical applications for various conditions, including wounds, colds, cancer, kidney dysfunction, and skin disorders, as well as in the cosmetics, pharmaceuticals, plastics, and petroleum sectors (Jacoboni; Standardi, 1987; Mills; Benzioni, 1992).Plants exhibit remarkable resilience to drought conditions (*Al-Ani et al.*, 1972), and their leaves serve as a valuable source of nutritious forage for livestock such as sheep, goats, and cattle, in addition to wild ungulates and smaller herbivores like rabbits. In arid and marginal regions, only a limited number of crops are cultivated, primarily for subsistence. These areas are devoid of cash crops that can withstand drought. In recent years, there has been significant interest in the cultivation of multipurpose crops capable of enduring stress, such as jojoba (*Thagana et al., 2004*) and (Weiss, 1983). Native to the Sonoran Desert, jojoba thrives in climates characterized by annual precipitation ranging from 80 to 450 mm and temperatures between 9 and 54°C (Gentry, 1958). Jojoba is regarded as a versatile plant and holds promise as a cash crop for impoverished communities. Additionally, it has potential applications in rehabilitation efforts, particularly in marginal areas. Jojoba is an effective species for combating and preventing desertification in arid regions, such as the Thar Desert in India (Patel, 2017) and around 6 October City in Egypt (Abobatta, 2017). The initial research on the propagation of jojoba through tissue culture was conducted by (*Salah et al., 2005*). Direct seeding results in genetic variability, with approximately half of the seedlings being male. However, a male population of 8–10% is essential for effective pollination (Reddy and Chikara, 2010). Establishing a plantation using asexual propagules incurs higher costs compared to seed propagation, yet it offers advantages in terms of time efficiency for replanting and ensures the production of plants with known sex and lineage. Vegetative propagation methods, including layering, grafting, or rooting semi-hardwood cuttings, can be employed; however, the maximum number of propagules is constrained by the size of the plant and the season (*Mills et al., 1997*) and (Reddy and Chikara, 2010).

The micropropagation of elite plant individuals capitalizes on the totipotency of plant cells, presenting a viable approach for the commercial mass production of pathogen-free superior clones. Jojoba plants derived from in vitro methods exhibit more vigorous growth compared to both seedlings and rooted cuttings, resulting in significantly larger

specimens after the first year of cultivation (National Academy of Sciences (U.S.) Advisory Committee on Technology Innovation, 2002; *Mills et al., 1997*; Reddy and Chikara, 2010; Chaturvedi and Sharma, 1989). Jojoba is recognized as a multipurpose crop and serves as a promising cash crop, providing income opportunities for impoverished communities. Additionally, it plays a role in combating and preventing desertification in regions such as the deserts of India (Alsharhan, 2003) and the 6 October desert in Egypt (Abobatta, 2016).

Vegetative propagation addresses the challenge of the disproportionate male-to-female ratio in the field, as fewer males are needed in comparison to females, allowing for the selection of only desirable plants. This method also enables growers to adhere to specific planting plans. Various asexual propagation techniques for jojoba are employed, including stem cuttings (Cao and Gao, 2003), air-layering (Palzkill and Feldman, 1993), grafting (Singh et al., 2003), and tissue culture (Llorente and Apóstolo, 1998). These techniques help mitigate the high male-to-female ratio in jojoba farms and establish the desired plant ratios. The advantages of these methods in commercial jojoba plantations include the production of genetically uniform plants and earlier fruiting (Lee and Paskill, 1984). Furthermore, they enable growers to reduce cultivation costs, optimize the male-to-female plant ratio according to cultivation plans, and facilitate earlier seed crop production compared to traditional seedy plants. Additionally, vegetative seedlings tend to produce seeds sooner, resulting in a quicker return on initial establishment and maintenance investments.

Jojoba can be propagated through various methods, including vegetative propagation techniques such as stem cuttings, grafting, air-layering, root cutting, and tissue culture, as well as through seed propagation methods (*Abramovich et al., 1978*). During in vitro propagation, shoots displayed varying morphogenic responses when subjected to different growth regulators and adjuvants. Notable differences were observed in the explants regarding the percentage of shoot regeneration, proliferation rates, shoot lengths, callus formation, and rooting behavior (Llorente and Apóstolo, 1998*; Prakash et al.,* 2003; Tyagi and Prakash, 2004). Conversely, certain genotypes did not exhibit significant differences in bud initiation, rooting, and survival rates in greenhouse conditions (*Singh et al.,* 2008). This chapter outlines a fundamental protocol for the micropropagation of jojoba, which serves as a foundational reference for all genotypes. However, it is advisable to create optimized protocols tailored to each genotype, as the in vitro responses of different clones can be unpredictable.

Consequently, large-scale micropropagation facilities are supplying millions of plants for both the commercial ornamental sector and the agricultural market for clonally propagated crops (Brown and Thorpe, 1995). In tissue culture, the application of plant growth regulators is crucial, as they significantly influence various plant processes, including growth, differentiation, and development, such as culture establishment, shoot initiation, multiplication, and rhizogenesis (Hobbie, 1998).

Benzyl aminopurine (BAP) is more effective than kinetin (Kn) in promoting the growth of primary explants (Llorente and Apóstolo, 1998). Among the jojoba cultivars, the most favorable results were observed with treatments involving NAA and BA at concentrations of 2.0 mg/L for J15 and 0.5 and 2.0 mg/L for J14, which yielded the highest productivity in terms of branches (shoots), shoot length, and node formation This research paper focuses on examining the effects of various concentrations of growth regulators on nodes, internodes, and identifying the optimal concentrations for propagating jojoba using plant tissue culture techniques.

MATERIALS AND METHODS

The research focused on the impact of various concentrations of specific growth regulators and their combinations, as detailed in Table (1), on the micropropagation of

Simmondsia chinensis (Link) Schn. (jojoba) plantlets. This study utilized node and internode segments as explants and was carried out at the Plant Tissue Culture Laboratory within the Plant Protection Department, Arid Land Cultivation Research Institute (ALCRI), as well as the Tissue Culture Laboratory of the Pharmaceutical Bio-products Research Department and the Central Lab Department at the Genetic Engineering and Biotechnology Research Institute (GEBRI), located in the City for Scientific Research and Technology Application (MUCSAT), New Borg El-Arab City, Alexandria, Egypt, over the period from 2019 to 2022.

Table 1: Various combinations of BAP (Benzyl aminopurine) and NAA (Naphthaleneacetic acid) plant growth regulators (PGRs) utilized for the initiation of the Jojoba plant (Simmondsia chinensis (Link) Schn.).

Treatments	**Media composition for Jojoba plant (*Simmondsia chinensis (Link) Schn*)** with PGRs (BA, NAA)
T1	0.0
T2	(0.5mg/l NAA)
T3	(1 mg/l NAA)
T4	(2mg/l NAA)
T5	(0.5 mg/l BA)
T6	(0.5 mg/l BA & 0.5mg/l NAA)
T7	(0.5 mg/l BA & 1.0mg/l NAA)
T8	(0.5 mg/l BA & 2.0mg/l NAA)
T9	(1.0 mg/l BA)
T10	(1.0 mg/l BA & 0.5 mg/l NAA)
T11	(1.0 mg/l BA & 1.0 mg/l NAA)
T12	(1.0 mg/l BA & 2.0mg/l NAA)
T13	(2.0 mg/l BA)
T14	(2.0 mg/l BA & 0.5 mg/l NAA)
T15	(2.0 mg/l BA & 1.0mg/l NAA)
T16	(2.0 mg/l BA & 2.0mg/l NAA)

Plant Materials:

The explant material, consisting of cuttings, was sourced from mature Simmondsia chinensis (Link) Schn. (jojoba) plants, which were generously provided by Elmasriah Elkhc7caligia Co., a commercial farm located along the El-Hamam highway, as illustrated in Figure (1). The healthy parent plants were cultivated in a controlled environment within a plastic house. Upon collection, the material was transported to the laboratory and thoroughly washed under running tap water for a duration of 30 minutes. Only 1 cm long nodal segments, each containing a single node as described by Bhattacharya et al. (1990), were prepared using forceps and a scalpel. For sterilization of the nodal segments, the excised explants were immersed in 70% ethanol for 60 seconds. Following this ethanol treatment, the explants were rinsed twice with double distilled water to mitigate any potential toxic effects of the ethanol. Subsequently, the nodal segments underwent surface sterilization using a solution of 0.1% mercuric chloride (HgCl2) combined with a few drops of the wetting agent Tween-80 for five minutes. After the surface sterilization process, the mercuric chloride solution was discarded, and the explants were rinsed three times with double distilled water to further reduce the toxic effects of $HgCl_2$, rendering them ready for culturing.

Propagation of Jojoba Plant by Tissue Culture

Fig. (1): Segregations of Jojoba plant, A: Male, B: Female J14, C: Female Invitro.

Experimental Phases:

1. Initiation Phase:

Explants were cultivated on a solid medium (Murashige and Skoog, 1962) that was solidified using gel rite (3g/l). The pH of the media was adjusted to 5.7 prior to the addition of gel rite, followed by sterilization through autoclaving at 121°C for 20 minutes. Subsequently, the explants were placed into the prepared MS medium, which contained varying concentrations of cytokinin (BA) 6-Benzyladenine at four levels: 0.0, 0.5, 1.0, and 2.0 mg/l, in conjunction with auxin (NAA) Naphthalene acetic acid at three concentrations: 0.0, 0.5, and 1.0 and 2.0 mg/l.

2. Callus Induction Phase:

Axillary shoots, derived from the original explants (J1 male, J14 female, J15 female), were utilized for callus induction. The explant consisted of internodes cut into segments of 0.5 cm in length, while leaves were excised from the shoots and cut into approximately 0.5 cm^2 segments. Both types of explants were cultured on MS medium enriched with various concentrations of BA, specifically at 2.0 mg/l, in combination with NAA and 2.0 mg/l (2,4 D). The pH of the media was adjusted to 5.7, followed by the addition of agar (7g/l) before autoclaving. Each treatment was replicated three times, with each replication containing one explant. The percentage of callus formation and the size of the callus were recorded after 35 days of culture. Callus was routinely sub-cultured on fresh media (basil) to maintain the callus stock, and fresh weights (g) were measured after one month of incubation. The percentage and appearance of callus or shoots were documented after four weeks. The size of the callus was visually assessed and categorized as "-" = no callus, "+" = minimal or slight callus, "++" = small callus, "+++" = medium callus, "++++" = large callus, with the color of the callus also being noted. The studied characteristics.

The following parameters were assessed for each propagule at the initiation and callus stages after 35 days of culture:

1. Average number of shoots developed per propagule.
2. Average shoot length (cm) per propagule.
3. Average number of nodes produced per propagule.
4. Size, induction, and color of the callus formed per propagule.

Experimental Design And Statistical Analysis:

The experiments conducted in this study were structured using a completely randomized design (Gomez and Gomez, 1984). The collected data were subjected to statistical analysis through the analysis of variance (ANOVA) method, and mean values were compared using Duncan's multiple range test (Steel *et al*., 1997), with significance established at $p \leq 0.05$.

RESULTS AND DISCUSSION

Initiation Stage:

The data illustrated in Table (2) and Figure (2), indicate the initiation phase of *Simmondsia* chinensis (Link) Schn. (jojoba) in male specimens, as well as in female specimens J14 and J15, under in vitro conditions. This study examines the responses of three Jojoba in vitro nodal segments and shoot tip cultures subjected to various combinations of growth regulators. The growth parameters assessed included the number of nodes, shoot length (cm), and the number of shoots across treatments (T1-T16), which incorporated different combinations and concentrations of BAP (Benzyl aminopurine) and NAA (Naphthaleneacetic acid), with concentrations ranging from 0.0 mg/L (control) to 2.0 mg/L. It was noted that for male varieties, treatment T1 (without growth regulators) yielded the highest average number of nodes (1.67), whereas T16 (2.0 mg/L BA & 2.0 mg/L NAA) resulted in no node production. In contrast, female J14 demonstrated the highest node production in T14 (1.00 nodes) and T16 (0.67 nodes), while female J15 achieved the highest node production in T5 and T16, both yielding 1.00 nodes. Regarding shoot length in male varieties, the longest shoots were recorded in T8 and T9 (1.17 cm each). Female J14 produced the longest shoots in T3 (1.33 cm), and female J15 exhibited the longest shoots in T16 (1.00 cm). Lastly, concerning the number of shoots for male varieties, T3 (1.33 shoots) and T16 (1.00 shoots) were identified as the most effective treatments. Female J14 displayed the highest number of shoots in T1 and T2 (1.00 shoots each), while female J15 recorded the highest number of shoots in T16 (2.00 shoots).

The table reveals that certain treatments exhibit significant variations in their effects on node count, shoot length, and the number of shoots across various jojoba varieties, while others do not. For the Male variety, the highest node production (1.67 nodes) was recorded with the control treatment (T1), which did not utilize any growth regulators. This implies that the absence of growth regulators may be beneficial for node production in the Male variety. Conversely, treatments T5, T10, T11, T12, T13, T15, and T16 resulted in no node production, suggesting that elevated concentrations or specific combinations of NAA and BA could hinder node production in this variety. Regarding shoot length, treatments T8 and T9 both achieved the longest shoot length (1.17 cm), indicating that BA at concentrations of 0.5 mg/l (T8) and 1.0 mg/l (T9) may be optimal for promoting shoot elongation in the Male variety. However, treatments such as T5, T10, T11, and T15 did not yield any shoot growth, indicating that these combinations may not be conducive to shoot elongation in this variety. In terms of the number of shoots, T3 (1.33 shoots) and T16 (1.00 shoots) were the most effective treatments, suggesting that a higher concentration of NAA (1.0 mg/l in T3) and a balanced combination of BA and NAA (2.0 mg/l each in T16) could enhance shoot proliferation. However, T5, T10, T11, and T15 showed no shoot production, further highlighting the potential inhibitory effects of certain BA concentrations or their combinations with NAA on shoot proliferation in the Male variety. For the Female J14 variety, the highest node count was noted with T14 (1.00 nodes) and T16 (0.67 nodes), indicating that BA at 2.0 mg/l combined with 0.5 mg/l NAA (T14) or equal concentrations of both (T16) may be advantageous for node production in the Female J14 variety. Treatments such as T6, T9, T11, T12, and T13 resulted in no node production.

Ultimately, the maximum number of shoots (1.00) was attained with treatments T1, T2, T7, T8, and T15, suggesting that lower concentrations of BA and NAA, or the absence of growth regulators, may enhance shoot production. Conversely, treatments such as T6, T12, and T13 did not yield any shoots, indicating their ineffectiveness in promoting shoot development in the Female J14 variety. In the case of the Female J15 variety, the highest node count (1.00 nodes) was observed with treatments T5 and T16, implying that 0.5 mg/l BA alone (T5) or in conjunction with 2.0 mg/l NAA (T16) could be optimal for node

production. Several treatments, including T3, T7, T10, T11, and T12, resulted in no node production, which may suggest an inhibitory effect of these combinations on node development. Regarding shoot length, the longest shoots (1.00 cm) were recorded with T16, indicating that a combination of 2.0 mg/l BA and 2.0 mg/l NAA may be ideal for shoot elongation in Female J15. However, treatments T3, T7, T10, T11, T12, T13, and T15 did not promote shoot elongation, indicating their ineffectiveness for in enhancing shoot growth in this variety. Finally, the highest number of shoots (2.00) was achieved with T16, demonstrating that this combination is particularly effective for promoting shoot proliferation in Female J15. In contrast, treatments such as T3, T7, T10, T11, and T12 produced no shoots, suggesting that these combinations may be ineffective or even suppressive for shoot production. Statistical significance, denoted by letters (a, ab, b, c), reflects the results of Duncan's Multiple Range Test ($P < 0.05$) utilizing the Statistical Package for the Social Sciences (SPSS).

Table 2: Effect of different combination concentrations of NAA and BA (mg/l) in the initiation stage of *Simmondsia chinensis (Link) Schn. (jojoba)* cultured *in vitro* for 35 days.

Treatments	Jojoba varieties								
	Male			Female J14			Female J15		
	No. of nodes	Length of shoot (cm)	No. of Shoots	No. of nodes	Length of shoot (cm)	No. of Shoots	No. of nodes	Length of shoot (cm)	No. of Shoots
T1	1.67^{a}	1.00^{a}	0.67^{b}	0.33^{a}	1.00^{ab}	1.00^{a}	0.67^{ab}	0.83^{a}	0.67^{b}
T2	0.67^{ab}	0.67^{ab}	0.67^{b}	0.33^{a}	1.00^{ab}	1.00^{a}	0.00^{b}	0.50^{a}	0.67^{b}
T3	0.67^{ab}	1.00^{a}	1.33^{a}	0.67^{a}	1.33^{a}	0.67^{ab}	0.00^{b}	0.00^{a}	0.00^{b}
T4	0.67^{ab}	1.00^{a}	1.00^{ab}	0.67^{a}	0.67^{ab}	0.67^{ab}	0.33^{ab}	0.33^{a}	0.33^{b}
T5	0.00^{b}	0.00^{b}	0.00^{c}	0.67^{a}	1.00^{ab}	0.67^{ab}	1.00^{a}	0.83^{a}	0.33^{b}
T6	0.00^{b}	0.50^{ab}	0.67^{b}	0.00^{a}	0.00^{b}	0.00^{b}	0.33^{ab}	0.67^{a}	0.33^{b}
T7	0.67^{ab}	1.00^{a}	1.00^{ab}	0.67^{a}	1.00^{ab}	1.00^{a}	0.00^{b}	0.00^{a}	0.00^{b}
T8	0.67^{ab}	1.17^{a}	1.00^{ab}	0.67^{a}	1.00^{ab}	1.00^{a}	0.33^{ab}	0.33^{a}	0.33^{b}
T9	0.67^{ab}	1.17^{a}	1.00^{ab}	0.00^{a}	0.33^{ab}	0.33^{ab}	0.33^{ab}	0.33^{a}	0.33^{b}
T10	0.00^{b}	0.00^{b}	0.00^{c}	0.33^{a}	0.33^{ab}	0.33^{ab}	0.00^{b}	0.67^{a}	0.67^{b}
T11	0.00^{b}	0.00^{b}	0.00^{c}	0.00^{a}	0.50^{ab}	1.00^{a}	0.00^{b}	0.33^{a}	0.33^{b}
T12	0.00^{b}	0.50^{ab}	1.00^{ab}	0.00^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.33^{a}	0.33^{b}
T13	0.00^{b}	0.50^{ab}	1.00^{ab}	0.00^{a}	0.33^{ab}	0.33^{ab}	0.33^{ab}	0.67^{a}	0.67^{b}
T14	0.00^{b}	0.33^{ab}	0.67^{b}	1.00^{a}	0.83^{ab}	0.67^{ab}	0.33^{ab}	0.67^{a}	0.67^{b}
T15	0.00^{b}	0.00^{b}	0.00^{c}	0.67^{a}	1.00^{ab}	1.00^{a}	0.33^{ab}	0.33^{a}	0.33^{b}
T16	0.00^{b}	0.50^{ab}	1.00^{ab}	0.67^{a}	0.33^{ab}	0.67^{ab}	1.00^{a}	1.00^{a}	2.00^{a}
Duncan's	*	*	*	ns	*	*	*	ns	*

- (T1:(0.0mg/l),T2:(0.5mg/LNAA),T3:(1.0mg/lNAA),T4:(2.0 mg//l NAA),T5(:0.5mg/l BA),T6:0.5mg/lBA,0.5mg/l NAA),T7:(0.5 mg/l BA,1.0 mg/l NAA),T8:(0.5mg/l BA,2.0 mg/l NAA) , T9: (1.0 mg/l BA) ,T10 :(1.0 mg/l BA & 0.5 mg/l NAA) T11:(1.0 mg/l BA & 1.0 mg/l NAA) ,T12 :(1.0 mg/l BA& 2.0mg/l NAA) T13: (2.0 mg/l BA),T14 (2.0 mg/l BA & 0.5 mg/l NAA) ,T15: (2.0 mg/l BA & 1.0mg/l NAA) ,T16 :(2.0 mg/l BA & 2.0mg/l NAA)).
- The letters (a, ab, b, c) indicate significant differences within the same column, meaning that treatments with the same letter are not significantly different from each other according to Duncan's Multiple Range Test ($P < 0.05$).

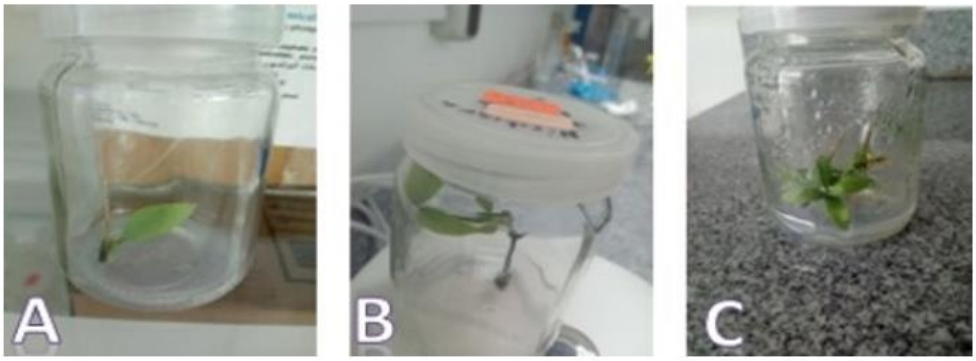

Fig. 2: Initiation of Simmondsia chinensis (Link) Schn. (jojoba) in A (male), B (j14 female), C(J15) cultured on MS medium supplemented with (BA, NAA).

The results discussed above generally suggest that a reduction in the mean values of the examined traits was associated with an increase in benzyladenine (BA). This phenomenon may be linked to the accumulation of cytokinin at supra-optimal levels within the tissues, which negatively impacts growth performance (George *et al.,* 2008). Consequently, the Murashige and Skoog (MS) medium devoid of BA yielded the highest mean value for shoot length. This observation can be attributed to the action of auxin (NAA) in cultured tissues, which regulates various essential processes, including cell growth and elongation (George *et al.,* 2008). Conversely, the lowest concentration of NAA employed was beneficial for the initiation of *Simmondsia chinensis (Link) Schn.* (jojoba). This may be explained by the fact that higher concentrations of NAA are typically ineffective in promoting shoot proliferation (Waseem *et al*., 2011). Additionally, the role and mechanism of action of auxin are well-documented for their capacity to enhance root formation, as noted by George *et al.* (2008) and Waseem *et al.* (2011). Regarding the interaction between the growth regulators at 0.00 BA and 0.50 mg/l NAA, significant effects were observed on the various traits assessed.

Callus Induction Stage:

The findings presented in Table (3) and Figure (3), indicate that nodes and internodes of *Simmondsia chinensis* were utilized as explants for both male and female specimens, employing various combinations of growth regulators (2, 4-D, NAA, and BA, NAA) at a concentration of 2.0 mg/l. It was observed that no callus formation occurred when the explants were cultured on MS medium (for male and J15 female) without the addition of plant growth regulators (PGRs), which served as the control for callus production. The formation of callus in Jojoba exhibited significant variability, with both the color and texture being influenced by the type of explant and the concentrations of PGRs used. The calli developed with 2 mg/l of (BA, NAA, and BA, 2,4-D) were characterized by a yellowish-green hue and a compact structure.

Conversely, Table (3) and Figure (4), illustrate the response to callus induction when utilizing a combination of (BA, NAA) in the nutrient medium at a concentration of 2.0 mg/l, which yielded the highest callus induction rate of 42.85% in male Jojoba. In contrast, the lowest response was recorded at the same concentration for female J14, resulting in a value of 27.27%. The callus size for female J15 was rated as ++++, with a yellowish-green coloration. The interaction between 2.0 mg/l of BA and 2, 4-D produced a maximum response of 83.3% in male Jojoba, while the lowest response of 68.75% was noted in female J15, with the callus size rated as +++ and exhibiting a yellowish-green color, as detailed in Table (3). Effective callus induction necessitates a balanced ratio of auxins and cytokinins, as noted by Skoog and Miller (1957). In several plant species, the induction of callus is more favorable with higher levels of auxins compared to cytokinins, as discussed by Ramawat (2004).

Table 3: illustrates the impact of two combinations of growth regulators, namely BA and 2,4-D, as well as BA and NAA, at a concentration of 2 mg/L on the induction of callus in segregations of the Jojoba plant, specifically male 15J and 14J female.

Segregations (s)	**% callus Induction**		**Callus Size**		**Callus color**	
	(BA, NAA)	**(BA, 2,4D)**	**(BA, NAA)**	**(BA, 2,4D)**	**(BA, NAA)**	**(BA, 2,4D)**
Male ♂	42.85	83.3	++	++	Yellowish green	Green weight,
14 J Female♀	27.27	80	+++	++	Yellowish green	Yellowish green, weight
15J Female♀	35.71	68.75	++++	+++	Yellowish green	Yellowish green
Total %	**34.375%**	**85%**				

The percentage and characteristics of callus or shoots were documented after a duration of four weeks. The size of the callus was assessed visually and categorized as follows: "-" indicating no callus, "+" representing minimal or slight callus, "++" denoting small callus, "+++" indicating medium callus, and "++++" signifying large callus. Additionally, the color of the callus was noted.

Cell enlargement, along with the synthesis of proteins and nucleic acids, are integral components of auxin-induced growth, which also influences the plasticity of plant cell walls and enhances apical dominance. These processes are crucial and occur rapidly during growth and elongation (Wilkins, 1989). The average number of leaves per shoot and the number of nodes is significant growth parameters that exhibit a direct proportional correlation with shoot length; as the length of the shoot increases, so too does the number of leaves and nodes (Waseem *et al.*, 2009). Our findings were further substantiated by the earlier research conducted by Waseem *et al*. (2011).

Fig. 3: A: Male,B:Female(J14),C:Female(J15) consecutively callus induction with (BA,2,4,D) media combination on MS medium at 2.0 mg/l in *Simmondsia chinensis (Link) Schn. (jojoba)* plant.

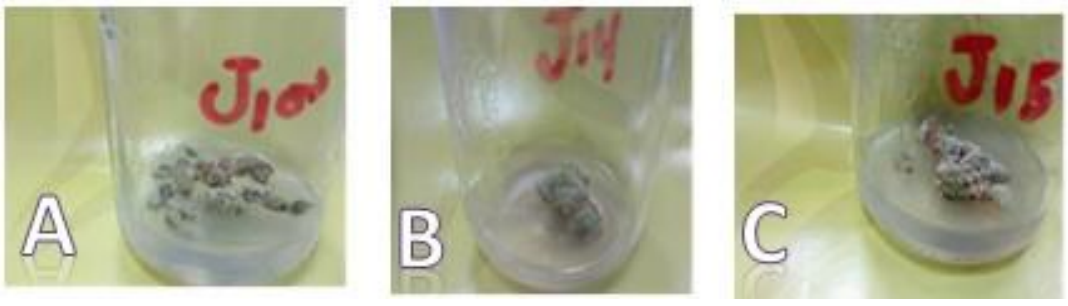

Fig. 4: A: Male,B:Female(J14),C:Female(J15) consecutively callus induction with (BA,NAA) media combination on MS medium at 2.0 mg/l in Simmondsia chinensis (Link) Schn. (jojoba)

CONCLUSION

In this study, as detailed, effective concentrations of growth-regulating hormones for callus initiation and stimulation of jojoba plants were determined. Initially, nodes and internodes were used as plant parts in MS medium with treatment T16 (2.0 mg/L BA and 2.0 mg/L NAA). This treatment consistently yielded positive results across all parameters evaluated, especially for the female cultivar J15, which showed the highest number of nodes, longest shoot length, and largest number of buds. In contrast, the control treatment (T1, 0.0 mg/L) surprisingly yielded the highest number of nodes for the male cultivar and showed satisfactory bud production in the female J14, suggesting that some cultivars may respond more favorably without the use of growth regulators. In addition, T3 (1.0 mg/L NAA) was significantly effective in enhancing shoot length and number of buds in the male cultivar, as well as enhancing shoot length in the female J14. Also, The best combination of plant growth regulators (NAA, BA) to stimulate callus production was selected at a concentration of 2 mg/L. This treatment offers a promising approach for improving, conserving and disseminating S. chinensis on a large scale.

RECOMMENDATIONS

This study recommends the propagation of jojoba cuttings utilizing a concentration of 2.0 mg/l of the interaction between NAA and BA. Nevertheless, there remains significant potential for additional research into the interactions among different plant growth regulators (PGRs) and female genotypes to identify optimal combinations for enhancing callus production.

Declarations:

Ethical Approval: No animal model(s) or human subjects were recruited directly for the current study. Consequently, no ethical considerations are necessary.

Conflict of interest: The authors declare no conflict of interest.

Authors Contributions: I hereby verify that all authors mentioned on the title page have made substantial contributions to the conception and design of the study, have thoroughly reviewed the manuscript, confirm the accuracy and authenticity of the data and its interpretation, and consent to its submission.

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Availability of Data and Materials: All datasets analysed and described during the present study are available from the corresponding author upon reasonable request.

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Propagation of Jojoba Plant by Tissue Culture

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Propagation of Jojoba Plant by Tissue Culture

ARABIC SUMMARY

إكثار نبات الجوجوبا (Simmondsia Chinensis (Link) Schneider)من خلال زراعة الانسجة تحت تركيزات مختلفة من منظمات النمو

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يعتبر نبات الجوجوبا (*Simmondsia chinensis*) نبات ممتاز لتنمية الأراضي الهامشية، يتكاثر الجوجوبا بطرق خضرية مختلفة منها (العقل الجذعية، التطعيم، الترقيد الهوائي، العقل الجذرية والأنسجة) أو التكاثر المباشر بالبذور. الجوجوبا المزروعة لزيتها الثمين ومكوناتها الحيوية النشطة التي تستخدم في مختلف الصناعات مثل المنتجات الصيدلانية ومستحضرات التجميل وإنتاج وقود الديزل الحيوي وكذلك زيوت التشحيم القابلة للتحلل البيولوجي، يعتبر الجوجوبا بمثابة حل جديد للوقود الحيوي في العصر القادم. الهدف من هذا العمل هو دراسة تركيزات مختلفة من منظمات النمو لاختيار أفضل تركيبة لإنتاج أفضل تحريض للكالس من خلال تعزيز تكاثر البراعم الإبطية من العقد المزروعة على وسط MS كامل القوة، 30 جم / لتر سكروز، 4 جم / لتر جل رايت معزز بتركيزات مختلفة من منظمات نمو النبات (T1: T16) مع مزيج بين BA و NAA، معقمة بكلوريد الزئبق بنسبة 1.0٪ لمدة خمس دقائق ثم تم شطفها ثلاث مرات بالماء المقطر المعقم. بالنسبة لمرحلة البدء، تمت زراعة الطعوم المعقمة السطحية على وسط موراشيج وسكوج (MS) المعزز بتركيزات مختلفة من كل من منظمات نمو النبات المطبقة عند 0.0 و 0.5 و 1.0 و 2.0 مجم / لتر، وبالاشتراك مع حمض النفتالين الأسيتيك (NAA) عند 0.0 و 0.5 و 1.0 و 2.0 مجم / لتر. تمت دراسة معلمات مختلفة بما في ذلك متوسط عدد البراعم وطول البراعم (سم) وعدد العقد لكل وحدة تكاثر خلال هذه المرحلة، وبدء وتطوير زراعة أنسجة الكالس باستخدام كل من النباتات الذكور ونباتين أنثويين، وتحديدًا ذكر J1 وأنثى J14 وJ15. كانت أفضل نتيجة مهمة في المعالجة بـ NAA و BA (2.0 مجم / لتر) من J15 و (0.5 و 2.0 مجم / لتر) من J14، مما أعطى أعلى إنتاجية في الفروع (البراعم) وطول البراعم والعقد. تشير البيانات إلى أن بعض المعالجات يمكن أن تعزز مرحلة البدء، مما يؤدي إلى المزيد من البراعم وطول البراعم الأكبر، في حين أن البعض الآخر قد لا يساهم بشكل كبير في نمو نبات الجوجوبا الذكر. أفضل النتائج في تحريض الكالس في 2.0 مجم / لتر (2، 4 د) مع تركيزات مختلفة من BA في التركيزات. تم تعديل الرقم الهيدروجيني إلى 5.7 من وسط MS الأساسي. تم تسجيل نسبة تحريض الكالس والحجم واللون بعد 35 يومًا في الثقافة. تحت ظروف معقمة، كانت استجابة تحريض الكالس مع مزيج (D2،4،BA) في وسط MS الأساسي، 2.0 ملغم / لتر أعلى قيمة (42.85٪) في ذكور الجوجوبا ولكن ظهرت أقل استجابة عند نفس التركيز معطية قيمة (27.27٪) في الإناث (J14)، كان حجم الكالس في الإناث (J15) صغيرًا (+++) وكان لونه أخضر مصفر ولكن التفاعل بين BA و NAA (2.0 ملغم / لتر) أظهر استجابة قصوى بلغت 83.3٪ في ذكور الجوجوبا ولكن كانت أقل قيمة (68.75٪) في الإناث (J15) ولكن حجم الكالس كان كبيرًا (++++) وكان لونه أخضر مصفر. فيما يتعلق بمرحلة تحريض الكالس، تم تسجيل أفضل النتائج عندما تم زراعة الطعوم وزراعتها الفرعية على وسط MS بالإضافة إلى BA و NAA بمعدل 2.0 ملغم / لتر، كل على حدة.