



Micropropagation of *Erodium olympicum* Endemic to Turkey

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Abstract: *Erodium* genus belonging to Geraniaceae has fifty species that are distributed over Balkan Peninsula, Mediterranean, Middle East, Central Europe and Central Asia and most of them are herbaceous annual plants. Members of the genus naturally grow in sandy soil with good drainage, foothills, pebbled places, and desert and semi desert areas. It is reported that the genus has more than two-hundred antimicrobial effective compounds and some species belong to the genus has been used in ethnomedicine since very ancient time. At present study, the effects of different plant growth regulators (PGR) on micropropagation of *Erodium olympicum* endemic to Turkey were investigated. Therefore, Murashige and Skoog (MS) media supplemented with different BA (0.1, 0.5, 1.0 mg L⁻¹) and GA₃ (0.1, 0.2 mg L⁻¹) concentrations and combination were used. Plant materials were collected from Uludağ Mountains in Turkey and surface sterilization was performed applying tap water for 30 min, EtOH 70% for 2 min, rinsing with distilled water for 3-4 times, NaOCl 20% for 20 min, rinsing with distilled water for 3-4 times, respectively. The highest shoot formation (58%) was obtained from MS medium with 0.5 mg L⁻¹ BA + 0.2 mg L⁻¹ GA₃. The best rooting was determined on MS medium containing 0,5mg L⁻¹ IBA as 25%.

Keywords: Biodiversity, Genetic Resources, Herbaceous Plant, *In vitro*

1. Introduction

Turkey is one of the richest countries in terms of large flora and endemic genetic resource due to its geographic location with different climates; Continental, Mediterranean and Oceanic [1]. Especially, Mediterranean basin is the centre of this floral diversity for Geraniaceae family. *Erodium*, *Palergonium*, *Geranium*, *Monsonia* and *California* are well known genus in *Geraniaceae* family. *Erodium* species, mainly herbaceous perennials or subshrubs, are native to the Mediterranean and Eurasia and grow in

mountainous areas in rocky, calcareous soil [2]. This genus is represented by 74 species. Among these species, 62 taxa occur in Mediterranean region, 31 of them are grown naturally in Anatolia and 16 of them are endemic to Turkey [3, 4]. *E. olympicum*, distributed to Uludağ Mountain, known as one of the important endemic species and under the threat of extinction due to human activity (grazing and urbanization, winter sports).

To get rid of the extinction risk, some innovative methodologies have emerged to preserve genetic resources recently. Propagation and protection of these endemic species by biotechnological methods, especially tissue culture

techniques are remarkable as much as conventional techniques [5]. Micropropagation is an effective method for vegetative and clonal production using different plant tissues in aseptic conditions [6]. Efficiency of micropropagation depends on culture medium, growth regulators and genotypes [2]. Micropropagation with nodes, shoot tips, axillary buds, leaf and stem segments are widely accepted methods for medicinal and aromatic plants [7]. Tissue culture has been investigated in a few study on *Erodium* sp. and using shoot tips of *E. sibthorpiatum* subs. *sibthorpiatum* as an explant source and MS medium containing 1 ppm BA were found very significant (%100) for micropropagation [2].

In this research, an efficient micropropagation protocol was developed for endemic *E. olympicum*, sampled from Uludağ Mountain in Bursa in the north-western part of Turkey, testing and effects of different PGRs concentrations on micropropagation. This is the first report about *in vitro* propagation of Turkish endemic *E. olympicum* species.

2. Material and Methods

2.1. Plant Material

For starting material, 75 plants were collected from Uludağ Mountain (NW Turkey) (40°05'27.55" N (long.), 29°10'26.26" E (lat.), 1600 m above sea level during August, 2012. Plants were cultivated individually in pots filled with peat, sand and perlite (1:1:1, v/v/v) in ornamental plant greenhouse (30°C; 2-11 MJ m⁻² day⁻¹) at Çukurova University and irrigated three times a week with tap water.

2.2. Surface Sterilization

2 cm long nodes were taken from young plants and they were washed under tap water for 30 min., then dipped in 0.1% HgCl₂ (Merck KGaA, Darmstadt, Germany) for 20 min. in a fume hood. Nodes were rinsed 4-5 times with distilled water and taken into laminar flow hood. Finally, explants dipped in 70% ethanol for 1-2 min, soaked in 20% NaOCl (4.6% active chlorine) (Domestos, Unilever, Turkey) for 20 min, then rinsed with sterilized distilled water 4-5 times.

2.3. Multiplication Medium

Nodes of the plantlets were cultured on MS medium supplemented with 3% sucrose, 7.5 g L⁻¹ agar (Duchefa,

P1001) and different combinations of BA (0, 0.5, 1.0, 1.5, 2.0, 2.5 mg L⁻¹) and GA₃ (0, 0.1, 0.2, 0.3, 0.4, 0.5 mg L⁻¹). Nodes were cultured in petri dishes (9 cm diameter) under 25°C, 40 μmol m⁻² s⁻¹ (F36W/54-765 daylight 2350 Lm, Philips Lighting Holding B.V.), 16^h photoperiod conditions and subcultured every 4 weeks. After 2 subcultures, 1 cm length explants were transferred to the rooting medium containing MS with 3% sucrose, 7.5 g L⁻¹ agar and different concentrations of IBA (indole-3-butyric acid) (I0902, Duchefa) (0, 0.5, 1, 1.5, 2, 2.5, 3 mg L⁻¹).

2.4. Statistical Analysis

The experiment was set up as a completely randomized design (5 replicates×6 PGR combinations). Data was statistically analysed using the JMP® program ver. 5.00 (SAS Institute, Cary, NC). Means were separated according to the least significant difference (LSD) test at the 0.05 level of probability. Percentage values were all arcsine transformed prior to analyses.

3. Results and Discussion

The nodes with 1 cm long of *E. olympicum* were cultured on MS medium containing different concentration of BA and GA₃ to obtain efficient *in vitro* multiplication and influential micropropagation protocol was developed. Considerable propagation coefficient was acquired on MS medium including 0.5 mg L⁻¹ BA + 0.2 mg L⁻¹ GA₃ (Table 1). At the first subculture, numerous shoot formations (1.33±1.28a) were observed in response to 0.5 mg L⁻¹ BA + 0.2 mg L⁻¹ GA₃. After the second subculture, propagation coefficient was detected as 1.42±1.54a on the same medium (Table 1). After two subculture, shoots were formed in 58% of explants (Figure 1). During *in vitro* cultivation, vitrification was detected in some node explants cultured on MS medium with 0.1 mg L⁻¹ BA + 0.1 mg L⁻¹ GA₃, 0.1 mg L⁻¹ BA + 0.2 mg L⁻¹ GA₃. Then, regenerated shoots were transferred to rooting medium after 8 weeks of cultivation. Limited root formation was observed from all MS medium supplemented with IBA (Table 2). In control medium, there was no rooting due to absence of auxin and cytokinin. MS medium containing 0.5 mg L⁻¹ IBA showed better root formation than the other concentrations (Table 2). Callus induction was observed on MS medium containing 2.5 mg L⁻¹ and 3 mg L⁻¹ IBA.

Table 1. Micropropagation of *E. olympicum*.

PGR combinations	1 st subculture Propagation coefficient (no±S.E)	2 nd subculture Propagation coefficient (no±S.E)	Shoot formation (%)
0.0 mgL ⁻¹ BA + 0.0 mgL ⁻¹ GA ₃	0	0	0
0.1 mgL ⁻¹ BA + 0.1 mgL ⁻¹ GA ₃	0.54 ±0.38c	0.11±0.40c	20c (26.42)
0.1 mgL ⁻¹ BA + 0.2 mgL ⁻¹ GA ₃	0.92±0.66c	0.28±0.66c	20c (26.19)
0.5 mgL ⁻¹ BA + 0.1 mgL ⁻¹ GA ₃	1.08±0.77b	1.03±0.44ab	31b (33.79)
0.5 mgL ⁻¹ BA + 0.2 mgL ⁻¹ GA ₃	1.33±1.28a	1.42±1.54a	58a (49.65)
1.0 mgL ⁻¹ BA + 0.1 mgL ⁻¹ GA ₃	0.95±1.48	1.22±0.84a	25bc (29.95)
1.0 mgL ⁻¹ BA + 0.2 mgL ⁻¹ GA ₃	0.92±1.37ab	1.05±1.14ab	24bc (29.26)

S.E: Standard error, Statistical analyzes were realized with ANOVA. (LSD test: p<0.05)

LSD_{shootformation}=4.9747 (p≤0,001)

Values in parentheses indicate arcsine transformed % values

Table 2. Rooting of *E. olympicum*.

IBA concentration	Rooting of plantlets derived from nodes (%)
0.0 mgL ⁻¹	0
0.5 mgL ⁻¹	25a (29.91)
1.0 mgL ⁻¹	7bc (15.12)
1.5 mgL ⁻¹	15ab (22.67)
2.0 mgL ⁻¹	3cd (7.75)
2.5 mgL ⁻¹	3cd (7.75)
3.0 mgL ⁻¹	3d (6.27)

LSD_{rooting}=7,6516 (p<<0.001)

Values in parentheses indicate arcsine transformed % values

There have been a lot of researches on micropropagation of other medicinal and aromatic plants spread around Mediterranean basin such as *Origanum* sp. [8-15], *Sideritis* sp. [16-24] etc., but limited reports were presented about *in vitro* regeneration of *Erodium* species [2, 25]. In this study, the effects of BA+GA₃ combinations on *E. olympicum* were investigated. MS medium including 0.5 mg L⁻¹ BA + 0.1 mg L⁻¹ GA₃ was found as the best PGR combination for shooting and MS medium containing 0.5mg L⁻¹ IBA was prominent media for rooting. Akin *et al.* [2] obtained significant shoot formation from the seedlings of *E. sibthorphanum* subs. *sibthorphanum* on MS medium containing 1 ppm BA. In contrast to this study, explants, cultured on MS medium supplemented with 1 mg L⁻¹ BA+0.2 mg L⁻¹ GA₃ was not effective as 0.5 mg L⁻¹ BA combined with 0.2 mg L⁻¹ GA₃. In the other study, micropropagation of *E. somanum* H. Peşmen was investigated and the most efficient explant for shoot formation was found as leaf and medium was MS medium involved 2 mg L⁻¹ BAP and 1 mg L⁻¹ IBA. GA₃ is known as effective plant growth regulator to increase the length of shoots during multiplication [27]. But in the present study, length of the shoots was nearly the same during the 8 week cultivation. On the other hand, Akin *et al.* [2] implied that hormone free MS medium was the best for the highest shoot length for *E. sibthorphanum*. Although there is a few studies on *Erodium* genus, there has been so many paper about other species in *Geraniaceae* family especially *Palergonium* genus. For instance, Saxena *et al.* [29] indicated that MS medium including 5 mg L⁻¹ KIN and 1 mg L⁻¹ NAA provided efficient regeneration for leaf explants while 8 mg L⁻¹ KIN and 1 mg L⁻¹ NAA gave impulsive regeneration for nodes of

Palergonium. Similarly, Pattanik and Chand [26] reported that the combination of BA and GA increased the effect of bud break and shoot multiplication for nodal explants of *Morus cathayana*. In tissue culture studies, cytokinin is necessary for plant cell due to regulator effect on protein synthesis [27]. BA is the most effective and low priced cytokinin for tissue culture studies [26]. As indicated Ueno and Shetty [28], the best micropropagation was obtained from the medium with 1 mg L⁻¹ BA in *Origanum vulgare*. GA₃ is known as one of the important shoot stimulator for some plants and it was reported that combination GA₃ with BA increased organ and shoot regenerations [27]. Rooting is provided by auxin without any combination with other PGR. Secondly, level of auxin is one of the most important factors for root induction. In the present study, efficient rooting was observed on MS medium containing 0.5 mg L⁻¹ IBA. IBA known as significant auxin for root induction due to it's effect on lateral root stimulation. In our study, while low concentrations of IBA promoted rooting, 2.5 mg L⁻¹ and 3 mg L⁻¹ IBA caused callus induction. Comparably, impressive root formations were observed on MS medium supplied with 0.5 mg L⁻¹ and 1 mg L⁻¹ IBA for *E. somanum* [25]. Likewise, *E. sibthorphanum* rooted best on modified MS medium with the presence of 0.1 mg L⁻¹ and 0.5 mg L⁻¹ IBA on modified MS medium [2]. Contrary to our study, researchers indicate that rooting was spontaneous in *O. vulgare* [10].

4. Conclusion

Tissue culture is one of the most important preserving methods for rare endemic genetic resource of Turkish *E. olympicum*. The combination of BA and GA₃ is very effective to stimulate shoot formation from node explants. After shoot formation, IBA has significant effects on rooting. Shoot formation protocol and rooting protocol was developed for Turkish endemic *E. olympicum* species.

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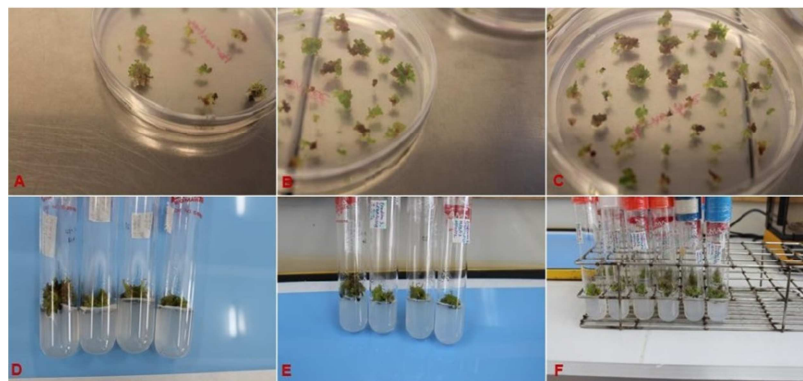


Figure 1. Micropropagation stages of *E. olympicum*. A-B-C) Regeneration of nodes on MS medium containing 0.5 mg L⁻¹ BA + 0.2 mg L⁻¹ GA₃, D) Shoot formations of *E. olympicum*, E) Multiplication of shoots, F) Plantlets obtained from node explants.

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