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In vitro PROPAGATION OF *Gypsophila pilulifera*, AN ENDANGERED ENDEMIC ORNAMENTAL PLANT SPECIES

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ABSTRACT

Gypsophila pilulifera Boiss. & Heldr., an endemic and endangered species is an economically important plant that has a potential used in medicine thank to the saponins it contains. It is also an ornamental plant. In this study, the production was carried out under *in vitro* conditions in order to protect and economically evaluate the species, which is in danger of extinction due to its distribution in a very narrow area open to anthropogenic effects. For this purpose, seed and shoot tip explants of *G. pilulifera* were cultured in Murashige and Skoog (MS) medium containing different concentrations of 6-benzylaminopurine (BAP), indole-3-acetic acid (IAA) and naphthaleneacetic acid (NAA). Explants were obtained from two different locations of the natural habitats of *G. pilulifera* and more plants were obtained in the tissue culture from both explant types taken from the 1st location. Propagation from seed gave better results, and 96.67% of the seeds cultured in hormone-free MS medium were germinated and rooted. The shoot tip showed lower regeneration *in vitro* than the seed explant. Especially the rooting percentage is quite low and the highest rooting and shoot formation was obtained in MS medium containing 2 mg/L BAP + 2 mg/L IAA. In this medium, 10.50 ±0.17 shoots were obtained per plant, and rooting (1.67 ±0.09 per plant) was also achieved only in this medium. Seedlings obtained in tissue culture continue to develop successfully in field conditions, and this method has been determined as a suitable method for *ex-situ* conservation and economically rapid production of the species.

Key words: *Gypsophila*, micropropagation, seed, explant, hormones, danger of extinction

INTRODUCTION

The genus *Gypsophila* L., a member of the *Caryophyllaceae* family, consists of annual, biennial and perennial herbs, and is represented by 55 species in Türkiye, 33 of which are endemic [Işık et al. 2015].

The members of the *Gypsophila* genus which has an important place in the floristry industry, are used both as an ornamental plant in gardens and as a filling material in flower. In addition to being important for the ornamental plant sector, some of the species are also commercially importance as a source of saponins used in haemolytic tests [Delay et al. 1997, Rahman 2002]. The roots of *Gypsophila* are rich in triterpenoid saponins used in the structure of film emulsions and fire extinguishers, in the manufacture of drugs, cosmetics and detergents [Hostettmann and Marston 1995, Rahman 2002, Gevrenova et al. 2010, Arslan et al. 2012]. In addition to its use in the treatment of upper respiratory tract diseases such as cough and cold in traditional medicine, it also has a therapeutic use in gastrointestinal system diseases [Rahman 2002, Simeonova et al. 2013]. The production of this genus, which is so significant commercially, is very important in terms of obtaining it quickly and economically and protecting the species in its natural life.

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The most basic problems encountered in the production of *Gypsophila* are that the varieties cannot be produced with seeds because they are generally unisex, and the cuttings have a low rooting rate in vegetative production. Therefore, the commercial cultivars used in the production of cut flowers are very limited. In addition to these, phytoplasma disease is another factor limiting production. When all these are evaluated together; it has become a necessity to use tissue culture techniques for plants production and breeding studies, to obtain different flower colors and to develop disease-resistant varieties of *Gypsophila* species [Shillo 1985, Ahroni et al. 1997, Zucker et al. 1997, Gera et al. 2007, Rashid et al. 2012]. Besides, *in vitro* culture system allows for the isolation of saponins in large quantities, which are of great economic importance, without harming natural populations of the *Gypsophila* species [Gevrenova et al. 2010]. Tissue culture studies in *Gypsophila* have focused mainly on *Gypsophila paniculata* and especially explants that were used for *in vitro* propagation of this species, which were leaves [Zucker et al. 1997], stem segments [Ahroni et al. 1997], axillary buds [Kapchina-Toteva and Stoyanova 2003], shoots [Rady and Bekheet 2008] and, apical meristems [Rashid et al. 2012].

One of the most striking species of this genus, *Gypsophila pilulifera* Boiss. & Heldr. which grows in a very narrow area open to anthropogenic effects within the borders of Antalya province is in the CR category [Muca 2017]. The crude saponin extract obtained from this endangered species has a scavenging effect on free radicals [Chima et al. 2014]. It was also determined that the composition of a cytotoxic triterpenoid saponin isolated from the underground parts of the species showed significant cytotoxicity against the IC A549 cell line (lung carcinoma cell line) [Arslan et al. 2012]. Studies on *G. pilulifera* are focused at determining the saponin content of the species, and there is no previous study on the propagation of the species by tissue culture. In this study, it is aimed to reveal the data of the *in vitro* production of the species, which has an important potential for use in the medical and ornamental plants sectors. In the medium and treatments suggested in this study, the rapid production of the species under *in vitro* conditions will ensure that this species under the threat of extinction is preserved for the next generations and the saponins it contains will be brought into the production from the natural populations, without harming. In addition, this study contains a potential to serve as a model for closely related groups.

MATERIAL AND METHODS

Collection of plant material from the field and disinfection

The plant material was obtained from two different locations with different ecological characteristics in the Lara region of Antalya province, Turkey which is the natural distribution area of the species. The mature seeds and cuttings of *Gypsophila pilulifera* were used for the *in vitro* multiplication experiments. The seeds collected in October 2019 were ventilated on blotting paper at room temperature (24 ±2) for 7 days. Leafy cuttings (15–20 cm) were taken from the fresh shoot tips of the plant in April 2020.

Before disinfection, explants taken into a jar were thoroughly washed with detergent and rinsed in running tap water. Explants were kept in 20% sodium hypochlorite (NaOCl) solution with continuous stirring for 15 min. Disinfection was completed by treating with 70% ethyl alcohol for 5 min and rinsing with sterile distilled water 3 times for 5 min.

After that, for axillary shoot proliferation, stem cuttings containing apical meristem were prepared to be approximately 5 cm long and then taken into the culture medium.

Culture medium and treatments

MS [Murashige and Skoog 1962] basal medium containing 30 g/L sucrose, 7 g/L agar and different combinations of plant growth regulators (PGR) [(0.5–4 mg/L NAA), (0.5–4 mg/L IAA), (1–2 mg/L BAP)] was used in the experiments. The pH of all media was adjusted to 5.7 before autoclaving. The cultures were maintained at 24 ±2 and a 16/8 h (light/dark) photoperiod with fluorescent light.

In vitro germination and vegetative growth parameters of *G. pilulifera*

The experiments were continued for 75 days and at the end, the number of shoots and roots formed, shoots

length, roots length, number of leaves and the number of axillary shoots were determined for both explant types. In addition to these, germination rate and germination time was determined for seed explants.

The plants obtained in *in vitro* conditions were transferred to pots containing 1 : 1 (peat : perlite) and subjected to acclimatization in the plant growth chamber. After 2 months of acclimatization, the surviving plants were counted and transferred to the field. At the end of 10 months, survival and flowering rate in the field were determined. In the evaluation of all these data, abbreviations are used for parameters in statistical analysis: GP – germination pertentage, GT – germination time, SL – shoot length, RL – root length, NL – number of leaves, NS – number of shoot, RN – root number, in the text.

Data evaluation and statistical analysis

The trials were conducted with three replicates and ten or five explants were used per repeat for seed and shoot tip culture respectively. For all data obtained, before the analyzes were performed in the study, it was investigated whether the data showed normal distribution or not with the tests of conformity to the normal distribution and all data correctness possibilities have been verified (Shapiro-Wilk and Kolmogorow-Smirnov). Distributions conforming to the normal distribution and providing the parametric test assumptions were evaluated. Data was evaluated with Student's-t Test and One Way ANOVA tests ($\alpha = 0.05$) in the SPSS package program (IBM, version 21.0).

According to the dataset and pattern: For the data obtained from the *in vitro* production and culture trials, the t-test was firstly used to analyze whether there were any differences between two localities (loc. 1 and loc. 2) from Lara region with respect to the relevant variables, and then the ANOVA test was used to evaluate whether there were any differences among the trials (plant growth regulators) in each location. Subsequently, similar/different trials were exhibited with multiple comparison tests (MCT: Duncan) in each localities. Finaly, for trials results in the tables created for these analyses, "the ones shown with the same letter are similar to each other, while the ones shown with different letters are different from each other".

RESULTS

Germination parameters of seed explants

The effect of plant growth regulators on the germination capacity of *Gypsophila pilulifera* seeds were determined under *in vitro* condition (Tab. 1). A statistically significant difference was found in terms of germination rate and germination days of seeds collected from two different locations, both according to the medium used and the location where the seeds were collected. The highest germination rate for both locations was obtained from hormone-free MS medium. While 96.67% of the seeds collected from the 1st location germinated, this rate was determined as 76.67% for the 2nd location in this medium (Tab. 1). In terms of germination rates, the seeds collected from the 1st location generally gave better results, and no germination could be obtained in some media for both locations. In terms of the day until germination, a statistical difference was determined between the two locations according to the media used ($tGT = 9.31$; $df = 1588.56$; $p < 0.0001$). The shortest germination time was determined on MS medium containing 2 mg/L BAP + 2 mg/L IAA (3.00 day), and 1 mg/L BAP (3.70 day) for the 1st and 2nd location, respectively (Tab. 1).

Morphological parameters of the seed explants

A statistical difference was found between the two locations in terms of vegetative parameters such as SL, RL and NL of the plants obtained from seeds germinated in *in vitro* conditions, according to the media used ($tGT = 9.31$; $df = 1588.56$; $p < 0.0001$, $tSL = 11.97$; $df = 952.08$; $p < 0.0001$, $tRL = 4.58$; $df = 856.11$; $p < 0.0001$, $tNL = 12.01$; $df = 1157.29$; $p < 0.0001$). Vegetatif qualities of the plants obtained from the seed explants collected from the 1st location were more superior than the 2nd location. The highest SL (34.00 ±1.21 mm), RL (39.00 ±1.40 mm), and NL (12.93 ±0.53) averages were obtained in hormone-free MS medium, and it was determined that it was significantly higher than the other applications according to Duncan test. Similarly, the vegetative parameters of the plants obtained from the 2nd location also differed according to the medium used and were found to be statistically significant ($p < 0.001$) (Tab. 2). Although the highest SL (4.57 ±0.49 mm), RL (5.87 ±0.62 mm) and NL (4.30 ±0.34 number) were also obtained from the

Table 1. Germination parameters of the seed explants of *Gypsophila pilulifera* under *in vitro* condition

Plant growth regulators (mg/L)	GP (%)		GT (day)	
	loc. 1	loc. 2	loc. 1	loc. 2
MS O	96.67 ±3.33 ^{j}	76.67 ±3.33 ^{d}	6.13 ±0.23 ^{d}	4.73 ±0.49 ^{bcd}
1 BAP	83.33 ±3.33 ^{i}	56.67 ±3.33 ^{b}	5.20 ±0.44 ^{cd}	3.70 ±0. 61 ^{b}
2 BAP	80.00 ±0.00 ^{hi}	0.00 ±0.00 ^{a}	5.23 ±0.49 ^{cd}	0.00 ±0.00 ^{a}
0.5 IAA	76.67 ±3.33 ^{hi}	76.67 ±3.33 ^{d}	5.10 ±0.53 ^{cd}	5.10 ±0.53 ^{cd}
1 IAA	83.33 ±3.33^{i}	83.33 ±3.33 ^{d}	5.53 ±0.47 ^{cd}	5.67 ±2.60 ^{d}
2 IAA	66.67 ±3.33 ^{ef}	0.00 ±0.00 ^{a}	4.37 ±0.54 ^{bc}	0.00 ±0.00 ^{a}
4 IAA	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}
0.5 NAA	70.00 ±0.00 ^{fg}	53.33 ±3.33 ^{b}	5.83 ±0.71 ^{cd}	4.80 ±0.83 ^{bcd}
1 NAA	60.00 ±0.00 ^{d}	50.00 ±5.77 ^{b}	5.63 ±0.86 ^{cd}	4.50 ±0.84 ^{bcd}
2 NAA	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}
4 NAA	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}
1 BAP + 0.5 IAA	80.00 ±0.00 ^{hi}	66.67 ±3.33 ^{c}	5.07 ±0.48 ^{cd}	4.60 ±0.61 ^{bcd}
1 BAP + 1 IAA	76.67 ±3.33 ^{hi}	56.67 ±3.33 ^{b}	5.03 ±0.52 ^{cd}	3.87 ±0.63 ^{bc}
1 BAP + 2 IAA	63.33 ±3.33 ^{de}	0.00 ±0.00 ^{a}	4.27 ±0.60 ^{bc}	0.00 ±0.00 ^{a}
1 BAP + 4 IAA	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}
2 BAP +0.5 IAA	80.00 ±0.00 ^{hi}	0.00 ±00 ^{a}	5.17 ±0.49 ^{cd}	0.00 ±0.00 ^{a}
2 BAP + 1 IAA	73.33 ±3.33 ^{gh}	0.00 ±0.00^{a}	4.83 ±0.55 ^{cd}	0.00 ±0.00 ^{a}
2 BAP + 2 IAA	46.67 ±3.33 ^{bc}	0.00 ±0.00 ^{a}	3.00 ±0.60 ^{b}	0.00 ±0.00 ^{a}
2 BAP + 4 IAA	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}
1 BAP + 0.5 NAA	80.00 ±0.00 ^{hi}	56.67 ±3.33 ^{b}	6.40 ±0.59 ^{d}	5.10 ±0.83 ^{cd}
1 BAP + 1 NAA	50.00 ±0.00 ^{c}	50.00 ±10.00 ^{b}	5.07 ±0.93 ^{cd}	4.97 ±0.92 ^{bcd}
1 BAP + 2 NAA	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}
1 BAP + 4 NAA	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}
2 BAP + 0.5 NAA	43.33 ±3.33 ^{b}	0.00 ±0.00 ^{a}	4.33 ±0.92 ^{bc}	0.00 ±0.00 ^{a}
2 BAP + 1 NAA	46.67 ±3.33 ^{bc}	0.00 ±0.00 ^{a}	4.33 ±0.92 ^{bc}	0.00 ±0.00 ^{a}
2 BAP + 2 NAA	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}
2 BAP + 4 NAA	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}
ANOVA	F = 281,80*	F = 121.86*	F = 23.44*	F = 30.77*

* df1 = 26, df2 = 783, $p < 0.0001$, n = 30

Table 2. Shoot and root length and the number of leaves of the plants obtained from *G. pilulifera* seeds under *in vitro* condition

Plant growth regulators (mg/L)	SL (mm)		RL (mm)		NL	
	loc. 1	loc. 2	loc. 1	loc. 2	loc. 1	loc. 2
MS O	34.00 ±1.21 ^{i}	4.57 ±0.49 ^{c}	39.00 ±1.40 ^{b}	5.87 ±0.62 ^{b}	12.93 ±0.53 ^{h}	4.30 ±0.34 ^{c}
1 BAP	6.23 ±0.53 ^{ghi}	3.20 ±0.52 ^{c}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	4.13 ±0.38 ^{fg}	2.27 ±0.37 ^{c}
2 BAP	7.20 ±0.69 ^{i}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	3.93 ±0.40 ^{efg}	0.00 ±0.00 ^{a}
0.5 IAA	6.53 ±0.68 ^{hi}	3.27 ±0.34 ^{cd}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	3.07 ±0.31 ^{cde}	3.07 ±0.31 ^{de}
1 IAA	5.50 ±0.46 ^{fgh}	4.00 ±0.34 ^{de}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	4.53 ±0.40 ^{g}	3.33 ±0.28 ^{c}
2 IAA	5.47 ±0.67 ^{fgh}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	4.20 ±0.51 ^{g}	0.00 ±0.00 ^{a}
4 IAA	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}
0.5 NAA	3.27 ±0.40 ^{cde}	1.70 ±0.32 ^{b}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	1.40 ±0.17 ^{b}	1.07 ±0.19 ^{b}
1 NAA	2.77 ±0.43 ^{bcd}	1.70 ±0.32 ^{b}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	1.20 ±0.18 ^{b}	0.00 ±0.00 ^{b}
2 NAA	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}
4 NAA	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}
1 BAP + 0.5 IAA	6.20 ±5.89 ^{ghi}	3.83 ±0.50 ^{cde}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	4.20 ±0.42 ^{efg}	2.67 ±0.35 ^{cd}
1 BAP + 1 IAA	6.00 ±0.62 ^{ghi}	3.30 ±0.54 ^{cd}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	3.93 ±0.43 ^{efg}	2.27 ±0.37 ^{c}
1 BAP + 2 IAA	5.10 ±0.73 ^{fgh}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	3.80 ±0.54 ^{defg}	0.00 ±0.00 ^{a}
1 BAP + 4 IAA	0.00 ±0.00 ^{a}	3.30 ±0.54 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}
2 BAP +0.5 IAA	5.13 ±0.49 ^{fgh}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	3.20 ±0.30 ^{cdef}	0.00 ±0.00 ^{a}
2 BAP + 1 IAA	4.73 ±0.54 ^{efg}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	2.93 ±0.33 ^{cd}	0.00 ±0.00 ^{a}
2 BAP + 2 IAA	4.00 ±0.80 ^{def}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	2.80 ±0.56 ^{c}	0.00 ±0.00 ^{a}
2 BAP + 4 IAA	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}
1 BAP + 0.5 NAA	4.37 ±0.41 ^{ef}	2.23 ±0.36 ^{b}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	1.60 ±0.15 ^{b}	1.13 ±0.18 ^{b}
1 BAP + 1 NAA	1.83 ±0.35 ^{bc}	1.77 ±0.34 ^{b}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	1.00 ±0.19 ^{ab}	1.00 ±0.19 ^{b}
1 BAP + 2 NAA	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}
1 BAP + 4 NAA	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}
2 BAP + 0.5 NAA	1.90 ±0.40 ^{bc}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.87 ±0.18 ^{ab}	0.00 ±0.00 ^{a}
2 BAP + 1 NAA	1.43 ±0.31 ^{ab}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.87 ±0.18 ^{ab}	0.00 ±0.00 ^{a}
2 BAP + 2 NAA	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}
2 BAP + 4 NAA	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}
ANOVA	F = 171.24*	F = 38.85*	F = 780.23*	F = 89.26*	F = 81.93*	F = 45.78*

* $df_1 = 26$, $df_2 = 783$, $p < 0.0001$, $n = 30$

Table 3. Number of shoots and roots and the shoot length of the plants obtained from shoot tip explant of *G. pilulifera* under *in vitro* conditions

Plant growth regulators (mg/L)	NS		SL (mm)		RN	
	loc. 1	loc. 2	loc. 1	loc. 2	loc. 1	loc. 2
MS O	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}
1 BAP	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}
2 BAP	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}
0.5 IAA	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}
1 IAA	2.60 ±0.23 ^{c}	0.70 ±0.16 ^{b}	0.39 ±0.03 ^{c}	0.14 ±0.03 ^{c}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}
2 IAA	3.57 ±0.09 ^{d}	2.63 ±0.12 ^{d}	0.62 ±0.01 ^{d}	0.54 ±0.09 ^{f}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}
4 IAA	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}
0.5 NAA	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}
1 NAA	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}
2 NAA	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}
4 NAA	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}
1 BAP + 0.5 IAA	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}
1 BAP + 1 IAA	3.80 ±0.09 ^{e}	2.57 ±0.10 ^{d}	0.65 ±0.01 ^{d}	0.42 ±0.01 ^{e}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}
1 BAP + 2 IAA	6.17 ±0.09 ^{f}	4.30 ±0.08 ^{e}	0.86 ±0.01 ^{f}	0.35 ±0.009 ^{d}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}
1 BAP + 4 IAA	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}
2 BAP +0.5 IAA	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}
2 BAP + 1 IAA	3.50 ±0.10 ^{d}	2.20 ±0.10 ^{d}	0.70 ±0.02 ^{e}	0.70 ±0.02 ^{g}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}
2 BAP + 2 IAA	10.50 ±0.17 ^{f}	6.83 ±0.17 ^{f}	1.71 ±0.01 ^{g}	0.99 ±0.02 ^{h}	1.67 ±0.09 ^{b}	1.07 ±0.10 ^{b}
2 BAP + 4 IAA	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}
1 BAP + 0.5 NAA	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}
1 BAP + 1 NAA	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}
1 BAP + 2 NAA	0.50 ±0.16 ^{b}	0.50 ±0.16 ^{b}	0.10 ±0.04 ^{b}	0.10 ±0.04 ^{bc}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}
1 BAP + 4 NAA	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}
2 BAP + 0.5 NAA	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}
2 BAP + 1 NAA	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}
2 BAP + 2 NAA	0.50 ±0.21 ^{b}	0.50 ±0.21 ^{b}	0.08 ±0.04 ^{b}	0.08 ±0.04 ^{cd}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}
2 BAP + 4 NAA	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}	0.00 ±0.00 ^{a}
ANOVA	F = 878.69*	F = 426.66*	F = 765.84*	F = 298.48*	F = 362.50*	F = 20.01*

*$df_1 = 26$, $df_2 = 783$, $p < 0.0001$, n = 30

hormone-free MS medium for this location, they were quite low compared to the 1st location (Tab. 2). The rooted plants were first transferred to pots and then to the field (Fig. 1), and their survival and flowering rates were determined.

Morphological parameters of the shoot tip explants

A statistically significant difference was found in between 1st and 2nd locations in terms of the averages of NS, SL and RN frequencies in tissue culture experiments established with shoot tip explant (tNS = 3.86; df = 1415,82; $p < 0.0001$, tSL = 3.93; df = 1396,92; $p < 0.0001$ tRN = 3.55; df = 1093,02; $p < 0.0001$). The highest shoot formation for both locations was obtained in MS medium containing 2 mg/L BAP + 2 mg/L IAA (Tab. 3, Fig. 1). 10.50 ±0.172 and 6.83 ±0.17 new shoots were obtained in this medium for the 1st and 2nd location, respectively. Similarly, the highest shoot length for both locations was obtained in the same medium. The second best medium in terms of shoot number was determined as 1 mg/L BAP + 2 mg/L IAA medium (6.17 shoot per explant) (Fig. 1), and new shoot formation did not occur in some of the tested media. When the seed and shoot tip explants are evaluated in terms of shoot length, it is seen that the shoot length obtained from the seed explant was higher (34.00 ±1.21mm). In addition, there was a significant loss of shoot tip explants due to vitrification (Fig. 2). Root formation occurred in only MS medium containing 2 mg/L BAP + 2 mg/L IAA for both locations and differed from other media according to Duncan test.

Flowering and survival rates of the plants

Only plants obtained in MS hormone-free medium from seed explants of *G. pilulifera* were able to survive in tissue culture conditions and transferred to the field condition. The shoot tips cultured in MS media containing different plant growth regulators were able to remain survive in MS medium containing only 2 mg/L BAP + 2mg/L IAA and were transferred to the soil. It was determined that both explants obtained from the 1st location were superior in terms of survival rates in both tissue culture and soil and the total number of plants obtained. In field conditions, the flowering rate for both locations was 100% for the seed explant, and it is twice as high as for the shoot tip explant (Fig. 3, Tab. 4).

DISCUSSION

The use of natural species for commercial purpose is quite limited in Türkiye [Kaya et al. 2012]. The genus *Gypsophila* which is an economically very important genus, contains endemic species that should be evaluated for this purpose. *In vitro* culture is considered as an alternative and rapid method due to the difficulties encountered in propagation by seed and vegetative methods [Rashid et al. 2012]. For this purpose, the most widely produced *Gypsophila* species *in vitro* condition is *Gypsophila paniculata*, which is very important for the floristry industry. Shoot tip and apical meristem explants of *G. paniculata* were cultured in MS medium containing NAA and 6-BA for *in vitro* production [Rady 2006]. Different combination and concentrations of BAP, NAA and Kin were also used for the same purpose [Rashid et al. 2012]. In the study in which the apical meristem was used as an explant, the highest shoot formation and shoot length were obtained in MS medium supplemented with 1 mg/L BAP. The best rooting was achieved in medium containing 0.5 mg/L NAA [Rashid et al. 2012]. In our study, seed and shoot tip explants of *Gypsophila pilulifera*, which

Table 4. Survival and flowering rates of the explants in tissue culture and field conditions for *G. pilulifera*

Loc.	Explant type	Survival percentage of the explants in tissue culture (%)	Survival percentage of the plants in field condition (%)	Flowering percentage (%)
1	Seed	96.7	89.7	100
	Shoot tip	100	26.7	50
2	Seed	76.7	17.4	100
	Shoot tip	100	53.3	50

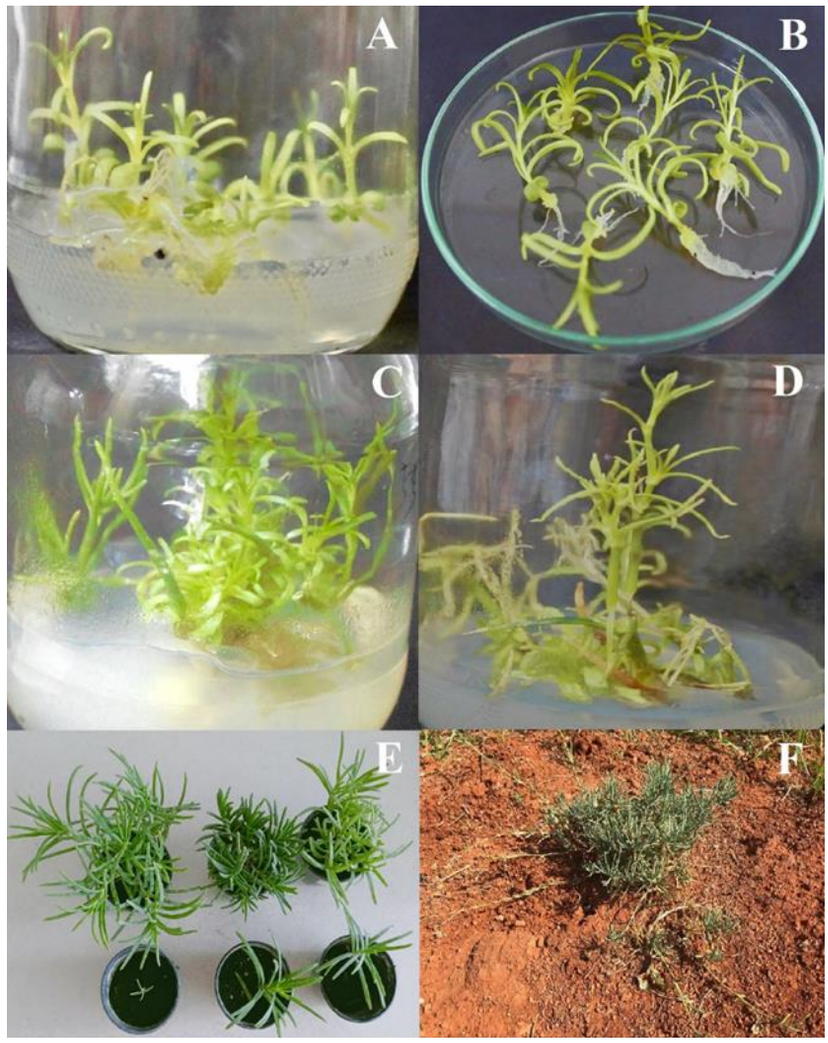

Fig. 1. A. The appearance of *G. pilulifera*, which germinated on hormone-free MS medium of the seeds collected from the 1st location, after 30 days. B. Rooted plants obtained in the same medium before transferred to the soil. C. Shoot formation from shoot tip explants collected from the 1st location on MS medium containing 2 mg/L BAP + 2mg/L IAA after 30 days. D. The plantlets formed from shoot tip explant on MS medium containing 1 mg/L BAP + 2 mg/L IAA. E. The appearance of the plants transferred to the soil in the pot after 30 days (upper: 1st loc.; bottom: 2nd loc.). F. The plants after 30 days transferred to the field

was produced for the first time *in vitro* were used for rapid multiplication. The cell division, differentiation and morphogenesis in explants cultured *in vitro* are closely related to the type and concentration of plant growth regulators used. Activation of lateral shoot formation *in vitro* is associated with the concentration of cytokinin added to the medium [Kapchina-Toteva and Stoyanova 2003]. Contrary to the above study, the most multiple shoot formation from shoot tip explants was obtained when auxin and cytokinin group hormones were used in combination in our study. Similarly, the longest shoot formation and the best rooting were obtained on MS medium containing 2 mg/L BAP + 2 mg/L IAA. In addition, the seed explant, which

Fig. 2. Appearance of vitrified (right) and non-vitrified (left) *G. pilulifera* plants in tissue culture

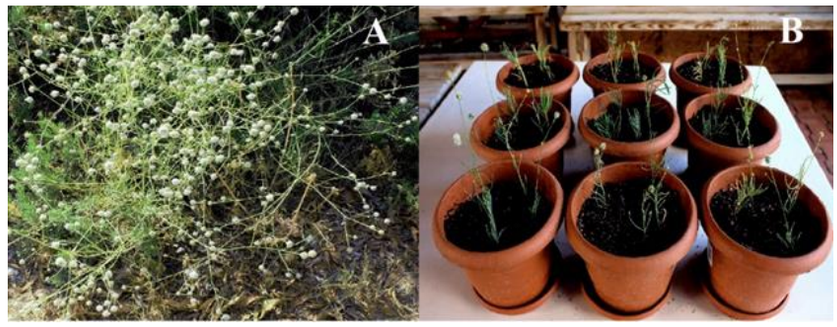

Fig. 3. Flowering of seed (A) and shoot tip (B) explants of *G. pilulifera* in field condition and pots

was not used in other studies, gave better results than the shoot type explant for *in vitro* production of *G. pilulifera*. In the study using shoot type explant of *G. paniculata*, generally high and low BA and NAA caused a decrease in shoot number and shoot length [Rady 2006]. A similar result was obtained in our study, and low or high IAA and NAA concentrations had a negative effect on shoot formation and length. On the other hand, the best results obtained in terms of both shoot and root parameters were obtained with germination of the seed explant in hormone-free MS medium.

Vitrification, which is an abnormal physiological condition seen in tissue cultured plants, greatly reduces survival rates when plants produced in tissue culture are transferred to field conditions. Enlarged leaves and stems in vitrified plants, which have a glassy appearance, are translucent and fragile [Gribble 1999]. The most serious problem limiting the production of *Gypsophila* in tissue culture is vitrification. The rate of vitrification also decreases in media containing lower concentrations of the plant growth regulators [Rady 2006]. The highest survival rate of plants grown in hormone-free MS medium in our study supports this result. In terms of vitrification, the seed explant gave better results, and most of the shoot tip explant died after a while as vitrified. In addition, the plants were rooted in the same medium without the need to be transferred to a special rooting medium. This is an advantageous situation in terms of reducing costs when considered on a commercial scale.

The explants used were obtained from two locations within a very narrow area where the species is

distributed in this study. It is noteworthy that the results obtained from the 1st location are quite high in terms of survival in both tissue culture and field conditions. If the locations are analyzed in more detail, it has been determined that there is intensive greenhouse agriculture around the 2nd location. It is thought that because of the mixing of pesticides and fertilizers used in this region into the soil and groundwater, the yield of explants taken from this region is reduced due to soil pollution. However, a more detailed soil analysis and studies would be more informative in this regard.

CONCLUSIONS

Among the biodiversity conservation strategies, ex situ conservation is the fastest method to ensure the medium and long-term survival of endangered, rare, or endangered species [Copaci et al. 2018]. In this study, the production potential of the *G. pilulifera* which is economically valuable and endangered species was investigated under *in vitro* conditions in order to protect and economically evaluate of the species. It was determined that the most effective and fastest method for rapid multiplication of *G. pilulifera* under *in vitro* condition was germinated of the seed explant in hormone-free MS medium and transfer to the field. In this way, the populations of the species, which is economically very valuable, will be protected in nature, and it will be possible to gain a significant income by introduced it to the ornamental plant sector and the pharmaceutical industry.

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