Optimizing micro propagation of artemisia dracunculus using tissue culture and growth regulators

Jawad Shakir Kadhim*, Sarab A. Almukhtar, Zaid K. Kahdim

Horticulture and Landscape Department, College of Agriculture, University of Kerbala, Karbala, Iraq.
Corresponding author e-mail: jawad.sh@s.uokerbala.edu.iq
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Abstract

The tarragon (*Artemisia dracunculus*) is on one of the most important medicinal plants that has a useful role in the field of pharmacy. The aim of this study was production of micro propagation the tarragon plant by applying the plant tissue culture technique in establishing vegetative branch cultures. The first experiment included testing the ability of NaOCl at different concentrations and durations to sterilize the explant used as a starting point for establishing tissue cultures, the second experiment included testing different concentrations of BA and NAA at concentrations of (0, 1, 1.5) mg L^{-1} and (0, 0.1) mg L^{-1} respectively for the establishment of tissue cultures, the third experiment included testing two types of cytokinines BA and 2IP at concentrations of (0, 1, 2, 3) mg L^{-1} and (0, 0.1, 0.2, 0.3, 0.4) mg L-1 respectively with presence of a fixed concentration of NAA 0.1 mg l^{-1} for the purpose of multiplying shoot cultures. The 1% concentration of the sterilizing substance NaOCl at the 15 minute period achieved the lowest contamination rate of 0% without affecting the vitality of the explant, The results of the second experiment included the superiority of the concentration of 1.5 mg L^{-1} BA in achieving the highest response rate of 65%, while the concentration of 0.1 mg L^{-1} NAA achieved the highest response rate of 60.33%, The results of the third experiment included the superiority of BA at the concentration of 2 mg L^{-1} in achieving the highest rate of branch number, lengths, and fresh and dry weight of the vegetative system, which reached 35.21 branch $plant^{-1}$, 4.54 cm, 3.16 mg, and 1.05 mg respectively. While the 2IP achieved the highest rates of branch number and fresh and dry weights of the vegetative system at the concentration of 0.3 mg L^{-1} which reached 31.05 branch $plant^{-1}$, 2.83 mg, and 0.93 mg, respectively, the highest rate of branch length was at the concentration of 0.2 mg L^{-1} which reached 3.31 cm.

Keywords: *Artemisia dracunculus*, micro propagation, BA, NAA, 2IP.

Introduction

The scientific name for tarragon *Artemisia dracunculus* L., there are two main types of tarragon: Russian tarragon and French tarragon. Russian tarragon has a much milder flavor than French tarragon and is native to Siberia and western Asia[1]. Tarragon belongs to the Asteraceae family, and the essential oils extracted from its aerial parts have been widely used in folk medicine. Research has shown that this herb has numerous pharmacological activities. In addition to its pleasant, spicy aroma, its essential oil also exhibits antibacterial and antifungal activity [2] "Characterized by a bright green appearance, a warm aroma, and a flavor profile similar to anise, tarragon leaves contain 0.3 to 1.0% essential oil, the main ingredient of which is methyl chavicol. The common name tarragon is believed to be a corruption of the Arabic word tarkhum, meaning "little dragon." Its generic name, Artemisia, is derived from the Greek goddess Artemisia, the moon goddess. Its generic names include tarragon, estragon, snakeweed, and dragon. Tarragon is an important medicinal plant. It has been used in folk medicine to treat many different ailments, including infections, digestive problems, and as a pain reliever. It also has antioxidant properties, exhibiting strong antioxidant activity and is beneficial for overall health [3]. This plant has also been used to relieve indigestion, detoxify the body, treat insomnia, and improve liver function [4]. Tissue culture is a fundamental component of plant biotechnology because it is an alternative means of vegetative propagation in plants. This technique has been applied to entire plants with the aim of rapid propagation and producing pathogen-free plants and multiplying the plant throughout the year by controlling environmental conditions and increasing the production of secondary metabolite compounds. This technique has also been used in genetic improvement programs and producing plants similar to the mother plant to solve many agricultural crop problems [5,6]. Plant tissue culture is a versatile technique essential for plant propagation, developing plant capacity, and producing bioactive compounds. It al-lows entire plants, organs, tissues, or cells to be grown under controlled, sterile conditions in the laboratory. Tissue culture provides all the nutrients, energy, and water necessary for plant growth in the medium, as well as optimal light and temperature conditions to promote growth. Plant growth can be manipulated by adding plant growth regulators at specific stages of growth or maturity [7]. Plant growth regulators affect the vital, physiological and morphological processes of plant growth and development, as cytokinins are one of the axis through which the growth and multiplication of vegetative branches can be increased and the concentrations of secondary metabolites increased [8]. The most commonly used cytokinines in tissue culture are benzyl adenine, kinetin, and 2IP, which play a essential role in cell division, stimulating the production of DNA and RNA by activating proteins and genes responsible for enzyme formation. Studies have shown that cytokinines stimulate the movement and transport of nutrients within plant tissues and organs, as well as cell division and differentiation, breaking apical dominance, increasing chlorophyll content, delaying aging, and stimulating the emergence and multiplication of vegetative branches [9]. In light of the above, the importance of the plant from a medical perspective, and the lack of studies related to tissue propagation of tarragon, the study aimed to test the ability of NaOCl at different concentrations and time periods to sterilize the explant and then test the ability of different types and concentrations of growth regulators to develop and multiply vegetative branches to establish tissue cultures of tarragon in vitro.

Materials and Methods

This study was conducted in the Plant Tissue Culture Laboratory of the Department of Horticulture and Landscape Engineering, College of Agriculture - University of Karbala in the fall of 2024.

Preparation of the culture media

The culture media was prepared by adding 4.9 g L^{-1} Murashige and Skoog (MS) media [10] and 30 g L^{-1} sucrose , the volume was made up to 800 ml of distilled water, the pH was then adjusted to 5.7±1 using hydrochloric acid (HCl) or sodium hydroxide (NaOH). The final volume was made up to 1000 mL, and 7 g L^{-1} agar was added to the culture medium. The culture medium was heated using a vibrating heater until homogeneous and then distributed into culture bottles, averaging 10 ml of medium per bottle. The bottles were then sealed with airtight caps and sterilized in a steam autoclave at 121 °C for 15 minutes. After sterilization, the bottles were removed to solidify at room temperature before use for culture. All tools used in the transplantation process including forceps scalpels and Petri dishes, were sterilized in a steam sterilizer at 121°C and 1 bar pressure for 45 minutes. Blades and forceps were sterilized before and during use using 98% ethyl alcohol and burned with a Bunsen lamp to eliminate any contaminants that might be transferred to them during transplantation.

Sterilization experiment of the explant

The vegetative parts (shoot tips) were placed under a stream of water for 30 minutes in a glass beaker. They were then washed with water and liquid soap (bright soap). They were then washed with water to ensure the removal of dust and some surface contaminants. They were then washed once with distilled water and transferred to a laminar air flow cabinet. The plant parts were superficially sterilized with 95% ethyl alcohol for three seconds with continuous agitation. After that, they were washed once with sterile distilled water for five minutes with continuous agitation to ensure the removal of the harmful effect of alcohol. Afterward, they were sterilized using a commercial bleach containing sodium hypochlorite (NaOCl) at 6% concentration, following the preparation of different concentrations (0%, 1%, and 2%) for 5, 10, and 15 minutes under continuous agitation., then they were washed with sterile distilled water three times to ensure the removal of the bleach effect, sterilized explant were planted on MS media free of growth regulators at a rate of 10 replicates for each concentration and time period. The plants were incubated in a growth chamber with a light intensity of 1000 lux for 16 hours of light/day and 8 hours of darkness at a temperature of 25 ± 2. The results were recorded approximately 10 days after planting based on the percentage of contamination.

Initiation experiment

Based on the results of the sterilization experiment, the best treatment obtained was selected. 1cm-long shoot tips were planted on MS medium prepared with different concentrations of BA (0, 1, 1.5) mg L^{-1} mixed with NAA (0, 0.1) mg L^{-1}, with ten replicates for each concentration. The plants were incubated in a growth room at 25°C ± 2°C and 1000 lux illumination for 16 hours per day. The percentage response was calculated four weeks after planting.

Multiplication Experiment

Based on the results obtained from the emergence stage, the best treatment was selected. The vegetative branches were cut to a length of 1 cm and planted on MS medium prepared with BA at concentrations of (0, 1, 2, and 3) mg L^{-1} combined with 2IP at concentrations of (0, 0.1, 0.2, 0.3, and 0.4) mg L^{-1} with the addition of a fixed concentration of NAA at a concentration of 0.1, with 10 replicates for each concentration. The plants were incubated in a growth chamber under the same conditions as before. The study indicators were taken after 6 weeks including the number and length of vegetative branches as well as the fresh and dry weight of the vegetative mass.

Statistical analysis

The results were analyzed statistically according to the statistical program CRD [SAS] [11]. and the averages were compared using the least significant difference (LSD) and the probability level was 0.05.

Results and Discussion

The effect of sodium hypochlorite and the duration of time in sterilizing the explant Table 1 shows the effect of the used sodium hypochlorite concentrations and different sterilization periods on the contamination rate in the vegetative growths of tarragon plants grown on MS medium free of plant growth regulators after 15 days of cultivation, where the contamination rate reached 100% in the control treatment and for all periods, indicating the inefficiency of sterilization in the absence of the disinfectant. With increasing the concentration of the bleach, the contamination rate decreased significantly to reach (28.33, 10.05) respectively at concentrations of (1, 2 %) respectively. The period of soaking the vegetative parts in the solution recorded significant differences, as the contamination rate decreased to the lowest level when the vegetative parts were left in the solution for 15 minutes, where it became (33.33) while it was (65.05)% when the sterilization period was 5 minutes. As for the effect of interaction between sodium hypochlorite concentration and sterilization period in reducing the contamination rate of green parts, the comparison treatment recorded the highest contamination rate of 100% with different sterilization duration. While treatments 1 and 2% for a 15-minute soaking period gave plants completely free of contamination (0.00)%, and a 2% concentration of sodium hypochlorite for 10 minutes gave a contamination rate of 0.00%. It was noted that increasing the concentration of 2% sodium hypochlorite for a period of (15) minutes led to the appearance of white plants devoid of chlorophyll and others dead compared to the 2% concentration of sodium hypochlorite for 10 minutes, which gave the lowest contamination rate (0.00) without affecting the vitality of the plant parts. The effect of sodium hypochlorite and its work as a sterilizing material for the plant part is due to the fact that hypochlorous acid (HOCl) is a strong oxidizing material with high efficiency in the process of surface sterilization of seeds, as this acid is formed as a result of dissolving chlorine in water as in the following equation [12, 13]

$$Cl_2 + H_2O \rightarrow HCl + HOCl$$

These results are consistent with those of several researchers who used NaOCl for sterilization. [13] The results showed that adding a 0.003% active chlorine concentration to the culture media provided complete control of contaminants when growing gerbera in the laboratory.[14] concluded that the best treatment for sterilizing vegetative parts of the Chrysanthemum hortorum hort plant was using a 4.5% concentration of sodium hypochlorite (NaOCl) for 15 minutes. [15] demonstrated that sodium hypochlorite treatment (4%) for different periods of time in in vitro propagation of Annona muricata resulted in the best results and high vitality of the plant parts used (nodes, leaves).

This discrepancy may be due to differences in exposure duration; a short exposure to a 4.5% concentration may not cause harm, while a 15-minute exposure to a 2% concentration is sufficient. The type and sensitivity of tissues, the temperature of the solution, and the method of assessing damage may also differ between the two experiments[16, 17].

Table (1): Effect of sodium hypochlorite concentrations and sterilization period on the percentage of contamination (%) of vegetative growths of tarragon plant after 15 days of cultivation on MS media.

Time	NaOCl (%)			Mean
	0	1	2	
5	100.00	65.00	30.15	65.05
10	100.00	20.00	0.00	40.00
15	100.00	0.00	0.00	33.33
$L.S.D_{(0.05)}$	1.155			0.667
Mean	100.00	28.33	10.05	
$L.S.D_{(0.05)}$	0.667			

The effect of benzyl adenine and naphthalene acetate on the percentage of explant initiation.

The results of Table 2 showed that BA at a concentration of(1.5 mg L^{-1}) was superior in achieving the highest response rate of 65% compared to the control treatment, which achieved a response rate of 31.91%. The data in the same table also indicated that there was a significant effect of NAA on the response rate at a concentration of(0.1 mg L^{-1})which reached 60.33% compared to the control treatment, which achieved a response rate of 34.55%. As for the effect of the interaction between BA and NAA concentrations on the response rate, the concentration of(1.5 mg L^{-1}) BA at a concentration of(0.1 mg L^{-1}) NAA achieved the highest response rate of 85.00%, while the control treatment achieved the lowest response rate of 23.33%.

Table (2): Effect of BA and NAA concentrations on the response of the plant part to initiation (%) after 6 weeks of cultivation on MS media.

BA con. mg L^{-1}	NAA con. mg L^{-1}		Mean
	0	0.1	
0	23.33	40.50	31.91
1	35.33	55.50	45.41
1.50	45.00	85.00	65.00
L.S.D (0.05)	0.18		0.23
Mean	34.55	60.33	
L.S.D (0.05)	0.28		

Effect of BA and 2IP on the number of vegetative branches

Table (3) shows that BA was significantly superior at a concentration of (2 mg L^{-1}) giving the highest average number of branches, reaching (35.21 branches plant-1 while the lowest average was achieved in the control treatment, which reached (16.02 branches $plant^{-1}$). The same table also indicated that 2IP was significantly superior at a concentration of(0.3 mg L^{-1}) recording the highest average of(31.05 branches $plant^{-1}$) compared to the control treatment which gave the lowest average of(21.76 branches $plant^{-1}$). As for the effect of the two-way interaction, the concentration of 2 mg L-1 BA was superior at a concentration of(0.3 mg L^{-1}) 2IP achieving the highest average number of branches, reaching (42.35 branches $plant^{-1}$) compared to the control treatment which achieved the lowest average of (10.12 branches $plant^{-1}$).

Table (3): Effect of BA and 2IP concentrations and their interaction on the number of branches (plant $branch^{-1}$) of tarragon plants after 6 weeks of cultivation on MS media.

BA con. mg L^{-1}	2IP con. mg L^{-1}					Mean
	0.0	0.1	0.2	0.3	0.4	
0	10.12	15.40	16.09	20.18	18.33	16.02
1	25.70	30.01	35.17	32.50	29.17	30.51
2	28.15	31.09	34.30	42.35	40.16	35.21
3	23.10	29.00	31.15	29.17	19.15	26.31
L.S.D (0.05)	0.22					0.10
Mean	21.76	26.37	29.17	31.05	26.70	
L.S.D (0.05)	0.11					

Effect of BA and 2IP concentrations on the length of vegetative branches

The results of Table 4 show that there are significant differences in the average branch length of tarragon plants, as BA was superior at a concentration of (2 mg L^{-1}) in achieving the highest average of (4.54 cm) compared to the control treatment, which achieved the lowest average length of (1.75 cm). The data of the same table also indicated the significant superiority of 2IP at a concentration of (0.2 mg L^{-1}) which gave the highest average length of (3.31 cm) compared to the control treatment, which reached (2.38 cm). As for the effect of the interaction between BA and 2IP concentrations on the average of the same trait, the concentration of (2 mg L^{-1}) BA at a concentration of (0.2 mg L^{-1}) achieved the highest average branch length of (5.25 cm), while the lowest average was (1.35 cm) in the control treatment.

Table (4): Effect of BA and 2ip on the average length of vegetative branches (cm) after 6 weeks of cultivation on MS medium.

BA con. mg L^{-1}	2IP con. mg L^{-1}					Mean
	0.0	0.1	0.2	0.3	0.4	
0	1.35	1.70	2.10	2.26	1.34	**1.75**
1	2.66	3.20	4.37	2.70	2.46	**3.07**
2	4.14	4.05	5.25	5.00	4.30	**4.54**
3	1.40	1.48	1.55	2.03	1.75	**1.64**
L.S.D (0.05)	0.04					**0.01**
Mean	2.38	2.60	3.31	2.99	2.46	
L.S.D (0.05)	0.02					

Effect of BA and 2IP on fresh weight.

The data in Table (5) indicate that there are significant differences in the average fresh weight of the vegetative group according to the different concentrations of BA added to the MS medium, as the concentration of (2 mg L^{-1}) was significantly superior in achieving the highest average fresh weight of(3.16 mg), while the lowest percentage of fresh weight was reached in the control treatment, which was(1.67 mg). The same results also indicated that there is a significant effect of 2IP when added to the nutrient medium, as the concentration of (0.3 mg L^{-1}) achieved the highest average fresh weight of (2.83 mg) compared to the control treatment, which achieved the lowest average weight of (2.00 mg). As for the effect of the second interaction between the concentrations of BA and 2IP, it is noted from the results of the table that BA was significantly superior at the concentration of(2 mg L^{-1}) with 2IP at the concentration of(0.3 mg L^{-1}) in achieving the highest average of (3.81 mg), while the control treatment achieved the lowest average fresh weight of(1.18 mg).

Table (5): Effect of BA and 2IP on the fresh weight of vegetative growths (mg) after 6 weeks of cultivation on MS medium.

BA con. mg L^{-1}	2IP con. mg L^{-1}					Mean
	0.0	0.1	0.2	0.3	0.4	
0	1.18	1.44	1.62	1.98	2.16	**1.67**
1	2.23	2.70	3.17	2.93	2.63	**2.73**
2	2.53	2.80	3.09	3.81	3.61	**3.16**
3	2.08	2.61	2.80	2.63	1.73	**2.37**
L.S.D (0.05)	0.04					**0.01**
Mean	2.00	2.38	2.67	2.83	2.53	
L.S.D (0.05)	0.02					

The effect of BA and 2IP on the dry weight

Table (6) shows that there is a significant superiority in the average dry weight of the vegetative group with increasing concentrations of BA added to the nutrient medium, as the concentration of (2 mg L^{-1}) achieved the highest average dry weight, reaching (1.05 mg), then the response decreased with increasing concentration to(3 mg L^{-1}) which achieved an average of(0.79 mg), while the lowest percentage of dry weight was in the control treatment, reaching (0.65 mg). The results of the same table also showed the superiority of 2IP with increasing its concentrations added to the nutrient medium, as the concentration of (0.3 mg L^{-1}) achieved the highest average dry weight, reaching (0.93 mg), then the response decreased with increasing its concentration to(0.4 mgL^{-1}) which reached (0.80 mg), while the lowest dry weight was recorded in the control treatment, which reached (0.65 mg). As for the effect of interaction between BA and 2IP concentrations, it is noted from the results of the same table that BA at a concentration of(2 mgL$^{-1)}$was significantly superior to 2IP at a concentration of (0.3 mg L^{-1}) in achieving the highest dry weight rate of (1.27 mg) while the comparison parameter achieved the lowest weight rate of (0.30 mg).

Table (6): Effect of BA and 2ip on the dry weight of vegetative growths (mg) after 6 weeks of cultivation on MS medium**.**

BA con. mg L^{-1}	2IP con. mg					Mean
	0.0	0.1	0.2	0.3	0.4	
0	0.30	0.46	0.48	0.60	0.55	0.47
1	0.77	0.90	1.05	0.98	0.88	0.91
2	0.84	0.93	1.03	1.27	1.20	1.05
3	0.69	0.87	0.93	0.88	0.58	0.79
L.S.D (0.05)	0.03					0.01
Mean	0.65	0.79	0.87	0.93	0.80	
L.S.D (0.05)	**0.01**					

According to the results presented in Table (2), at the emergence stage, there was a significant response in the percentage of tarragon emergence with increasing concentrations of BA added to the nutrient medium. This may be due to the stimulating effect of BA in inducing cell division and development. Furthermore, cytokinines aid in indirect cell division, followed by cell division, stimulating the growth of vegetative buds from meristematic tissues, and also the synthesis of RNA, DNA, enzymes, and proteins [14, 15]. Cytokinins are essential in regulating cell division and promoting the growth of vegetative shoots from meristematic tissues. They also interact with auxins to control apical dominance and lateral branching [16] . These results are consistent with the findings of [17 ,18 ,19].

The results of Tables (3-6) generally indicated the superiority of BA in the studied traits, which included the number and length of vegetative branches and the fresh and dry weight of the tarragon plant compared to the control treatment. The reason for this may be attributed to the stimulating action of BA in urging cells to divide and develop, or the reason for the superiority of the studied traits may be attributed to the role of cytokinines in being stable compounds that do not decompose easily and their high efficiency in breaking the apical dominance, as they work to reveal and expand the vessels that transport both the wood and the bark and prevent the decomposition of chlorophyll, thus stimulating cell division and increasing the production of nucleic acids, in addition to their role in attracting dissolved and solid nutrients[20]. Cytokinins also have an important and major role in the metabolism of nutrients, carbon assimilation, and chloroplast development, which is positively reflected in the increase in the fresh and dry weights of differentiated vegetative growths [21 ,22]. The reason for the superiority of BA in vegetative characteristics may be due to the fact that its side chain is linked to three double bonds, which makes it superior in its activity to the rest of the cytokinines, unlike 2IP, which contains two bonds, as well as containing a Benzyl ring. For this reason, the role of BA was greater in the multiplication of vegetative branches compared to 2IP [23]High levels of cytokinines also led to decreased growth rates due to hormonal imbalance within plant tissues, which leads to decreased growth of plant parts. This decrease does not necessarily mean cell death, but it usually results in growth retardation [24]. The results are consistent with those of [25] when applying growth regulators to Artemisia nilagirica in vitro [26] in their study on chrysanthemum, and [27] in their study on the effect of different growth regulators on Artemisia annua, which demonstrated that BA significantly outperforms 2IP in improving the vegetative characteristics of tarragon (Artemisia dracunculus L.), including the number and length of shoots, and fresh and dry weights. This superiority is attributed to the high ability of BA to stimulate cell division, break apical dominance, and activate lateral growth, compared to the lower effectiveness of 2IP in these physiological functions. BA is also effective in enhancing nutrient uptake efficiency, leading to better growth results compared to 2IP [28] The results of this study are consistent with those of [29] in a comparative study of the effect of BAP and 2-iP on the propagation of lemon citrus (Cymbopogon citratus), where it was found that BAP was more effective in stimulating shoot proliferation, while 2-iP showed a lesser effect and was associated with the stimulation of rooting, through the activation of growth-promoting genes, while 2-iP tends to activate growth-inhibiting genes and stimulate rooting, which explains the superiority of BAP in micro propagation compared to 2ip. I agree with what was proven by[30] when using cytokines, including benzyl adenine, to propagate thyme in vitro, giving it the best growth indicators.[31] studied the effectiveness of different auxins and cytokinins on Artemisia amygdalina , (MS) medium fortified with 2 µM of α-napthaleneacetic acid together with 1 µM of 6-benzyladenine produced Highest number of buds. [32] demonstrated that MS medium supplemented with growth regulators BA or KIN was more effective than hormone-free MS medium in producing a higher number of shoots Sideritis raeseri subsp. Attica. In tarragon, genetic variations may influence the expression and activity of auxin and cytokinin signaling components, resulting in species-specific hormonal responses. Tarragon may possess different types of hormone receptors, suggesting that variations in tarragon's genes are the primary reason for its distinct hormonal responses. These genetic variations specifically affect its hormone receptors and how hormonal signals are transduced within its cells, making it interact with hormones (such as auxins and cytokinins) in a unique way compared to other plants, enhancing growth parameters. The interaction between auxins and cytokinins is essential for tarragon growth and development and occurs through complex regulatory networks. Auxin also regulates cytokinin levels, often by inhibiting its biosynthesis. In turn, cytokinins influence auxin transport and distribution, particularly by regulating PIN proteins [33] . The genetic makeup of tarragon (e.g., variations in AUX/IAA genes and cytokinin response regulators) shapes the nature and strength of these hormonal interactions [34].

The results of this study indicated the possibility of employing plant tissue technology and propagating medicinal plants in large numbers within short periods of time and free of pathogens. The results of the study also indicated the possibility of providing the nutritional medium with different concentrations of cytokinines to stimulate axillary and lateral buds and obtain vegetative branch farms in large numbers, but high and inappropriate concentrations of cytokinines negatively affected most of the studied traits.

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