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Araştırma Makalesi/*Research Article*

Multiple Shoot and Callus Formation in Different Explants of The Medicinally Important *Peganum harmala* L.

Burcu Çetin[1]*, Halil İbrahim Koçak[1]
[1]Department of Biology, Faculty of Art and Science, University of Dumlupinar, Kütahya
(orcid.org/0000-0003-1465-1464); (orcid.org/0000-0003-2605-7550)
*e-mail: burcu.cetin@dpu.edu.tr

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Abstract: In this study, the micropropagation potential of the medicinally important *Peganum harmala* L. plant containing valuable metabolites has been examined. For this purpose, hypocotyl and shoot tips isolated from *P. harmala* plants were applied different concentrations and combinations of plant growth regulators of benzyl amino purine (BAP), naphthalene acetic acid (NAA), and indole acetic acid (IAA). Best callus growth were obtained in hypocotyl explants in Murashige and Skoog (MS) medium that contained 1 mg L^{-1} NAA and 1 mg L^{-1} BAP. Best results concerning shoot count per explant (5.42±0.313 count) and shoot length (2.95±0.06 cm) were determined in MS medium which contained 1.5 mg L^{-1} BAP. IBA applications were performed in order to root the obtained shoots. The rooted plants were transferred to the soil with a success rate of 72%.

Keywords: *Peganum harmala* L., plant growth regulator, plant tissue culture, micropropagation

Tıbbi Öneme Sahip *Peganum harmala* L. Bitkisinin Farklı Eksplantlarında Çoklu Sürgün ve Kallus Oluşumu

Öz: Bu çalışmada içerdiği metabolitler nedeni ile tıbbi öneme sahip olan *Peganum harmala* L. bitkisinin mikroçoğaltım potansiyeli çalışılmıştır. Bu amaçla, *P. harmala* bitkilerinden izole edilen hipokotil ve sürgün ucu eksplantlarına farklı konsantrasyonlarda ve kombinasyonlarda benzil amino pürin (BAP), naftalen asetik asit (NAA) ve indol asetik asit (IAA) bitki büyüme düzenleyicileri uygulanmıştır. Yapılan uygulamalar sonucunda, en iyi kallus gelişimi hipokotil eksplantlarında 1 mg L^{-1} NAA ve 1 mg L^{-1} BAP içeren Murashige ve Skoog (MS) besin ortamında elde edilmiştir. Eksplant başına düşen sürgün sayısı (5.42±0.32 adet) ve sürgün uzunluğu (2.95±0.06 cm) en iyi 1.5 mg L^{-1} BAP içeren MS besin ortamında belirlenmiştir. Elde edilen sürgünlerin köklendirilmesi amacıyla IBA uygulamaları yapılmıştır. Çalışmalar sonucunda elde edilen köklenmiş bitkiler %72 başarı ile toprağa aktarılmışladır.

Anahtar Kelimeler: *Peganum harmala* L., bitki büyüme düzenleyici, bitki doku kültürü, mikroçoğaltım.

1. Introduction

Peganum harmala L. is an important medicinal plant which was included in Zygophyllaceae family before, but lately it has been included in Nitrariaceae family. It spreads across West India, North Africa, Central Asia and Mediterranean (Baytop 1999; Goel, et al. 2009). Its pharmacologically active compounds are; a few alkaloids, β-carbolines (such as harmine, harmaline, harmane and harmalol) and quinozaline derivatives vasicine and vasicinone (Mirzaie, et al. 2007). Because of the metabolites it includes it has been reported that it has diuretic (Al-Saikhan, et al. 2016), antifebrile, emetic, hallucinogenic, hypertonic, antispasmodic, (Nirouman, et al. 2015), analgesic, antiseptic (Asgarpanah and Ramezanloo, 2012), antibacterial (Shafiei, et al. 2011), antifungal (Diba, et al. 2015), antiparasitic (Tanweer, et al. 2014), and antitumoral (Wang, et al 2015) effects. Aside from its medicinal importance, the dried capsules of this plant are used as a red dye for carpets and wool, as incense, or as charm for evil eye by superstitious people (Kırıcı, et al. 2018; Koyuncu, et al. 2008; Kartal, et al. 2003).

Lately, it has been reported that there are many studies in the literature concerning the use of plant tissue cultures in medicinal plant production (Yue, et al. 2016; Jeevan, et al 2017; Moharana, et al. 2018). Plant tissue culture

techniques make it possible to produce many plants, tissue or plant metabolites, by providing the necessary light, heat and basic nutritional elements *in vitro* conditions. The products obtained in controlled conditions can be used as raw material in medicine, food and cosmetic industries. In this study, the effects of plant growth regulators over the forming of callus and shoots which are used on medically and economically important *Peganum harmala* L. plant's different explants have been researched.

2. Material and Methods

2.1. Sterilization of *Peganum harmala* seeds and *in vitro* germination

First, the seeds of *P. harmala* L. were immersed in 70% ethyl alcohol for 4 minutes and then in %10 commercial bleach (containing 5% NaOCl) for 6 minutes for surface sterilization and then rinsed in distilled sterile water three times for three minutes each. The seeds were taken on sterile napkins and excess water on them was filtered and after that they were germinated in Murashige and Skoog (MS) nutrient medium (Murashige and Skoog 1962). Culture procedure was continued at 24±2 °C in a plant growth compartment, which provided 4000 lux for 16 hours of light and 8 hours of darkness in photoperiod and 50% humidity. Hypocotyl explants isolated from the seeds and germinated *in vitro* condition in a sterile way were used in callus research and shoot tip explants were used in shoot research.

2.2. Callus studies in *Peganum harmala* explants

Hypocotyl explants which were obtained from the seeds of *P. harmala*, which were planted in MS nutrient media, were cut approximately 1 cm long and transferred to 9 different concentrations of MS nutrient media which include BAP and NAA. As a control group, a MS nutrient medium with no plant growth regulator was used. The cut hypocotyl explants were weighted in precision scales before being transferred to nutrient media. The experiment continued for 6 weeks and callus development was determined by weighing during the subculture procedures, which were performed once in two weeks.

2.3. Shoot studies on *P. harmala* shoot tip explants

The shoot tips of plantlets of approximately 0.5 cm length were excided from *in vitro* germinated *P. harmala* seeds that were planted in MS nutrient medium and were transferred to MS nutrient media containing 7 different concentrations and combinations BAP and IBA for shoot propagation studies. Shoot studies were continued for 6 weeks with subculture studies performed at 3-week intervals. The control group consisted of MS nutrient medium without any plant growth regulator. As a result of the observations the number of the shoot per explant and length of the shoots per explants were determined.

2.4. Rooting *P. harmala* shoots obtained *in vitro* studies

Multiple shoots obtained from shoot tip explants were separated from each other and transferred to rooting media. MS nutrient media containing different concentrations of IBA were used as the rooting medium, and MS nutrient media containing no IBA were used as the control group. In order to alter the hormone content to promote the root growth, shoots were incubated in MS nutrient medium that contains 1.5 mg L^{-1} IBA for 2 weeks, in ½ MS nutrient containing 1.5 mg L^{-1} IBA for 2 weeks and ½ MS nutrient containing no IBA for 4 weeks, respectively. Rooting studies continued for a total of 8 weeks.

2.5. Acclimatization and transferring to soil of *P. harmala* plantlets

The jar lids were opened and closed for short periods of time for one week in order to provide adaptation to the external environment before the *P. harmala* plants obtained in the studies were transferred to the soil. The plantlets were taken from the nutrient media and the agar was removed under the tap water. After this process, the plantlets were planted in small containers with sterile soil and covered with transparent plastics for greenhouse effect. The transparent

plastics were opened every three days to control and to add water. At the end of the second week, plantlets were unboxed and transferred to larger pots and protected from direct sunlight during their growth.

3. Results

3.1. Findings on *in vitro* germination of the *P. harmala* seeds

In plant tissue studies, *in vitro* germination yields more successful results when compared to external environment to obtain sterile explants (Kocaçalışkan 2017). It is common to use NaOCl as a sterilant during the sterilization process (Perez-Tornero, et al. 2010; Ebad, et al. 2015; Nunes, et al. 2018). As a result of this study, planlets that were 80% sterile and 10% contaminated were obtained after the sterilization of the *P. harmala* seeds by 70% ethyl alcohol for 4 minutes and %10 commercial bleach (containing 5% NaOCl) for 6 minutes.

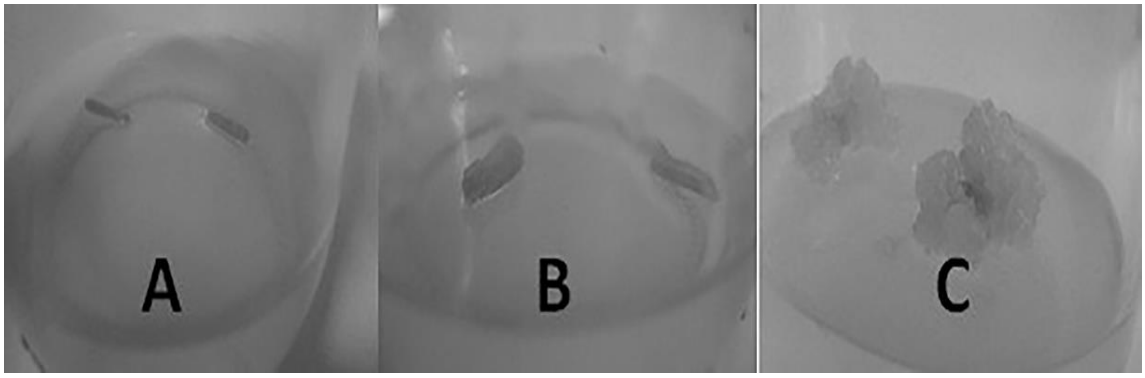

Figure 1. Callus formation on *P. harmala* hypocotyl explant (1mg L^{-1} NAA+1mg L^{-1} BAP MS)
A: First week **B:** Two weeks old explants **C:** Six weeks old explants
Şekil 1. *P. harmala hipokotil eksplantlarında kallus oluşumu (1mg L^{-1} NAA+1mg L^{-1} BAP MS)*
A: *İlk hafta* ***B:*** *İki haftalık eksplantlar* ***C:*** *Altı haftalık eksplantlar*

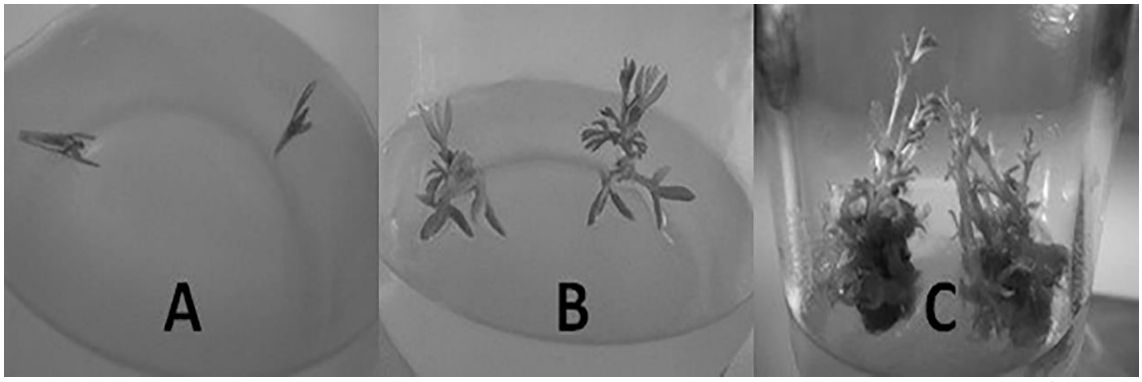

Figure 2. Multiple shoot formation on *P. harmala* shoot tip explant (1.5 mg L^{-1} BAP)
A: First week, **B:** Two weeks old explants, **C:** Six weeks old explants
Şekil 2. *P. harmala sürgün ucu eksplantlarındaki çoklu sürgün oluşumları (1.5 mg L^{-1} BAP)*
A: *İlk hafta* ***B:*** *İki haftalık eksplantlar,* ***C:*** *Altı haftalık eksplantlar*

3.2 Findings on excided callus from plant extracts of *P. harmala*

Hypocotyls of *P. harmala* plantlets which were excited as sterile were cut approximately 1cm long and transferred to 10 different concentrations of MS media which include BAP and NAA. Calluses were obtained from the explants which had stayed in culture medium for 6 weeks (Figure 1). The results of the researches on calluses that were obtained from *P. harmala* plant were evaluated with F and TUKEY-HSD tests. According to the analysis, it has been determined that plant growth regulators have statistical importance over the growth of callus.

The most major callus development was observed in the MS medium which consists of 1 mg L^{-1} NAA + 1 mg L^{-1} BAP while the slowest callus development was detected in the 5 mg L^{-1} NAA + 0.1 mg L^{-1} BAP treatment. The result of our application of equal amounts of auxin and cytokine is in harmony with several other research results that have been reported (Yokoya 2000; Verma, et al. 2016; Khan, et al. 2017). Besides, it has been noted that the callus development was high in the applications in which the rates of cytokinin and auxin were close, and that it was low in the applications in which high cytokine and low auxin treatments were carried out (Table 1).

Table 1. Weights of calli formed in *P. harmala* hypocotyl explant (p<0,05).

Çizelge 1. *P. harmala hipokotil eksplantlarında oluşan kallusların ağırlıkları*

Plant Growth Regulators (mg L^{-1})		Callus Weights Mean ± SE
BAP	NAA	(gr)
-	-	0.08 ± 0.003 f
1.0	0.1	0.76 ± 0.066 d
1.0	0.5	1.05 ± 0.086 bc
1.0	1.0	1.97 ± 0.066 a
3.0	0.1	0.71 ± 0.033 d
3.0	0.5	1.27 ± 0.061 b
3.0	1.0	0.82 ± 0.070 c
5.0	0.1	0.39 ± 0.030 e
5.0	0.5	1.12 ± 0.038 b
5.0	1.0	1.12 ± 0.059 b

3.3. Findings on shoot obtainment from the plant explants of *P. harmala*

Shoot tips isolated from the *P. harmala* plantlets which were obtained in a sterile way were used as explants. For shoot studies, 12 different MS media which contain BAP only and combinations of BAP and IBA were preferred. As a result of the observations, the number and length of shoot per explant were determined. The results of the researches on shoot obtainment from *P. harmala* plant were evaluated with F and TUKEY-HSD tests. According to the analysis, it has been determined that plant growth regulators have statistical importance over the formation of the shoots. As a result of this study, it has been found out that shoots in the highest number and length can be obtained from the shoot tip explants of the *P. harmala* plants in MS media containing 1.5 mg L^{-1} BAP. Besides, shoots in the lowest number and length were acquired in the treatments of equal amounts of cytokinin and auxin (Figure 2, Table 2).

Table 2. The number and length of shoots obtained from shoot tip explants of *P. harmala* (p<0,05)

Çizelge 2. *P. harmala'nın sürgün ucu eksplantlarında elde edilen sürgünlerin sayı ve uzunlukları (p<0,05)*

Plant Growth Regulators (mg L^{-1})		Shoot Number Mean ± SE (number)	Shoot Length Mean ± SE (cm)
BAP	IBA		
-	-	1.67 ± 0.225 d	1.63 ± 0.025 g
0.5	-	3.25 ± 0.131 bc	1.74 ± 0.051 f
0.5	0.5	2.67 ± 0.142 c	1.53 ± 0.032 h
0.5	1.0	3.08 ± 0.150 c	1.67 ± 0.043 g
1.0	-	4.00 ± 0.174 b	1.93 ± 0.037 e
1.0	0.5	3.42 ± 0.150 bc	2.09 ± 0.062 d
1.0	1.0	2.92 ± 0.150 c	1.64 ± 0.041 g
1.5	-	5.42 ± 0.313 a	2.95 ± 0.057 a
1.5	0.5	5.25 ± 0.250 a	2.63 ± 0.035 b
1.5	1.0	5.08 ± 0.193 a	2.43 ± 0.052 bc
2.0	-	3.42 ± 0.150 bc	2.29 ± 0.041 c
2.0	0.5	3.25 ± 0.131 bc	2.26 ± 0.073 cd
2.0	1.0	3.33 ± 0.142 bc	2.03 ± 0.063 c

3.4. Results of rooting and acclimatization of *P. harmala* shoots

The use of IBA plant growth regulators in plant tissue culture researches to root shoots has been reported in many different studies in the literature (Aina, et al. 2015; Muttaleb, et al. 2017; Ghimire, et al. 2018). In this study, the multiple shoots that had been obtained were separated one by one and transferred to MS media which contained IBA as one of the plant growth regulators. Since the shoots were in shoot medium for 6 weeks during the shoot researches, the hormones in the shoots were gradually changed to induce root development. In the first phase of the rooting, the shoots were transferred to MS nutrient medium which contained 1.5 mg L^{-1} IBA for 2 weeks and cultivated. After that process, during the transfer to a sub-cultivation nutrient medium was changed to ½ MS and then the shoots were transferred to ½ MS which contained 1.5 mg L^{-1} IBA for another 2-week

cultivation period. In the next phases of the process, a medium without IBA was preferred, and it was used as ½ MS. Explants were left to root for 4 weeks in ½ MS medium and they were transferred to sub-cultivation every two weeks. During a total of 8 weeks of rooting process, first roots formed at the 4th week and at the end of this process all explants were rooted (Figure 3).

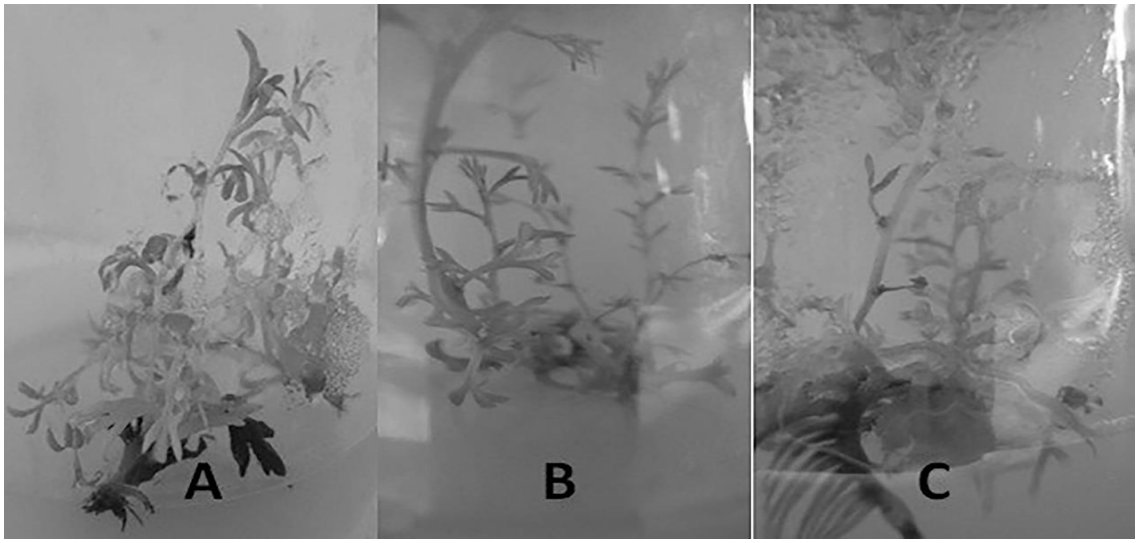

Figure 3. Root formation of regenerated shoot (1.5 mg L^{-1} IBA+MS) **A:** four weeks old roots, **B:** six weeks old roots, **C:** eight weeks old roots
Şekil 3. *Rejenere sürgünlerde oluşan kökler (1.5 mg L^{-1} IBA+MS)* ***A:*** *Dört haftalık kökler,* ***B:*** *Altı haftalık kökler,* ***C:*** *Sekiz haftalık kökler*

Plantlets acquired at the end of the research were acclimatized for two weeks to external environment. At the end of the 2 weeks, plantlets were transferred to larger pots and left to grow with no cover and no direct sunlight in the laboratory. Rooted plants were transferred to soil after acclimatization to external environment with 72% success rate (Figure 4).

Figure 4. Regenerated *P. harmala* plants transferred to soil
Şekil 4. *Toprağa aktarılmış rejenere P. harmala bitkileri*

4. Discussion

The studies over the controlled production of a lot of plants which have medical and industrial importance with plant tissue culture method has gained speed. In this study, the effect of the application of plant growth regulators to different explants of *Peganum harmala* L., which has medical and economic importance, was determined. The data obtained from this study has the ability to show the way to many studies which investigate the production of tissue, cell, and secondary metabolites.

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