Iraqi Journal of Agricultural Sciences –2026:57(2):402-414
P-ISSN: 0075- 0530/ E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/fkvbje80

FACTORS INVOLVED IN MICROPROPAGATION OF CHRYSANTHEMUM (*CHRYSANTHEMUM MORIFOLIUM*)

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ABSTRACT

Various concentrations of plant growth regulators added to MS (Murashige & Skoog) media were used to multiply chrysanthemum shoots. pH, sugar and licorice extract. Before being used as attest material, using the *ex vitro* obtained plant, a nodal piece was sterilized with 1.0% mercuric chloride ($HgCl_2$) for five minutes.MS medium supplemented with BA at a lower concentration (1.0 mg.l^{-1}) showed the best results when compared to other concentrations (8.7 shoots/explants, 7.5 cm, 17.1 leaves/explants and 4.0 nodes/explants)were studied. The average shoot length, nodes per explants, leaves per explants, and number of shoots per explants were all greater in the medium containing 30 g.l^{-1} sucrose, with values of 6.0 shoots per explants, 4.83 cm, 14.2 leaves per explants, and 3.6 nodes per explants, respectively Results showed that the effect of licorices extract gave the highest response percentage at concentration 50 ml.l^{-1} (13.5 shoots /explants, 8.5 node/explants, 3.5 leaves / explants and 4.82cm) respectively. It was shown that media with a pH adjustment of 5.7 produced the maximum nodes per shoot, leaves per shoot, average length of shoot, and number of shoots per explants (3.3 nodes per shoot, 14 leaves per shoot, 5.77 cm and 6 shoots per explants, respectively).Concerning the root formation stage, the concentration (0.1 mg.l^{-1}) IBA with 1/4 MS salt strength gave the highest value for all parameters. While, the acclimatization stage showed the culture media supplemented with soil + Peat moss (1:1) gave the highest response percentage 100%. The best treatment was recorded at 1/2 MS salt strength with (2.5 mg.l^{-1}) licorices extract for most characterize.

Key words: BA, Chrysanthemum, Kinetin, Licorice extract, Sucrose, pH and Tissue culture.

Received: 15/12/2023, Accepted: 3/3/2024, Published: 28/02/2026

INTRODUCTION

Autum Queen is the popular name for *Chrysanthemum morifolium*. It is a member of the (*asteraceous)* compositeae family. It is a short-day plant that blooms both annually and perennially and is highly beautiful and charming. The plant can grow up to three feet tall. The blooms have a broad variety of colors, shapes, and sizes, and they bloom in the early winter (Banno *et al*., 2007). 35% of the overall production of cut flowers is accounted for by chrysanthemums. The second-most important cut flower in terms of profitability is chrysanthemums, according to a research from the Flowers and Plants Association from 2001. One of the most popular ornamentals planted in greenhouses, pots, and as cut flowers worldwide is the chrysanthemum. Chrysanthemums for florists are grown all over the world as potted plants as well as for cut flowers. Because it lasts a long time in a vase, it is frequently used for flower arrangements. Growing chrysanthemums from seed produces a complicated hybrid that separates into a variety of flower types. According to Verma *et al.* (2009), commercial cultivars are typically grown through vegetative cuttings, suckers, or

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terminal cuttings. Shoot cutting traditionally is a rather slow process (Nhut *et al*., 2005). The rate of multiplication can be considerably accelerated by clonal propagation in *in vitro* culture. With this method, a large number of genetically homogeneous, disease-free plants can be produced quickly and reliably. However, clonal proliferation in *in vitro* culture will increase the rates of multiplication (Sauvaire and Galgy, 1978). Sucrose is the primary source of carbon and energy for optimal proliferation and growth in *in vitro* growing cultures. Because they are unable to grow autotrophic ally in a growth media, plant cells and tissues require external carbon sources for energy (Razdan, 1993). The addition of an external carbon source may enhance the medium's capacity to maintain cell development and the regeneration of new green shoots. The proper concentration of sucrose in a medium should be able to provide all the energy required for cell division and differentiation without having an adverse effect on the growth of new shoots due to osmotic effects. This indicates that sucrose that serves as an osmotic source of carbon energy in a medium is one of the factors regulating the induction and growth of shoots. (Nowak *et al*., 2004). An ideal pH is necessary for the growth and development of plant cells and tissues in cultures. pH affects not only food uptake but also plant hormone and enzymatic activities. The optimal pH level maintains the functionality of the cell membrane and the buffered pH of the cytoplasm without interfering with cytoplasmic activity that affects cell division and shoot growth. The ability of a medium to solidify can also be influenced by the pH; a pH greater than 6 produces an extremely hard medium, while a pH lower than 5 does not consolidate the medium to the proper degree (Ebrahim and Ibrahim, 2000). The ions taken in from the medium cause a change in pH in the cells or organs (Sakano, 1999). Since both the pH and sugar concentration have an effect on the regeneration of new shoots, it is imperative to achieve the ideal levels for both in order to produce the maximum amount of shoot regeneration. Lower and higher pH levels inhibit multiple shoot proliferation. Studies and research indicate the importance of natural plant extracts which are extracted from plants and added to MS media used in tissue culture, has been noted the positive effect of these materials in the tissue culture of many plant species, and these materials such as coconut Milk in the tissue culture of citrus (Soliman *et al*., 2010), and extract some of the weed, including the baker (*Malva rotundifolia* L.) in the tissue culture of potatoes and cucumber (Haider, 2002). And licorice extract in the tissue culture in shrub rose (Al-Mamori, 2009), and extracts of potatoes and banana (Smith, 2013).where these extracts stimulate plant parts on growth and the formation of roots and increase branches vegetative them. The licorice plant (*Glycyrrhiza glabra*) belong to *Leguminosae* family where characterized by its roots sweetness extracts which is estimated at 50 times the sweetness of sugarcane (Chorpa, and Choi, 1956), and containing plants sweat Iraqi licorice on glycyrrhizic acid increased by 4.56% and sugar stenographer 3.13% and sugar is stenographer 3.53% and starch 12.87%, as well as the number of large metal salts of the major and minor, which play a big role in the doubling of vegetative branches and increasing number of shoot (Al-Drisi *et al.*, 2022). Many researchers have been working in the field of tissue culture and to conduct studies and research on how to add natural plant extracts to MS media used in tissue culture for the purpose of improving the nutrient media and increase efficiency. One of plant tissue culture's significant and significant contributions to commercial plant propagation is micropropagation. Therefore, the aim of the current study was to determine how in vitro shoot development and chrysanthemum multiplication throughout the cultural stage were affected by plant growth regulators, sucrose, licorice extract, and pH. Finding the best culture conditions for producing stable, many explants shoots is the goal of this study.

MATERIALS AND METHODS

Chrysanthemum plants have been grown in the Department of Horticulture garden at the University of Duhok's College of Agriculture Engineering Sciences. During the multiplication stage, explants are used to produce stock plant cultures. The collected

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materials were transported to the lab, where the shoots were defoliated and thoroughly washed for 30 minutes under running water to remove soil and other surface contaminants, washed for another 20 minutes under running water with liquid soap, and rinsed for three to five minutes under sterile distilled water. The lengthy nodal segments (1 cm) produced a single node. Surface sterilization with 1.0% mercuric chloride ($HgCl_2$) was used to sterilize the explants for five minutes. Healthy cultures were achieved and maintained for the following micropropagation stages. Explants (nodal segment) were placed into MS media that had been enhanced with 1 $mg.l^{-1}$ GA_3 at the multiplication stage and evaluated with various concentrations of BA and kinetin (0.0, 1.0, 2.0, 3.0, and 4.0 $mg.l^{-1}$, respectively). The following studies used the multiplication stage's best outcome (concentration):

-To determine the effects of the sugar, we used MS full strength with 0.7% agar at pH 5.7 and different amounts of sucrose (15, 30, 45, 60, and 75 $gm.l^{-1}$). The effects of the licorice extract were investigated using MS full strength with 0.7% agar at pH 5.7 at different concentrations (10, 20, 30, 40 and 50 $gm.l^{-1}$). For preparation root extract of licorice were take (500g) of licorice root plants. Cut roots into small pieces by a sharp knife and then milled quantitative Herbaúa device and turned into a fine powder, dissolved 100g of powder in a liter of distilled water and then leave in the laboratory to the next day, the solution was filtered by filter paper and save it in the refrigerator until use. Finally, multiple pH values were utilized to examine the effects of pH (3.7, 4.7, 5.7, 6.7, and 7.7) at 3% sucrose, 0.7% agar, and MS full strength. The treatment that proved to have been most effective was selected, and its shoots were grown into jars containing a rooting media composed of a medium with a 1/4 MS salt strength supplemented with different concentrations of IBA (0.0, 0.1, and 0.3 mg.l-1). This was carried out following the application of the multiplication experiments and the recording of all information concerning the amount of grown shoots, their length, the number of leaves, and the number of nodes per explants. The data on the rooting percentage (%), average number of roots, and average root length were gathered six weeks after the plants were moved to the rooting media. All cultures used in the research were kept in a culture room with white fluorescent lights that provided a 16-hour photoperiod and an 8-hour darkness period, which was kept at 25°C and 60°RH. The photoperiod was maintained by a timer system that worked automatically. After six weeks, chrysanthemum plantlets that had established roots were rinsed in tap water to remove any potential contamination sources such as agar. It is imperative to avoid cutting the roots in any way during washing. The plantlets are then submerged for 10 minutes in a Benlate fungicide solution (0.1%) to prevent fungus infections before being planted in plastic pots. The plantlets are then grown in various culture medium mixtures in the plastic pots. (Soil 100%, Peat moss 100%, Perlite 100%, Peat moss + Perlite 1:1, soil + Perlite 2:1 and Soil + Peat moss + Perlite 1:1:1) v:v. To keep the humidity levels high in the cultural setting, the pots were covered with transparent, light-colored beakers. The potted plants were kept in the incubator chamber for four weeks while this parameter was tracked:

1- Survival rate as a percentage.
2. The number of shoots per plantlet
3- Mean shoot length (as a cm)
4- Stem diameter (mm)
5- Number of leaves

The plantlets were then treated to licorice extracts at various concentrations (0, 2.5 and 5 $g.l^{-1}$) and MS salt strength (¼ MS and ½ MS) in addition of control. The best result of culture media mixtures were used in these experiments. After 10 weeks of treatment this parameter were taken.

1. Number of shoot per plantlet
2. Stem diameter (mm)
3. Number of leaves
4. Mean length of shoot (cm)
5. Leaf area (cm2)
6. Pigments (i- chlorophyll)

a. Chlorophyll a (fresh weight, $mg.g^{-1}$).
b. Chlorophyll b (fresh weight, $mg.g^{-1}$).
c. Chlorophyll total (Chl. a + Chl. b)

ii- Total Carotenes (fresh weight, $mg.g^{-1}$)
iii- Total Anthocyanin (fresh weight $mg.g^{-1}$).

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The experiment was designed using a Completely Randomized Design (CRD), with 10 duplicates being assigned for each level of treatment. The Duncan's multiple range test (P 0.05) was applied to compare the means using a computerized SAS (2001) program.

RESULTS AND DISCUSSION

The maximum number of shoots per explant (8.0), leaves per explant (17.1), shoot length (7.5 cm), and nodes per explant (4.0) were observed in 1.0 mg.l^{-1} of BA (Table 1). This was followed by 2 and 3 mg.l^{-1} BA producing (7.1 and 6.2 shoots/ explant, 16.6 and 15 leaves/ explant, 5.43 and 4.87 cm long shoots, and 3.5 and 2.6 nodes/ explant, respectively). Control had an insignificant effect on all factors that were recorded (Figure 1).

Table 1. Influence of BA on multiplication stage of *Chrysanthemum morifolium* grown on MS medium enriched with 1.0 mg.l^{-1} GA_3 after six weeks in culture.

BA concentrations mg.l^{-1}	No. of shoots/ Explant	No. of leaves/explant	Average length of shoots (cm)	No. of node/explant
0	1.7 e	6.2 d	2.39 d	1.4 c
1	8.7 a	17.1 a	7.5 a	4 a
2	7.1 b	16.6 a	5.43 b	3.5 a
3	6.2 c	15 b	4.87 b	2.6 b
4	5.3 d	11.3 c	4.08 c	2.5 b

***Means within a column followed by different letters are significantly different according to Duncan's Multiple Range Test (p<0.05).**

Figure 1. Shoots multiplication from single node on MS salt strength medium containing (1mg.l^{-1}) of BA

According to Mohammed *et al*. (2010), this difference in BA level may be due to the amount of endogenous hormones present in the plants under study as well as their genetic make-up. The findings are similar to those obtained by Karim *et al.* (2002) who reported that 1.0 mg.l^{-1} BA was the ideal BA concentration based on the greatest shoot initiation it produced in chrysanthemum when using shoot tips as explant. Similar results were also noted by Ali *et al*. (2009)**,** who also observed that BA used alone at a dosage of 1.0 mg.l^{-1} produced the greatest number of shoots overall in the carnation culture. The rate of shot multiplication was slowed down by a higher BA concentration. According to the results above, 1.0 mg.l^{-1} BA is the perfect amount for this particular hormone to function at its best. And Yaseen (2022) also noted that BA used at 2 mg.l^{-1} in multiplication of *Viburnum opulus* obtained the high shoots per explant (3.33 shoots/explant).

In Table 2 the nodal segment of the chrysanthemum cultivated on MS media with a high concentration of Kinetin 4mg.l^{-1} had the most shoots per explant (3.3), leaves per explant (10.1), average shoot length (7.0 cm), and nodes per explant (3.05), as shown by Table (2)'s findings (Figure 2)

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Table 2. Influence of kinetin on multiplication stage of *Chrysanthemum morifolium* grown on MS medium enriched with 1.0 mg.l^{-1} GA$_3$ after six weeks in culture.

Kinetin concentrations mg.l^{-1}	No. of shoots/ Explant	No. of leaves/explant	Average length of shoots (cm)	No. of node/explant
0	1.2 d	4.8 c	3.0 c	1.45 b
1	2.0 c	6.3 b	5.5 b	2.71 a
2	2.4 bc	7.5 b	6.1 ab	2.82 a
3	2.7 b	9.1 a	6.7 a	2.93 a
4	3.3 a	10.1 a	7.0 a	3.05 a

***Means within a column followed by different letters are significantly different according to Duncan's Multiple Range Test (p<0.05).**

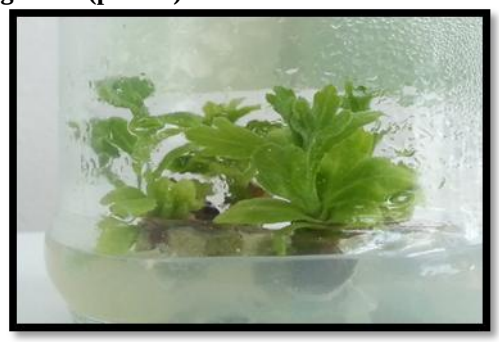

Figure 2. Shoots multiplication from single node on MS salt strength medium containing (4 mg.l^{-1}) of kinetin

On chrysanthemum (Karim *et al.*, 2002 and Long *et al*., 2006) and cotton (Meloni *et al*., 2001), kinetin has been applied to promote shoot proliferation. While compared to the regeneration of shoots from chrysanthemum explants, BA surpassed kinetin, as reported by Karim *et al*. (2003). According to Mok and Mok (2001), cytokinins which are known for their significant roles as plant hormones in a wide range of activities associated with plant growth and development, such as cell division and shoot initiation, perform the functions of BA and kinetin. The superiority of BA for the induction of shoots may be explained by the plant tissues' ability to absorb it more quickly compared to other synthetic growth regulators or by BA's ability to stimulate the tissue's production of natural hormones like zeatin (Kajla *et al*., 2018 and Aziz and ALtaweel, 2019). Table (3) shows that varied sucrose concentrations were used in MS media to produce and regenerate a variety of shoots. The highest values for the number of shoots per explant, node per explant, leaves per explant, and mean length of shoots (6 shoots/explant, 3.6 nodes/explant, 14.2 leaves/explant, and 4.83 cm) were obtained in the medium supplemented with 30 g.l^{-1} sucrose (Figure 3). The medium containing 15 gm.l^{-1} sucrose, it should be pointed out, produced the lowest values, including (2.2 shoot/explant, 1.3 node/explant, 9.6 leave/explant, and 2.25 cm).

Table 3. Influence of Sucrose on multiplication stage of *Chrysanthemum morifolium* grown on MS medium supplemented with 1 mg.l^{-1} BA and 1.0 mg.l^{-1} GA$_3$ after six weeks in culture.

Sucrose concentrations g.l^{-1}	No. of shoots/ Explant	No. of node/explant	No. of leaves/explant	Average length of shoots (cm)
15	2.2 c	1.3 d	9.6 d	2.25 c
30	6 a	3.6 a	14.2 a	4.83 a
45	5.5 a	3 b	12.3 b	4.45 a
60	4.2 b	2.4 c	11.2 bc	3.68 b
75	3.5 b	2 c	10.1 cd	3.25 b

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Figure 3. On MS salt strength medium with 30 g.l^{-1} Sucrose, shoots multiply from a single node

In tissue cultures of numerous plant species, including *Paederia foetida*, Amin *et al.* (2003) discovered that 30 g.l^{-1} sucrose is a desirable sucrose level for application as an efficient carbon source. which increased shoot growth and development. It has been reported that adventitious shoot development is less effective at lower sucrose concentrations. On the other hand, high sucrose content has a negative impact on shoot formation since it may prevent further shoot development due to the medium's osmotic level. Therefore, it appears that high sucrose concentrations inhibit the growth and development of shoots. Another reports (Ahmad *et al*., 2007 and Misal and Chavan, 2025) supports up these observations. Additionally, an increase in sugar content causes one or more of these processes to break down or behave abnormally, which results in an unorganized digestion of carbohydrates in the tissue culture. At higher sucrose concentrations (60 and 90 g.l^{-1}), the decrease in shoot length and aberrant development, which result in the cultured plantlets losing water as a result, may be caused by the nutritional medium's osmotic variations, which can also promote plant dehydration. Karim *et al*. (2003) obtained similar results. The results indicated in Table (4) that there is considerable important of adding different concentration of licorice solution which led to increase all characterized of chrysanthemum when increased solution concentration. The high concentration (50 ml.l^{-1}) gave the higher rate of all traits recorded, (8.5) shoots per explant, (3.5) nodes per explant, (13.5) leaves per explant and an average shoot length of (4.82) cm (Figure 4). The result was low shoots (3.6, 1.5, 9.2, and 2.61 cm), limited nodes per explant, little foliage per explant, and average shoot length on MS medium containing 10 ml.l^{-1} licorice extract, respectively.

Table 4. Influence of Licorice extract on multiplication stage of *Chrysanthemum morifolium* grown on MS medium enriched with 1 mg.l^{-1} BA and 1.0 mg.l^{-1} GA$_3$ after six weeks in culture.

Licorice extract concentrations ml.l^{-1}	No. of shoots/ Explant	No. of node/explant	No. of leaves/explants	Average length of shoots (cm)
10	3.6 c	1.5 c	9.2 d	2.61 d
20	5.9 b	2.2 b	9.5 dc	2.38 d
30	6.7 b	2.6 b	11.2 bc	3.18 c
40	6.9 b	3.4 a	12.9 ab	3.88 b
50	8.5 a	3.5 a	13.5 a	4.82 a

***Means within a column followed by different letters are significantly different according to Duncan's Multiple Range Test ($p<0.05$).**

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Figure 4. Shoots multiplication from single node on MS salt strength medium containing 50 g.l^{-1} licorice extract

According to Hasan *et al*. (2021), the explanation is due to the solution licorice's important function in containing elements such potassium salts, calcium, phenol compounds, proteins and amino acids, lactin, and sugars. Al-Mamori (2009) achieved a good doubling of shoot numbers, an appropriate percentage of rooting, and a good number of roots of two types of shrub roses planted on the MS medium containing 20 ml.l^{-1} of root licorice extract plants when compared to the other concentration. It confirmed (George *et al*., 2008) that the extract of licorice similar behavior to the behavior of GA_3 when sprayed on plants in the fields, as well as contain the extract energy and nutrients that promote the growth and development of the cultivated variety' shoots. The media adjusted to pH 5.7 gave the maximum numbers of shoots per explant (6), nodes per explant (3.3), leaves per explant (14), and mean length of shoots (5.77 cm), as indicated in **Table (5).** In media with a pH adjustment of 3.7, the rate of explant formation proliferation was the lowest (**Figure 5**).

Table 5. Influence of pH on multiplication stage of *Chrysanthemum morifolium* grown on MS medium supplemented with 1 mg.l^{-1} BA and 1.0 mg.l^{-1} GA_3 after six weeks in culture.

pH levels of the medium	No. of shoots/ Explants	No. of node/explant	No. of leaves/explants	Average length of shoots (cm)
3.7	2.4 c	1.2 c	8.3 c	2.41 d
4.7	4.3 b	2.3 b	12.8 a	3.13 cd
5.7	6 a	3.3 a	14 a	5.77 a
6.7	4.9 b	2.3 b	11 b	4.23 b
7.7	2.8 c	2 b	9.7 bc	4.05 bc

***Means within a column followed by different letters are significantly different according to Duncan's Multiple Range Test ($p<0.05$).**

Figure 5. Shoots multiplication from single node on MS salt strength medium at pH level 5.7

Our research revealed that the combination responded best at a pH of 5.7 throughout each stage (**14**), while pH values that were either too low or too high led to substantial abnormalities, including shorter shoots with thin, curled, and sharply pointed leaves. The pH of the culture media has significant effects on shoot growth in vitro. The ionization of acidic and basic groups results in significant structural changes that have an influence on their biological function in the absence of pH regulation (Sakano, 1990). Every species needs a pH level that can maximize shoot

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development. Rather of cytokinin, auxin, and gibberellins, different substances are required for *in vitro* multiple shoot formation (Bhatia and Ashwath., 2005).Based on **Table (6)**, IBA at 0.1 mg.l^{-1} of a low concentration produced the highest response percentage (100%), mean root length (15 cm), and number of roots per explant (9).

Table 6. Effect of IBA on root formation stage of *Chrysanthemum morifolium* grown after 6 weeks in culture on MS salt strength medium.

MS Salt Strength	IBA (mg.l^{-1})	Rooting percentage (%)	Mean length of roots (cm)	Number of roots/ explant
¼(Quarter Strength)	0.0	95 b	9c	5 h
	0.1	100a	15 a	9a
	0.3	100a	12 b	7b

***Means within a column followed by different letters are significantly different according to Duncan's Multiple Range Test (p<0.05).**

These results are in accordance with that Danial *et al.* (2010) and Soliman *et al.* (2010) and have shown about the efficiency of IBA and NAA in improving fig root formation.

The results in Table (7) demonstrated that after 4 weeks of culture, Chrysanthemum plantlets grown from single node *in vitro* cultures in a variety of culture media mixtures (soil + peat moss + perlite 1:1, soil, peat moss and perlite are combined in a 1:1:1 ratio) did not exhibit any acclimatization response. The maximum number of shoots (6) and mean length of shoots (40 cm), as well as the highest stem diameter (0.35 mm) and high number of leaves (35) were obtained on culture media supplemented with soil + peat moss (1:1). This table (7) further shows that the highest response percentage (100%) was obtained on these culture media.

Table 7. Acclimatization stage of (*Chrysanthemum morifolium*) plantlets produced from *in vitro* after 4 weeks.

Culture media mixture	Survival percentage (%)	Number of shoots/ plantlet	Mean length of shoots (cm)	Stem diameter (mm)	Number of leaves
Soil 100%	90 b	2 e	22 c	0.16c	15 c
Peat moss 100%	100 a	3 d	35 b	0.29b	28b
Perlite 100%	70 d	3 d	26 bc	0.27 b	27 b
Soil + Peatmoss1:1	100 a	6 a	40 a	0.35a	35a
Soil + Perlite 1:1	85 c	3 d	30 b	0.25 b	29 b
Peatmoss+ Perlite 2:1	80 c	4 c	29 bc	0.25 b	25 b
Soil + Peatmoss + Perlite 1:1:1	95 b	5 b	42 a	0.28 b	24 b

***Means within a column followed by different letters are significantly different according to Duncan's Multiple Range Test (p<0.05).**

This step determines whether *in vitro* research is successful or not, which is the most critical one in micropropagation. Chrysanthemum plantlets gradually acclimatize to their environment with 95% success following healthy growth and the absence of morphological defects. These vegetative micropropagation processes are consistent with the findings of numerous other studies on plants that were later transplanted to open fields (Banno *et al.*, 2007; Misal and Chavan, 2025; Hasan *et al.*, 2021 and Yildirim *et al.*, 2007). After 10 weeks in culture, the acclimatization responses of Chrysanthemum plantlets grown in soil and peat moss with varying concentrations of MS salt strength (0, 1/4 and 1/2 MS salt) and extracts of licorice (0, 2.5, and 5 g.l^{-1}) alone and in interaction with

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P-ISSN: 0075- 0530/ E-ISSN: 2410-0862
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each other are shown in **Tables (8 and 9)**. It can be noticed that the using ½ MS salt strength with 5 g.l^{-1} licorice extracts obtained high number of shoot (7) and leaves number (39), high Chl.b content (9.34 mg.g^{-1}).While the high mean length of shoot (44 cm), Total chlorophyll (65.83 mg.g^{-1}) and chlorophyll a content (57.40 mg.g^{-1}) and high contain of total Anthocyanin contain (0.38 mg.g^{-1}) were recorded at ½ MS salt strength with 2.5 g.l^{-1} licorice extract.

Table 8. Acclimatization stage of (*Chrysanthemum morifolium*) plantlets produced from *in vitro* grown in soil + peatmoss culture media with different concentrations of licorice extracts and MS salt strength after 10 weeks.

Treatments		Number of shoots/ plantlet	Mean length of shoots (cm)	Stem diameter (mm)	Number of leaves	Leaf area (cm^2)
MS salt strength	Licorice extracts (g.l^{-1})					
Control	0	3 e	25 bc	0.25 c	19 d	5.45 c
	2.5	3 e	35 ab	0.35 ab	26 c	6.24 b
	5	4 d	36 ab	0.32 b	28 bc	6.75 b
¼ MS	0	6 b	39 ab	0.38 ab	28bc	8.58 a
	2.5	5 c	32 b	0.33 b	30 bc	8.29 a
	5	4 d	30 b	0.42 ab	27 bc	8.84 a
½ MS	0	7 a	37 ab	0.39 ab	38 a	8.24 a
	2.5	5 c	44 a	0.34 ab	34 b	7.87 ab
	5	7 a	25 bc	0.26 c	39 a	8.34 a

***Means within a column followed by different letters are significantly different according to Duncan's Multiple Range Test (p<0.05).**

Table 9. Acclimatization response of (*Chrysanthemum morifolium*) plantlets produced from *in vitro* grown in soil + peatmoss culture media with different concentrations of licorice extracts and MS salt strength after 10 weeks.

Treatments		Chl.a (mg.g^{-1} fresh weight)	Chl.b (mg.g^{-1} fresh weight)	Total chlorophyll	Total Carotenes (mg.g^{-1} fresh weight)	Total Anthocyanin (mg.g^{-1} fresh weight)
MS salt strength	Licorice extracts (g.l^{-1})					
Control	0	18.23 d	3.64 e	22.87 d	3.67 ab	0.22 ab
	2.5	45.43 b	8.43 b	53.86 bc	3.91 ab	0.25 ab
	5	38.12 bc	5.93 cd	44.05 bc	3.37 ab	0.21 ab
¼ MS	0	42.40 b	5.62 cd	48.02 bc	4.33 ab	0.27 ab
	2.5	54.43 a	5.72 cd	56.95 bc	3.89 ab	0.24 ab
	5	42.81 b	5.32cd	48.13 bc	3.92 ab	0.35 a
½ MS	0	48.33 b	6.43 cd	54.76 bc	3.22 ab	0.26 ab
	2.5	57.40 a	8.56 b	65.83 a	4.32 ab	0.38 a
	5	34.23 c	9.34 a	43.57 c	3.42 ab	0.22 ab

***Means within a column followed by different letters are significantly different according to Duncan's Multiple Range Test (p<0.05).**

CONCLUSION

Based on the results in this study it was found that the MS medium supplemented with BA at concentration (1.0 mg.l^{-1}) significant increase in mostly studied characters. MS medium containing 30 g.l^{-1} sucrose, showed the best results when compared to other concentrations in most parameters recorded. Results about the effect of licorices extract gave the highest response percentage at MS medium congaing

50 ml.l^{-1} in all parameters studied. Concerning the root formation stage, the concentration (0.1 mg.l^{-1}) IBA with 1/4 MS salt strength gave the highest value for all parameters. Finely the acclimatization stage showed the culture media supplemented with soil + Peatmoss (1:1) gave the highest response percentage 100%. The best treatment was recorded at 1/2 MS salt strength with (2.5 mg.l^{-1}) licorices extract for most characterize.

ACKNOWLEDGEMENT

The quality of this work has been enhanced by the authors from the Department of Horticulture at College of Agricultural Engineering Sciences / Duhok University.

CONFLICT OF INTEREST

The authors state that there are no conflicts of interest with the publication of this work.

DECLARATION OF FUND

The authors declare that they have not received a fund.

AUTHOR/S DECLARATION

We confirm that all Figures and Tables in the manuscript are original to us. Additionally, any Figures and images that do not belong to us have been incorporated with the required permissions for re-publication, which are included with the manuscript.

Author/s signature on Ethical Approval Statement.

Ethical Clearance and Animal welfare

Funds:

AUTHOR'S CONTRIBUTION STATEMENT

REFERENCES

Ahmad, T., Abbasi, N. A., Hafiz, I. A., & Ali, A. (2007). Comparison of sucrose and sorbitol as main carbon energy sources in microprogation of peach rootstock GF-677. Pakistan Journal of Botany, 39(4), 1269-1275. https://www.researchgate.net/profile/Ansar-Ali-5/publication/228508728_Comparison_of_sucrose_and_sorbitol_as_main_carbon_energy_sources_in_micropropagation_of_peach_rootstock_GF-677/links/58c637c192851c0ccbfba007/Comparison-of-sucrose-and-sorbitol-as-main-carbon-energy-sources-in-micropropagation-of-peach-rootstock-GF-677.pdf.

Ali, A., Ahmad, T. N., Abbasi A. and Hafiz, I. A. (2009). Effect of different media and growth regulators on *in vitro* shoot proliferation of olive cultivar '*Moraiolo*'. Pak. J. Bot., 41(2): 783-795. Doi. http://www.pakbs.org.

Al-Mamori, K. H. A. (2009). Micropropagation of Rosu sp. In vivo. Master Thesis, Department of Life Technologies plant, Musayyib technical college.

Amin, M.N., Rahman, M.M. and Manik, M.S. (2003). *In vitro* clonal propagation of *Paederia foetida* L: A medicinal plant of Bangladesh, *Plant Tissue Cult.*, 13, 117-123. https://www.baptcb.org/view-details-article/241.

Arora, J. S. (1990). Introductory Ornamental Horticulture. Kalyani Publishers, New Delhi, p. 48. Flowers and Plants Association.

Aziz, R.A and Al-Taweel, S.K. (2019). Effect of plant growth retadants on stevia (*Stevia rebaudiana Bertoni*) acclimatization produced *in vitro*. Plant Archives, 19 (1): 1275-1284. https://www.cabidigitallibrary.org/doi/pdf/10.5555/20209904253.

Banno, K., Yoshida, K., Hayashi, S. and Tanabe, K. (2007). *In vitro* Propagation of Japanese Pear Cultivars. Japanese Society for Hort. Sci., vol. 58 (1): 37-42. https://www.semanticscholar.org/paper/In-Vitro-Propagation-of-Japanese-Pear-Cultivars-Banno-Yoshida/17bada7a66c35559220beaec55b120d2975feba9.

Bhatia, P. and Ashwath, N. (2005). Effect of medium pH on shoot regeneration from cotyledonary explants of tomato, *Biotechnol.*, 4, 7-10. DOI: 10.3923/biotech.2005.7.10.

Chorpa, R. N. and Choi, I. C. (1956). Medicals plants, 2nd ed. USDA. A forest service northern experiment. Georgia, USA. (11) 1-27.

Danial, G.H., Ibrahim, D. A., Barket, S.A. and Khalil, B.M. (2010). Multiple shoots production from shoot tips of fig tree (*ficus carica*) and callus induction from leaf segments. Int. J.Pure Apple. Sc. Techno., 20(1):117-124.Available online www.ijopaasat.in.doi. https://www.researchgate.net/profile/Diaa-Ibrahim-4/publication/362813925_Multiple_Shoots_Production_from_Shoot_Tips_of_Fig_Tree_Ficus_carica_L_and_Callus_Induction_from_Leaf

Iraqi Journal of Agricultural Sciences –2026:57(2):402-414
P-ISSN: 0075- 0530/ E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/fkvbje80

_Segments/links/6300b7dfe3c7de4c346ee341/Multiple-Shoots-Production-from-Shoot-Tips-of-Fig-Tree-Ficus-carica-L-and-Callus-Induction-from-Leaf-Segments.pdf?origin=publication_detail&_tp=eyJjb250ZXh0Ijp7ImZpcnN0UGFnZSI6InB1YmxpY2F0aW9uIiwicGFnZSI6InB1YmxpY2F0aW9uRG93bmxvYWQiLCJwcmV2aW91c1BhZ2UiOiJwdWJsaWNhdGlvbiJ9fQ&__cf_chl_tk=4Dk3hzGUZXuV0pVgtoaMAARk9KqG.BWv4RgIqhBUfow-1769869372-1.0.1.1-WAUrssCk21BMImV1xDZ2irUJE115M4_Ds2Lf5YvrEYwEbrahim, M. K.H. and Ibrahim, A. I. (2000). Influence of medium solidification and pH value on in vitro propagation of *Maranta leuconeura* cv. Kerchoviana. Scientia Horticulturae. Volume 86, Issue 3, 3 November, Pages 211-221. https://doi.org/10.1016/S0304-4238(00)00148-5.

George, E. F., Hall, M. A. and De Klerk, G. J. (2008). Plant Propagation by Tissue Culture. Volume 1. The Background, 3rd Edition, Published by Springer, Dordrecht, the Netherlands.

Haider, H. J. A. (2002).The use of some herbs extracts (weeds) to improve the storage ability and tissue culture of potato (*Solanum tuberosum* L.).PhD. thesis. College of Agriculture, University of Baghdad. Iraq.

Hasan, M.K., Ara, I., Mondal, M.S.A. and Kabir, Y. (2021).Phytochemistry, pharmacological activity,and potential health benefits of *Glycyrrhiza glabra*. *Heliyon 7*(6): 1-10. https://doi.org/10.1016/j.heliyon.2021.e07240.

Kajla S., Shashi, K., Anil, K. , Hidayatullah, M. and Manoj, K. S.(2018). Effect of Growth Regulators on *in vitro* Shoot Multiplication and Plant Regeneration of *Rosa hybriad* L. from Nodal Explants. International Journal of Current Microbiology and Applied Sciences ISSN: 2319-7706 Special Issue-7 pp. 3804-3811. https://www.researchgate.net/publication/328477494_Effect_of_Growth_Regulators_on_in_vitro_Shoot_Multiplication_and_Plant_Regeneration_of_Rosa_hybriad_L_from_Nodal_Explants/link/5e216c7f92851cafc38aa36d/download?_tp=eyJjb250ZXh0Ijp7ImZpcnN0UGFnZSI6InB1YmxpY2F0aW9uIiwicGFnZSI6InB1YmxpY2F0aW9uIn19.

Karim, M.Z., Amin, M.N., Asad, Z.U., Islam, S., Hassin, F. R and Alam, R. (2003). Rapid multiplication of *Chrysanthemum morifolium* through *in vitro* culture. Pak. J. Biol. Sci. 5(11): 1170-1172. DOI: 10.3923/pjbs.2002.1170.1172.

Karim, M.Z., Amin, M.N., Azad, M.A.K., Begum, F., Islam, M.M. and Alam, R. (2002). Effect of different Plant Growth regulators on *in vitro* Shoot Multiplication of *Chrysanthemum morifolium*. Online J. Biol. Sci. 3(6): 553-560. DOI: 10.3923/jbs.2003.553.560.

Long, V., Vinh, B., Don, N., Thuy, D. and Nhut, D. (2006). Microponic technology in disease free chrysanthemum production. J Appli Hort. 7. 67-73. DOI:10.37855/jah.2005.v07i02.18. https://www.horticultureresearch.net/jah/2005_7_2_67_71.PDF.

Meloni, A., Oiva, M.A., Ruiz, H.A. and Martinez, C.A. (2001). Contribution of praline and inorganic solutes to osmotic adjustment in cotton under salt stress. J Plant Nutri 24: 599-612. https://doi.org/10.1081/PLN-100104983.

Misal, V.D. and Chavan, A.M.(2025). Effect of Sucrose Concentration on In Vitro Shoot Regeneration in Stevia rebaudiana Bertoni. International Journal of Science and Research (IJSR). Volume 14 Issue 2, February. DOI: https://dx.doi.org/10.21275/SR25222145814.

Mohamad, S. A., Al-Fauri, A., Megdadib, H., Kair, M., Rida, A., Abu-Rommana, S. S. and Al-Ghzawi, A. (2010). *In Vitro* Multiplication of *Chrysanthemum morifolium* Ramat and it is Responses to NaCl Induced Salinity. Jordan Journal of Biological Sciences. *Volume 3, Number 3, June, doi. ISSN 1995-6673 Pages 101 – 110.* https://jjbs.hu.edu.jo/files/v3n3/In%20Vitro%20Multiplication%20of%20Chrysanthemum.pdf.

Dok, D.W. and Mok. M.C. (2001). Cytokinin metabolism and action. Annual Review in Plant physiology. Plant Mol. Biol. 52: 89-118.DOI: 10.1146/annurev.arplant.52.1.89.

Nhut, D.T., Don, N.T., An, T.T.T., Van T.P.T, Vu, N.H., Huyen, P.X. and Khiem, D.V. (2005). Microponic and hydroponic techniques

Iraqi Journal of Agricultural Sciences –2026:57(2):402-414
P-ISSN: 0075- 0530/ E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/fkvbje80

in disease free chrysanthemum (*Chrysanthemum* sp.) production. J Applied Hort 7: 67-71. https://www.horticultureresearch.net/jah/2005_7_2_67_71.PDF.

Nowak, B., Miczynski, K. and Hudy, L. (2004).Sugar uptake and utilization during adventitious bud differentiation on *in vitro* leaf explant of Wegierka Zwykla plum (*Prunus domestica*), *Plant Cell, Tissue Organ Cult.*, 76, 255-260. DOI:10.1023/B:TICU.0000009247.94819.02.

Razdan**,** M.K., (1993). Introduction and Techniques: An Introduction to Plant Tissue Culture, Oxford and IBH Publishing, New Delhi, Bambey, Calcutta. https://books.google.co.in/books?id=oe_liIY_tVsC&printsec=frontcover#v=onepage&q&f=false.

Sakano, K., (1990). Proton/phosphate stoichiometry in uptake of inorganic phosphate by cultured cells of *Catharanthus roseus* (L.) G. Don, *Plant Physiol.*, 93, 479-483. https://www.jstor.org/stable/4276536.

SAS, (2001). SAS/ STAT User's Guide for Personal Computers, Release 6.12, SAS Institute Inc.

Sauvaire, D. and Galgy, R. (1978). Multiplication of vegetative dela Cannal a sourse par bounturage *in vitro.* CRA Cad D Sci. Se., 287: 446-470.

Sedlak**,** J. and Paprstein, F. (2003). Influence of growth regulators on *in vitro* propagation of *Pyrus communis* cv. Koporecka. Acta Hort., (616): 379-382. https://www.ishs.org/ishs-article/616_57.

Smith, R.H.(2013).Plant Tissue Culture . Techniques and Experiments. 2nd Edition .Academic Press .San Diego, Newyork. PP (53-54). https://scholar.google.com/scholar_lookup?&title=Plant%20Tissue%20Culture%3A%20Techniques%20and%20Experiments&publication_year=2000&author=Smith%2CR.H.

Soliman, F, Glatt, C.E., Bath, K.G., Levita, L., Jones, R.M., Pattwell, S.S, Jing, D., Tottenham, N. and Amso, D. (2010): A genetic variant BDNF polymorphism alters extinction learning in both mouse and human. *Science* 327: 863–866.

Taha**,** F. H. (2002) .Some factors affecting the growth and multiplication of rootstock lemon Citrus *Jambhiri Lush* and Citrus *volkameriana pasq in vivo*. Master Thesis. College of Agriculture, University of Baghdad, Iraq.

Verma, O.P., Abha-Singh, A. Verma, S.K., Meetu, C. and Shukla, A. K. (2009). Standarization of growth regulators for rapid shoot proliferation in *Chrysanthemum morifolium*. *Asian Journal of Bio-Science*, 4(2): 337-339. doi. https://www.researchgate.net/publication/319643852_Standarization_of_growth_regulators_for_rapid_shoot_proliferation_in_chrysanthemum_morifolium.

Yaseen, S. A. (2022). Micorpropagation of *Viburnum opulus* (roseum) by using Single nodes. Iraqi Journal of Agricultural Sciences 53(6):1388- 1396.

Yildirim, H., Tilkat, E., Onay, A. and Ozen, H. Ç. (2007). *In vitro* Embryo Culture of Apricot (*Pruns armeniaca* L.) cv. Hacıhaliloğlu. International J. Sci. & Techn. 2(2): 99-104. https://www.researchgate.net/publication/237240997_In_Vitro_Embryo_Culture_of_Apricot_Prunus_armeniaca_L_cv_Hacohalililolu.

Iraqi Journal of Agricultural Sciences –2026:57(2):402-414
P-ISSN: 0075- 0530/ E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/fkvbje80

العوامل المؤثرة في التكاثر الدقيق لنبات الأقحوان (*Chrysanthemum morifolium*)

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المستخلص

تم استخدام تركيزات مختلفة من منظمات نمو النبات المضافة إلى وسائط MS (Murashige & Skoog) لمضاعفة براعم الأقحوان. الرقم الهيدروجيني، السكر ومستخلص عرق السوس. قبل استخدامها كمادة معتمدة، باستخدام النبات الذي تم الحصول عليه خارج المختبر، تم تعقيم القطعة العقدية باستخدام كلوريد الزئبق ($HgCl_2$) بنسبة 0.1% لمدة خمس دقائق.أظهر الوسط MS المضاف إليه BA بتركيز أقل (1.0 ملغم/لتر) أفضل النتائج بالمقارنة مع التراكيز الأخرى المدروسة (8.7 فرع/نبات، 7.5 سم، 17.1 ورقة/نبات، و4.0 عقد/نبات).كان متوسط طول الأفرع، والعقد لكل نبات، والأوراق لكل نبات، وعدد البراعم لكل نبات، كلها أكثر في الوسط المحتوي على 30 غم/لتر سكروز، بقيم 6.0 براعم لكل نبات، 4.83 سم، 14.2 ورقة لكل نبات، و3.6 العقد لكل نبات، على التوالي.أظهرت النتائج أن تأثير مستخلص عرق السوس أعطى أعلى نسبة استجابة عند التركيز 50 مل.لتر (حيث أعطى 13.5 فرع/ نبات، 8.5 عقدة/ نبات، 3.5 ورقة/ نبات و 4.82 سم) على التوالي .لقد تبين أن الوسائط ذات ضبط الرقم الهيدروجيني 5.7 أنتجت الحد الأقصى للعقد لكل فرع، والأوراق لكل فرع، ومتوسط طول الفرع، وعدد البراعم لكل نبات (3.3 عقد لكل فرع، 14 ورقة لكل فرع، 5.77 سم و6 براعم لكل فرع). الزرع، على التوالي وفيما يتعلق بمرحلة تكوين الجذر فإن التركيز 0.1 ملغم/لترمن IBAمع MS ربع قوة الأملاح أعطى أعلى قيمة لجميع الصفات المدروسة. بينما أظهرت مرحلة التأقلم أن الوسط الزراعي المضاف للتربة + البيتموس (1:1) أعطى أعلى نسبة استجابة 100%. وأفضل معاملة سجلت عند التركيزMS نصف قوة الأملاح مع مستخلص عرق السوس (2.5 ملغم/لتر) لأغلب الصفات.

الكلمات المفتاحية: الكينتين، السكروز، بنزيل أدنين، درجة الحموضة، زراعة الأنسجة، مستخلص عرق السوس ونبات الأقحوان .