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In vitro propagation of *Gypsophila germanicopolitana* Hub.-Mor. an endangered and edaphic endemic in Çankırı

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Research Article

ABSTRACT

In this study, in vitro propagation of *Gypsophila germanicopolitana* HUB.- MOR. (Çankırı çöveni), which grows on gypsum hills within the limits of Çankırı province was taken as an aim. The species is included in the list of plants under "critical extinction (CR)" according to the International Union for Conservation of Nature and Natural Resources red list category. Shoot tips and internodes of the plant were used as explant sources for in vitro propagation, as basal medium 1) Murashige and Skoog (MS) 2) Nitsch & Nitsch (NN) were chosen, as plant growth regulators 1) 6-benzylaminopurine (BAP) and 2) Kinetin (KIN) (0 mg/L, 0.5 mg/L, 1 mg/L) were used as cytokinin source as well as 3) Indole-3-butyric acid (IBA) and 4) α-naphthalene acetic acid (NAA) (0 mg/L, 0.25 mg/L, 0.5 mg/L) were as auxin sources. After that 36 combinations with different doses were established. As a result, the best shoot regeneration was observed in NN basal medium with a combination of 0 mg/L KIN + 0.5 mg/L NAA. Root regeneration was more successfully present within the explants cultured in MS than in NN. It is important that no in vitro propagation studies of this endemic species have been encountered before, and that the findings obtained can be propagated and disseminated in the laboratory.

Key Words: Çankırı, *Gypsophila germanicopolitana*. in vitro techniques, internode, shoot tip

Çankırı ilinde yok olma tehlikesi altındaki ve edafik endemik *Gypsophila germanicopolitana* HUB.-MOR.'un in vitro koşullarda çoğaltımı

ÖZ

Bu çalışmada, Çankırı ilindeki jipsli tepeler üzerinde yayılış gösteren ve Uluslararası Doğa ve Doğal Kaynakları Koruma Birliği kırmızı liste kategorisine göre "kritik yok olma tehlikesi (CR)" altındaki bitkiler listesinde yer alan *Gypsophila germanicopolitana* HUB.-MOR. (Çankırı çöveni) bitkisinin in vitro koşullarda çoğaltımı amaçlanmıştır. Eksplant kaynağı olarak bitkinin sürgün uçları ve sürgünler üzerindeki boğum araları kullanılmıştır. In vitro çoğaltım için temel besin ortamları olarak 1) Murashige ve Skoog (MS) 2) Nitsch & Nitsch (NN), sitokinin kaynağı olarak 1) 6-benzylaminopurin (BAP) ve 2) Kinetin (KIN) (0 mg/L, 0.5 mg/L, 1 mg/L) yanı sıra oksin kaynağı olarak da 3) Indol-3-bütirik asit (IBA) ve 4) α-naftalen asetik asit (NAA) (0 mg/L, 0.25 mg/L, 0.5 mg/L) dozları ilave edilmiştir. Sonuç olarak en yüksek sayıda sürgün rejenerasyonu, 0 mg/L KIN + 0.5 mg/L NAA kombinasyonu ilave edilmiş Nitsch & Nitsch (NN) besin ortamında gözlenmiştir. MS besin ortamında kültüre alınmış eksplantlarda ise köklenme NN besin ortamına göre daha başarılı olmuştur. Daha önce bu endemik türe ait herhangi bir in vitro çoğaltım çalışmasına rastlanmamış olması ve elde edilen bulguların türün laboratuvar ortamında çoğaltılabilmesi ve yaygınlaştırılması bakımından önemlidir.

Anahtar Kelimeler: Çankırı, *Gypsophila germanicopolitana*, in vitro teknikler, boğum arası, sürgün ucu

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1. Introduction

Turkey is one of the leading countries in the world with its richness in terms of biodiversity. It draws attention with its richness, plant diversity and high endemism rate (35%) (Ataşlar, 1999). Anatolia is the homeland of many plants, and it is recognized as the gene center of some economic plant species (such as *Astragalus*, *Verbascum* and *Bolanthus*). However, this situation has been threatened by factors such as agriculture, animal husbandry, erosion, fires, logging, settlement, industrialization, and excessive pesticide use over the last 50 years (Avcı, 2005). For this reason, it is stated that twelve rare and endemic plant species are extinct. Caryophyllaceae (Carnations) is one of the largest angiosperm families. The family has about 100 genera and 2200 species (Korkmaz, 2012). Individuals of the Caryophyllaceae family are generally found in temperate regions of the northern and southern hemispheres and in tropical mountains. Although it is reported that the gene center of this family is the Mediterranean Basin; Eastern Anatolia, Sinai peninsula, Syria, Palestine, Armenia, Iran, Jordan, upper Mesopotamia, arid and semi-arid parts of eastern and southern Transcaucasia, south of Hindu Kush, southern part of Northern Himalaya, south of Volga river, Gobi Desert, it has also spread in the areas surrounding Turkey and called Iran-Turanian regions (Korkmaz, 2007). The plant species found in these regions are heliophytes that emerged in habitats with dry and intense sunlight. On the other hand, some species are limited to mountainous regions only. The Caryophyllaceae family is important for its medicinal and ornamental plant properties.

Gypsophila sp., the third largest genus of the Caryophyllaceae family, which is distributed in different regions of the world, grows in thirty-five genera and around four hundred and seventy species concentrated in Iran-Turanian and Eastern Anatolia regions in Turkey, and more than half of them are endemic and Anatolia is the gene source for this species (Yıldız, 2012). "*Gypsophila*" is the name of plants that show optimal growth in gypsum media. The name *Gypsophila* is derived from the Greek words "gypsums" meaning gypsum and "Philos" meaning loving (İnan, 2006). The species of the genus adapt to grow in many different habitats (Korkmaz and Özçelik, 2011). These plants are available in annual, biennial, or perennial forms. Turkey, the gene source feature of *Gypsophila*, which is an indicator of gypsum fields, is a very necessary species to be introduced due to the biological characteristics of the taxon (Korkmaz, 2012).

The province of Çankırı, which has semi-arid and arid areas, possesses herbaceous and medicinal aromatic plants that grow locally endemic. *Gypsophila germanicopolitana* HUB.-MOR., (Figure 1a-1b-1c) is on the list of critically endangered plants International Union for Conservation of Nature (IUCN), Threatened Plant Committee (TPC), World Wildlife Foundation (WWF), as well as organizations such as the Ministry of Agriculture and Forestry in Turkey (Ekim ve ark., 2000) (Figure 1d).

Known by local names in different regions of Turkey, *Gypsophila* attracts attention in many production sectors due to its different characteristics. Some *Gypsophila* species (*G. arrostii* Guss., *G. bicolor* Freyn & Sint.), (*G.rossh*., *G. eriocalyx* Boiss), (*G. arrostii* var. *nebulosa*) were used in the medical field, food products, cleaning, and cosmetic products. It can also be used as an ornamental plant in parks and gardens, and it is widely used in the fields of agricultural sector, landscape, textile, jewelry, chemistry, and pharmacists (İnan, 2006).

This study is unique in that it is the first study on the in vitro propagation of *G. germanicopolitana*. In addition, it is aimed to reproduce in vitro conditions by using explants such as shoot tip and node of *G. germanicopolitana* plant with tissue culture method, which is an important biotechnological method and provides intensive, rapid and clonal propagation. The aim of this study is to ensure the propagation and acclimatization of the plant with the help of in vitro techniques.

2. Materials and Methods

Study area: The research area is within the "Gypsum Hills of Çankırı" area, which is ranked 89th in Turkey's Important Plant Area (IPA). The IPA is located in the North of Kızılırmak, on large gypsum deposits close to Çankırı province. The IPA flora does not show rich vegetation diversity and forms a mosaic of steppe and mesotrophic pasture habitats. There are 41 taxa endemic to Turkey in the IPA flora. The IPA is not officially protected, conversion to agriculture and grazing threaten the area (Çerçi ve Göl, 2021; Öz ve Göl, 2021).

Plant material: This experiment was carried out in the Laboratory of Biotechnology in 2021-2022. For the in vitro propagation of the endemic plant *G. germanicopolitana* was collected from the field and brought to the Graduate School of Natural and Applied Science, Biology Dept. laboratory of Çankırı Karatekin University. *G. germanicopolitana* is located on the gypsum hills on the Çankırı-Ankara highway, at an altitude of 700-750 m, at the coordinates of 40º33'24" N and 33º33'41" E. Spreading on the gypsum hills of Çankırı province, *G. germanicopolitana* is present in the field in the last week of May 2021 and has been found in full flowering and fruiting from the second week of June. The most physiologically and morphologically mature state of a plant is when it is fully flowering (Xiong et al., 2000). *G. germanicopolitana* was collected in limited quantities from its gypsum habitats (Figure 1c) and brought to the laboratory aseptic environment on 20 June in a way that does not interfere with the natural habitats and propagation of the plant.

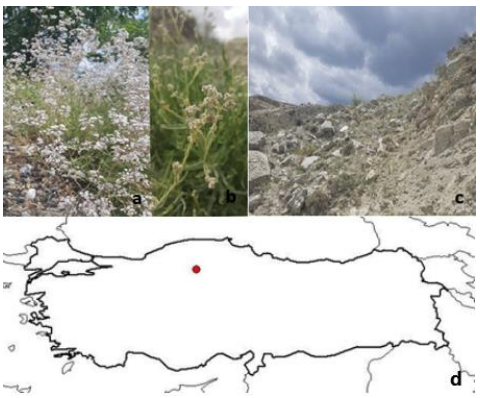

Fig. 1. *G.germanicopolitana* a-b) Flowers c) Habitat d) Distribution area of *G. germanicopolitana*-Çankırı/Türkiye

Sterilization and explant preparation: *G. germanicopolitana* plant shoot tip and internodes were used as explant sources. Sterilization of *G. germanicopolitana* plant was performed (Figure 2). For this, a superficial sterilization protocol was applied. Defoliated shoots were cut, and apical meristems tissues and internodes were used as explant sources. Then, the explants were kept in 70% ethanol for 3 minutes and rinsed with sterile distilled water 2 times for 5 minutes. These explants were treated for 10 minutes in a 20% NaOCl solution (min 15% active chlorine) in which 3 or 4 drops of Tween 20 were added. In order to remove the sodium hypochlorite with increased activity from the tissues, it was rinsed with sterile distilled water 3 times for 5 minutes under aseptic conditions (Kapdan and Sezgin, 2021).

Culture preparation and conditioning: In the preparation of the culture medium, 2 basal media, gelrite as a solidifier, and plant growth regulators (PGR) prepared in different doses and combinations were used.

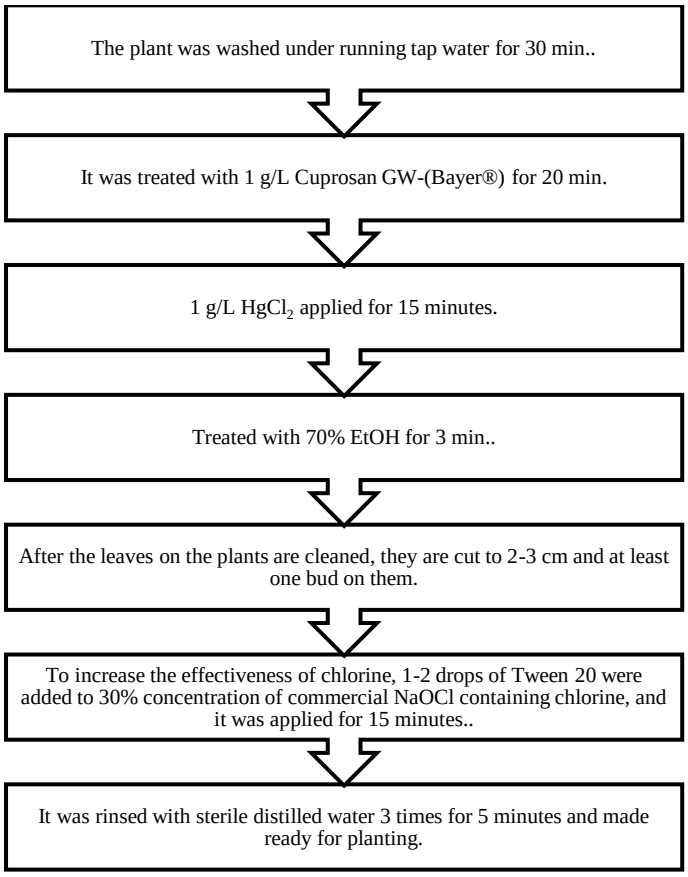

Fig. 2. Sterilization stages of *G. germanicopolitana*

Basal media

a) Murashige and Skoog (MS) (Murashige and Skoog, 1962)
b) Nitsch & Nitsch (NN) (Nitsch and Nitsch, 1969)

Growth regulators and their combinations

a) 6-benzylaminopurine (BAP)-(0 mg/L, 0.5 mg/L, 1 mg/L)
b) Kinetin (KIN)-(0 mg/L, 0.5 mg/L, 1 mg/L)
c) Indole-3-butyric acid (IBA)-(0 mg/L, 0.25 mg/L, 0.5 mg/L)
d) α-naphthaleneacetic acid (NAA)-(0 mg/L, 0.25 mg/L, 0.5 mg/L)

MS and NN basal media were used as the solidifier with gelrite as the basal medium for the in vitro micropropagation of *G. germanicopolitana*. Plant growth regulators were prepared in 36 different combinations including 6 cytokines and 6 auxin doses and 30 g/L sucrose as a carbon source was added to these media. Plant Preservative Mixture (PPM-Duchefa®) was added as 1 mL/L to prevent possible contamination that may occur after planting the explants in basal media. The pH of the basal media was adjusted to 5.7.

Each combination was prepared as 10 replications. A total of 720 De Wit tubes (Duchefa®) were prepared for the two primary basal media. Under aseptic conditions, the apical nodes on the explants were planted in the tubes in a vertical position, taking care to settle into the medium. Only one explant was planted in each De Wit tube to avoid and reduce the possibility of contamination.

A controlled autoclave was used for sterilization of the basal media, for this it was carried out at 121ºC, 1.2 atm pressure for 20 minutes. The sterilized basal media were taken into a direct laminar air flow cabinet and immediately distributed into sterile De Wit tubes (130x10 mm) as 12 mL.

Incubation: Cultures were incubated in a growth chamber where optimal living parameters were set to ensure plant regeneration. All cultures were cultured at 25±2 ºC, 16 hours of light (35 µmol·m-2·s-1), and 8 hours of darkness.

Subculture: *G. germanicopolitana* explants, which were planted, were incubated in the climate chamber and their growth was checked daily. After optimal growth conditions were adjusted, the explants were subcultured 2 times in De Wit tubes and 3 more times in ECO2BOX/Green Filter® (Duchefa) 125x65x80 mm dishes at 4 weeks intervals (Figure 3a). Subculture is done to maintain the freshness of the media and to continue the development of the plant (Figure 3b). In subculture studies, the same basal media and PGR combinations used in the initial stage were used to continue the development of the plant. Root length, root number, shoots length and shoot number of explants showing shoot and root development were measured.

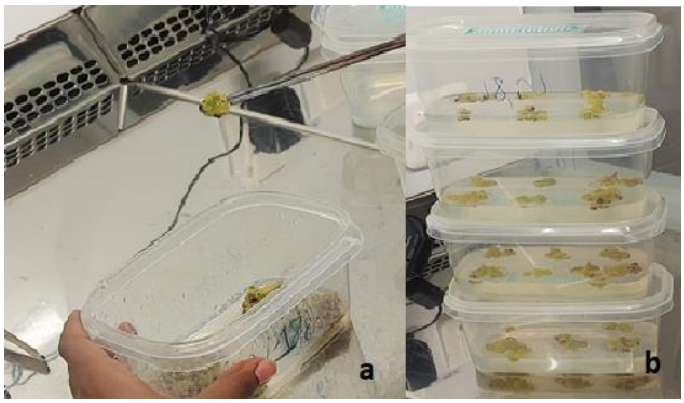

Fig. 3. Subculture of callus a) Planting calluses in ECO2BOX/Green Filter®(Duchefa) b) Subcultured explants

Statistical analysis: In this study, the mean of the data obtained from the results was checked with the analysis of variance method (ANOVA) and SPSS Package Program according to the F-test ($P < 0.05, 0.01$). Significant differences were made with the Game-Howell Nonparametric Post-Hoc multiple comparison tests on a 5% margin of error basis.

3. Results

In this study, in vitro propagation and transformation of *G. germanicopolitana* into plants were aimed by tissue culture, which is one of the important methods of biotechnology. The shoot tip and internodes of the plant were used as an explant source. Plant regeneration via direct organogenesis was not achieved in *G. germanicopolitana*. However, plant regeneration was targeted from the callus obtained by the indirect organogenesis method. Since the *G. germanicopolitana* plant is on the list of plants under critical extinction (CR) according to the International Union for Conservation of Nature and Natural Resources red list category, a limited amount was taken from the field. Combined with the in vitro propagation stage without embryo culture and the problems in the plant's ability to regenerate, a complete transformation into a plant could not be achieved. However, it was observed that the concentration, combination, and types of plant PGRs used in the study had a positive effect on callus formation. Based on the organ-forming potential of callus from explants, abundant organogenic callus was obtained that were not capable of regeneration. From the callus obtained by the indirect organogenesis method, shoot and root formation were targeted (Figure 4). The sterilization protocol used in our study was very effective and the incidence of infection was very low.

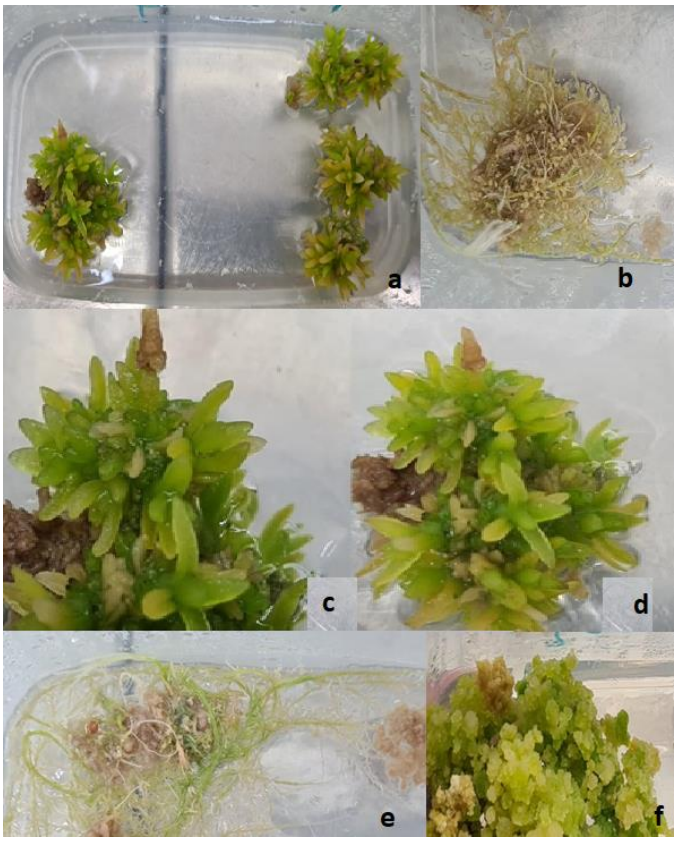

Fig. 4. Shoot, root and callus formation in *G.germanicopolitana* a) KIN (0 mg/L) + NAA (0.5 mg/L) shoot formation in MS b) BAP (1 mg/L) + IBA (0.5 mg/L) root formation in MS c) KIN (0.5 mg/L) + NAA (0.5 mg/L) shoot formation in NN d) BAP (1 mg/L) + NAA (0,5 mg/L) shoot formation in NN e) KIN (0 mg/L) + IBA (0.5 mg/L) root formation in MS f) KIN (0.5 mg/L) + IBA (0.5 mg/L) callus formation in NN

Organogenic Callus Formation: The data on callus formation obtained in the study were evaluated statistically. It was aimed to monitor the differences in shoot and/or root formation from callus between MS and NN basal media and different PGR combinations. Due to the scarcity of data obtained on root formation, statistical evaluations could not be made. However, since the amount of shoot formation is higher, the statistical significance of the differences between the basal medium and PGR combinations according to the shoot formation status of the explants was investigated (Table 1). The independent variable "PGR combination" was found to be statistically significant ($p<0.05$). The other independent variable, "basal media", was found to be statistically insignificant on its own. Based on the statistical difference in the data obtained in the study, it was observed that 36 different PGR combinations had different effects on shoot formation. In order to indicate the meaning of the statistical difference, a pairwise comparison was presented. The interactions of the 36 PGR combinations are investigated. Only the significant PGR combinations on shoot formation were included in Table 1. The mean number of shoot formation for each PGR combination and basal media were included in the below table.

Table 1. Interaction between basal media and some combinations of PGR in terms of shoot formation from organic callus

	NN		MS	
PGR Combinations	**Sample number**	**Mean± standard error**	**Sample number**	**Mean± standard error**
BAP 0.5 mg/L + IBA 0 mg/L	10	0.60±0.5*	10	0
BAP 0.5 mg/L + IBA 0.5 mg/L	10	0	10	0.30±0.4*
BAP 1 mg/L + IBA 0.5 mg/L	10	0	10	0.50±0.5*
BAP 0 mg/L + NAA 0.5 mg/L	10	0.60±0.5*	10	0.10±0.3
BAP 0.5 mg/L +NAA 0.25 mg/ L	10	0.40±0.5*	10	0
BAP 0.5 mg/L + NAA 0.5 mg/L	10	0.60±0.5*	10	0
BAP 1 mg/L + NAA 0.5 mg/L	10	1.0*	10	0.20±0.4
KIN 0 mg/L + IBA 0.5 mg/L	10	0.10±0.3	10	0.20±0.4*
KIN 0.5 mg/L + IBA 0 mg/L	10	0	10	0.10±0.3*
KIN 1 mg/L + IBA 0.25 mg/L	10	0	10	0.30±0.4*
KIN 0 mg/L + NAA 0.25 mg/L	10	0.20±0.4*	10	0.20±0.4
KIN 0 mg/L + NAA 0.5 mg/L	10	0.80±0.4*	10	0.50±0.5
KIN 0.5 mg/L + NAA 0 mg/L	10	0	10	0.30±0.4*
KIN 0.5 mg/L + NAA 0.25 mg/L	10	0.50±0.5*	10	0.30±0.4
KIN 0.5 mg/L + NAA 0.5 mg/L	10	1.0*	10	0.30±0.4
KIN 1 mg/L + NAA 0 mg/L	10	0.60±0.5*	10	0
KIN 1 mg/L + NAA 0.25 mg/L	10	0.40±0.5*	10	0
KIN 1 mg/L + NAA 0.5 mg/L	10	0.40±0.5*	10	0.10±0.3

(*)Represents the statistically significant interactions between PGR combination and basal media

In the in vitro propagation of *G.germanicopolitana*, shoot formation showed a better development in NN compared to the MS. In addition to the basal medium, it was statistically revealed that PGRs were highly effective on shoot growth. In terms of highest shoot number obtained, 1 mg/L BAP + 0.5 mg/L NAA (35 units), 0.5 mg/L KIN + 0.5 mg/L NAA (40 units) and 0 mg/L NN containing KIN + 0.5 mg/L NAA (50 units) PGR combinations were found in the NN basal media. Shoot formation also took place in MS in which 0 mg/L KIN + 0.5 mg/L NAA, 1 mg/L BAP + 0.5 mg/L IBA was added (Figure 4a and 4b). The sizes of the shoots here vary between 0.1 cm and 2 cm. The lowest shoot number was obtained from NN containing combinations of 0 mg/L KIN + 0.25 mg/L NAA (20 units) and 0 mg/L KIN + 0.5 mg/L NAA (10 units), PGR and 1 mg/L NAA. It was obtained from MS containing combinations of L KIN + 0.5 mg/L NAA, 0.5 mg/L KIN + 0 mg/L IBA. The sizes of the shoots developed here vary between 0.1 cm and 0.5 cm. There was no shoot formation in the roots developed from the callus. Although callus was found in environments containing both auxin and cytokinin group PGR, they could not be fully planted. However, roots with different lengths emerged. The length of the roots varies between 0.5 cm and 8 cm. Root formation was obtained in MS (Figure 4b). The highest root formation rate was found in MS and the combination that gave the best results was the combination containing 0 mg/L KIN + 0.5 mg/L IBA, 1 mg/L BAP + 0.25 mg/L IBA (Figure 4e).

Non-Organogenic Callus Formation: Simple callus or root formation was achieved in most of the explants cultured in MS and NN basal media. While targeting organogenesis from callus, initial PGR combinations used in the NN basal medium were added. The callus without organ-forming ability continued to proliferate. No organogenic formation was obtained at this stage. The most abundant callus development was obtained in combinations of 0 mg/L BAP + 0.25 mg/L NAA, 0.5 mg/L KIN + 0.5 mg/L IBA (Figure 4f). The lowest amount of callus was observed in the combination of 0.5 mg/L KIN + 0.25 mg/L IBA. NN medium in our study and it was noticed that root development was obtained from MS medium. It was observed that the selected PGR combinations had different effects when added to each medium.

Shoot Number: The highest shoot number obtained from organogenic callus was 35, 40 and 50 numbers, respectively, with 1 mg/L BAP + 0.5 mg/L NAA, 0.5 mg/L KIN + 0.5 mg/L NAA and 0 mg/L KIN + 0.5 mg. NN to which the /L NAA combination was added was found in the basal (Figure 4c and 4d). Shoot formation was also found in MS medium where 0 mg/L KIN + 0.5 mg/L NAA, 1 mg/L BAP + 0.5 mg/L IBA was added. The dimensions of the shoots vary between 0.1 cm and 2 cm (Figure 4a). The lowest number of shoots is 20 and 10, and 0 mg/L KIN + 0.25 mg/L NAA, 0 mg/L KIN + 0.5 mg/L, from NN medium containing their combinations and 1 mg/L KIN + 0.5 mg/L NAA, obtained from MS medium containing combinations of 0.5 mg/L KIN + 0 mg/L IBA. The sizes of the shoots vary from 0.1 cm to 0.5 cm. The highest root count was found in MS medium and it was noticed that the combination that gave the best results was the combination containing 0 mg/L KIN + 0.5 mg/L IBA, 1 mg/L BAP + 0.25 mg/L IBA (Figure 4f). The length of the roots varies between 0.5 cm and 8 cm. The most abundant non-organogenic callus development was obtained in combinations of 0 mg/L KIN+ 0.25 mg/L NAA, 0.5 mg/L KIN+ 0.5 mg/L IBA (Figure 4a). The lowest amount of callus was observed in the combination of 0.5 mg/L KIN + 0.25 mg/L IBA (Figure 4e).

4. Discussion

It is the first and only in terms of in vitro propagation of the plant. It is a very comprehensive optimization study in terms of testing two different basal media used in the initial phase, 36 different PGR combinations. This subject is suitable for looking at the medicinal and cosmetic properties of the shoots obtained from the plant *G. germanicopolitana*, which will be studied in the future.

For the in vitro micropropagation of plants of the Caryophyllaceae family, shoot tips and nodes have been successfully used as explant sources in many studies (Ahroni et al., 1997; Miranda et al., 1999; Morariu et al., 2008; Çördük and Akı, 2010; Aslam et al., 2012; Kiani et al., 2012; Toaima et al., 2013; Tejavathi and Indira, 2013; Thiem et al., 2013; Shaulo et al., 2014; Teteryuk and Mikhovich, 2020). The superficial sterilization protocol applied to prepare the plant for planting under in vitro conditions was effective for the plant, which was taken from the field and brought directly to the laboratory conditions, and infection formation was very rare during the study. However, because of the very slow regeneration rate of the plant, the first callus formation appeared only after 2 months, and the shoot and root developments obtained by indirect regeneration could be observed after 6 months. There have also been cases in the study where some explants did not show any vital signs even though they were not infected. After sterilization, the explants were planted in MS and NN basal media prepared with 36 different PGR combinations. All combinations were prepared as 10 replications in the experiments. Effective and strong surface sterilization, which was carried out in order to prevent possible infection in explants brought to laboratory conditions and prepared for in vitro micropropagation, caused a decrease in the regeneration ability of the plants.

In *G.germanicopolitana*, shoot growth of NN medium gave better results than MS medium, while MS medium provided callus development and root formation. The same PGR combinations were used in both media, but different growth results were obtained.

The data obtained from the in vitro propagation of *G.germanicopolitana* were evaluated statistically. The differences between the growth effects of the basal media and PGR combinations were compared statistically and the differences between the averages were found to be significant The Games-Howell test was used to make multiple comparisons between PGR combinations. “PGR combination” was statistically significant among the independent variables ($p<0.05$). It turned out that 36 different PGR combinations have different effects on shoot formation. Pairwise comparison has been put forward in order to indicate the meaning of statistical difference. For this purpose, it was investigated whether there was a statistical difference between PGR combinations in terms of average shoot formation rate.

Unlike the number of shoots we obtained in our study, Aslam et al. (2012), obtained in MS medium supplemented with 1 mg/L BAP high shoot formation and maximum shoot length from the

apical meristems of *G. paniculata*. The best medium for root growth 85% was observed in MS medium containing 0.5 mg/L NAA. Toaima et al. (2013), on the other hand, shoot tips of *Gypsophila perfecta*, *Gypsophila paniculata* L. plants were cultured in MS medium containing different doses of BAP and NAA PGRs in micropropagation. The highest shoot formation was obtained from MS medium containing 0.5 mg/L each of BAP and NAA. Ahroni et al. (1997) tried to achieve shoot regeneration by using internodes of Arbel cultivar for in vitro propagation of *Gypsophila paniculata* L. TDZ, BAP, KIN, or Zeatin were used as the cytokinin source and NAA as the auxin source. 100% of the explants cultured in MS medium containing TDZ PGR provided shoot formation, and 19 shoots developed per explant. Teteryuk and Mikhovich (2020) used tissue culture method to obtain callus from *Gypsophila uralensis* less seedlings. A combination of 1 mg/L BAP + 0.1 mg/L IAA PGR was added to MS and Woody Plant Medium (WPM) media. Indirect shoot regeneration was obtained from 90% of the callus. MS medium with different doses of IAA was used for the rooting step. Aslam et al. (2012) apical meristems of *Gypsophila paniculata* L. were used as explants. Different concentrations of NAA (0.1, 0.2, 0.3, 0.4 & 0.5 mg/L) were used for the rooting medium of the developing shoots. Root development was observed in MS medium containing 0.5 mg/L NAA with a rate of 85%. Kiani et al. (2012), shoot tips of seeds obtained from the plant of *Matthiola incana* were used in this study. The highest root number (1.85) and the longest root (5.2 cm) of the developing shoots were observed in the medium containing 2 mg/L NAA. In our study, however, the highest root count was found in MS medium, and it was noticed that the combination that gave the best results was the combination containing 0 mg/L KIN + 0.5 mg/L IBA, 1 mg/L BAP + 0.25 mg/L IBA (Figure 4f). The length of the roots varies between 0.5 cm and 8 cm. While the best shoot and root development was obtained from MS basal medium in other studies, the best shoot formation was found in NN medium in our study and it was noticed that root development was obtained from MS medium. It was observed that the selected PGR combinations had different effects when added to each medium.

As a result, in the National Science and Technology Policies 2003-2023 Strategy Document (TÜBİTAK, 2011) prepared by TUBITAK, in line with the goal of sustainable development, the goal of "Developing technologies for the characterization and conservation of gene resources and the protection of biological diversity" has been stated. In this study, in addition to its medicinal and aromatic properties, *Gypsophila germanicopolitana* (Ekim et al., 2000), which is locally endemic and included in the "critically endangered (CR)" plants list by IUCN. It is the first and only in terms of in vitro propagation of the plant. It is a very comprehensive optimization study in terms of testing two different media, 36 different PGR combinations and one type of main solidifier used in the initial phase. This subject is suitable for looking at the medicinal and cosmetic properties of the shoots obtained from the plant *G. germanicopolitana*, which will be studied in the future.

Saponins, a type of glycoside, belong to the class of organic chemical compounds, generally toxic and plant-derived. This saponin extract, which is obtained from the rhizomes of the calyx, is widely used in the treatment of cough, respiratory system, and some other diseases, in the production of soaps and liquors, in film emulsions and chemical cleaners, in the production of fire extinguishers as a foaming agent. It is used as an additive in the production of sweet varieties called "Tahin Helvası", "Koz Helva" and "Paşa Lokumu" (Özçelik ve Yıldırım, 2011). Although saponins are grouped as amphiphilic glycosides, they are also structurally called lipophilic steroids or triterpenoid derivatives. In addition, they are also called glycosides with one or more carbohydrate side chains combined (Kocaoğlu, 2004).

Some *Gypsophila* species are resistant to some stress factors such as cold, drought, salinity, and accumulation of high boron elements. They determined that *Gypsophila perfoliata L.* and *Gypsophila sphaerocephala* Fenzl ex Tchihat plants have the potential to be hyperaccumulatory in terms of boron, and that the plant develops healthily even though there is approximately 3500 mg/kg Boron in the leaves of *G. sphaerocephala* (Babaoğlu et al., 2004). Therefore, determining the hyperaccumulatory potential of *G. arrosti* which is the same species as *G. sphaerocephala* and *G. perfoliata* plants which are easier to produce in terms of agriculture is important for agricultural areas in terms of boron toxicity problem. In addition, it is thought that boron toxicity caused by the use of artificial fertilizers causes' loss in crop yield and this damage can be eliminated with *Gypsophila* species growing in the same area (Özçelik ve Yıldırım, 2011).

Warm water obtained by boiling the roots of many species of *Gypsophila* is used for cleaning silk and delicate fabrics. İnan (2006) reported that after the root stems are thoroughly boiled with water, silk and precious fabrics kept in this water have their color and brightness of the fabrics cleaned without deterioration. It states that it is also used in medicine production and gold bleaching (Özçelik ve Özgökçe, 1999). The juice obtained from the decoction of the rhizomes of *Gypsophila* has diuretic effects. In addition, it has been reported that the liquid has a spot and acne removal effect in the field of cosmetics (Ayeh et al., 2009).

British scientists Dr. David Flavell and his wife Bee who lost their son to leukemia made an important study on *Gypsophila*, according to the research, this plant, which is also called 'Spring Star', has been determined to be a door of hope for most leukemia patients. There is a substance in the flowers of the Çöven medicinal plant that will increase the effect of leukemia drugs. Thanks to this substance, many patients with leukemia can be cured. Studies are ongoing to ensure that the research is fully clarified.

In the study, direct plant regeneration target from shoot tip and internode explants of *G. germanicopolitana* plant could not be fully achieved. However, plant regeneration was targeted by the indirect organogenesis method from the obtained callus. Due to the fact that *G. germanicopolitana* is included in the list of plants in critical danger of extinction according to the International Union for Conservation of Nature and Natural Resources (IUCN) red list category (Ekim et al., 2000), it can be taken from the field in limited quantities and the plant has been directly in vitro propagated accordingly. Regeneration could not be achieved due to problems in regeneration ability. However, it was observed that the concentration, combination, and types of PGRs used in the study had a positive effect on callus formation.

4. Conclusion

In this study, it was aimed to micropropagation of the *Gypsophila germanicopolitana*, which is in the category of locally endemic and endangered plants, by using shoot tips and nodes as explant sources, by culturing in vitro conditions in different basal media and plant growth regulators at different doses. This study aims to establish the optimum propagation and regeneration protocol of the selected endemic plant.

Although no in vitro propagation studies have been found on the edaphic endemic *Gypsophila germanicopolitana*, the findings obtained from the study are important in terms of in vitro propagation and dissemination of the species.

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