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Investigation of *Erodium hoefftianum* (Geraniaceae) production by tissue culture methods

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Abstract

This current and novel approach was designed to occur the production method of Erodium hoefftianum via direct organogenesis. Lateral meristems obtained from seeds germinated in Murashige and Skoog (MS) basal medium were preferred as explants. Then, MS medium supplemented with individually 1.0/0.1 mg/L zeatin (ZEA)/indole-3-butyric acid (IBA), 1.0/0.1 mg/L kinetin (KIN)/IBA, 1.0/0.1 mg/L N6-[2-isopentenil]adenine (2iP)/IBA, 1.0/0.1 mg/L 6- Benzyladenine (6-BA)/IBA, 1.0/0.1 mg/L thidiazuron (TDZ)/IBA and without plant growth regulators (PGRs) were investigated for the effective shoot multiplication. 1.0/0.1 mg/L 6-BA/ IBA combination was found to be the most appropriate PGRs in all cases except shoot length. Zeatin/IBA combination was determined to be most ideal for shoot length with 37.28 mm. The rooting capability was also studied by using MS with IBA, indole-3- butyric acid (IAA), and 1-naphthaleneacetic acid, (NAA) (0.5 mg/L) or without auxin (control). The control group has the highest values in all cases with the rooting percentage at 100%. Well rooted and healty plantlets were transplanted into rich peat: perlite (2:1) substrates and subsequently acclimatized carefully under climate room conditions and transfer to the botanical garden conditions.

Keywords: *Erodium hoefftianum*, acclimatization, *ex situ*, *in vitro*, micropropagation, PGR

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Erodium hoefftianum (Geraniaceae)'un doku kültürü yöntemleriyle üretiminin araştırılması

Özet

Bu yeni yaklaşım, doğrudan organogenesis yoluyla *Erodium hoefftianum*'un üretim yöntemini belirlemek için tasarlanmıştır. Eksplant olarak Murashige ve Skoog (MS) besin ortamında çimlenen tohumlardan elde edilen yan meristemler kullanılmıştır. Daha sonra, ayrı ayrı 1.0/0.1 mg/L zeatin (ZEA)/indol-3- bütirik asit (IBA), 1.0/0.1 mg/L kinetin (KIN)/IBA, 1.0/0.1 mg/L N6-[2-isopentenil]adenin (2iP)/IBA, 1.0/0.1 mg/L 6- Benziladenin (6-BA)/IBA, 1.0/0.1 mg/L thidiazuron (TDZ)/IBA ve bitki büyüme düzenleyicisi olmadan desteklenen MS besin ortamı etkin sürgün çoğalması için araştırılmıştır. 1.0/0.1 mg/L 6-BA/IBA kombinasyonu sürgün uzunluğu hariç tüm durumlarda en uygun bitki büyüme düzenleyicileri olarak bulundu. Zeatin/IBA kombinasyonu 37.28 mm ile sürgün uzunluğu için en ideal olarak belirlendi. Köklenme kabiliyeti ayrıca IBA, indol-3-bütirik asit (IAA) ve 1-naftalen asetik asit (NAA) (0,5 mg/L) veya oksinsiz (kontrol) ile MS kullanılarak da incelenmiştir. Kontrol grubu, %100 köklenme yüzdesi ile tüm durumlarda en yüksek değerlere sahiptir. Turba: perlit (2:1) ortamlarına nakledilen köklü bitkicikler daha sonra iklim odası koşulları altında iklimlendirildi ve botanik bahçesi koşullarına transfer edildi.

Anahtar kelimeler: *Erodium hoefftianum*, iklimlendirme, *ex situ*, *in vitro*, mikroçoğaltma, PGR

1. Introduction

This current and novel approach was designed to occur the production method of Erodium hoefftianum via direct organogenesis. Lateral meristems obtained from seeds germinated in Murashige and Skoog (MS) basal medium were preferred as explants. Then, MS medium supplemented with individually 1.0/0.1 mg/L zeatin (ZEA)/indole-3-

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butyric acid (IBA), 1.0/0.1 mg/L kinetin (KIN)/IBA, 1.0/0.1 mg/L N6-[2-isopentenil]adenine (2iP)/IBA, 1.0/0.1 mg/L 6- Benzyladenine (6-BA)/IBA, 1.0/0.1 mg/L thidiazuron (TDZ)/IBA and without plant growth regulators (PGRs) were investigated for the effective shoot multiplication. 1.0/0.1 mg/L 6-BA/ IBA combination was found to be the most appropriate PGRs in all cases except shoot length. Zeatin/IBA combination was determined to be most ideal for shoot length with 37.28 mm. The rooting capability was also studied by using MS with IBA, indole-3- butyric acid (IAA), and 1-naphthaleneacetic acid, (NAA) (0.5 mg/L) or without auxin (control). The control group has the highest values in all cases with the rooting percentage at 100%. Well rooted and healty plantlets were transplanted into rich peat: perlite (2:1) substrates and subsequently acclimatized carefully under climate room conditions and transfer to the botanical garden conditions.

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Anahtar kelimeler: *Erodium hoefftianum*, iklimlendirme, *ex situ*, *in vitro*, mikroçoğaltma, PGR

2. Introduction

Plants are natural resources and attract the attention of people all over the world, so phytochemicals in their structures are frequently used in modern medicine or alternative medicine applications. Studies at the point of reaching these phytochemicals by collecting them from nature accelerate the endangerment of plants. *Erodium* L'Herit, one of the six genera belonging to the Geraniaceae family, spreads all over the world and is represented by 74 species [1-3]. The biggest share in this diversity is the Mediterranean region with 63 species. Other species are distributed in America (1), South America (1), Australia (5) and Asia (4) [4, 5]. Turkey occupies a large place in this diversity with 30 taxa, 16 of which are endemic [6-8]. *Erodium hoefftianum* C. A. Mey. is one of these taxa. Phytochemicals that are heavily involved in *Erodium* species have generally been reported as quinones, tannins, saponins, alkaloids, flavanoids, coumarins, anthracenosides, mucilages, sterols, triterpenes and phenolic derivatives [9, 10]. Antioxidant, antiviral, antimicrobial, anticancer, antiallergic, anti-inflammatory, antitumor, antimutagen, anti-gout, insecticidal, antifeedant, antibiotic and fungicidal effects of these valuable chemicals have been revealed before in modern medicine [11-16]. In alternative medicine, it is known that species belonging to this genus are used in many applications such as wound healing, urinary tract infections, eczema, diabetes and skin diseases [17-20].

All endemic taxa are endangered according to the Red Book of Plants of Turkey. Others are predicted to be endangered in the near future [8, 21]. The Red List extinction data of species are directly related to lower competitiveness and anthropogenic factors such as uncontrolled collection, overgrazing, big fires, trampling and fragmentation, as well as other climatic changes created by anthropogenic effects in recent years. It is predicted that the world will pass into the age of fire due to the great fires experienced especially in our country and all over the world in recent years. Therefore, populations of many plant species will face extinction in the near future if in situ and/or *ex situ* conservation strategies are not activated as soon as possible.

One of the most popular *ex situ* preservation methods in recent years in tissue culture studies because of the several advantages such as controlled environment, faster production of difficult growing species, growing disease-free plants, enabling effective clonal propagation, and shortening the growth cycle as well as rapid mass production of high-quality planting material [22]. In addition to enabling the production of many new plants similar to the ancestral characters of medicinally and aromatically valuable plants, tissue culture applications come to the fore as an alternative application, as it allows the production of biologically active phytochemicals in these plants. Because of all these factors, this study supports our country's goals of producing its own pharmaceutical raw materials from natural resources with alternative methods and bringing them into the country's economy. The aim of the present study is to

develop an efficient *in vitro* micropropagation protocol for *E. hoefftianum* and occur *ex situ* collections. The absence of any study on tissue cultures of this species in the literature makes the study original and innovative.

2. Materials and methods

2.1. Plant material

E. hoefftianum seeds were collected from indigenous populations of Yağmurdere-Gümüşhane (40° 35′ 09″ N, 39° 52′ 1″ E; 1744 m), and followed by storage in darkness at 4 °C until use. Before surface sterilization, seeds were washed with tap water nearly 30 min, followed by treated with 70% ethanol (EtOH) for 30 seconds. After the removal of methanol, seeds were disinfected with 20% commercial bleach (Domestos®, Unilever, Istanbul, Turkey), for 10, 11, 12, 13, 14, and 15 min. Disinfected seeds were washed with sterile distilled deionized water 3 times for nearly 15 min in sterile cabinet (Biohazard SafeFAST Permium 212, Ferrara, Italy) to thoroughly remove chemical residue. Finally, all sterile seeds cultured on approximately 35 mL nutrient basal media in 98.5 × 59 mm glass containers (Magenta, Sigma-Aldrich Ltd., St Louis, MI, USA). Cultured seeds were stored in growth chamber until germination.

2.2. Experimental

2.2.1. Seed germination

Murashige and Skoog basal medium (MS, Duchefa Biochemie, Haarlem, The Netherlands) [23] individually supplemented with two different GA_3 concentrations (1.0 and 3.0 mg/L) and without PGR (control) were tested to determine the most ideal culture conditions for seed germination. To support these two different GA_3 concentrations in the same medium, 1.0 mg/L activated charcoral (AC) was individually added to each medium. The cultivated seeds were stored in the climate chamber for 30 days. The most ideal germination medium was determined by calculating the germinated seed number and shoot length data in the culture medium. Germinated seeds were subcultured and made ready for shoot propagation studies.

2.2.2. Shoot proliferation

Nodal segments were excised from the shoots of seedlings and placed on MS basal medium containing 2% (w/v) sucrose (Duchefa), 0.8% (w/v) phyto agar (Duchefa) supplemented with individually different plant growth regulators (PGRs), including 1.0 mg/L zeatin (ZEA), 1.0 mg/L 6- Benzyladenine (6-BA), 1.0 mg/L N6-[2-isopentenil]adenine (2iP), 1.0 mg/L kinetin (KIN), and 1.0 mg/L thidiazuron (TDZ) in combination with 0.1 mg/L indole-3- butyric acid (IBA). All PGRs used in this study were sourced from Sigma-Aldrich Ltd., St Louis, MI, USA and were filter-sterilized with 0.22 µm filters and added to the cooled media after autoclaving. For these media, the sucrose ratio was 2% (w/v, Duchefa) and the agar ratio was 0.8% (w/v, (Duchefa). Stock solution of 1 N HCl or 1 N NaOH was used to adjust the pH of the media to 5.8 before sterilization process. All cultures were maintained in grooth room at 24 ± 2 °C under a 16 h photoperiod at a photosynthetic flux density of 50 µmol m^{-2} s^{-1}, provided by cool daylight fluorescent lamps (Philips HO 49W/840, Poland. The subcultures were carried out at 4-week intervals. After one month, the samples in the trial sets were evaluated in terms of evaluating the number of shoots per explant (fold), length of shoots (mm), number of leaves (fold), callus (%), and biomass yield based on fresh and dry weight (g) on each shoot.

2.2.3. Root induction

MS media, each individually supplemented with IBA, Indole-3- acetic acid (IAA) and 1-naphthaleneacetic acid, (NAA) at a concentration of 0.5 mg/mL and without growth regulators (control), was again selected for root induction studies. The well-developed healty and sufficiently elongated shoots (≥ 20 mm) were preferred for rooting studies. At the end of the four weeks, rooting parameters, which are rooting rate (%), root number (fold), root length (mm), secondary root number (fold), were calculated to evaluate the rooting success of *E. hoefftianum*. Each rooting treatment contained a total of 16 healthy wwll-developed shoots and each experiment was carried out in triplicate.

2.2.4. Acclimatization

The roots of the plantlets were gently treated with running tap water for a while to remove any media residue. Then these microshoots were transferred in 72 mesh plastic containers containing peat: perlite (2: 1) mixture. These microshoots transferred to peat: perlite (2:1) (v/v) mixture in 72 mesh plastic containers. The plants were subjected to the first acclimatization process for four weeks in climate room conditions by gradually decreasing the humidity. All plantlets were held on at 24 ± 2 °C under a 16-h photoperiod at a photosynthetic flux density of 50 µmol m−2 s−1, provided by cool daylight fluorescent lamps (Philips HO 49W/840, Poland). The survival rates of the plants were determined at the end of the 4th week and transferred to botanical garden conditions. The healthy plants were kept in the botanical garden among 30 days, and then they were transfer to a field next to the botanical garden. The survival successes of the plants were calculated individually in both the botanical garden and field conditions.

2.3. Statistical analysis

For all germination experiments, five achenes were placed into each Magenta glass containers, and six vessels were prepared per treatment. For all shoot proliferation experiments, four nodal explants were cultured into each magenta glass containers and again six vessels were preferred for each treatment. Each rooting treatment contained a total of 16 healthy shoots. All data were analyzed using SPSS 21.0 (IBM Corp., Armonk, NY, USA). For shoot multiplication, mean shoot length, mean number of shoots, mean number of leaves, callus percentage rate, mean number of fresh and dry weight were analyzed using Duncan's multiple range test from one-way analysis of variance (ANOVA). Data in tables were given as mean ± standard deviations (SD). Each treatment was performed in triplicate.

Shoot-forming capacity (SFC) [24] and root-forming capacity (RFC) [25] were also calculated to determine the percentage of shoots regeneration and root induction as follows:

SFC index = (average number of shoots per regenerating explant) × (% of regenerating explant)/100.

RFC index = (average number of roots per shoot) × (% of multiplying roots)/100.d.

3. Results

3.1. Seed Germination

In seed germination studies, significant data were obtained only in 12, 13 and 14 min sterilization applications in MS basal medium supplemented with 3.0/1.0 mg/L GA_3/AC at the end of the 30th day. The highest germination percentage was obtained from MS medium supplemented with 3.0/1.0 mg/L GA_3/AC at 13 min sterilization time with 55.57%. Germinated shoots obtained from this culture medium and sterilization time had an average shoot length of 14.19 mm and appeared healthy for shoot multiplication studies. These percentages were determined as 34.43% and 17.78% in MS medium supplemented with 3.0/1.0 mg/L GA_3/AC at 12 and 14 min sterilization time, respectively. Although the germination percentage was high in the sterilization time of 12 min, the average length of the germinated seeds was shorter (10.08 mm), and the stem thickness was weaker compared to the 14 min application. No significant germination data could be obtained in any of the other sterilization time and germination media.

3.2. Shoot Proliferation

Among the tested five different PGRs; namely ZEA, 6-BA, 2iP, KIN, and TDZ were individually combination with 0.1 mg/L IBA and compared with basal medium without PGR (control) for this purpose, 1.0/0.1 mg/L ZEA/IBA and 1.0/0.1 mg/L 6-BA/IBA were detected more convenient for shoot proliferation of this species (Table 1, Figure 1A-G). Among these two PGRs, 1.0/0.1 mg/L ZEA/IBA was more effective than 1.0/0.1 mg/L 6-BA/IBA only in terms of shoot length with 37.28 ± 2.87 mm. While this efficacy of ZEA was valid for other PGRs, there was no statistical difference between itself and the control group with an average shoots length of 36.52 ± 4.07 mm ($P < 0.05$). The lowest data in terms of shoot length was calculated as 19.53 mm, from media containing 1.0/0.1 mg/L TDZ/IBA. The most effective shoot number was determined in MS medium supplemented with 6-BA as mentioned above, and this yield was 3.0 ± 0.59 per shoot. While this data statistically differentiated 6-BA from other PGRs and control group, the closest value to 1.0/0.1 mg/L 6-BA/IBA was obtained from MS medium supplemented with 1.0/0.1 mg/L ZEA/IBA, with an average of 2.5 shoots per shoot (Table 1). Culture media containing 2iP and the control group were less effective than other PGRs for this parameter with a 1.71 ± 0.46 mean shoot number.

The highest leaf number was 13.58 ± 1.25 in MS containing 1.0/0.1 mg/L 6-BA/IBA. Among the other all tested PGRs, 1.0/0.1 mg/L TDZ/IBA gave the other effective mean leaf number per shoot with 11.5 ± 1.53. Although this result creates a statistical difference with 6-BA and other PGRs, the weak leaves of the obtained plantlets do not make TDZ stand out in terms of healthy leaves ($P < 0.05$). Table 1 shows that 1.0/0.1 mg/L 2iP/IBA also has a lower effect on the number of leaves per shoot with 5.71 ± 0.69. In terms of fresh and dry weight, which is especially important at the point of secondary metabolite production, 1.0/0.1 mg/L 6-BA/IBA gave the highest values with an average of 1.514 ± 0.039 and 0.086 ± 0.002, respectively. There were significant statistical differences between 6-BA and other PGRs and control group in terms of both fresh and dry weight values ($P < 0.05$). However, the dry weight per fresh weight of the shoots obtained from MS medium supplemented with this cytokinin was the lowest with 5.68%. The highest dry weight per fresh weight was obtained from the control media with 12.65%. Among the PGRs tested, more callus formation occurred in MS media fortified 1.0/0.1 mg/L TDZ/IBA with 77.8%. Control group did not give a significant result in terms of this parameter. Based on all these data, it was inevitable that 1.0/0.1 mg/L 6-BA/IBA would be more effective than other cytokinins and control in terms of shoot forming capacity, and this was achieved with 2.875 (Table 1).

Table 1. The effects of different cytokinin in the presence of IBA (0.1 mg/L) combination and control group on shoot proliferation of *E. hoefftianum*

Cytokinin/Auxin (mg/L)	*Shoot Number/ Explant*	*Shoot Length/ Explant (mm)*	*Leaf Number/ Explant*	*Fresh Weight/ Explant (g)*	*Dry Weight/ Explant (g)*	*Callus (%)*	*Shoot Forming Capacity (SFC)*
CONTROL (0.0/0.0)	*1.71 ± 0.46d*	*36.52 ± 4.07a*	*8.31 ± 1.0d*	*0.332 ± 0.034cd*	*0.042 ± 0.004c*	*nd*	*0.854*
ZEA/IBA (1.0/0.1)	*2.5 ± 0.51b*	*37.28 ± 2.87a*	*9.29 ± 0.95c*	*0.292 ± 0.022d*	*0.030 ± 0.002d*	*50 ± 0.0b*	*1.875*
KIN/IBA (1.0/0.1)	*1.83 ± 0.38bd*	*29.95 ± 2.48b*	*7.5 ± 0.83e*	*0.303 ± 0.021d*	*0.028 ± 0.002c*	*18.1 ± 2.4d*	*1.451*
2IP/IBA (1.0/0.1)	*1.71 ± 0.46d*	*31.21 ± 2.95b*	*5.71 ± 0.69f*	*0.354 ± 0.034c*	*0.028 ± 0.003d*	*50 ± 0.0b*	*0.996*
6-BA/IBA (1.0/0.1)	*3.0 ± 0.59a*	*21.88 ± 1.67c*	*13.58 ± 1.25a*	*1.514 ± 0.039a*	*0.086 ± 0.002a*	*27.8 ± 2.4c*	*2.875*
TDZ/IBA (1.0/0.1)	*2.13 ± 0.34c*	*19.53 ± 1.71d*	*11.5 ± 1.53b*	*1.144 ± 0.166b*	*0.071 ± 0.010b*	*77.8 ± 2.4a*	*1.682*

Data were recorded 4 weeks after the culture and represents a total of 24 explants per treatment on MS. Each experiment was performed in triplicates. Values having the same letter(s) in the same column are not significantly different according to Duncan's multiple range test at $P < 0.05$. nd = Not detected, ZEA = Zeatin, 6-BA = 6-Benzyladenine, 2iP = N6-[2-isopentenil]adenin, KIN = Kinetin, TDZ = Thidiazuron, IBA = Indole-3- butyric acid.

3.3. Root Induction

Enough elongated shoots (> 20 mm) were excised and carefully transferred to MS media individually supplemented with 0.5 mg/L IBA, IAA, NAA, and without auxin (control). MS basal media without auxin stimulated the rooting more efficiently than fortified above mentioned auxins at the end of the four weeks with 100% (Figure 1H). The first root induction was seen on the 17th day in the control medium. The fact that the first root formation was earlier in the control group compared to the applied auxins encouraged higher root number, root length, secondary root number and root forming capacity values at the end of the 4 weeks application period. These values were calculated as 4.75 ± 0.68, 105.02 ± 7.49 mm, 6.5 ± 0.63 and 4.8 per shoot, respectively. Among the tested auxin groups, the order of efficiacy of root induction rates was IBA (91.7%) > IAA (81.3%) > NAA (77.1%) (Figure 1I-L). The only parameter that differed during the effect of auxin applications was the number of root. This result was negatively reflected in the root forming capacity of the MS medium supplemented with IBA with 3.54. MS media supplemented with IBA reached a lower root number value of 3.86 ± 0.50 per shoot than MS media containing IAA or NAA (Table 2). Root numbers were calculated as 4.54 ± 0.46 and 4.50 ± 0.58, respectively, in MS fortified with IAA or NAA, and there were no statistically differences between them and the control group ($P < 0.05$). In terms of the second highest root length, the efficiency of IBA came to the fore with 69.43 ± 4.49 mm. The lowest root length success was determined in MS medium containing NAA with 11.31 ± 1.26 mm, and this created a significant statistical difference with the other test groups ($P < 0.05$). The efficiacy of IBA among the auxin groups did not change in terms of the number of secondary roots, and this value was calculated as 4.93 ± 0.57 per shoot (Table 2).

Table 2. The effects of different auxin types (0.5 mg/L) on *in vitro* rooting of *Erodium hoefftianum* shoots

PGRs (mg/L)	Rooting rate (%)	Root Number (No/Plant)	Root Length (mm)	Secondary Root Number (No/Plant)	Root Forming Capacity (RFC)
CONTROL	100 ± 0.0a	4.75 ± 0.68a	105.02 ± 7.49a	6.5 ± 0.63a	4.8
IBA	91.7 ± 3.6b	3.86 ± 0.50b	69.43 ± 4.49b	4.93 ± 0.57b	3.54
IAA	81.3 ± 0.0c	4.54 ± 0.46a	64.99 ± 3.18c	4.46 ± 0.64c	3.69
NAA	77.1 ± 3.6c	4.50 ± 0.58a	11.31 ± 1.26d	2.00 ± 0.37d	3.47

Data were recorded on the four weeks after the culture and represent a total of three replicates of 16 plants per treatment for root induction. Values having the same letter(s) in the same column are not significantly different according to Duncan's multiple range test at $P < 0.05$. PGRs = Plant Growth Regulators, IBA = Indole-3- butyric acid, IAA = indole-3- acetic acid, NAA = 1-naphthaleneacetic acid

3.4. Acclimatization and ex situ collection

The well developed and seen healty *in vitro* rooting plantlets were transferred in plastic containers containing peat: perlite (2: 1) and covered with plastic bags to provide necessary humidity. These pots were transferred firstly in climate room conditions to increase the viability of *in vitro* rooting plantlets in *ex vitro* (Figure 1M and N). By opening the mouths of the plastic containers intermittently, the humidity rate was reduced, the plantlets were not rotted and their

adaptation to the *ex vitro* condition was facilitated. The viability percentages of seedlings were determined as 90% in climate room conditions.

At the end of the 4 weeks, acclimatized, well developed, and healthy plants were transferred again in botanical garden conditions and were obtained about 86.7% survival percentage. After a month, these plants produced fresh leaves and developed considerably (Figure 1O). All plants formed buds, bloomed, and produced seeds within the first season. The *ex situ* collection of *E. hoefftianum* has been established at the end of the spring 2020 and completed in the same summer of the same year.

4. Conclusions and discussion

Ex situ conservation strategies are particularly important for small plant populations or plant populations with low seed yield and/or low seed viability. *In vitro* techniques are among the most successful *ex situ* conservation methods beause of the above mention many advantages and used for the reproduction and preservation of such taxa [24, 25]. From this perspective, the production of plants with medicinal, aromatic, and economic value by *in vitro* techniques has accelerated [28-31]. A limited number of studies have been carried out on the production of *Erodium* taxa, which are valuable in the field of modern and alternative medicine, by tissue cultures [32-34]. But, there is no comprehensive *in vitro* production report about *E. hoefftianum.* Therefore, it is notable to study micropropagation and *ex situ* conservation of this species.

In tissue culture studies on *Erodium* taxa, researchers generally preferred commercial bleach or sodium hypochlorite (NaOCl) as a sterilization agent. The application that differs in these studies is the chemical agent used for the pre-sterilization process, the sterilization time, the concentration of the sterilization agent, and explant type [33, 34]. In the study carried out on *Erodium somanum* H. Peşmen, the researchers applied 5% commercial bleach on the seeds for 5 min and achieved a 70% germination success at the end of the second week [33].

Figure 1. *In vitro* micropropagation of *E. hoefftianum* (**A)** Shoot proliferation after 4 weeks; **(B)** on culture medium from nodal explant on MS free-medium, **(C)** supplemented with 1.0/0.1 mg/L ZEA/IBA, **(D)** with 1.0/0.1 mg/L KIN/IBA, **(E**) with 1.0/0.1 mg/L 2iP/IBA. **(F**) with 1.0/0.1 mg/L 6-BA/IBA. (H) with 1.0/0.1 mg/L TDZ/IBA. **(H)** Root induction after 4 weeks; **(I)** MS free-medium, **(J)** 0.5 mg/L IBA, **(K)** 0.5 mg/L IAA, **(L)** 0.5 mg/L NAA, **(M)** flower form, (N) Climate room conditions, **(O)** Botanical garden conditions, Bars: B, C, D, E, F and G = 1.47 cm, I, J, K and L = 5.65 cm

In another study, researchers this time 0.5%, 1%, and 2% concentrations of NaOCl were tested on *Erodium sibthorpianum* Boiss. subsp. *sibthorpianum* seeds for 10 min and determined that 1% NaOCl application was the most ideal [32]. These researchers achieved germination success between 67% and 28% in their studies. These results show that species within the same genus differ in terms of sterilization processes and germination percentage in tissue culture studies. The fact that the highest germination percentage we obtained from the seeds of *E. hoefftianum* was 55.57 % with a optimum sterilization time of 13 min is a comparative proof of these results.

Although all PGRs mentioned in our study were used in tissue culture studies of different plant species [30, 35], researchers generally tried different concentrations of 6-BA or KIN, either alone or in combination with any auxin or GA_3, in MS medium for shoot propagation studies of *Erodium* taxa [32-34]. Researchers have reported that high 6-BA and low NAA combinations can be effective in their shoot propagation studies on *Erodium* species [32]. However, in cytokinins combined with IAA or NAA, it was determined that *E. hoefftianum* did not form healthy shoots. The shoots remained weak and did not grow sufficiently. These findings reveal the differences with our study. Another weak indicator of the difference with our study is that the same researchers obtained an average of 11.33 ± 0.44 shoots per explant depending on the species difference in these media. On the other hand, the obtained highest mean number of shoots per explant from *Erodium olympicum* was 1.42 ± 1.54. This result showed the strength of our study. When the shoot length data obtained from our study were compared with *E. sibthorpianum* subsp. *sibthorpianum*, it was determined that close results supported each other. The highest shoot length was found in MS medium absence of PGRs with 35.97 ± 0.55 [32]. The highest shoot length was found in MS medium absence of PGRs with 35.97 ± 0.55 [32]. This result was 3.64% lower than the highest shoot length obtained from *E. hoefftianum*. The maximum average shoot length of 2.06 ± 0.04 mm in micropropagation studies on *Pelargonium graveolens* L. belonging to the *Geranium* genus supports the low shoot length of these family members in culture studies [36]. The shoot length, leaf number, fresh and dry weight parameters were not evaluated in other tissue culture studies on *Erodium* taxa. These data obtained from our study enrich the content of tissue culture studies on these taxa and shed light on future studies on this genus.

Researchers tested IBA at a concentration of 0, 0.5, 1, 1.5, 2, 2.5, 3 mg/L in MS medium for root induction of *E. olympicum*. The most effective results were obtained from MS medium supplemented with 0.5 mg/L IBA with 29.91% [34]. While our preference for 0.5 mg/L auxin concentration is supported by this study, the fact that we achieved 70% more rooting success according to the researchers reveals the strength of our study. The lower results of IAA compared to other tested auxins in the reported study were consistent with our study. Various concentrations of IBA and IAA (0, 0.5, 1, 1.5, and 2 mg/L) in MS medium and various concentrations of NAA (0, 0.1, 0.5, 1, and 2 mg/L) in modified MS medium (ModMS) have been tested for root induction of *E. sibthorpianum* subsp. *sibthorpianum*. The most influential rooting percentage has been obtained in ModMS media containing 0.1 or 0.5 mg/L NAA with 100%. When compared in terms of the highest root number and root length, the same researchers reported the average values of 21.17 ± 0.43 and 44.03 ± 0.58 mm per shoot, respectively [32]. Calculation of these values as 4.75 ± 0.68 and 105.02 ± 7.49 mm per shoot in our study revealed the strengths and weaknesses of our findings in this study. These differences can be caused by many factors such as explant source, explant type, explant collection time, environmental conditions of the explant, media types and culture time, number of subcultures, culture conditions in tissue culture studies [29, 37].

As a result, this *in vitro* protocol can be used as an effective protocol in future *ex situ* conservation studies for the restoration of plant populations.

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