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In Vitro Propagation of Medicinal and Ornamental Plant Oxalis triangularis A.st.-Hil.

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ABSTRACT: *An in vitro propagation protocol of Oxalis triangularis A.st.-Hil, a valuable medicinal and ornamental plant, has been developed. In experimental studies, α-Naphthalene acetic acid (NAA) (0-0.5-1- 2 mg/L) and 6-Benzylaminopurine (BAP) (0.5-1 mg/L) plant growth regulators were applied together or alone to O. triangularis leaf and petiole explants. The best shoot formation per explant and the best leaf formation were obtained as 5.67 ± 2.43 and 4.00 ± 1.86 from leaf explants cultured in Murashige and Skoog (MS) medium containing 2 mg/L NAA and 0.5 mg/L BAP plant growth regulators, respectively. The shoots obtained were transferred to MS medium containing 0.5 mg/L NAA for root development. The plants obtained as a result of micropropagation processes were successfully acclimatized.*

Keywords: *Plant tissue culture, micropropagation, auxin, cytokinin, Oxalis triangularis.*

Tıbbi ve Süs Bitkisi Oxalis triangularis A.st.-Hil. 'in In Vitro Çoğaltımı

ÖZ: *Tıbbi ve süs bitkisi olarak değerli bir bitki olan Oxalis triangularis'in in vitro çoğaltım protokolü belirlenmiştir. Deneysel çalışmalarda Oxalis triangularis yaprak ve petiol eksplantlarına, α-Naftalin asetik asit (NAA) (0-0.5-1- 2 mg/L) ve 6-Benzilaminopurin (BAP) (0.5-1 mg/L) bitki büyüme düzenleyicileri birlikte veya tek başına uygulanmıştır. Eksplant başına en iyi sürgün oluşumu ve en iyi yaprak oluşumu, sırasıyla 2 mg/L NAA ve 0.5 mg/L BAP bitki büyüme düzenleyicilerini içeren Murashige ve Skoog (MS) besiyerinde kültüre alınan yaprak eksplantlarında 5.67 adet ± 2,43 ve 4.00 ± 1,86 olarak elde edilmiştir. Elde edilen sürgünler kök gelişimi için 0.5 mg/L NAA içeren MS besin ortamına aktarılmıştır. Mikroçoğaltım işlemleri sonucunda elde edilen bitkiler başarıyla aklimatize edilmiştir.*

Anahtar Kelimeler: *Bitki doku kültürü, mikroçoğaltım, oksin, sitokinin, Oxalis triangularis.*

INTRODUCTION

Plant tissue culture methods are used to obtain plant or plant metabolites. Unlike other known classical production methods, it involves culturing sterile root, stem, leaf, or apical and meristem tissues called explants in a sterile nutrient medium containing plant nutrients, under controlled light, humidity and temperature conditions. This technique has many advantages over traditional production. The applied culture conditions are not affected by geographical and seasonal differences and environmental conditions and are determined independently. Moreover, production is done in constant quality, quantity and speed and is not affected by agricultural policies. In addition, changing culture conditions allows the synthesis of new compounds that are not naturally produced in the parent plant. As a result of studies conducted in areas such as plant development and organogenesis, metabolite production, and gene transfer studies under *in vitro* conditions, much basic biological information such as physiology, biochemistry, genetics and molecular biology can be obtained (Babaoğlu *et al*., 2001; Gürel *et al*., 2013; Kocaçalışkan, 2021).

The first commercial studies in plant tissue culture were related to the production of ornamental and food plants. Plants are produced using very small plant tissues or cells with the micropropagation method. This technique allows rapid production of genetically homogeneous and healthy individuals, and the applicability of the method varies depending on the plant species and the techniques used. This process is usually carried out with methods such as meristem

culture, organogenesis and somatic embryogenesis. Careful selection of factors such as plant growth regulator, nutrient medium and explant type increases the success of micropropagation processes (Preil, 2003).

Oxalis triangularis, commonly known as "false clover", is an important member of the Oxalis genus. This species is a decorative plant with its aesthetic structure. Its dark red/purple leaves, which emerge from an underground bulb, and its unique morphological structure are the elements that make it stand out among other Oxalis species. In recent years, research on the positive health effects of natural compounds contained in this plant has increased. In a study conducted by Pazmiño-Durán *et al.* (2001), the content and profile of anthocyanins obtained from this plant were examined and a monomeric anthocyanin content of 195 mg/100 g per leaf was determined based on malvidin-3,5-diglucoside. Considering that anthocyanins and flavonoids in particular are known for their antioxidant, anti-inflammatory and anticancer properties, the pharmaceutical potential of *O. triangularis* becomes even more important. Its high anthocyanin content and edible character increase the potential of *O. triangularis* to be used as a natural food colorant. In addition, in a study conducted by Huh *et al.* (2010), it was found that fatty acid alkyl esters isolated from *O. triangularis* have skin whitening potential by inhibiting melanin production. This feature is also important for the cosmetic industry. However, *O. triangularis* populations show morphological variation and are affected by variables such as climate, soil structure and other environmental factors in different geographical regions. For example, in a study conducted by Luo *et al.* (2022), it was reported that three different anthocyanin compounds found in the plant differed depending on environmental conditions. In a study investigating the effects of the genetic structure of the plant and environmental factors on anthocyanin production, the molecular mechanisms of anthocyanin biosynthesis were examined with RNA-seq and qRT-PCR applications (Luo *et al.*, 2022).

O. triangularis reproduces abundantly with bulbs and, like other bulbous plants, regularly goes through a dormant period. Traditionally, propagation is done with bulbs, with a low propagation rate (Klaocheed and Rittirat, 2024). When we review the literature, we can find several research results reporting various explant types and plant growth regulator applications on micropropagation of *O. triangularis*. (Klaocheed *et al.*, 2024; Chen *et al.*, 2024; Rittirat *et al.*, 2021). Plants have also been grown *in vitro* in studies on specific topics such as suspension culture (Teng and Ngai, 1999), synthetic seeds (Taha *et al.*, 2013), phenotypic mutation with ionizing radiation (Ren *et al.*, 2017), and gene transfer via Agrobacterium (Xiao *et al.*, 2023). The effects of petiole explants and high cytokinin applications have generally been reported in the literature. In our study, we investigated the effects of only BAP and high auxin-containing BAP and NAA combination applications using petiole and leaf explants of *O. triangularis*, which has medicinal and ornamental plant properties and has insufficient studies on its *in vitro* potential in the literature.

MATERIALS AND METHODS

Plant materials

The study material, *O. triangularis* A.St.-Hil., was purchased from a private company and grown for two years in the plant growth room. It was used as an explant source for leaf and petiole explants to be used in the study.

Culture conditions

MS, the nutrient medium described by Murashige and Skoog (Murashige and Skoog, 1962), was used as nutrient medium. MS nutrient medium (Duchefa, MS salt) was prepared in accordance with the company protocol. In addition, 30 g/L sugar was added to the nutrient medium as a carbon source and 7 g/ L agar was added to make it semi-solid, the pH of the solution was adjusted to 5.7-5.8 using 0.1N NaOH and/or 0.1N HCl. The prepared nutrient medium was distributed in 212 ml glass jars with 30 ml per jar, the jar lids were closed and the nutrient medium was sterilized by autoclaving at 121 ºC, 1.06 kg/cm² pressure for 15 min. Culture was carried out in a plant growth room where 24±2º C, 4000 lux, 16-hour day, 8-hour night photoperiod was applied.

Culture procedures

Plant leaf and petiole explants were sterilized by keeping them in 70% ethanol for 1 min and 0.5% NaOCl for 1 min. Then, they were rinsed with sterile distilled water 3 times for 3 min each. The sterilized explants were transferred to MS nutrient medium containing cytokinin (BAP) and auxin (NAA) at 5 different concentrations: 0.5 mg/L BAP, 0.5 mg/L BAP + 0.5 mg/L NAA, 0.5 mg/L BAP + 1 mg/L NAA, 0.5

mg/L BAP + 2 mg/L NAA, 1 mg/L BAP. Leaf explants were divided into three with the abaxial side facing down and placed in the nutrient medium with 3 explants in each jar and leaf petiole explants were placed in the nutrient medium with 5 explants of 0.5 cm length in each jar. Treatments were made in 3 replicates. Shoots obtained from leaf and petiole explants were cultured in MS medium containing 0.5 mg/L NAA for root development.. Plant explants were observed every week.

Acclimatization study

The obtained shoots were transferred to MS nutrient medium containing 0.5 mg/L NAA for root development and then acclimatization processes were started. For this purpose, firstly, jar lids were loosened for 5-10 min two days before the plantlets were removed from the nutrient medium. Then, the nutrient medium was washed under running water and transferred to jars containing sterile peat. Jar lids were kept closed for two days. Starting from the second day, jar lids were loosened and adaptation of the plant to the humidity in the external environment was gradually provided.

Statistical Analyses

The data obtained as a result of the experiments were analyzed using the Minitab Statistical Analysis program and "p" values were calculated. Significant values were shown with "*" and standard deviations were shown with "±". F test and TUKEY-HSD multiple comparison test were used to determine the effects of applied plant growth regulators on leaf and petiole explants.

RESULTS AND DISCUSSION

In order to obtain explants to be used in culture processes, a non-dormant, young and disease-free mother plant should be selected (Kocaçalışkan, 2021). Considering the literature, instead of bulb parts that cause high contamination problems, leaf and petiole explants, which are frequently used in plant tissue culture applications and constitute the above-ground parts of the plant, were selected as explants (Zhou *et al.*, 2023; Pan *et al.*, 2024). In order to start the culture processes of *O. triangularis*, sterilization applications at different concentrations and times were tested on the explants. However, it was observed that applying sterilant to the explants for a long time such as 3-5 minutes during the sterilization processes caused necrosis in the tissues. In the study conducted by Teng and Ngai (1999), it was reported that *O. triangularis* leaf and petiole explants died due to the applied surface sterilization. As a result of this experience and knowledge, it was decided to apply short-term sterilization processes in the study and to use a mother plant grown in the plant growth room, isolated from the external environment. As a result of the many sterilization processes, completely sterile and 80% healthy explants were obtained as a result of the application of 1 min 70% ethanol and 1 min 0.5% NaOCl to the leaf and petiole explants.

After sterilization, leaf and petiole explants were cultured in MS nutrient medium containing BAP and NAA plant growth regulators at six different concentrations and combinations, and regeneration in the explants was determined by observations made at weeks 2, 3, 4 and 5. Plant growth regulators caused morphological changes in the explants at the end of the 2nd week; 0.5 mg/L BAP plant growth regulator applied to leaf explants induced callus and adventitious shoot formation (Figure 1a); 0.5 mg/L BA + 0.5 mg/L NAA plant growth regulator applied to petiole explants induced callus formation at the tips of the explants (Figure 1b). Callus and root formation were induced at the edges of the leaf explants in medium containing 0.5 mg/L BAP + 2 mg/L NAA (Figure 1c). It was observed that 1 mg/L BAP plant growth regulator applied to leaf explants induced shoot and root formation (Figure 1d). These data are consistent with the results of studies in which shoot formation was observed in *O. triangularis* petiole explants at the end of the 2nd week and in *O. corniculata* nodal explants on the 10th day (Klaocheed *et al.*, 2024; Prasuna and Srinivas, 2015). The applied plant growth regulators caused creamy green callus formation at the tips of the petiole explants in the medium containing 0.5 mg/L BAP and green callus formation in the medium containing 0.5 mg/L BAP+2 mg/L NAA. As reported by Martin (2002) , the color and texture of the obtained calli varied according to the auxin:cytokinin ratio.

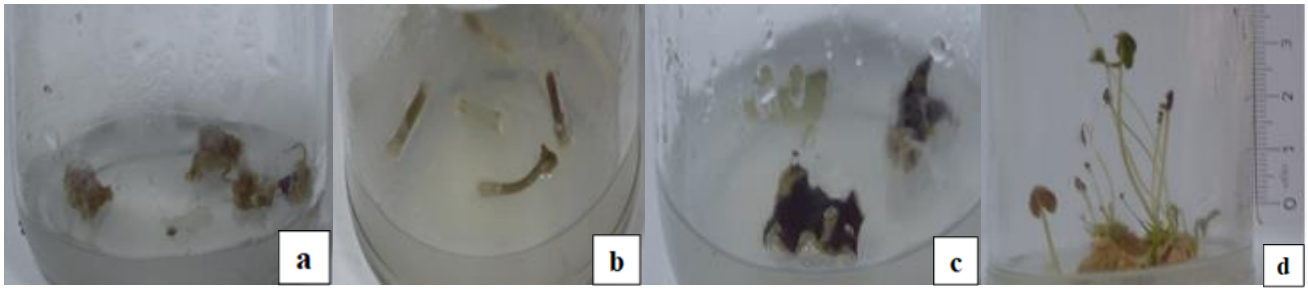

Figure 1. Leaf (L) (a) and petiole (P) (b) explants cultured in MS medium containing BAP and NAA plant growth regulators.
a) 0.5 mg/L BAP, (L) b) 0.5 mg/L BA + 0.5 mg/L NAA, (P) c) 0.5 mg/L BAP + 2 mg/L NAA, (L) d) 1 mg/L BAP, (L)

As a result of the observations made in the 3rd, 4th and 5th weeks, shoot formation was observed in all treatments. The highest shoot formation in petiole explants was obtained as 70% in the nutrient medium containing 0.5 mg/L BAP + 0.5 mg/L NAA (Table 1). In the study conducted by Klaocheed *et al.*, (2024), 100% shoot formation was obtained in medium containing 0.5 mg/L BAP + 0.5 mg/L NAA using petiole explants. This result reported in the literature is close to the results of our study. The best shoot formation percentage in leaf explants was again obtained in the nutrient medium containing 0.5 mg/L BAP + 0.5 mg/L NAA (78%) (Table 1). Prasuna *et al.* (2022) reported that *O. corniculata* leaf explants in MS medium containing 0.5 mg/L BAP + 0.5 mg/L NAA, gave 75% shoot formation, which is similar to the results of our study. As a result of the observations, the highest shoot formation (5.67 ± 2.43) and the highest leaf formation (4.00 ± 1.86) in leaf explants were obtained in the nutrient medium containing 0.5 mg/L BAP + 2 mg/L NAA. No difference was found as a result of the statistical analysis of the number of shoots and leaf number obtained per leaf explant in the different treatments (Table 2).

Table 1. Effects of BAP and NAA plant growth regulators on shoot formation in *O. triangularis* leaf and petiole explants (%).

Nutrient Medium	BAP (mg/L)	NAA (mg/L)	Number of shoots per explant (%)					
			Leaf Explant			Petiole Explant		
			3. Week	4. Week	5. Week	3. Week	4. Week	5. Week
MS	0.5	0	22	56	56	20	20	40
	0.5	0.5	33	67	78	70	70	70
	0.5	1	00	20	20	20	20	20
	0.5	2	56	67	67	00	20	40
	1	0	44	56	56	20	20	20

Table 2. Statistical comparison of the effects of plant growth regulators applied to *O. triangularis* leaf explants on shoot number.

Media	BAP (mg/L)	NAA (mg/L)	Number of shoot per explant ± SE	Number of leaf per explant ± SE
MS	0.5	0	1.89[a] ± 0,61	0.56 [a] ± 0,30
	0.5	0.5	2.89 [a] ± 0,84	1.22 [a] ± 0,37
	0.5	1	1.00[a] ± 0,76	1.00 [a] ± 0,76
	0.5	2	5.67 [a] ± 2,43	4.00 [a] ± 1,86
	1	0	5.22 [a] ± 3,00	2.67 [a] ± 1,83
P Value			0.301	0.278

Differences between mean values with the same letters in a column are not found to be significant at P≤0.05 level by TUKEY test.
SE: Standard Error

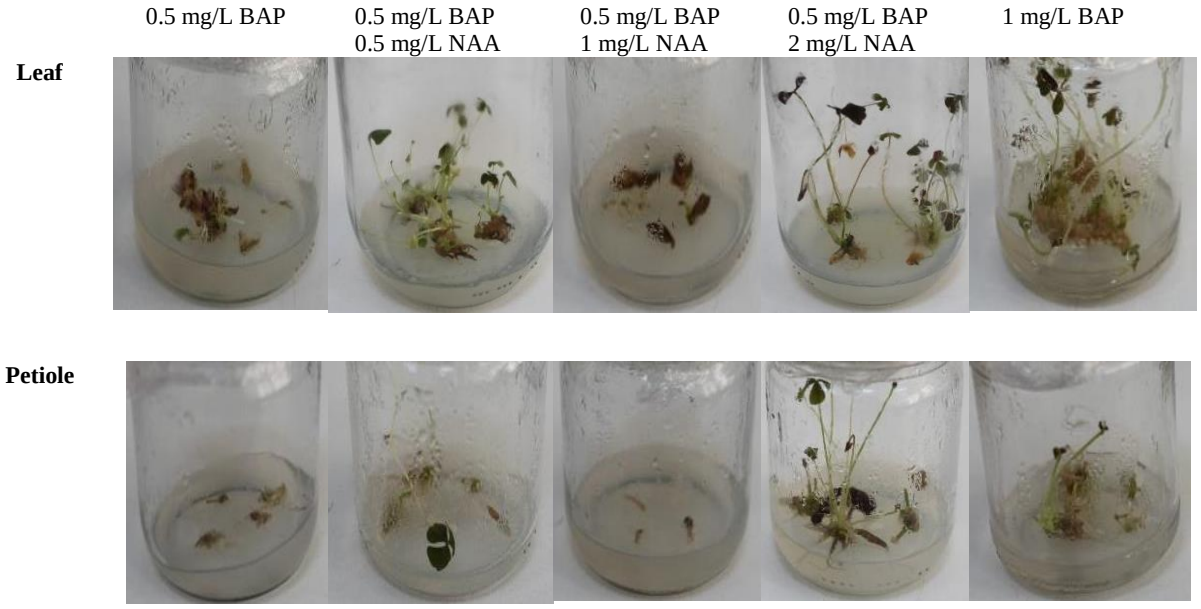

Figure 2**.** Effect of plant growth regulators applied to *O. triangularis* leaf and petiole explants.

In our experiments, first shoot and then flower formation were observed in leaf explants applied with 0.5 mg/L BAP + 2 mg/L NAA and 1 mg/L BAP. Flower formaton is not commonly seen in plant tissue culture applications. These data are compatible with the literature reported by Taha *et al.*, (2009) that *O. triangularis*, *Celosia cristata* and Begonia × hiemalis can flower in MS medium supplemented with various plant growth regulators such as BAP and NAA. Determination of the necessary plant growth combinations and concentrations that ensure flowering *in vitro* conditions facilitates basic studies on molecular, morphological and physiological changes in flower formation of *O. triangularis* and various basic research on solving problems such as premature fruit drop and poor seed formation.

Plants that completed their root development were 70% adapted to the external environment as a result of the acclimatization process. The plants grew with a normal growth cycle without showing any morphological changes (Figure 3).

Figure 3. Acclimatization of *O. triangularis.*

CONCLUSION

As a result of experimental data and statistical analyses, it was determined that *O. triangularis* leaf explants could be used for micropropagation in nutrient medium containing 0.5 mg/L BAP+2 mg/L NAA. Leaf explants, which were reported to be unusable in the literature due to high mortality rates, were successfully sterilized by immersing them in 70% ethanol and 0.5% NaOCl solutions for 1 min each. Moreover, it was shown that they could be used for shoot formation as an alternative to petiole explants. As a result of the research, shoot and root formation were obtained in both leaf and petiole explants in 0.5 mg/L BAP+2 mg/L NAA application with the plantlets cultured in medium containing 0.5 mg/L NAA for the development of roots and shoots before acclimatization. The plantlets obtained through micropropagation were successfully transferred to the soil. The results of this study conducted with *O. triangularis* A.St-Hil, an interesting and valuable plant with its aesthetic appearance, metabolite content and nyctinastic behavior, provide basic information for many future studies such as basic biology, metabolite production and gene transfer, as well as for the use of the plant in commercial production.

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