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Somatic Embryogenesis on Turkish Lentil (*Lens culinaris* Medik.) Cultivars

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Abstract
Somatic embryogenesis studies were carried out on 11 Turkish lentil cultivars. Lentil seeds were sterilized and germinated in Plant Growth Regulator (PGR) free MS (Murashige and Skoog) medium. Cotyledon, hypocotyl, root, shoot tip, leaf and nod excised from sterile grown seedlings and embryos were excised from sterilized seed and incubated in MS, B5(Gamborg's medium), SH (Shenk and Hildebrandt) and WH (White) media with different PGRs. Six of 11 cultivar gave potentially embryogenic callus. Solid media studies were carried on with the six cultivars. Four embryologically best callus producing cultivars of 6 were used in suspension culture studies. 2,4-D (2, 4-dichlorophenoxyacetic acid), NAA(1-Naphthaleneacetic acid), BAP(6-Benzylaminopurine) were used in different concentrations and combinations to produce callus and somatic embriyo. Zygotic embryo was found to be best potentially embryogenic callus producing explant. MS with NAA and 2,4-D were determined to be embryogenic callus reporoductive conditions. BAP produced adventitious shoot rather than embryogenic callus. As a result, calli were tranferred to liquid culture and somatic embryos of Yerli Kırmızı, Sazak 91, Kafkas and Pull 11 cultivars gave best globular, heart and torpedo shapes embriyos in MS medium supplemented with 0.3-1 mg/l NAA.
Key words: somatic embryo, lentil

INTRODUCTION

Lentil (Lens culinaris Medic.) is a pulse crop that belongs to the family Leguminosae. Two main types of are grown in many parts of the world including India, Canada, Turkey, USA, Australia, China and Nepal [1]. The nutrient database of the United States Department of Agriculture (USDA) shows that lentils are rich in proteins and have 18 of the 20 amino acids including all 8 essential amino acids [2]. Lentils continue to occupy an important place in human diet, especially in developing countries, as a rich source of protein, vitamins and minerals. Lentils also provide other health benefits and promote general well-being due to their richness in protein, dietary fibre, soluble fibre, anti-oxidants, phytoestrogens and folate, and their low glycaemic index [3]. Seed protein content varies from 22 to 35% , with relatively high levels of lysine, leucine and sulfur- amino acids [4]. Lentils are sometimes called "poor man's meat", referring to their high protein content and low price [1]. Lentils are grown for their high protein, carbohydrate, vitamin and mineral containing grains and mainly for use in the human diet. In the world, 22% of vegetable protein , 7% of carbohydrates in the human diet are provided legumes, 8% of vegetable protein and 5% of carbohydrates in animal nutrition come from food legumes [5].

Rapid increase in the world population and inadequate agricultural land compared to the rising population reveals the need to get more product per unit area. Besides the development of high yielding varieties, appropriate breeding techniques need to be developed for each plant product [6]. In recent years, climate change, increase or decrease in temperature, lead to significant yield losses on the plants cultivated. Since it is not possible to increase production by increasing the planting area, It is necessary to increase the grain yield per unit area. This will be possible with the development of good varieties with high yield potential and appropriate cultivation techniques [7]. In agriculture, the selection of high yield potential commercial varieties has an important part . Using plant tissue culture techniques, with in vitro selection method carried out under controlled conditions, the selection of genotypes in the population, having the desired properties is possible. In recent years, in vitro cell and tissue culture techniques is being developed as an adjunct to conventional plant breeding program.

Plant tissue culture, utilizing the ability totipotency that plants owned, is to develope a new individual plant from cell, tissue, organ parts taken from the donor plant under aseptic conditions in an artificial nutrient media. Cell, tissue, organ parts taken from the plant and moved to artificial media for growth called explant. In vitro formation of plants can be achieved by encouraging the explants to produce organs, callus or embryos. With plant tissue culture techniques, embryo cultures, meristem culture, callus culture, haploid culture, suspension culture, protoplast culture can be established. Plant tissue culture studies aims to overcome the difficulties that the plants face in their natural environment and to produce the plants with high economic value in large quantities without depending on the environmental conditions. It also aims to lower cost and more profitable commercial production [8,9].

Today, many crops were produced embryos in the plant tissue culture and these embryos turned into synthetic seeds and their mass production are carried out with technological methods. Unlike the natural environment, tissue culture enables many manipulations. Creating stress on the explants, the desired shoot, tissue, organ development can be obtained. In this technology, plant growth regulators, natural plant hormones and their synthetic derivatives has an important role. These materials are the most important factor in the manipulation of the culture medium used in very low concentrations. Associated with relevant applications, these substances are thought to activate or repress genes.

The deterioration of environmental conditions, excessive industrialization, global warming, negative effect of synthetic fertilizers on the soil is narrowing day by day the amount of planting area. Therefore, there is a need for technological method. Somatic embryo production is a method of providing a very wide range of opportunities for synthetic seed and mass production. However, this method does not has routine protocols. For each plant species even their variations, media conditions varies. Researchers must evaluate the clues offered by literature to create media conditions for their study materials.

In the majority of Legumes, new growth regulators such as chlorophenoxyasetik acid and Thidiazuron have been reported to provide great advantages on obtaining high proportion of direct regeneration from somatic embryos. In still other hand, the high rate of somaclonal variation in Legumes has been reported the limiting factors for somatic embryogenesis[10].

Therefore, this study will investigate the embryogenic potential of indigenous breeds in lentils and using Plant Growth Regulators believed to activate genes associated with this potential and we attempted to establish a suitable protocol for somatic embryogenesis in lentil plants.

MATERYAL AND METOD

In this study, Sultan 1, Yeşil 21, Sazak 91, Kafkas, Özbek, Yerli Kırmızı, Pul 11, Emre 20, Kırmızı 51, Malazgirt 89 and Çiftçi were used. Lentil seeds were obtained from Faculty of Agriculture , Yuzuncu Yi l University. In applications, MS, SH, B5 and White basic nutrient media contained 3% sucrose and solidified with 0.6% agar were used and nutrient media was prepared bidistilled pure water. Plant growth regulators such as NAA, 2,4-D, BAP were added at different concentrations to the nutrient media. The pH of the nutrient medium was adjusted to 5.8 using 1 M NaOH and 1 M HCl. All cultures were incubated in air-conditioning cabinet at 25 ± 2 ° C and 24 hours white fluorescent light .

As a first step, for surface sterilization of lentil seeds, seeds were held 70% alcohol for 30 second. Immediately after, the seeds were treated with 4 minutes 40% commercial bleach NaOCl. After this period, the seeds rinsed sterile distilled water for 5 times. Since this dose of disinfectant had the lowest dose of contamination and the lowest rate of tissue damage was found to be %40 NaOCl for 4 minutes, in the study, it is preferred to use this sterilization method.

Murashige and Skoog [11], White , Gamborg's B5 [12], and Schenk and Hildebrandt [13] media used this study were sterilized holding 20 min at 121 ° C under 1.5 atm pressure in autoclave. Before being autoclaved, 2.4-D, NAA and BAP were added to nutrient medium.

Mature zygotic embryos and various explants of lentil plants grown in vitro were encouraged to callus production on 4 different nutrient medium modified with different concentartions of 3 PGR. Obtained callus cultures were taken to a subculture after 3-4 weeks. Calli reached a sufficient size were transferred to nutrient media broth prepared with the same content in the flask. Calli were incubated at shaker 80 -120 rev / min at 25 ± 2 ° C for 15-20 days.

The tissue in liquid media with an increased number were placed to embryo development medium. Auxins and cytokinins provide continuous cell division was removed from this medium for embryo maturation.

RESULTS

Determination of the lentil type has the most capability to produce callus

In the literature, due to the most extensively studied nutrient media is MS medium has been studied primarily in that medium. Zygotic mature embryos of Sultan 1, Yeşil 21, Sazak 91, Kafkas, Özbek, Yerli kırmızı, Pul 11, Emre 20, Kırmızı 51, Malazgirt 89 ve Çiftçi were cultured on MS medium modified with different combinations and concentrations NAA and 2,4-D for the the purpose of testing their ability to generate callus. The objective of this application is to continue working with lentils, which have the potential to produce the best callus.

As a result of this application, Sultan 1, Yeşil 21, Sazak 91, Kafkas, Özbek, Yerli kırmızı varieties, when treated with NAA and 2,4-D, have been found to produce amounts of callus greater than others.

Determination the best plant growth regulators for callus production

Lentil varieties identified high potential to produce callus were cultured in MS, WH, B5 and SH medium modified with 2,4-D and BAP in various concentrations and combinations. These applications's aim is to continue to work with plant growth regulators determined to have potential to generate the highest callus.

When they were taken on MS medium modified with different concentrations and combinations plant growth regulators, shoot growth of these 6 kinds of lentils was found to be dominant in the medium where modified with BAP . A small amount of calli was observed to be produced on the medium modified with 2,4-D . It was observed that a large amount of loose-yellow translucent callus were produced in the Medium modified with NAA (Table 1).

Determining the best food media applications with NAA

From the previous stage, food environment modified with NAA gave better results than the medium modified with 2,4-D and BAP in terms of the potential to produce callus from mature zygotic embryo explants taken to the culture. At this stage of the study, It has tried to determine the most successful food environment among MS, SH, B5 ve WH medium modified with low concentrations NAA.

Applications with MS medium

Food environment modified with NAA gave better results than the medium modified with 2,4-D and BAP in terms of the potential to produce callus from mature zygotic embryo explants taken to the culture. Also, in low concentrations, large amounts of callus production was observed in addition root development. Decreased concentration of NAA increases the amount of callus, root development is also gradually decreasing even no root growth was observed in the medium modified with 2 mg / l NAA. Sultan 1 cultivar, was the largest producer of callus in all kinds of examples. However, unlike a lot of the callus production, in microscopic observation, embryogenic callus cells weren't observed. Özbek was observed to produce shoots in the environment modified with 0.5 mg / l NAA. It was observed that variety of Çiftçi produce gray calli in all kinds of concentration. Sazak 91, Emre 20, Malazgirt 89 varieties have been observed to produce uncommon gray callus not found in previous practice.

Kırmızı 51 were seen as the most resistant genotype for development. Besides, in microscopic observations,Sazak 91, Kafkas, Yerli Kırmızı ve Pul 11 were seen to produce embrionic callus.

Applications with WH medium

Mature zygotic embryo explants was found to yield higher amounts of callus in the medium modified with NAA at lower concentrations than the medium containing high concentrations of NAA. Also, at low concentrations, along with a high amount of callus manufactured root growth was also observed. Decreased concentration of NAA increases the amount of callus, root development is also gradually decreasing, and even no root growth was observed in the medium modified with 2 mg / l NAA. Sazak 91, Özbek, Kırmızı 51, Malazgirt 89 ve Çiftçi varieties was observed to produce gray callus. Sultan 1 variety showed high levels of callus productiın also root growth. In microscopic observation, Sazak 91, Kafkas, Yerli Kırmızı ve Pul 11 varieties was observed to be more successful than other kinds of samples in terms of producing embryogenic callus.

Applications with B5 medium

Root formation observed at low concentrations of NAA in MS and WH medium, was only observed Kafkas, Emre 20 ve Malazgirt 89 varieites in B5 medium suplemented with 0.5 mg of / l NAA.hovewer, no root formation observed 1 mg / l NAA modified environments, root development was observed in all samples except Kafkas, Emre 20, Kırmızı 51 ve Çiftçi varieties in the medium suplemented with 2 mg/l NAA.

Tablo 1. Determination plant growth r egulators with the highest potential to produce callus on MS medium

MS Medium	1 mg/l NAA	1 mg/l NAA + 1 mg/l 2,4-D	2 mg/l NAA + 1 mg/l 2,4-D	1 mg/l NAA + 1 mg/l BAP	1 mg/l 2,4-D + 1 mg/l BAP	0.2 mg/l NAA + 2.25 mg/l BAP
Sultan 1	Transparent, yellow callus, root	Transparent, yellow callus, root	White, Yellow Callus	Shoot, transparent Callus	Shoot, transparent Callus	Shoot
Yeşil 21	Transparent, yellow callus, root	Transparent, yellow callus, root	yellow Callus	Shoot, yellow Callus	Shoot, Callus	Shoot
Sazak 91	Transparent, yellow callus, root	Transparent, yellow callus, root	Yellow callus, root	Shoot, yellow Callus	Shoot, Callus	Shoot
Kafkas	Transparent, yellow callus, root	Transparent, yellow callus, root	Yellow callus, root	Shoot, Brown Callus	Shoot, Callus	Shoot
Özbek	Transparent, yellow callus, root	Transparent, yellow callus, root	Yellow callus, root	Shoot, Brown Callus	Shoot, Callus	Shoot
Y.Kırmızı	Transparent, yellow callus, root	Transparent, yellow callus, root	Transparent, yellow callus, root	Sürgün Gelişimi, Sarı Kallus	Shoot, Callus	Shoot

Table 2. The practice to determine the best concentration of NAA that has the highest capacity of producing callus on MS medium

MS Medium	0.5 mg/l NAA	1 mg/l NAA	2 mg/l NAA
Sultan 1	Yellowish callus, root	Yellowish callus, root	Yellowish callus
Yeşil 21	Brown callus, root	Yellowish callus	Yellowish callus
Sazak 91	Yellowish callus	Grey Callus	Yellowish callus
Kafkas	Yellowish callus, root	Brown callus	Brown callus
Özbek	Shoot	Brown callus, root	Brown callus
Y. Kırmızı	Yellowish callus, root	Yellowish- brown callus	Brown callus
Pul 11	Yellowish callus, root	Brown callus, root	Brown callus
Emre 20	Yellowish- brown callus	Grey Callus	Yellowish callus
Kırmızı 51	No development	No development	Yellowish callus
Malazgirt 89	Brown callus, root	Grey Callus, root	Brown callus
Çiftçi	Grey Callus, root	Grey Callus	Grey Callus

Applications with SH medium

In applications with 0.5 mg / l NAA, Çiftçi didn't produce any callus in SH medium. Root production seen in other applications, wasn't observed Sultan 1 ve Kırmızı 51 varieties. Especially, production of cream-brown callus by Kafkas, Yerli Kırmızı ve Pul 11 seen as an important marker of embryogenic callus. Embryogenic cells were observed in microscopic observation. NAA application in low concentrations was observed almost give the same reaction in the environments that we use to determine the best medium. On the other hand, 0.5 mg / l NAA has been found to be more successful acording to 1 mg / l NAA and 2 mg / l NAA of application. Sazak 91, KAzak , Yerli kırmızı and Pul11 were identified as particularly significantly successful in microscopic observation .

Somatic embryos production practices on MS medium

Callus production aplications were made with Sazak 91, Kazak, Yerli Kırmızı and Pul 11 lentil varieties with low concentrations (0.3 mg / l 0.5 mg / l and 1 mg / l) NAA on MS medium. The applications are shown in Table 3.

Especially at lower concentrations of NAA applications was known to be produced transparent, loose, yellow callus, in a previous stage of the study.The aim of this application is to stimulate to produce embryos from high amounts of callus in liquid culture. Transparent, loose yellow callus and root development was also observed in all medium modified with 0.3 mg/l NAA . In the medium modified with 0.5 mg/l NAA, white, yellow callus was observed with root development from Pul 11 variety. Besides the formation of root growth, it was observed in transparent yellow calluses from Yerli kırmızı variety. Sazak 91 ve Kafkas varieties white, yellow callus was observed with root growth. Almost no root growth was observed in the culture suplemented with 1 mg/l NAA .

Practice for determining callus production capability of in vitro grown lentil plants

Root, hypocotyl, cotyledon, nodes, internodes and leaf stem + leaf blade explants were taken from in vitro grown young plant parts of Sazak 91, Kafkas, Yerli Kırmızı and Pul 11 lentil varieties which they had the highest potential to produce somatic embryo and the applications were made to explore the potential of producing somatic embryos on MS medium suplemented with NAA and 2,4-D.

Determine the potential of producing callus on MS medium modified with NAA from the explants taken from lentil plants grown in vitro

Explants taken from Pul 11, Sazak 91, Kafkas and Yerli Kırmızı, found the most successful lentil varieties by means of somatic embryo production grown, were subjected to applications at low concentrations NAA on MS medium. The applications of observations are shown in Table 4.

It was observed that, stem explants produced transparent callus in all applications, the petiole of the leaf blade explants didn't response to any treatment . In any of explants taken from Pul 11, somatic cells were not observed. Cotyledon explants of Sazak 91 were found to produce transparent yellow callus in the medium suplemented with 1 mg/l NAA. Cotyledon explants of Kazak variety was observed to produce transparent yellow callus in the medium modified with 0.5 ve 1 mg/l NAA, hypocotyl, nodes and internodes explants of Kazak variety was observed to produce transparent yellow callus in the medium modified with 1 mg/l NAA. Cotyledon explants of Yerli Kırmızı variety was observed to produce transparent yellow callus in the medium modified with 0.5 mg/l NAA.

Determine the potential of producing callus on MS medium modified with 2,4-D from the explants taken from lentil plants grown in vitro

It was observed that stem explants produced transparent callus in all applications, while petiole, the leaf blade explant didn't give response any applications. Cotyledon explants and internodes explants of Pul 11 was observed to generate transparent yellow callus on MS medium suplemented with 1 mg/l 2,4-D and 0.3 mg/l 2,4-D, respectively. Hypocotyl explants and cotyledon explants of Sazak 91 was observed to generate transparent yellow callus on MS medium suplemented with 1 mg/l 2,4-D. Hypocotyl explants and nodes and internodes explants of Kafkas variety was observed to produce transparent yellow callus on MS medium suplemented with 1 mg/l 2,4-D and 0.3 mg/l +1 mg/l 2,4-D, respectively . Cotyledon explants of Kafkas variety was observed to produce yellow callus in all concentrations of PGR. Cotyledon explants of Yerli Kırmızı variety was observed to produce yellow callus in all concentrations of PGR.

Embryo Development Applications in Liquid Culture

Embryogenic callus obtained with NAA at lower concentrations also were grown in liquid MS medium having the same content. Cultures were shaken at 25 ± 2 ° C for 15 days, at 90-100 rev / min. At this stage, aim of the study is to make the calli interact with each other in all proportions in liquid cultures.

Embryo Maturation Applications in Liquid Medium

Objective in this step, is maturation of the embryos produced in nutrient media supplemented with PGR and incubated on a shaker for 15 days. After the nutrient medium free from content PGR, hhey were incubated on a shaker for 7 days. During this time, it was observed that the embryos matured without the need for any other factors. Maturing embryos were examined under a microscope.

Table 3. Applications producing callus on MS medium

	0.3 mg/l NAA	0.5 mg/l NAA	1 mg/l NAA
Pul 11	Transparent, loose ,yellow callus, root	White, yellow callus, root	Tranparent, yellowish callus
Sazak 91	Transparent, loose ,yellow callus, root	Transparent, White, yellow callus, root	Transparent, yellow callus
Kafkas	Transparent, loose ,yellow callus, root	Transparent, white callus, root	White, yellowish callus
Yerli Kırmızı	Transparent, loose ,yellow callus, root	Transparent, Yellow callus, root	Transparent, yellowish callus

Table 4. Determining the potential of producing callus from plant parts with NAA in MS medium

Eksplant	PGR	Pul 11	Sazak 91	Kafkas	Y. Kırmızı
Root	MS-0.3 mg/l NAA	Transparent callus	Transparent callus	Transparent callus	Transparent callus
	MS-0.5 mg/l NAA	Transparent callus	Transparent callus	Transparent callus	Transparent callus
	MS-1 mg/l NAA	Transparent callus	Transparent callus	Transparent callus	Transparent callus
Hypocotyl	MS-0.3 mg/l NAA	No development	Yellow callus	Yellowish- white callus	No development
	MS-0.5 mg/l NAA	No development	Yellow callus	Transparent callus	No development
	MS-1 mg/l NAA	No development.	Transparent callus	Transparent , yellow callus	No development
Cotyledone	MS-0.3 mg/l NAA	No development	No development	No development	No development
	MS-0.5 mg/l NAA	No development	No development	Transparent , yellow callus	Transparent , yellow callus
	MS-1 mg/l NAA	Transparent callus	Transparent , yellow callus	Transparent , yellow callus	Transparent callus
Nod	MS-0.3 mg/l NAA	No development	No development	Transparent , white callus	Transparent callus, root
	MS-0.5 mg/l NAA	Transparent callus	No development	Transparent callus	No development
	MS-1 mg/l NAA	No development	No development	Transparent , yellow callus	No development
İnternod	MS-0.3 mg/l NAA	No development	No development	White callus	No development
	MS-0.5 mg/l NAA	No development	No development	Transparent callus	White callus
	MS-1 mg/l NAA	No development	No development	Transparent , yellow callus	No development
Petiole+ leaf	MS-0.3 mg/l NAA	No development	No development	No development	No development
	MS-0.5 mg/l NAA	No development	No development	No development	No development.
	MS-1 mg/l NAA	No development	No development	No development	No development

Table 5. Determining the potential of producing callus from explants with 2,4-D

Eksplant	BBD	Pul 11	Sazak 91	Kafkas	Y. Kırmızı
Root	MS-0.3 mg/l 2,4-D	Transparent callus	Transparent callus	Transparent callus	Transparent callus
	MS-0.5 mg/l 2,4-D	Transparent callus	Transparent callus	Transparent callus	Transparent callus
	MS-1 mg/l 2,4-D	Transparent callus	Transparent callus	Transparent callus	Transparent callus
Hypocotil	MS-0.3 mg/l 2,4-D	No development	Transparent callus	White, yellow callus	No development
	MS-0.5 mg/l 2,4-D	No development	White callus	White, yellow callus	No development
	MS-1 mg/l 2,4-D	No development	Transparent, yellow callus	Transparent, yellow callus	Transparent callus
Cotyledon	MS-0.3 mg/l 2,4-D	No development	No development	Transparent, yellow callus	Transparent, yellow callus
	MS-0.5 mg/l 2,4-D	No development	No development	Transparent, yellow callus	Transparent, yellow callus
	MS-1 mg/l 2,4-D	Transparent, yellow callus	Transparent, yellow callus	Transparent, yellow callus	Transparent, yellow callus
Nod	MS-0.3 mg/l 2,4-D	No development	No development	Transparent, yellow callus	No development
	MS-0.5 mg/l 2,4-D	No development	White, yellow callus	White callus	White callus
	MS-1 mg/l 2,4-D	Transparent callus	No development	Transparent, yellow callus	Transparent callus
İnternod	MS-0.3 mg/l 2,4-D	Transparent, yellow callus	No development	Transparent, yellow callus	No development
	MS-0.5 mg/l 2,4-D	No development	No development	Transparent callus	Transparent callus
	MS-1 mg/l 2,4-D	No development	No development	Transparent, yellow callus	Transparent callus
Petiole, leaf	MS-0.3 mg/l 2,4-D	No development	No development	No development	No development
	MS-0.5 mg/l 2,4-D	No development	No development	No development	No development
	MS-1 mg/l 2,4-D	No development	No development	No development	No development

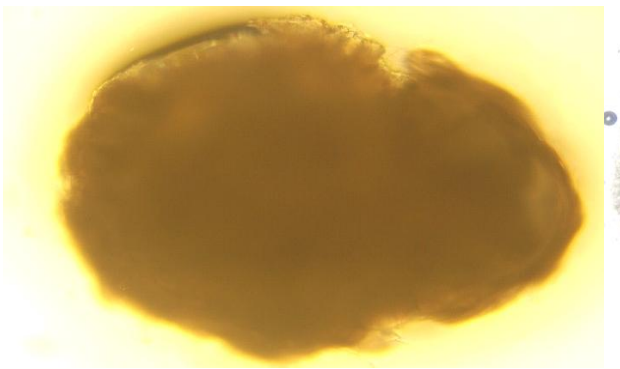

Figure 1. MS-0.5 NAA Yerli Kırmızı "globular" structure.

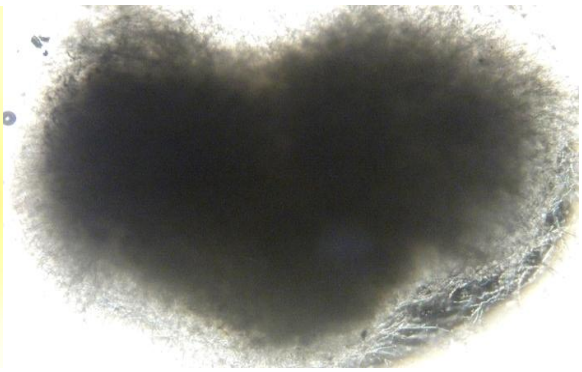

Figure 2. MS-0.3 NAA Sazak 91 "heart" structure.

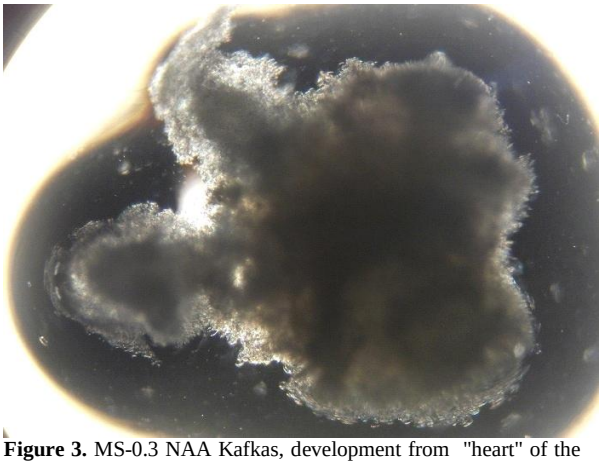

Figure 3. MS-0.3 NAA Kafkas, development from "heart" of the structure towards "torpedo"

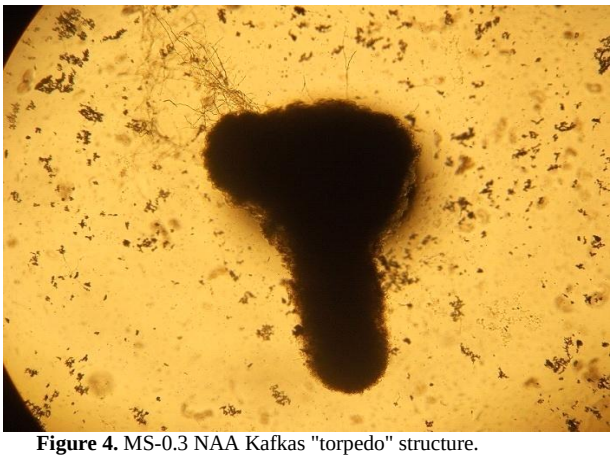

Figure 4. MS-0.3 NAA Kafkas "torpedo" structure.

DISCUSSION AND CONCLUSIONS

This study for producing somatic embryos in vitro from 11 native lentil varieties belong to *Lens culinaris* Medik. species cultured on MS, B5, SH,WH medium modified with 2,4-D, NAA ve BAP, takes a total of 45 ± 7 days including , sterilization the explants, hold in bidistilled pure water (24 hours), planting the explants on nutrient medium modified with PGR (2 hours), incubation the solid nutrient medium on the conditioning cabinet (21 days), incubation of the broth on shaker (14 days) and liquid culture without PGR for embryo development (7 days). Erksan and Witcombe [14], in their study of the lentils materials , reported that , the number of days to flowering was to be 115-158 days. Working in vitro reduces production time and also ensures independent of the seasons and environmental conditions.

Although we use mostly mature zygotic embryos as explant source in our study , hypocotyl, cotyledon, nodes, internodes and leaf stem + leaf blade of plants grown in vitro for 10 days are used as explant source. De Jong et al. [15] and Parrot et al. [16] reported that arious plant parts could use for somatic embryos. Especially immature zygotic embryos are an important source for the production of somatic embryos. Welander [17], Fasolou et al. [18], Yepes et al.[19] and Pierik [20] reported that young plants and tissues showed larger morphogenic potential than those aged in culture. Turhan and Base [21] reported in their study that the highest callus production occured on MS medium suplemented with 4 mg/l 2,4-D + 1 mg/l NAA from mature embryos received cultured in various PGR combination and concentration. In the same study, the highest embryogenic callus production has been found to occur from mature embryos without endosperm. It was observed to be taken better results from mature zygotic embryos in our study. Consequently, our observations is compatible with, Turhan and Base [21], Welander [17], Yepes et al. [19], Fasolou et al. [18] and Pierik [20]. It can not make an inference about notifications of De Jong et al. [15] and Parrot et al. [16]. Also, According to the report made by Kumari and Saradha [22], the cotyledons is the most convenient source of explants for callus formation. Cotyledons and roots explants taken from young plants grown in vitro for 10 days, were observed to form callus. Especially cotyledon explants producing loose yellow, transparent callus was identified as the most important source explants after zygotic embryo.

In our work, MS, Gamborgs B5, SH and White nutrient media were used as nutrient media. Cells, tissues and organs only can grow in the medium containing the appropriate agents. An obvious superiority among nutrient media we used to others weren't observed in our study. With microscopic examination, all genotypes capable of producing embryogenic was observed in all food environments. Advantages can only be measured by the amount of produced callus. Thus, MS medium used in this study can be said to be better than other nutrient media. Therefore, mainly MS medium was used. According to Berrios et al. [23], specific genotype explant reaction depends mainly on the interaction between the genotype and environment culture conditions. However, in our study, rather than the relationship between genotype and environment it was observed a high correlation between genotype and PGR. It was used 2,4-D and NAA as auxin BAP as cytokinin in our study. All plant growth regulators were performed, both alone and with various combinations.

Shoot growth, which generally feature of cytokinins was observed in all nutrient media modified with BAP. According to the report made by Kumari and Saradha [22], when explants are taken only BAP or combination of NAA + BAP subculture, shoot formation occurred. However, NAA has been reported to suppres the effect of the formation shoot of BAP. In our study, in the culture contained combination of NAA + BAP was observed the same situation. BAP was observed to suppress the effect of the formation callus of NAA. Nevertheless, compared with the amount of callus produced NAA alone was found to yield very little callus. According to the report made by Ozcan et al. [24] the highest plant regeneration in sainfoin was observed on MS medium modified with 0.5 mg / l BAP. İn our study, High amount of shoot formation was observed in the cultures modified with BAP alone. Bap applied at low concentrations was observed to create a high amount of shoots. On the other hand it was observed that the content of the nutrient medium is important in forming shoots. Nutrient media , can be listed as B5, SH, White and MS, in terms of success to create shoot. In the culture modified with BAP and 2,4-D no callus was observed unlike the culture modified with BAP and NAA.

2,4-D is the most commonly used PGR. In the nutrient medium modified with 2,4-D, the first week, no reaction was observed, after 2 weeks the production of callus and after 3 weeks quickly blackening is observed. Thus, the subculture of the culture medium was observed to be done before the start of blackening. On the other hand, callus produced by 2,4-D was observed a lower amount. According to the report made by Bezo ve Stefunova [25], callus formed on MS medium suplemented with 2,4-D

from the explants taken from Hypericum perforatum. According to the report made by Shah et al.[26], Alizadeh et al. [27], Delporte et al. [28], the culture with 2-4 mg / l 2,4-D was more effective for promoting callus, whereas the lower concentration of the 2,4-D has been reported to enhance the ability regeneration. In our study 1 mg / l 2,4-D was found to yield larger quantities of callus than 2 mg / l 2,4-D . According to the report made by Arı et al. [29] , Astragalus chrysochlorus is a spesices of Fabaceae was found to yield callus on MS medium with 0.5 mg / l 2,4-D. In this case, it can be said our studies with 2,4-D is incompatible with Shah et al. [26], Alizadeh et al. [27], Delporte et al. [28] declarations, is compatible with notifications of Arı et al. [29].

The most successful PGR of our study was found to be NAA. According to the report made by Ferrari et al. [30], callus fresh weight was observed to be higher in medium containing NAA. In our study, it was clearly seen that NAA is a PGR provides the maximum amount of callus production. According to the report made by Arı et al. [29] , Astragalus chrysochlorus which is a species of Fabaceae was observed to form root on MS medium containing 0.4 mg / l NAA. In our studies, the culture with 0.3 mg / l and 0.5 mg / l NAA, besides the large amounts of callus, root formation was observed . NAA concentration increases root formation is reduced. On the other hand NAA concentration increases, in the amount of callus it was observed to be decreased.

According to the report made by Werbrouck and Debergh [31], high ratio of cytokinin / auxin supports of shoot formation, high ratio of auxin / cytokinin supports of root formation, equal ratio of auxin and cytokinin supports formation of callus. In many reports,the increased cytokine concentrations have been reported to increase the formation shoot [32, 33, 34, 35, 36, 37, 38]. In our study, applications with high ratio of cytokinin / auxin shoot formation, with high ratio of auxin / cytokinin root formation was observed to be occured. In this case our work can be said to be compatible with notifications of Werboruck ve Debergh [31], Sagawa ve Kunisaki [32], Anderson ve ark. [33], Simpson ve Bell [34], Kris ve ark.[35], Eric [36], Al-Bahrany [37] , Saadat ve Hennerty [38]. However, in applications where auxin and cytokinin ratio is equal, callus production occurred in some instances very least, the majority observed that no callus produced.

In plant tissue culture, under specific culture conditions, Two different genotypes are reported not to give the same reaction [39], although all culture conditions at all stages were the same, it was observed that each genotype reacted differently in our study. There are wide differences in terms of plant regeneration capacity in the plant world. There are significant differences even within a single species in terms of cell division and regeneration capacity [20]. Hoque and Mansfield [40] , in the study investigated the effect of genotype on callus induction, stated that genotype was effective in the regeneration response. Our work is compatible with these notifications.

In our study, the potential to produce somatic embryos was observed that depended on the high correlation between genotype and plant growth regulators. The ability of regeneration and callus formation is also reported that vary according to the kind and lines harmonious Birsin ve ark. [41].

According to Finer [42] , achieving to get the fast-dividing embryogenic cells from explants cultured on is primarily required at higher concentrations auxin. After promoting embryo formation in nutrient medium containing auxin, cultures must be transferred into auxin-free medium. When explants are cultured in nutrient medium containing high auxin, before embryogenic callus formation is observed then the formation of pro-embryos on the callus is observed. This callus when transferred to auxin-free nutrient medium, pro-embryos, the bipolar embryos are formed and if conditions are suitable, the seedlings can be obtained from these embryos. Considering Finer [42] 's notification, before explants were cultured in nutrient media modified with auxin, then explants were are transferred to medium containing auxin to promote formation of embryos as reported by Tisserat [43]. After embryonic callus and pro-embryos produced in nutrient medium modified with oxcin, bipolar embryos was evolved from pro-embryos on PGR free nutrient medium.

The explants cultured in nutrient media in our study was performed in a certain number. It was observed differences in between a large number of explants (7-8) and a small number of explants (2-3) in terms of producing callus. Çiftçi [44] were reported that increasing planting density increased plant height and first pod height, the effects of plant density on yield of grain per unit area is statistically significant. In this regard, in the in vitro studies, It was found necessary to consider the sowing.

Explants were taken to culture in nutrient medium solidified with agar at first, then they also transferred to the liquid culture with the same content. Bornman ve Vogelman [45] reported that, the physical condition of the environment affected to the diffusion of nutrients and plant growth regulators. Liquid cultures are advantageous relative to the solid nutrient medium in many ways. Increasing density of agar in nutrient medium, cause to inhibition of uptake the nutrients and PGR by the tissue and reduction of growth rate Bornman ve Vogelman [45] ve Chawla [46]. According to Pierik [20], plants could get nutrients PGR and other substances, with all the parts in a liquid medium, thus, in liquid culture according to the agar-containing medium which explants only contact the bottom of the medium is generally observed with the better growth and development. According to the report made by Alvard et al. [47], liquid media usage in tissue culture is a factor that reduces the cost of micro-propagation.

According to the report made by Mohammed [48], meristem and shoot tip culture's growth regulatory requirements depends on the type of the plant and stage of culture. In our study, the removal of PGR from material received in liquid culture were observed to be an important step in production of somatic embryos. Pierik [20] , Kung [49] and Endress [50] is reported that in the first study for Somatic embryogenesis is made by Steward et al. [51] for carrot plant.

In our study, made 11 varieties of *Lens culinaris* Medic. species to produce somatic embryos in vitro , cotyledon structure has been reached on MS medium modified with 0.3 mg / l NAA from Yerli Kırmzı variety. According to the report made by Çiftçi ve Ülker [52] using Sazak-91, Kışlık Yeşil-21, Kışlık Pul-11 ve Yerli Kırmızı in Van ecological conditions, Yerli Kırmızı variety can be grown in Lake Van basin and have as high stability. In vitro conditions, in our study we have identified Yerli Kırmızı race as the most successful race and this consequence is compatible with the notifications made by Çiftçi ve Ülker [52].

Today, methods for producing a high rate of somatic embryos have been developed in many important crop plants such as wheat [53] ,corn [54, 55], rice [56], soybean [57] , peas [24], alfalfa [58]. However Lentils are quite

reluctant plant in that topic. It have not yet created a complete protocol for the production of somatic embryos in Lentils. Although somatic embryos preferences vary according to species of plants, can occur even between varieties of the same species (cultivar / genotype / strain) . Their requests can be quite different in terms of environment, macro and micro nutrients, PGR kind, concentration and combinations of light and other environmental conditions. Therefore, intensive research and development is required. In this study, under the guidance of earlier studies, it has tried to develop a protocol on lentils somatic embryos. In the results of this study, we can say the best embryogenic tissue was Yerli kırmızı varieties on MS medium modified with 0.3-1 mg / l NAA. Proembriyo, globular, heart, torpedo embryo and the cotyledons were obtained. Maintaining liquid culture studies, mature embryos can be obtained and carried out the regeneration.

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