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DETERMINATION OF THE EFFECTS OF DIFFERENT PROTOCOLS ON TISSUE CULTURE MICROPROPRIATION IN *EUPHORBIA MILII*

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Abstract
In recent years, with the emergence of some important species in the field of ornamental plants, the interest in some species naturally grown in nature has started to increase and the Euphorbia milii species has started to create related demands both in commercial and hobby terms. In order to meet these demands, seedlings produced by appropriate methods are needed. Clonal propagation is very important in Euphorbia milii as in other fruit species. Tissue culture techniques are the leading methods in clonal propagation. Although there are various studies on production by tissue culture technique in Euphorbia milii species, it is known that different protocols used in tissue culture have different micropropagation efficiency. However, when commercial production is started, determining the most effective protocol and increasing the micropropagation efficiency will contribute to the reduction of both time and inputs. Studies on in vitro micropropagation have been carried out in Euphorbia milii plant and successful results have been obtained in each study. However, it is still not known exactly which protocol is more successful in the materials to be studied. From this point of view, in this study, it was aimed to determine the effects of different protocols on the micropropagation efficiency of Euphorbia milii plants. In present study, 3 different plant tissue culture protocol were used and, as obtained results, although plantlets were obtained from 3 protocols, the most successful protocol was the P1 protocol. The obtained findings are of a quality that can contribute to tissue culture production of Euphorbia milii plant and other scientific studies.

Keywords: Euphorbia milii, in vitro micropropogation

1. INTRODUCTION

The production of ornamental plants in Turkey started in the 1940s, and the fact that country has different ecological regions, the climate and soil characteristics offer wide opportunities for ornamental plants production, and the gene source of many ornamental plants has enabled the sector to develop rapidly. This situation has made Turkey a important country in the production and cultivation of ornamental plants. Ornamental plants are produced in 55 provinces of Turkey. The provinces with the highest production are Izmir, Sakarya, Yalova, Antalya, Bursa and Istanbul, respectively. Cut flower production in the Marmara and Aegean Regions (Istanbul, Yalova, Izmir, Aydın) is generally for the domestic market. Export-oriented production is carried out, mostly in greenhouses in Antalya and its surroundings. As the production speed and quantity increased, the search for ornamental plants that are appealing to the eye, resistant to seasonal changes and at the same time different in appearance has increased. One of them is the Euphorbia milii plants(Turkey Ornamental Plants Industry Report, 2019).

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Euphorbia milii species, belonging to the *Euphorbia* genus of the *Euphorbiaceae* family and native to Madagascar, has become one of the important ornamental plants with its many hybrid and standard varieties. It is an endemic species of Madagascar. They mostly grow on granite cliffs, as well as in bushes and forest areas. But it was determined that it was grown in the Middle East two thousand years ago. It is also located in Kerala, India. According to rumors, some Jews who immigrated from Palestine took *Euphorbia milii* with them to Kerala. It has various local names such as Chinese rose, bride's mother-in-law, crown of thorns, crown of thorns. Like the rose and many ornamental plants, it has a long history. Its value in Europe only started in France in 1821. Thus, it has taken its permanent place in the ornamental plants sector in developed western countries. Species Euphorbia milii Des Moul. Varieties include *Euphorbia milii*. Lutea *Euphorbia milii* var. tananarivae.

It is a very thorny plant in the form of a bush with climbing and creeping features. Its spines are for water storage and climbing trees rather than for protection. If it is left without the sun among other plants, its branches are weak and frail, and thanks to its thorns, it stands upright by holding onto the surrounding plants and extends upwards towards the sun. Its body is not too thick. In the long time, it reaches 2 meters in length. It shows rapid and frail elongation in the absence of sun, which is an effort to reach a sunnier place. Its leaves are not very long lasting. Therefore, the leaves appear on the developing ends of the branches. Its body has 5-7 faces and dark brown color. While the dense branches coming out of the trunk give the plant the form of a bush, the dark, hard thorns on the branches are remarkable. It is in the succulent class with its succulent leaves. The leaves are especially concentrated at the ends of the branches and their average length is 2.5-3 cm. While the leaf length of hybrids produced in Thailand reaches up to 15 cm (Kapadiya et al. 2017).

It is among the plants with flower and leaf beauty. It is a plant that is easily preferred indoors and loves the sun very much. It is easy to maintain. In recent years, it has come to the fore among succulent plants with flower and leaf beauty. Reproduction is by seeds and cuttings. As with many succulent species, one of the rare but prominent methods in propagation methods is tissue culture techniques.

In recent years, with the emergence of some important species in the field of ornamental plants, the interest in some species naturally grown in nature has started to increase and the Euphorbia milii species has started to create related demands both in commercial and hobby terms. In order to meet these demands, seedlings produced by appropriate methods are needed. Clonal propagation is very important in Euphorbia milii as in other fruit species. Tissue culture techniques are the leading methods in clonal propagation. Although there are various studies on production by tissue culture technique in Euphorbia milii species, it is known that different protocols used in tissue culture have different micropropagation efficiency. However, when commercial production is started, determining the most effective protocol and increasing the micropropagation efficiency will contribute to the reduction of both time and inputs. It is very important for companies that want to invest in this field to start production with successful protocols, especially in production with tissue culture.

Studies on in vitro micropropagation have been conducted in the Euphorbia milii plants and successful results have been obtained in each study (Airò et al. 2004; Tunjit, 2008; Dewir et al., 2005). However, it is still not known exactly which protocol is more successful in the materials to be studied. From this point of view, in this study, it was aimed to determine the effects of different protocols on the micropropagation efficiency of *Euphorbia milii* plants.

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2. MATERIALS AND METHODS

1.Materials

Euphorbia milii species was used as material.

2. Methods

In the study, 15 plants belonging to the Euphorbia milii species were grown in a polyethylene greenhouse in pots. Spring (April-May-June, the flowers were taken and kept in 70% ethanol for 1 minute right after the flowers were taken. Then they were rinsed once with distilled water. Then they were kept in 1% hypochlorite for 8 minutes and then rinsed with pure water 3 times. Sterilization After the procedure was completed, the explants were cultured following 3 different protocols.

Protocol 1: Airò et al.(2004)
Protocol 2: Tunjit, P. (2008)
Protocol 3: Dewir et al. (2005)

In the study, the number of Cultured Explants (number), the Developing Explant Number (number), the Developing Explant rate (%), the number of siblings (number) the tillering rate (%), the number of rooted plants (number) and the Rooting rate (%) were determined.

3. RESULTS AND DISCUSSIONS

In the study carried out to determine the effects of different protocols on the in vitro micropropagation efficiency of Euphorbia milii, 3 different protocols were used and the results are given in Table 1. According to the findings, the highest growing explant rate was obtained from protocol 3 (85%), while the lowest growing explant rate was obtained from protocol 1 (16.4%). On the other hand, the highest tillering rate was obtained from protocol 2 (328%) and the lowest tillering rate was obtained from protocol 1 (210%). While the highest rooting was determined in protocol 3 with 90.1%, the lowest rooting was obtained from protocol 1 with 9.5%.

Table 1. Number of Cultured Explants (number), Growing Explant Number (number), Growing Explant rate (%), Sibling number (number) Tilling rate (%), Rooting plant number obtained in in vitro micropropagation of Euphorbia milii species of different protocols (number) and Rooting rate(%)

Protocol	Number of explants cultured (pieces)	Number of Developed Explants (pieces)	Growing Explant rate(%)	Number of multiplication (pcs)	Multiplication rate (%)	Rooted number of plants (pieces)	Rooting rate(%)
1	61	10	16.4	21	210.0	2	9.5
2	35	25	71.4	82	328.0	71	86.6
3	40	34	85,0	91	267.6	82	90.1

In the first periods, people started to cultivate plants while collecting plants from nature and using them, and then they selected from nature with or without rules. When the productions obtained as a result of these selections could not respond, breeding studies were put into action. It is aimed to increase the number of plants produced in plant production in Euphorbia milii, to increase rooting, to standardize the rooting medium, to increase the number of tillering plants and to standardize the tillering medium. According to the findings, the best shoot growth was obtained from the application of 5 mg/L BA, while the highest tillering was obtained from the application of 2 mg/L.

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(Tunjit, 2008). It is aimed to develop a protocol to increase knowledge about micropropagation in Euphorbia milii plant. In order to achieve this aim, while sufficient sibling was obtained in MS+0.30 mg/L BA medium, 0, 0.15, 0.30, 0.30 and 0.60 mg/L doses of IAA were used during the rooting stage and the highest rooting rate was 0, It was obtained from MS medium containing 15 mg/L IAA. 71.25% of the plants included in the exercise grew healthy (Airò et al. 2004).
Dewir et al. (2005) MS+1 mg/L BA and 0.3 mg/L IBA application was determined as the most effective dose in terms of shoot growth

4. CONCLUSIONS

Although the world is origine to many ornamental plant species, it lags behind in producing and gaining added value from these plant species. In addition, in order to increase the added value of the species used especially indoors, which are both adapted to and able to provide the ecology of our country, it is necessary to expand the production areas and production activities. This area can be expanded thanks to correctly produced seedlings. In this study, modification of tissue culture techniques for correct and effective seedling production, which is the first step in order to increase production and rooting especially in the crown of thorns plant, may contribute to the producers. Increasing the number of plants produced and rooted in production and offered to the market will be an important way in the production and sale of crown of thorns in the country.

5. ACKNOWLEDGMENT

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