The effect of growth regulators on micropropagation of medicinal plant *Chrysanthemum hortorum* hort by using plant tissue culture technology

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Abstract

This study was conducted to evaluate the impact of various concentrations of growth regulators added to the MS on the propagation of *Chrysanthemum* plants outside the living body, a study was conducted in the Plant Tissue Culture Laboratory at the Department of Horticulture, College of Agriculture, University of Kerbala. The findings of study can be summed up as follows:The best treatment for sterilizing the vegetative parts was by using a concentration of 4.5% of sodium hypochlorite (NaOCl) for 15 minutes. The concentration of the benzyl adenine gave 2 mg L^{-1} highest percentage in the number of shoots, length and number of sheets of 6.80 sections $^{-1}$ and 4.30 cm and 25.00 leaves, respectively, comparison by the comparative processing given by Lowest rates, when grow shoot tips measurement of 1 cm on the MS media. When growing vegetative shoots from the multiplication stage on the MS nutrient media, which is equipped with various concentrations of NAA for rooting, the concentration exceeds 1 mg L^{-1} of it to achieve the highest percentage of rooting, the number and length of roots, the length of the shoot, and the fresh and dry weight of the shoot reached 100%, respectively, 18.50 root, 5.50 cm, 13.30 cm, 2.80 and 0.98 mg), compared with the control.

Keywords: Chrysanthemum , in vitro, propagation, rooting, medicinal plant, growth regulators.

Introduction

The *chrysanthemum hortorum* is a perennial herbaceous plant. It is one of the short-day plants and its yearly renewal of cultivation, belongs plant to is an Asteraceae or Compositae family [1]. This plant was produced from the hybridization of the wild species *Chrysanthemum* morifolium Ramat and *Chrysanthemum* indicum Linn, as these two species originated in China and Japan, from which all the current varieties of multi-shape and color of flowers originated [2]. The genus chrysanthemum includes more than 150-200 species, and it is derived from the Greek word chrusos, which

means gold, and the word anthemom, which means flower, and for this reason it is called the golden flower. The flower of the *Chrysanthemum* plant is an inflorescence that bears disc flowers and in its center are radial flowers surrounding it. They can be called flowers with petals, and they are the ones that give the inflorescence its own colour. Its colors are multiplied and the characteristics of the inflorescences vary greatly, perhaps unparalleled in the rest of the ornamental flowering plants, which made *Chrysanthemum* is of great importance in the union of the world,م in many cases, the inflorescence is called a flower, as it is a common name in the producing markets [3]. from scientific side, the *Chrysanthemum* plant's flowers are preferred due to their multiplicity and radiance of colors, including red, yellow, purple, and white; their size and adaptability to be used as potted plants in gardens and building entrances; and their longevity after harvesting for a long time, up to two weeks, with good growth and variety in height [4]. Also, Chrysanthemum plants have many medicinal uses if recorded from medicinal plants, which relieves and treats diseases of the common cold, inflammation of the ear, eye, and mouth, and improves eyesight, as well as regulates blood pressure, tonic, vasodilator, and detoxification, if used as an antibacterial, antispasmodic, anti-inflammatory, cancer, carminative, diaphoretic, Diuretic and menstruation, and the plant drink helps to reduce blood sugar, and the ancient Greeks used it to treat joints, headaches, joint pain, and stomach reflux [5,6]. The science of cultivating plant cells, tissues, and organs that have been separated from the mother plant on nutrient media is known as plant tissue culture [7]. One of the biological technologies that has played a significant role in human life and continues to do so is tissue culture, particularly in the area of plant propagation. Because of the uniqueness of this process and its benefits, perhaps the most significant of which is the ability to produce large numbers of plants that are similar to the mother plant and free of pathogens in a short amount of time and at any time of year, as well as the use of this technology in research and applied fields such as plant breeding and improvement, as well as the production of drugs and medicines. Additionally, one of the really important uses is fast phylogenetic propagation. The aim of this study is to determine the optimal combination of growth regulators in plant propagation and obtain the highest rate of quality in the vegetative and root totals.

Materials and Methods

The experiment was carried out in the Micropropagation laboratory of the Horticulture and Landscape Department - College of Agriculture / University of Kerbala during the winter of 2022-2023.

Preparation and sterilization of media

Prepared the media additionally 4.9 gm. L^{-1} Murashige and Skoog (MS) media and 30 g L^{-1} sucrose and complete the volume to 800 ml distilled water, then adjusted the pH to 5.7 by adding drops of 1 N standard solution of NaOH or HCl acid, after that completing the final volume to 1000 ml with adding by 7 g L^{-1}. The nutritious media components were heated in an electric mixer to dissolve the acer with the components,

and once the media became homogeneous, it was distributed in glass bottles of 50 ml capacity, with 10 ml of media per vial. It was then sealed with tight-fitting caps and sterilized in an autoclave at 121 °C for 15 minutes. After sterilization, the bottles were removed from the autoclave and allowed to harden at ambient temperature before being used for cultivation. Other agricultural materials, such as tweezers, blades, and Petri dishes, were sterilized in an autoclave for 45 minutes at 121°C .

Experiment with sterilizing explants

The ends of the shoots were removed from the *Chrysanthemum* plant, 2 cm long, and then washed with water several times to get rid of dust and suspended matter, after which they were transferred to the laminated flow air cabinet which has been previously sterilized with alcohol at a concentration of 70%. The explants were immersed in NaOCl at a concentration of (1,2,3,4) % as an active substance for sterilization, as well as the comparison treatment, for periods (5,10,15) minutes with continuous stirring. After that, it was washed with sterilized distilled water three times to ensure that the harmful effect of the sterilized substance was removed from the surface of the plant parts. The tips of the explants were removed with the blade, so that they became 1 cm long. The explants were transferred to culture bottles with MS nutritional media without of growth regulators and cultured in the growth room for 16 hours at a temperature of 25 °C $^{+-2}$ and a light intensity of 1000 lux. Day^{-1}, with ten replications for each concentration and duration, the results were recorded on the percentage of contamination after 7 days of cultivation.

Multiplication stage

Following the determination of the optimal concentration and sterilizing period in the preceding paragraph, the tips of shoots with a length of 1 cm acquired from the stage of emergence were used then, they were planted on the (MS) media and in different concentrations of BA (4, 3, 2, 1, 0.5, 0) mg. L^{-1} when constant concentration is contains (0.2) mg L^{-1} of NAA was replicated 10 times for each concentration. The crops were kept in the growth chamber at a temperature of 25 m $^{\pm 2}$ and a light of 1000 lux for a period of 16 hours. day^{-1}, the study indicators were taken, after six weeks of cultivation, which comprised the number and length of vegetative shoots as well as the number of leaves for each plant.

Rooting stage

The tips of the shoots resulting from the doubling stage, with a length of 2 cm, were planted on MS medium equipped with different concentrations of IBA (2,1.5,0.5,0) mm L^{-1} and in the presence of (0.2) mm L^{-1} BA by ten repetitions for each concentration. The crops enjoyed the previous conditions to encourage them to root. Data were recorded on the percentage of rooting, the number and length of roots, and the fresh and dry weight of the root system after four weeks of planting.

Acclimatization of plants resulting from tissue culture

The rooted plants were washed with running water to remove the remnants of the nutrient media, which is a good source of microbe development, and then immersed for ten seconds in Benlate fungicide at a concentration of 0.1% for 10 seconds to limit the possibility of fungal infection. For 7 days to purify the plants, the plantlets were placed in a solution containing a quarter of the strength of MS salts, then the plants were planted. Rooted on stalks containing peat moss in a 1:2 ratios, moistened with water, and covered with glass covers to ensure an optimum amount of humidity [8], described how the covers were gradually removed. After two weeks, the success rate of acclimation was measured.

Statistical analysis

The Results were Analysed statistically according to CRD, statistical program (SAS, 2004). The means were compared using the Least Significant Difference (LSD) and the probability level was 0.05.

Results and Discussion

Effect of NaOCl and Durations on the Percentage of Plant Contamination Table (1) shows the effect of sodium hypochlorite and sterilization duration on the percentage of contamination of Chrysanthemum plants, as the percentage of contamination reached 100% in the neutral treatment and decreased significantly as the concentration of sodium hypochlorite increased, reaching (72.13, 40.06, 6.46, 0.0) at concentrations (1.5, 3.0, 4.5, 6.0) %, respectively.

As the percentage of contamination reduced, the length of sterilization showed substantial differences decreased by increasing the time period to 15 minutes, as it gave a pollution rate of 30.60%, while the percentage of pollution reached 57.48% when the sterilization period was 5 minutes. Significant differences appeared in the percentage of contamination when the period overlapped with sodium hypochlorite concentrations, reaching 100% for all sterilization periods in the neutral treatment. The least of these was in the sterilization period of 15 minutes at concentrations of 4.5 and 6%, and 5 and 10 minutes at a 6% sodium hypochlorite concentration, reaching 0.0%. It was noted that increasing the concentration to 6% of sodium hypochlorite at all periods led to the emergence of white seedlings free of chlorophyll, compared to a concentration of 4.5% of sodium hypochlorite at a period of 15 minutes, which gave the lowest pollution rate of 0.0% without affecting the vitality of the plants. The effect of sodium hypochlorite and its function as a sterilizer for plant tissues is due to (HOCl), which is a strong oxidizing substance. As this acid is formed as a result of chlorine dissolving in water, as in the following equation $Cl_2+H_2O \rightarrow HCl + HOCl$: [10]

These findings corroborated what was discovered by [11] when propagating Chrysanthemum plants in vitro and with [12] when sterilizer the shoot tips to induction the callus from *Vitis Vinifera* in-vitro and with [13] when sterilizer the shoot tips to induction the callus from *papaver somniferum* in vitro and with [14] When sterilizing the different explants *of Gardenia jasminoides* Micro propagation.

Table (1): shows the effect of NaOCl concentrations and sterilizing time on the percentage of contamination in *Chrysanthemum* plants after 14 days of growing on MS media.

Time (min)	Con. of NaOCl %					Mean
	0	1.5	3	4.5	6	
5	100.00	100.00	74.80	12.60	0.00	57.48
10	100.00	80.00	28.80	6.80	0.00	43.12
15	100.00	36.40	16.60	0.00	0.00	30.60
LSD (0.05)	1.76					0.78
mean	100.00	72.13	40.06	6.46	0.00	
LSD (0.05)	1.01					

The effect of varying BA concentrations on the multiplication of *Chrysanthemum* plant shoot tips

Table (2) shows the effect of different concentrations of AB (4.0, 3.0, 2.0, 1.0, 0.5, 0) with a constant concentration of NAA (0.2) mg L-1 on vegetative shoot tip multiplication after 4 weeks of growth on MS media. As the concentration of 2 mg L^{-1} achieved the highest rate for the number and length of shoots and the number of leaves, which reached (6.80 shoots, plant $part^{-1}$, 4.30 cm, and 25 leaves. $plant^{-1}$), respectively, the response was reduced by increasing the concentrations of benzyl adenine added to the nutritional media to 3 and 4 mg L-1 in the rate of studied trait.

Table (2): The influence of BA concentrations on the multiplication of *Chrysanthemum* plant shoot tips after 4 weeks of growth on MS media

BA con. (mg l^{-1})	N. shoots (per plant $^{-1}$)	L. shoots (cm)	N. leaves (per plant $^{-1}$)
0.0	1.00	1.00	2.00
0.5	2.50	1.95	12.20
1.0	5.60	2.50	17.35
2.0	6.80	4.30	25.00
3.5	1.60	1.60	4.20
4.0	1.30	1.25	3.40
LSD (0.05)	0.06	0.06	0.06

Table (3) shows the effect of different NAA concentrations added to the MS nutrient media, as well as the presence of BA at a fixed concentration of 0.2 mg L-1, on the

average of the root system's studied characteristics, which included the percentage of rooting, the number and length of roots, the length of the shoot, and the fresh and dry weight of the root system.

It was discovered that increasing the concentrations of Auxins added to the media resulted in a significant increase in the average of the traits, down to the optimal level of 1 mg L-1, which achieved the highest rate for the same traits (100%, 18.50 root branch-1, 5.50 cm, 13.30 cm, 2.50 and 0.98 mg) respectively, while the neutral treatment did not achieve any significant rate for the above characteristics.

Table (3): The effect of NAA concentrations on the roots of *Chrysanthemum* plant shoots after four weeks of cultivation on MS media

NAA con. (mg l^{-1})	Rooting percentage	N. roots ($branch^{-1}$)	L. Root (cm)	L. Branch (cm)	Soft weight (mg)	Dry weight (mg)
0.0	0.00	0.00	0.00	0.00	0.00	0.00
0.5	100	12.30	3.60	10.40	1.95	0.76
1.0	100	18.50	5.50	13.30	2.80	0.98
1.5	100	7.20	2.85	8.60	1.12	0.30
2.0	100	5.80	1.25	6.25	0.50	0.08
LSD (0.05)	0.20	0.06	0.65	0.06	0.06	0.04

Acclimatization of plants

To reduce the risk of pathogen infection, the acclimatization and transfer of plantlets from tissue culture to soil demonstrated the importance of washing the rooted plant from the nutrient media under running water and immersing it for ten seconds in the fungicide benlat at a concentration of 2.5%. Seven days had a great role in hardening the plants before transferring them to the soil and thus reducing the losses, and covering the plants with a glass cover helped to maintain the required moisture inside the cover surrounding it with the gradual lifting and then covering because it is characterized by the absence of the cuticle layer that covers the surface of the leaves, as well as the lack of The stomata function normally due to the growth of the plants in a high-humidity environment. After two weeks of cultivation, the cover was removed from the plants to live under field conditions. The percentage of acclimatization success reached 90%.

The multiplication stage results revealed a considerable superiority in the rate of the tested features. The reason could be that cytokines, especially BA, are used in tissue culture because they are constant compounds that do not decompose easily and have a high efficiency in breaking the apical dominance as they work to reveal and widen the vessels transporting both wood and phloem, promotes cell division and enhances nucleic acid production [15] The results of increasing the rates of response to the studied traits may be explained by the increase in BA concentrations and the presence of a constant concentration of NAA in the nutrient media to reach the optimal state, when achieving a state of hormonal balance that leads to the response of the cultivated plant parts, high levels regulators cause a drop in growth rates due to disruption of critical

processes inside tissues caused by hormonal imbalance, resulting in a decrease in growth rates of plant sections. This decline does not always indicate cell death, but it is frequently due to inhibited growth [16]. These findings matched with those of [17] when micropropagating *Catharanthus roses* in vitro, [18] when micropropagating *Gardenia jasminoides* in vitro, and [19] when propagating Banana Shoots Musa spp. in vitro.

The results also demonstrated that increasing the quantities of auxins applied to the nutrient media resulted in a considerable superiority in the average of the analyzed features of the root total. The explanation could be owing to the superiority at 1 mg L-1 concentration, as this concentration is thought to be the optimal in rooting the vegetative shoots of the Chrysanthemum plant; additionally, the reason could be due to physiological changes. Auxin concentrations, together with a high concentration of endogenous auxins, cause the development of root initiators at the bases of vegetative shoots [20]. Differentiation is the origins of the roots that grow and develop into the roots of the roots and then into adventitious roots emerging from the bases of the vegetative shoots forming the adventitious roots.

Furthermore, the effect of auxins on the average length of rooted vegetative shoots may be attributed to its role in the process of cell division and expansion, which leads to increase elongation and improved vegetative growth by increasing the root system in the vegetative system, which leads to increase absorption of nutrients that are transported to the top and leads to the growth and opening of buds, which leads to good vegetative growth.

This result agreed when rooting *Chrysanthemum* shoots in vitro [21,22] By rooting, the shoots of Bauhinia alba plant in vitro, as well as [23,24] when rooting *Digitalis lanata* in vitro.

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