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Research Article

# Ovule-Embryo Culture in Lentil *(Lens culinaris Medikus)*

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## Abstract

Tissue culture techniques are needed in many areas of plant studies. In this study, it is aimed to reveal the potential of a vegetable such as lentils, which is an essential plant in human nutrition, to be multiplied by the ovule-embryo culture technique, which is one of the tissue cultures methods. Ovule-embryo culture method minimizes embryo deterioration during the early stages of development, reduces breeding time, and allows for the effective multiplication of numerous plants from a single embryo. Lentil plant (*Lens culinaris* Medik.) ovule explants were used as plant material. Embryos extracted from ovular integuments and cultured on Murashige and Skoog's medium supplemented with various concentrations and combination of Kinetin and 2,4-D. As a result of the study, the best callus formation rate (60%) was obtained from MS nutrient medium containing Kinetin (1 mg $mL^{-1}$) and 2,4-D (0.5 mg $mL^{-1}$).

**Keywords:** Mersin; Ovule; Propagation; *Lens culinaris*

## Abbreviations

2,4-D: 2,4-Dichlorophenoxyacetic Acid.

## Introduction

Legumes (Fabaceae) are important in plant production due to their high protein content and high digestibility of dried grains [1]. In addition, legume family members are rich in iron, phosphorus, calcium and potassium minerals, and vitamin B [2]. Due to these features, legumes have an important place in human nutrition. *Lens culinaris* Medik. (lentil) is one of the four species included in the genus Lens in the legumes (Fabaceae) family. German botanist and physician Medikus named the plant *Lens culinaris* in 1787 [3]. Lentil is diploid (2n=2x=14), self-fertile, annual, and herbaceous [4]. A significant part of the human diet includes lentils, which are high in protein and fiber. It is also beneficial as animal feed, and because of its nitrogen-fixing properties, it plays an essential part in crop rotations. As a drought-resistant crop, it may grow in various soil types [5,6].

Lentils have 4,800,017 of the harvested area in the world. The production amount of lentils worldwide is 5,734,201, and Canada ranks first with 1,275,278.31 tonnes of production [7]. Canada, India, Turkey, Australia, the United States, Nepal, China, and Ethiopia are the top lentil-growing nations. In order to increase lentil production, it is crucial to develop resistant varieties to biotic and abiotic stress factors. Tissue culture techniques are frequently used in breeding studies. Tissue culture techniques are important for bringing new varieties to the sector in breeding studies of plants with commercial importance in the agricultural field, such as lentils. The use of plant cell, tissue and organ culture methods is vital for plant clonal propagation and genetic manipulations applied in

advanced molecular techniques. This study aims to provide plant regeneration through ovule culture in lentils and determine the most appropriate regeneration protocol.

## Materials and Methods

### Plant growth

*L. culinaris* seeds were used, and plants were grown from seeds provided by local breeders around Adana in Turkey. Thirty seeds of *L. culinaris* were sown in plastic pots filled with garden soil and turf (2:1) in the greenhouse, and each pot contained five seeds. For ovule culture, at least 60 fruits were collected from the *L. culinaris* plant.

### Surface sterilization

Collected fruits were taken to the laboratory for ovule culture. Collected plant fruits were first washed in tap water for 30 minutes to remove contaminants such as dust and soil. After washing in tap water, fruits of lentils were soaked in 70% ethyl alcohol for 2 minutes. Afterward, it was incubated for 15 minutes by shaking in 15% sodium hypochlorite (NaOCl) containing two drops of 2% tween 20. Samples were washed three times with sterile distilled water to remove sodium hypochlorite.

### Ovule-Embryo culture

Ovule samples were excised from the fruits in a sterile cabinet. Then, embryos at different growth stages were extracted from ovular integuments carefully. The isolated embryo samples were taken into the MS medium containing Kinetin and 2,4-D at different concentrations (0, 0.5, 1.0, and 2.0 mg $mL^{-1}$). Experiments were set up with three replicates of each nutrient medium and three samples per replicate. Samples were cultured at 25 ± 1°C and 16 hours photoperiod (30 µmol.$m^{-2}s^{-1}$ fluorescent white light). Weekly observations were taken from the cultured samples, and the samples with growth were viewed with a stereomicroscope (Olympus SZ61, Japan).

## Results and Discussion

Seed germination occurred after one week, and plants were maintained until the flowering stage (Figure 1). All of the legumes collected had good fruit seeds.

There was no contamination found using the sterilizing technique utilized in the research. Embryos at different developmental stages were cultured in MS medium containing kinetin and 2,4-D.

**Figure 1:** Images of lentil plants grown in the greenhouse; the left figure is the image of the plants germinating in the 1st week following the seed sowing; the right image is the images of the developing plants in the 1st month.

In this research, there was a wide range in the developmental stage of ovule-extracted embryos from torpedo to cotyledonary (Figure 2).

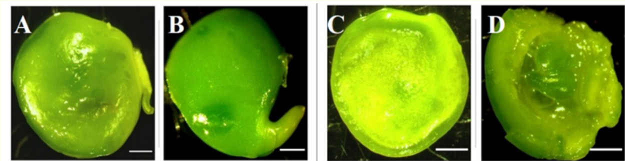


**Figure 2:** Embryos at different developmental stages and ovules from which they were extracted; (B) cotyledon stage embryo and (A) the ovule from which the embryo shown in B was isolated; (D) torpedo stage embryo and (C) the ovule from which the embryo shown in C was isolated.

While no improvement was observed in the control group cultured in MS medium without any plant growth regulator, callus formation was observed in the samples cultured in MS medium containing 2,4-D and Kinetin from the 2nd week. The developed callus was light yellow and compact (Figure 3). The best callus development from explants was in MS medium containing Kinetin (1 mg $mL^{-1}$) and 1,4-D (0.5 mg $mL^{-1}$) with a 60% regeneration rate.

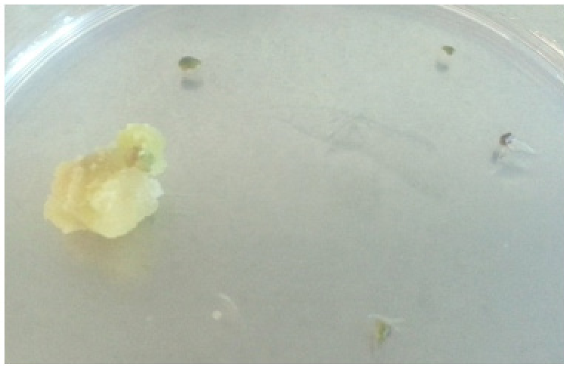

**Figure 3:** Observation of callus development in the 1st month of culture from the embryos cultured in MS medium containing kinetin (1 mg $mL^{-1}$) and 2,4-D (0.5 mg $mL^{-1}$) at the 1st month.

Plant growth regulators are highly effective on tissue cultur and are a factor affecting plant regeneration success. Kinetin is a cytokinin derivative plant growth regulator and is effective in cell division, plant regeneration, and redifferentiation. MS medium is a nutrient medium that is commonly used in plant tissue culture [8]. The tissue type, culture conditions, and nutrient media components selected in the tissue culture study are essential for obtaining an entire plant from the cultured samples [9]. Different tissue culture protocols are required for *in vitro* propagation for each plant species, cultivar, and genotype. The findings of this study show that the medium used was appropriate, and callus stimulation from lentil plant embryo samples was successfully performed. In this study, plant regeneration studies from callus samples are continuing. Abiotic (temperature, soil fertility, and drought) and biotic (insects, diseases, and weeds) limitations impact lentil production; therefore, it is important to propagate lentils by tissue culture method.

## Conclusion

The most successful medium for callus induction in the ovule-embryo culture of *L. culinaris* was MS, including Kinetin and 2,4-D. These findings suggest that the extraction of lentil embryos might be facilitated by developing an *in vitro* approach for cultivating embryos. Further studies are needed for plant regeneration from the calli obtained as a result of this study. If the plants are regenerated, the micropropagation protocol for lentils will be optimized, and this protocol can be a source for many studies on plant breeding and molecular genetics.

## Conflict of Interest

The author declares no conflict of interest.

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