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In vitro rejeneration potential of *Matricaria recutita* L. capitulum explant

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Abstract

Purpose: In this study, the *in vitro* regeneration potential of *Matricaria recutita* L., a medicinal and aromatic plant, was investigated
Method: Six different sterilization processes were tested to determine the sterilization protocol of capitulum explants isolated from plants grown under field conditions. For regeneration studies, plant growth regulators BAP (0.5-1-2 mg/L) and NAA (0-0.5-1-2 mg/L); KIN (0.5-1-2 mg/L) and 2.4-D (0-0.5 mg/L) were applied to capitulum explants at 24 different concentrations and combinations.
Findings: In the experiments carried out for sterilization purposes, the best results were obtained in 70% ethanol for 3 minutes, in 30% NaOCl for 10 minutes and in 1% $AgNO_3$ for 20 minutes. The best shoot lengths were determined as 3.02 and 3.00 cm in MS nutrient medium containing 0.5 mg/L BAP+0.5 mg/L NAA and 0.5 mg/L BAP+2 mg/L NAA, respectively; the best shoot number per explant was determined as 23.91 and 18.29 in MS nutrient medium containing 0.5 mg/L BAP+0.5 mg/L NAA and 0.5 mg/L BAP+2 mg/L NAA plant growth regulators, respectively.
Conclusion: According to the results obtained from *in vitro* regeneration studies, in terms of the applied plant growth regulator combinations, BAP and NAA gave more successful results than KIN and 2.4-D applications

Keywords: Auxin, cytokinin, *Matricaria recutita* L., plant growth regultor, tissue culture

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Matricaria recutita L. capitulum eksplantının *in vitro* rejenerasyon potansiyeli

Özet

Amaç: Bu çalışmada, tıbbi ve aromatik bir bitki olan *Matricaria recutita* L.' nin *in vitro* rejenerasyon potansiyeli araştırılmıştır.
Metod: Tarla koşullarında yetiştirilen bitkilerden izole edilen kapitulum eksplantlarının sterilizasyon protokolünü belirlemek amacıyla altı farklı sterilizasyon işlemi test edildi. Rejenerasyon çalışmaları için kapitulum eksplantlarına, 24 farklı konsantrasyon ve kombinasyonda BAP (0,5 -1-2 mg/L) ve NAA (0-0,5-1-2 mg/L); KIN (0,5-1-2 mg/L) ve 2,4-D (0-0,5 mg/L) bitki büyüme düzenleyicileri uygulanmıştır.
Bulgular: Sterilizasyon amaçlı yapılan deneylerde en iyi sonuçlar, %70'lik etanolde 3 dakika, %30'luk NaOCl'de 10 dakika ve %1'lik $AgNO_3$'te 20 ile elde edilmiştir. Rejenerasyon çalışmaları sonucunda; en iyi sürgün uzunlukları sırasıyla 0,5 mg/L BAP+0,5 mg/L NAA ve 0,5 mg/L BAP+2 mg/L NAA içeren MS besin ortamlarında 3,02 ve 3,00 cm olarak belirlenirken; en iyi eksplant başına sürgün sürgün sayısı sırasıyla 0,5 mg/L BAP+0,5 mg/L NAA ve 0,5 mg/L BAP+2 mg/L NAA bitki büyüme düzenleyicileri içeren MS besin ortamlarında 23,91 ve 18,29 olarak belirlenmiştir.
Sonuç*: In vitro* rejenerasyon çalışmalarından elde edilen sonuçlara göre uygulanan bitki büyüme düzenleyici kombinasyonları açısından, BAP ve NAA; KIN ve 2,4-D uygulamalarına göre daha başarılı sonuçlar vermiştir.

Anahtar kelimeler: Bitki büyüme düzenleyicisi, doku kültürü, oksin, *Matricaria recutita* L.**,** sitokinin

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1. Introduction

Chamomile (*Matricaria recutita* L.) belongs to the Asteraceae family and is of great importance as a raw material in the pharmaceutical, cosmetic, and food industries. It is native to eastern and southern Europe and western Asia and is found throughout much of Europe, including Türkiye, Iran, and Afghanistan. It can grow at elevations ranging from fields to mountain valleys (up to 1200 m). It is found in sun-loving or moderately shade-loving conditions. The species is generally found in very dry, cool soils with alkaline or slightly acidic pH. It can also grow in nutrient-poor soils [1].

Approximately 120 secondary metabolites, including 28 terpenoids and 36 flavonoids, have been identified in chamomile essential oil and its extract. The most important terpenoid compounds in chamomile essential oil are bisabolol and its oxides A and B, bisabolone oxide A, camulene, and farnesene. Phenolic compounds, including phenolic acids, flavonoids, and coumarins, predominate in chamomile extracts. Among the flavonoids, apigenin is the most promising compound [2]. The extract and essential oil of the flowers have been traditionally used medicinally to treat wounds, ulcers, eczema, gout, skin irritations, bruises, burns, sores, neuralgia, sciatica, rheumatic pain, hemorrhoids, hay fever, inflammation, muscle spasms, menstrual irregularities, insomnia, gastrointestinal disorders, and rheumatic pain [3, 4]. In the book El-Kanun fi't Tıb, written by Ibn Sina, it is reported that the plant was used for the treatment of colds, sores, and the common cold [5]. In current studies, its antibacterial, antioxidant, anti-inflammatory, antidepressive, anticancer, hepatoprotective, angiogenesis effects, and effects in the premenstrual period and antigastrointestinal disorders have been reported [6].

In addition to its pharmaceutical use, chamomile is reportedly added to various cosmetic formulations, including creams, soaps, detergents, perfumes, skin lotions, ointments, toiletries, hair products, and shampoos. Dried chamomile flowers are also in high demand for use in herbal teas, baby massage oil, to support gastric secretion, and to treat coughs and colds [7].

More than 7000-8000 tons of chamomile are produced annually in Europe, with *M. recutita* accounting for a large portion of this amount [8]. Different cultivation methods are needed to increase product yield to meet high consumption and increasing market demand [9]. Therefore, in recent years, there has been a need for research on plant, cell, and tissue culture methods, which represent alternative methods to traditional agriculture in the production of plants and plant metabolites. Plant, cell, and tissue culture, a modern biotechnology technique and soilless agriculture production model, has become one of the indispensable techniques that humanity will benefit from in many ways today. It is considered an effective method for clonal propagation and plant metabolite production. These techniques allow rapid and high-quality plant and plant secondary metabolite products to be obtained without being affected by seasonal or environmental conditions [10].

Plant growth regulators added to the nutrient medium play a role in plant tissue culture applications. The most commonly used are auxins and cytokinins, which play important roles in organ development and differentiation in plant tissues. The amount and type of plant growth regulator added to the medium varies depending on the plant species, the explant, and the purpose of the culture. Among the auxins, naphthalene acetic acid (NAA), indole acetic acid (IAA), and 2.4-Dichlorophenoxyacetic acid (2.4-D) are preferred. Among the cytokinins, benzyl adenine (BA) and kinetin (KIN) are preferred [11]. Auxins are a general class of plant growth hormones that promote cell elongation. Root meristems and apical shoots are considered the sites where auxins are produced. Phototropism develops as a result of unequal auxin distribution. In nature, they have physiological properties such as adventitious and lateral root formation, photoperiodism, apical dominance, stem and coleoptile development, delaying the onset of leaf abscission, and promoting fruit development. They increase plant cell wall flexibility, promote transverse growth in tubers, and promote cell and tissue differentiation. They also promote transverse growth in dicotyledons. Cytokinins have physiological effects that include cell division, redifferentiation, and components of the cell cycle. In plants, cytokinins regulate the growth of stems and lateral shoots, increase cell expansion in cotyledons and leaves, enhance chloroplast development, delay leaf yellowing, and stimulate the transport of mineral nutrients. They are also used in cut flower cultivation to reduce postharvest spoilage and promote seed germination [12].

Plant cells possess the capacity for rapid regeneration. *In vitro* plant regeneration is the process by which explants form organs and tissues during the growth phase following cell division and differentiation. *In vitro* plant regeneration can be achieved through somatic embryogenesis or organogenesis. Organogenesis is the process by which new organs, or even entire plants, form, often in response to injuries originating from other parts of the body. In somatic embryogenesis, a structurally similar cell to a zygotic embryo is formed first, followed by whole plant regeneration. This regeneration can occur directly or indirectly through callus tissue. However, plant regeneration capacity varies significantly among different genotypes. Therefore, further research is needed on this topic [13]. As a result of regeneration studies, the conditions required for *in vitro* plant callus, shoot, and root formation are determined, and this information will guide studies such as plant gene transfer, secondary metabolite production, and micropropagation.

This study investigated the response of capitulum explants of *Matricaria recutita* L., a plant with important metabolite content and significant applications in folk medicine, pharmaceutical, food, and cosmetic industries, to plant growth regulators such as BAP and NAA; KIN and 2.4-D.

2. Materials and methods

Matricaria recutita L. seeds, constituting the study material were supplied by Pharmasaat Company. They were grown in the medicinal and aromatic plants field of the Balıkesir Büyükşehir Belediyesi Çiftçi Eğitim Şube Müdürlüğü (Balıkesir Metropolitan Municipality Farmer Education Branch Directorate). *M. recutita* L. capitulum explants were used for plant tissue culture studies.

2.1. Sterilization studies

As a result of the preliminary applications, high contamination problems were encountered in the *in vitro* culture processes of capitulum explants. In order to solve this problem, 6 different surface sterilization processes were applied to the explants (Table 1). After the surface sterilization processes, the explants were incubated for 1 week in Murashige and Skoog (MS) [14] nutrient media containing 400 mg/L Amoxynil LA (Amoxynil LA; 150 mg amoxynil equivalent in 1 mL) in order to prevent problems caused by internal contamination.

Table 1. Sterilization procedures applied to *M. recutita* capitulum explants

Treatment No	Running water	Pre-application	70% Ethanol	NaOCl	1% $AgNO_3$	10% H_2O_2	25% Fungicide (0.25 gr/ml)
1.Treatment	10 min	18% sucrose, 1% NaOCl +4° C in the refrigerator for 72 h	3 min	10% 5 min	-	-	-
2.Treatment	10 min		3 min	10% 5 min	-	-	-
3.Treatment	10 min		3 min	10% 3 min		5 min	
4.Treatment	10 min		3 min	30% 10 min	10 min	-	for 2 min,
5.Treatment	10 min		3 min	30% 10 min	20 min	-	for 2 min
6.Treatment	20 min		3 min	30% 10 min	20 min	-	-

2.2. Effect of plant growth regulators on *M. recutita* capitulum explants

The explants obtained as a result of sterilization processes were transferred to MS nutrient media containing BAP and NAA plant growth regulators in 24 different concentrations and combinations (Table 2).

Table 2. Plant growth regulators applied to *M. recutita* capitulum explants

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
BAP (mg/L)	0.5	0.5	0.5	0.5	1	1	1	1	2	2	2	2
NAA (mg/L)	0	0.5	1	2	0	0.5	1	2	0	0.5	1	2

	13	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
KIN (mg/L)	0.5	0.5	0.5	0.5	1	1	1	1	2	2	2	2
2.4-D (mg/L)	0	0.5	1	2	0	0.5	1	2	0	0.5	1	2

2.3. Culture studies

Chemicals used in the studies were supplied by Sigma and Merck. A biosafety cabinet, autoclave, heated magnetic stirrer, distilled water device, precision balance, pH meter, digital camera, refrigerator, and micropipettors were used in plant tissue culture studies. The Murashige and Skoog (MS) Media was used as the plant nutrient medium.

To prepare MS nutrient media (for 1 L), 30 g sucrose and 0.1 g myo-inositol were added to the MS nutrient medium. The solution pH was adjusted to 5.7-5.8 with buffers (0.1 N NaOH and 0.1 N HCl). 7 g agar was added to the medium to make it semisolid. The prepared nutrient medium was divided into 175 mL jars, each containing 30 mL. The nutrient medium was sterilized by autoclaving at 121 °C and 1.06 kg/cm2 pressure for 15 minutes. It was then cooled to room temperature and stored in the dark.

To obtain sterile explants, antibiotics (Amoxynil LA; 1 mL equivalent to 150 mg amoxynil) were added to MS nutrient media after filter sterilization at 400 mg/L in a biosafety cabinet. For *in vitro* regeneration studies, the plant growth regulators listed in Table 2 were added to MS nutrient media.

Plant growth regulator stocks were prepared at a concentration of 1 mg /5 mL. For this purpose, 0.02 g of growth regulators were first dissolved in 2.5 ml of suitable solvents and then made up to 100 mL with distilled water. They were stored in dark bottles to prevent deterioration from light.

2.4. Culture conditions

Glass jars (175 ml) were used in the studies. Culture processes were carried out in the plant growth room at 24±5 ºC, under 4000 lux cold white fluorescent lamp light and 16h/8h photoperiod.

2.5. Statistical analysis studies applied to *in vitro* regeneration studies

IBM SPSS [15] Statistics 24 software was used in the analysis of data. Mean, Standard deviation, Minimum and Maximum values were used as descriptive statistics of the obtained data. The normality test of the data was tested with the Kolmogorov-Smirnov test (n>50). Kruskal Wallis H test was used in the analysis of data that did not show normal distribution. Pairwise Multiple Comparison Test was used to determine the source of the difference between the groups. The confidence interval was selected as 95% and values below $p<0.05$ were considered significant

3. Results

In order to obtain sterile explants to be used in *in vitro* studies, capitula of equal size surrounded by petals of *M. recituta* plants that had not completed flower formation were collected from the field. As a result of 6 different applications, the most successful sterile explant was obtained from the 6th treatment. The results of the surface sterilization processes applied to the explants are given in Table 3. After the surface sterilization process, *M. recituta* capitulum explants were cultured in MS nutrient medium containing 400 mg/L Amoxinil antibiotic for 1 week in order to prevent internal contamination that may originate from the explants. It was determined that the capitulum explants were not adversely affected as a result of the application.

Table 3. Results of sterilization procedures applied to *M. recutita* L. capitulum explants

Treatment number	Sterile Plant %
1.Treatment	50
2.Treatment	0
3.Treatment	60
4.Treatment	80
5.Treatment	10
6.Treatment	100

Plant growth regulators were applied to the sterile capitulum explants in different concentrations and combinations (Table 2) and the number and length of shoots formed on the explants were determined. As a result of the applications, darkening was observed after callus formation in 0.5 mg/L KIN + 1 mg/L 2.4-D, 0.5 mg/L KIN + 2 mg/L 2.4-D, 1 mg/L KIN + 1 mg/L 2.4-D, 1 mg/L KIN + 2 mg/L 2.4-D, 2 mg/L KIN + 0.5 mg/L 2.4-D, 2 mg/L KIN + 1 mg/L 2.4-D, 2 mg/L KIN + 2 mg/L 2.4-D applications. For these reasons, these applications were not evaluated in the statistical analyses.

According to the statistical analysis results, it was determined that there was a difference between the effects of plant growth regulators applied to *M. recituta* capitulum explants on shoot formation. The highest shoot number average was found as 23.91±18.00 and 18.29±7.65 in 0.5 mg/L BAP + 0.5 mg/L NAA and 0.5 mg/L BAP + 2 mg/L NAA plant growth regulator concentrations, respectively, and was different from all other groups ($p<0.05$) (Table 4-5).

Table 4. Descriptive statistics of the effects of different plant growth regulators on the number of shoots formed in *M. recutita* capitulum explants

PGRs Concentrations (mg/L)	*N*	Average	Standard Deviation (number)	Minimum (number)	Maksimum (number)
0.5 mg/L BAP	10	12.60	5.42	5.00	21.00
0.5 mg/L BAP+ 0.5 mg/L NAA	10	23.91	18.00	4.00	70.00
0.5 mg/L BAP + 1 mg/L NAA	11	11.20	3.11	7.00	14.00
0.5 mg/L BAP + 2 mg/L NAA	5	18.29	7.65	9.00	29.00
1 mg/L BAP	7	10.22	6.26	2.00	20.00
1 mg/L BAP + 0.5 mg/L NAA	9	12.60	4.16	8.00	19.00
1 mg/L BAP + 1 mg/L NAA	5	8.13	6.62	2.00	21.00
1 mg/L BAP + 2 mg/L NAA	8	6.71	3.25	3.00	12.00
0.5 mg/L KIN	7	5.46	1.47	3.00	8.00
0.5 mg/L KIN + 0.5 mg/L 2.4-D	24	10.42	6.57	3.00	23.00
0.5 mg/L BAP	12	9.36	4.96	2.00	21.00
0.5 mg/L BAP+ 0.5 mg/L NAA	14	12.00	-	12.00	12.00
2 mg/L KIN	25	8.96	4.34	2.00	23.00

Table 5. Effects of plant growth regulator applications on the number of shoots formed in *M. recutita* capitulum explants

PGRs Concentrations (mg/L)	*N*	Median Value	Rank Means	Chi-Square	*p*
0.5 mg/L BAP	10	12.00	48.113 ^{beg}	472.005	.001**
0.5 mg/L BAP+ 0.5 mg/L NAA	11	19.00	110.95 ^{ad}		
0.5 mg/L BAP + 1 mg/L NAA	5	12.00	89.20 ^{bfgh}		
0.5 mg/L BAP + 2 mg/L NAA	7	15.00	113.71 ^{dg}		
1 mg/L BAP	9	12.00	70.67 ^{cef}		
1 mg/L BAP + 0.5 mg/L NAA	5	11.00	94.60 ^{bfg}		
1 mg/L BAP + 1 mg/L NAA	8	6.00	53.19 ^{cef}		
1 mg/L BAP + 2 mg/L NAA	7	6.00	46.79 ^{cef}		
0.5 mg/L KIN	24	6.00	35.27 ^{eh}		
0.5 mg/L KIN + 0.5 mg/L 2.4-D	12	8.00	70.38 ^{cef}		
0.5 mg/L BAP	14	7.50	68.82 ^{cef}		
0.5 mg/L BAP+ 0.5 mg/L NAA	1	12.00	96.50 ^{begh}		
1 mg/L KIN	14	7.50	68.82 ^{cef}		
1 mg/L KIN + 0.5 mg/L 2-4D	1	12.00	96.50 ^{begh}		
2 mg/L KIN	25	9.00	66.20 ^{cef}		

*p< 0.05; **p< 0.01; abcdef: Groups containing different letters are different from each other

As a result of the statistical analyzes, it was determined that there were differences between the effects of different plant growth regulators applied to *M. recituta* capitulum explants on the lengths of shoots regenerated from the explants. Accordingly, the highest shoot length average was found in 0.5 mg/L BAP+0.5 mg/L NAA and 0.5 mg/L BAP+2.0 mg/L NAA plant growth regulator concentrations and was different from all other groups ($p<0.05$). It was found that shoot lengths decreased depending on the increasing NAA concentration. Darkening was observed in the explants at high BAP concentrations. In the applications of Kinetin and 2.4-D, it was observed that shoot regeneration from capitulum explants was much more successful as a result of applications containing only KIN than applications containing combinations of KIN and 2.4-D. The addition of 2.4- D, a plant growth regulator, to the nutrient medium caused a decrease in shoot formation and an increase in callus formation (Table 6-7).

Table 6. Effects of plant growth regulators on the length of shoots formed in *M. recituta* capitulum explants.

PGRs Concentrations (mg/L)	*N*	Average (cm)	Standard Deviation (cm)	Minimum (cm)	Maximum (cm)
0.5 mg/L BAP	126	1.22	.66	.30	3.00
0.5 mg/L BAP+ 0.5 mg/L NAA	263	3.02	1.55	.50	7.00
0.5 mg/L BAP + 1 mg/L NAA	56	2.66	1.09	1.00	6.00
0.5 mg/L BAP + 2 mg/L NAA	128	3.00	1.05	1.00	5.00
1 mg/L BAP	92	1.58	1.17	.30	6.00
1 mg/L BAP + 0.5 mg/L NAA	66	1.60	.57	.40	3.00
1 mg/L BAP + 1 mg/L NAA	65	1.29	.81	.10	3.50
1 mg/L BAP + 2 mg/L NAA	47	.99	.53	.20	2.50
0.5 mg/L KIN	131	1.58	.71	.30	4.10
0.5 mg/L KIN + 0.5 mg/L 2-4D	130	1.13	.75	.10	4.00
1 mg/L KIN	131	1.75	1.09	.10	5.00
1 mg/L KIN + 0.5 mg/L 2-4D	12	1.79	1.00	.20	3.00
2 mg/L KIN	224	1.62	.87	.30	4.00
Total	1471	1.92	1.26	.10	7.00

*p< 0.05; **p< 0.01; abcdef: Groups containing different letters are different from each other.

Table 7. Statistical analyses of the effects of plant growth regulators on the length of shoots formed from *M. recituta* capitulum explants.

PGRs Concentrations	*N*	Median	Standard Deviation	Chi-Square	*p*

(mg/L)		Value	(cm)		
0.5 mg/L BAP	126	1.00	488.42 ^{e}		
0.5 mg/L BAP+ 0.5 mg/L NAA	263	2.80	1058.09 ^{a}		
0.5 mg/L BAP + 1 mg/L NAA	56	3.00	1028.63 ^{b}		
0.5 mg/L BAP + 2 mg/L NAA	128	3.00	1129.84 ^{a}		
1 mg/L BAP	92	1.10	600.58 ^{ce}		
1 mg/L BAP + 0.5 mg/L NAA	66	1.55	681.05 ^{cd}		
1 mg/L BAP + 1 mg/L NAA	65	1.00	520.95 ^{def}		
1 mg/L BAP + 2 mg/L NAA	47	1.00	377.40 ^{e}	472.005	.001**
0.5 mg/L KIN	131	1.40	652.31 ^{cf}		
0.5 mg/L KIN + 0.5 mg/L 2.4-D	130	1.00	437.43 ^{e}		
1 mg/L KIN	131	1.70	689.37 ^{c}		
1 mg/L KIN + 0.5 mg/L 2-4D	12	2.00	738.83 ^{ce}		
2 mg/L KIN	224	1.50	657.67 ^{cd}		

4. Conclusions and discussion

Sterilization of plant material taken directly from the field or potted plants is usually difficult due to the intense microorganism infestation. Therefore, the most important step affecting the success of culture processes in *in vitro* studies is the explant sterilization step. The presence of exogenous and endogenous contamination and exudation of phenolic compounds in the culture medium are the main limiting factors for successful *in vitro* regeneration. To overcome this problem, the selection of appropriate sterilant, concentration and exposure time are important. Successful micropropagation of all plant species depends on the removal of contaminating agents. Commonly used disinfectants are sodium hypochlorite, calcium hypochlorite, ethanol, mercuric chloride, hydrogen peroxide and silver nitrate. Since these sterilants are toxic to plant tissue, they must be rinsed away from plant tissues [16]. In this study, ethanol, H_2O_2, $AgNO_3$, and fungicide applications were used for surface sterilization of capitulum explants. Ethanol and H_2O_2 sterilants were found to be insufficient for plant surface sterilization. In fungicide trials, it was observed that removing fungicide from explants was difficult, resulting in poor plantlet development. In treatment number 6, a 20-minute application of 1% $AgNO_3$ resulted in the healthiest and most sterile explants.

Antibiotics are added to the nutrient medium in *in vitro* applications to minimize the internal contamination problem encountered in plant tissue culture applications. It is reported in the literature that antibiotics positively affect explant growth and/or shoot and root differentiation, and that different types of β-lactam antibiotics (ampicillin, carbenicillin, cefotaxime) are successful in *Oryza sativa*, *Centella asiatica* L., *Daucus carota*, *Brassica pekinensis* L., *Tectona grandis* L., *Cucumis melo* L.. species [17]. As a result of literature information and experiences gained from the experiments, explants were cultured in MS nutrient medium for 1 week to prevent contamination that may come from the explant after surface sterilization. As a result of the observations, it was determined that antibiotic treatment supported cell development, similar to the reports in the literature.

The interaction between auxin and cytokinin has been reported to play an important role in triggering cellular differentiation and organ formation in tissue and organ cultures [18]. In this study, plant growth regulators BAP and NAA; KIN and 2.4-D, were applied to *M. recutita* capitulum explants. Based on measurements and statistical analyses, the best plant growth regulator combination in terms of shoot number and shoot length was determined to be 0.5 mg/L BAP+0.5 mg/L NAA. The results of our study are consistent with the results of literature studies in which shoot formation was achieved in *Matricaria chamomilla* shoot tip explants (0.2 mg/L BAP+0.2 mg/L NAA) [19] and *Anthemis nobilis* leaf explants (1.00 µM BAP+1.00 µM NAA) [20] treated with equal amounts of auxin and cytokinin. However, no shoot formation was observed in high BAP treatments; 2 mg/L BAP, 2 mg/L BAP+0.5 mg/L NAA, 2 mg/L BAP+1 mg/L NAA, and 2 mg/L BAP+2 mg/L NAA. Many researchers attribute this to the fact that continuously exposing explants to high BAP concentrations during shoot induction leads to high cytokinin accumulation, which in turn inhibits further shoot development [21, 22].

In the KIN and 2.4-D studies, shoot formation was observed from capitulum explants in applications containing 0.5 mg/L KIN, 1 mg/L KIN, 2 mg/L KIN and 0.5 mg/L KIN+0.5 mg/L 2.4-D, 1 mg/L KIN+0.5 mg/L 2.4-D. However, brown callus formation was observed in capitulum explants in applications containing plant growth regulators 0.5 mg/L KIN+1 mg/L 2.4-D, 0.5 mg/L KIN+2 mg/L 2.4-D, 1 mg/L KIN+1 mg/L 2.4-D, 1 mg/L KIN+2 mg/L 2.4-D, 2 mg/L KIN+0.5 mg/L 2.4-D, 2 mg/L KIN+1 mg/L 2.4-D and 2 mg/L KIN+2 mg/L 2.4-D. In a study in which NAA (0, 1, 2 and 4 mg/L) and KIN (0, 0.5, 1 and 2 mg/L) were applied to leaf explants of *Matricaria chamomilla* L., 0.5 mg/L KIN induced shoot formation via indirect organogenesis. In the same study, calli were observed to fail to grow on other nutrient media and to undergo necrosis after one month of culture. When the researchers examined the regeneration processes of the same line, they concluded that high auxin levels played an inhibitory role [23]. These reported results are consistent with those of our study.

This study demonstrated that auxin and cytokinins applied to capitulum explants of *M. recutita* can induce shoot organogenesis. Experimental results indicated that combinations of BAP and NAA applied to capitulum explants had a greater effect on shoot length and number compared to KIN and 2.4-D treatments. The study yielded data on the

sterilization protocol to be applied for *in vitro* study of the plant and the regeneration potential of capitulum explants. Furthermore, the obtained data provide guidance for studies on the morphological, physiological, and biochemical changes in the plant during regeneration.

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