Black Sea Journal of Agriculture
doi: 10.47115/bsagriculture.1722353

Open Access Journal
e-ISSN: 2618 – 6578

Research Article
Volume 8 - Issue 5 : 646-652 / September 2025

IN VITRO DIRECT SHOOT REGENERATION OF KAMAN 1 WALNUT VARIETY AND KAMAN 5 WALNUT GENOTYPE (*Juglans regia* L.)

Ebru ŞİRİN[1]*, Sevil SAĞLAM YILMAZ[2], Kenan YILDIZ[3]

[1]*Kırşehir Ahi Evran University, Faculty of Agriculture, Department of Horticulture, 40100, Kırşehir, Türkiye*
[2]*Kırşehir Ahi Evran University, Faculty of Agriculture, Department of Agricultural Biotechnology, 40100, Kırşehir, Türkiye*
[3]*Tokat Gaziosmanpasa University, Faculty of Agriculture, Department of Horticulture, 60250, Tokat, Türkiye*

Abstract: In this study, a simple and effective clonal micropropagation protocol was developed from axillary buds of Kaman 1 walnut variety and Kaman 5 walnut genotype. For surface sterilization of explants, they were first treated in 70% ethanol for 1 minute and then treated with 0.2% HgCl2 containing two drops of Tween 20 for 5 minutes. To prevent browning of the explants, 100 mg/l Ascorbic Acid, 100 mg/l Citric Acid and 100 mg/l Ascorbic Acid+100 mg/l Citric Acid were added to the MS nutrient medium. In addition, explants were subcultured at 3, 24 and 48 hour intervals. It was observed that the most effective treatment in both Kaman 1 walnut variety and Kaman 5 walnut genotype was 100 mg/l AA + 100 mg/l SA + 48 hours subculture. Within the scope of the shoot regeneration study, six different combinations of 0.5, 1.0 and 1.5 mg/l doses of BAP and 0.5 and 1.0 mg/l doses of IBA were tried. The highest average number of shoots per explant 2.67 was obtained from the Kaman 1 variety on MS medium supplemented with 0.5 mg/l BAP and 1.0 mg/l IBA. On the other hand, in the medium containing 0.5 mg/l BAP and 0.5 mg/l IBA, the average number of shoots was 2.00 for the Kaman 1 cultivar and 0.67 for the Kaman 5 genotype. After the shoots were kept in a solution containing 4 mg/l IBA for 10 minutes, they were planted in pots containing a peat: perlite (1:1) mixture, but rooting couldn't be achieved from the shoots.

Keywords: Walnut, *İn vitro*, Tissue culture, Micropropagation, Antioxidant, Subculture

***Corresponding author:** Kırşehir Ahi Evran University, Faculty of Agriculture, Department of Horticulture, 40100, Kırşehir, Türkiye
E mail: ebru.sirin@ahievran.edu.tr (E. ŞİRİN)
Ebru ŞİRİN https://orcid.org/0000-0002-7416-6367
Sevil SAĞLAM YILMAZ https://orcid.org/0000-0003-1302-9147
Kenan YILDIZ https://orcid.org/0000-0003-3455-5146

Received: June 23, 2025
Accepted: August 11, 2025
Published: September 15, 2025

Cite as: Şirin E, Sağlam Yılmaz S, Yıldız K. 2025. In vitro direct shoot regeneration of Kaman 1 walnut variety and Kaman 5 walnut genotype (*Juglans regia* L.) BSJ Agri, 8(5): 646-652.

1. Introduction

Walnut (*Juglans* regia L.) is a very rich type of fruit in terms of nutritional value. It is rich in many vitamins including Thiamine, vitamin B6 and Folate. In addition to vitamins; it is rich in iron, zinc, copper, magnesium, phosphorus and potassium. Walnut is the only fruit type containing silver in fruit species. Another important element is selenium (Akça, 2009). Turkey is the homeland of walnuts as well as many fruit species and has an important potential in terms of walnut production. When it comes to vegetative propagation of walnut trees, the first thing that comes to mind is grafting (Şen, 2011). Another vegetative propagation method is propagation by tissue culture. Tissue culture enables intensive production of desired lines with its advantage. For this reason, it becomes an important option against traditional propagation methods such as seed, cutting, branching and grafting in various plant species. However, micropropagation of fruit species and characteristics of hard-shelled species is quite difficult compared to micropropagation of herbaceous plants. For this reason, explant selection and the physiological period of the explant, overcoming the harmful effects of tannin and other toxic compounds in cultures, ensuring an acceptable shoot growth rate and obtaining rooted shoots are extremely important for micropropagation of selected hard shellfish (Babaoğlu et al., 2002). A group of plants that are vegetatively propagated from a single plant and that carry the same genotype form a "clone". Vegetative production using very small plant parts (explants) under sterile conditions in the laboratory is called "*in vitro*" propagation or microproduction (Emiroğlu and Gürel, 2005). Since it is essential to preserve the genotype in clonal propagation, it is important to use shoot tips or axillary buds in *in vitro* production and to know that meristem tissues are stable in terms of genotype (D' Amato, 1977). The most important step in *in vitro* tissue culture is the sterilization process. Bacteria are the most common source of contamination. Acute bacterial or fungal contaminants may appear several days after culture. Therefore, the sterilization process should be handled at the entire

laboratory level. Growth regulators (2,4-D, IBA, IAA, Kinetin, BAP, etc.) added to the *in vitro* medium significantly affect development (Babaoğlu ve ark., 2002). As with many woody plants, the most important problem encountered in the propagation of walnut by tissue culture is the browning that occurs in the culture medium. Browning occurs as a result of the oxidation of phenols leaking from the cut surface of the explant. To overcome this problem, antioxidant substances such as polyvinyl pyrrolidone (PVP), citric acid, ascorbic acid, activated charcoal, thiourea, L-cysteine, glutamine, asparagine, arginine are added to the medium or subculture is frequently used (Rout et al., 1999). In countries where walnut cultivation is developed, many studies have been carried out on the propagation of walnuts by tissue culture and important knowledge has been gained. Most of these studies focused on the appropriate concentration of growth regulators to be added to the culture medium (Revilla et al., 1989; Penuela et al., 1988; Rodriquez et al., 1993). Although unsuccessful results were obtained at the beginning in some countries where walnut cultivation was developed, studies were continued persistently and today a certain success has been achieved in propagating walnuts with tissue culture. In this study, it was aimed to determine the propagation possibilities of Kaman 1 walnut variety and Kaman 5 walnut genotype, which have an important place in walnut cultivation in Türkiye by tissue culture.

2. Materials and Methods

This study was carried out in the Plant Tissue Culture Laboratory of Ahi Evran University, Faculty of Agriculture, Department of Agricultural Biotechnology, in Kırşehir province. In our study, explants taken from grafted saplings of Kaman 1 walnut variety and Kaman 5 walnut genotype were used. Explants were taken from the axillary buds of 3-month-old grafted seedlings (Figure 1a). Explants were isolated and used starting from May, when shoot development in seedlings was most active. Explants approximately 1.0–3.0 cm long were used, but this caused increased secretion of phenolic compounds and negatively affected shoot regeneration. In our subsequent studies, explants containing at least two buds and approximately 6-7 cm in length were used (Figure 1b).

Figure 1. a) Explant isolation from three-month-old grafted walnut seedlings in the greenhouse b) Explants of 6-7 cm length carrying two nodes c) *In vitro* surface sterilization of explants with five min. in 0.2% $HgCl_2$ d) *In vitro* cultivation of explants in MS nutrient media e) Shoot regeneration of Kaman 1 walnut variety in MS nutrient media containing 0.5 mg/l BAP+1.0 mg/l IBA+100 mg/l ascorbic acid+100 mg/l citric acid+5 ml/l augmentin f) Treatment of shoots in 4 mg/l IBA solution for 10 min. before acclimatization.

2.1. Surface Sterilization of Explants

Two different methods were used for surface sterilization of explants. In the first treatment; the isolated explants were presterilized under tap water for 5 minutes. Then, sodium hypochlorite was applied at doses of 20%, 40% and 60% for periods of 10 minutes and 30 minutes. After rinsing with bidistilled water 3 times for 5 minutes, they were cultured in MS (Murashige & Skoog, 1962) nutrient medium. In the second treatment; explants were washed under running tap water for 10 minutes and with distilled water for 5 minutes. Then, presterilization was completed by keeping it in 70% ethanol for 5 minutes. In the biosafety cabinet, two drops of Tween 20 are added to 0.2% $HgCl_2$

(0.2% mercuric chloride was prepared by adding 0.2 g of mercuric chloride to 100 ml of water and mixing) and sterilized in the prepared solution for 5 minutes. Finally, the explants were rinsed with bidistilled water 3 times for 5 minutes (Figure 1 c, d).

2.2. Culturing Explants

MS nutrient medium containing 3% sucrose was used. The pH of the nutrient medium was adjusted to 5.6–5.8 the optimal value for plant growth, using 1N NaOH or 1N HCl. Before autoclaving, 0.6% agar was added to the medium and solidified. Sterilization of the environment is done in an autoclave at 1.2 atmospheres of pressure and 121 °C for 20 minutes preserved and provided. All cultures were kept under Philips white fluorescent light (Preheat daylight/42 µmol photons $m^{-2}s^{-1}$) at 24±2 °C with a photoperiod of 16 hours light and 8 hours dark.

2.3. Prevention of Browning

To prevent browning, explants were first subcultured into MS0 nutrient medium at 3, 24 and 48 h intervals. Secondly, different concentrations of antioxidants were applied. In this context, 100 mg/l Ascorbic Acid, 100 mg/l Citric Acid and 100 mg/l Ascorbic Acid+100 mg/l Citric Following autoclaving, ascorbic acid and citric acid were added to the nutrient media once it had cooled to a temperature suitable to avoid degradation of these substances.

2.4. Regeneration

Two different methods were used for regeneration of explants. In the first treatment; due to the high browning rate in 1.0–3.0 cm long explants, 6.0–7.0 cm long axillary buds were isolated from walnut seedlings. BAP and IBA at determined concentrations were added to the MS medium and autoclaved. When the MS0 nutrient medium reached 35 °C, 100 mg/l ascorbic acid + 100 mg/l citric acid was added to the medium after filter sterilization. The medium was poured into GA_7 magenta containers and allowed to solidify. The study was set up according to a random plots design with 3 replicates, 5 explants in each GA_7 magenta.

In the second treatment; the explants were sprayed with fungicide every 1 week for 3 weeks. Explants 6-7 cm tall with at least 2 buds were used. In order to increase the efficiency of sterilization, 5 ml/l augmentin was added to the MS medium after the medium reached the appropriate temperature. Explants were kept in MS nutrient medium containing 10 mg/l BAP + 100 mg/l ascorbic acid + 100 mg/l citric acid and augmentin for a week. Then, it was transferred to the 2nd medium containing 0.5 mg/l BAP+1.0 mg/l IBA+100 mg/l ascorbic acid+100 mg/l citric acid and augmentin and subcultured every days. In the other application, which was considered as the control group, explants were cultured directly in MS nutrient medium containing 0.5 mg/l BAP+1.0 mg/l IBA+100 mg/l ascorbic acid+100 mg/l citric acid and augmentin and subcultured every two days (Table 1).

2.5. Rooting

Shoots that reached a length of approximately 4-5 cm were placed in MS rooting medium containing 4 mg/l IBA. They were kept in this environment for a week and subcultured every two days. Microshoots removed from the culture medium were deagared under running tap water and treated with 4 mg/l liquid IBA solution for 10 minutes (Figure 1f). They were planted in pots containing an oven-sterilized peat: perlite (1:1) mixture. After the microshoots were planted in the pots, the pots were covered with bags. The obtained data were subjected to variance analysis in the MINITAB 12.0 package program.

Table 1. Regeneration treatments applied to walnut explants

Treatment	Explants	Pre-treatment	Growth Regulators	Ascorbic acid, citric acid, Augmentin®	Subculture
T1	1 bud	None	0.5, 1.0, 1.5 mg/l BAP + 0.5, 1.0 mg/l IBA	100 mg/l ascorbic acid+ 100 mg/l citric acid	No subculture
T2	2 buds	Explants were sprayed with fungicide once every week for 3 weeks and one week pretreatment in MS containing 10 mg/l BAP	After pretreatment, explants were placed in MS nutrient medium containing 0.5 mg/L BAP + 1.0 mg/L IBA	100 mg/l ascorbic acid+ 100 mg/l citric acid+ 5 ml/l Augmentin®	subcultured every 2 days
Control	2 buds	None	0.5 mg/l BAP + 1.0 mg/l IBA	100 mg/l ascorbic acid+ 100 mg/l citric acid+ 5 ml/l Augmentin®	Subcultured every 2 days

3. Results and Discussion

It was determined that the contamination rate in the 20% sodium hypochlorite application for surface sterilization in Kaman 1 walnut variety and Kaman 5 walnut genotype was higher than the 40% and 60% sodium hypochlorite applications. No significant difference was detected between the sterilization applications in terms of the browning rate. As a result of our studies, the desired results were not obtained from sodium hypochlorite solution for sterilization, and in the following stages, the sterilization protocol was changed and the sterilization process was carried out with a solution prepared with 0.2% $HgCl_2$. The results of subculturing at 3, 24, 48 hour intervals to prevent browning are given in Table 2.

In order to prevent browning in the culture medium, the effects of different antioxidant applications were also examined. For this purpose, ascorbic acid and citric acid were added to the culture medium separately and together. The results of ascorbic acid and citric acid application to prevent browning are given in Table 2. The difference between the browning rates obtained after all three antioxidant applications in Kaman 1 walnut variety and Kaman 5 walnut genotype was found to be statistically insignificant. The effects of different amounts of growth regulators on explant browning, number of shoots per explant, shoot length and number of leaves in Kaman 1 walnut variety and Kaman 5 walnut genotype were determined the results are given in Table 3.

Table 2. Effects of subculturing application and different antioxidant treatments on the browning rate (%) in Kaman 1 walnut variety and Kaman 5 walnut genotype

	Subculturing Application (hour)			Antioxidant Treatments (mg/l)			
Varieties	3	24	48	Control	100 AA	100 SA	100 AA+100 SA
Kaman 1NS	66.7	100.0	60.0	73.3	66.7	66.7	60.0
Kaman 5NS	86.7	93.3	86.7	100.0	73.3	80.0	66.7

The difference between the all application averages was found to be nonsignificant (NS).

Table 3. Effects of different amounts of growth regulators on explant browning, number of shoots per explant, shoot length and number of leaves in Kaman 1 walnut variety and Kaman 5 walnut genotype

Treatments		Browning Rate (%)		Number of shoots per explant		Shoot length (cm)		Number of leaves	
BAP	IBA	K1*	K5 NS	K1*	K5 NS	K1NS	K5NS	K1NS	K5NS
0.5	0.5	33.3c	60.0	2.0ab	0.67	1.7	1.1	3.7	0.0
0.5	1.0	00.0d	73.3	2.67a	0.0	1.7	0.0	3.9	0.0
1.0	0.5	93.3a	73.3	1.0bc	0.0	1.8	0.0	5.3	0.0
1.0	1.0	80.0a	93.3	0.3c	0.0	1.8	0.0	5.0	0.0
1.5	0.5	66.7ab	73.3	0.3c	0.0	1.5	0.0	3.0	0.0
1.5	1.0	53.3bc	100.0	1.0bc	0.0	1.8	0.0	3.0	0.0

*The differences between the means shown with different letters in the same column were found to be nonsignificant ($P<0.05$). NS Nonsignificant.

In Kaman 1 variety, considerable variation in browning rates was observed depending on the concentration of growth regulators in the culture medium. The browning rate reached high levels of 93.3%, 80.0% and %66.7 in media containing 1.0 mg/l BAP + 0.5 mg/l IBA, 1.0 mg/l BAP + 1.0 mg/l IBA and 1.5 mg/l BAP + 0.5 mg/l IBA respectively. In contrast, no browning was observed in the medium supplemented with 0.5 mg/l BAP + 1.0 mg/l IBA, while a browning rate of 33.3% was recorded in the medium containing 0.5 mg/l BAP + 0.5 mg/l IBA.

The highest shoot regeneration, with an average of 2.7 shoots per explant, was achieved on MS medium supplemented with 0.5 mg/l BAP and 1.0 mg/l IBA. This was followed by the medium containing 0.5 mg/l BAP and 0.5 mg/l IBA, which produced an average of 2.00 shoots per explant. Shoot length varied between 1.5 cm and 1.8 cm across all media, and no statistically significant differences were observed among treatments in terms of shoot elongation (Figure 1e)

In Kaman 5 genotype, 60.0-100.0% browning was observed across all treatments. The difference between the applications in terms of browning rates was not found to be statistically significant. Shoot development was observed only in the medium containing 0.5 mg/l BAP + 0.5 mg/l IBA. The number of shoots per explant was determined as 0.67 and the shoot length was 1.1 cm. No shoots were obtained in any of the other applications. The effect of 10 mg/l BAP and 100 mg/l AA+100 mg/l SA pretreatment shoot regeneration in Kaman 1 walnut variety and Kaman 5 walnut genotype was determined and the results obtained are given in Table 4.

Table 4. Effect of pretreatment on shoot regeneration in Kaman 1 walnut variety and Kaman 5 walnut genotype

Treatment	Browning rate (%)^{NS}		Number of shoots per explant^{NS}	
	Kaman 1	Kaman 5	Kaman 1	Kaman 5
10 mg/l BAP	43.3	53.3	1.0	0.0
100 mg/l AA+100 mg/l SA	46.7	53.3	0.0	0.0

The difference between the application averages was found to be nonsignificant.

As a result of all pretreatments, only one shoot was obtained with 10 mg/l BAP treatment in Kaman 1 walnut variety. No significant difference was observed in terms of browning rate observed in the culture medium. In all treatments, when the shoots of Kaman 1 walnut variety and Kaman 5 walnut genotype reached 4-5 cm in length, signs of necrosis and chlorosis were observed in the form of yellowing of the leaves and browning of the leaf tips and edges. Later, it caused the leaves to fall off and adventitious root formation wasn't observed. In previous studies on the propagation of walnuts by tissue culture, it was reported that explants were treated with sodium hypochlorite at rates ranging from 0.5% to 20.0% and for periods ranging from 5 to 30 minutes, for surface sterilization purposes (Zamir et al., 2004).

Rios-Leal et al. (2007) reported that contamination was an important problem in explants taken from walnut trees and that oxidation occurring in the tissues during the surface sterilization stage caused low shoot formation. In our study, high levels of contamination and browning were observed in the culture medium during surface sterilization with sodium hypochlorite, and shoot regeneration was negatively affected. It has been observed that mercuric chloride is more effective for sterilization of explants. Many researchers reported that they encountered browning problems in the propagation of walnuts by tissue culture and that frequent subculturing could be effective in reducing this problem (Leslie and McGranahan, 1992). In our study, explants were subcultured for three different periods at 3, 24 and 48 hour intervals. However, subculturing was not effective in preventing browning. This situation shows that subculturing alone is not sufficient to solve the browning problem in the propagation of these varieties by tissue culture. Çördük and Akı (2011), also reported that antioxidants should be added to the medium to prevent browning and that adding 100 mg/l AA and 50 mg/l SA to the MS0 medium was an effective method to prevent browning. Tekinsoy and Gülşen (1993) stated that browning decreased in nutrient media containing 150 mg/l SA + 100 mg/l AA, but no successful development was achieved. Myrselaj et al. (2020) reported that the browning rate is a parameter that is highly affected by the ascorbic acid concentration and exposure time, and that a low browning rate is achieved if 200 mg/l ascorbic acid solution is used for 15 or 20 minutes. In our study, when 100 mg/l AA+100 mg/l SA was added to the MS0 nutrient medium, browning was observed at a rate of 60% in Kaman 1 variety and 66.7% in the Kaman 5 walnut genotype. Despite the high rate of browning, a lower rate of browning was observed compared to the control group. It is stated that one of the factors affecting the browning rate is the sterilization of explants for a long time and with high doses of chemicals (Ellialtıoğlu, 1999). In our study, it was determined that sodium hypochlorite was not effective in preventing contamination in surface sterilization and caused high rates of darkening. In our study, $HgCl_2$ was used as an alternative sterilization agent. Another factor affecting success in tissue culture studies is determining the appropriate growth regulator combination and concentration for each plant species and variety. Vahdati et al. (2009) stated that the success of tissue culture depends on the chemical composition of the culture medium and that micropropagation of walnuts is partially limited due to the lack of a suitable culture medium. In our study, the effects of BAP and IBA at different doses and concentrations on walnut shoot regeneration were examined. It was determined that the average number of shoots per explant was 2.67 and the shoot length was 1.7 cm in the medium containing 0.5 mg/l BAP + 0.1 mg/l IBA in Kaman 1 variety. A high level of browning was observed in explants of the Kaman 5 walnut genotype in almost all environments. In the medium containing 0.5 mg/l BAP+0.5 mg/l IBA, the average number of shoots per explant was determined as 0.67 and the shoot length was 1.1 cm.

Many researchers working on the propagation of walnut by tissue culture have stated that the best cytokinin concentration at the shoot propagation stage is 1 mg/l BA (Penuela et al. 1988; Revilla et al. 1989; Rodriquez et al. 1993; Saadat and Hennerty, 2002; Türen ve ark., 2024).

Gruselle et al. (1987), investigated the effects of different types and concentrations of cytokinins on shoot formation in walnut tissue culture and reported that the best result was obtained with 1 mg/L BAP.

In this study, the Güleryüz, 1982 best results were obtained from the medium containing 0.5 mg/l BAP. Auxins promote root formation, while cytokinins promote shoot formation. They contribute to organ formation and development in tissue culture environments.

Tuan et al. (2016) reported in their study that the best combination of shoot elongation and production of new axillary shoots was achieved in Rugini and DKW medium with 4.4 µM BA and 0.2 µM IBA, and the highest rooting percentages were obtained in DKW medium containing 12 µM IBA. For this purpose our study, the effect of preapplication with 10 mg/l BAP on increasing the

number of shoots was examined. As a result of the study, no positive effect of pretreatment application on Kaman 5 genotype was observed; in Kaman 1 variety, one piece shoot formation per explant was observed. It was determined that there were significant differences between the varieties used in our study. Other researchers have also reported that significant differences occur between walnut varieties in tissue culture propagation (Navatel and Bourrain 2001; Vahdati et al. 2004). One of the most important problems encountered in the propagation of walnuts by tissue culture is the difficulty of rooting in the microshoots obtained. Among the shoots obtained from the axillary buds of Vlach, Chandler, Yalova-1 walnut varieties, only the Yalova-1 walnut variety was able to root in MS nutrient medium containing 4 mg/l IBA (Yıldırım, 2018). Kepenek and Kolağası (2016) reported that the best rooting environment for microshoots obtained from walnuts is nutrient media containing 5 mg/l IBA. Dirlik et al. (2022) reported that the most effective sterilization method in *in vitro* micropropagation from Paradox' (*Juglans regia*) node explants is 5 min 70% EtOH + 5 min 0.2% $HgCl_2$ (containing Tween-20) treatment. They reported that the highest number of shoots per explant (1.0 shoots/explant) was achieved in DKW medium containing 4mg/l BAP + 0.001 mg/l IBA + 200 casein hydrolyzate + 50 adenine hemisulphate. They stated that the best root induction was obtained in DKW medium containing 30 g/l sucrose and 4 mg/l IBA. In our study, 4 mg/l IBA was applied for rooting microshoots, but rooting could not be achieved.

4. Conclusion

In our study, the possibilities of propagation of Kaman 1 walnut variety and Kaman 5 walnut genotype by tissue culture were investigated. Procuring the plants used as explant sources from the external environment caused high contamination in the culture environment. Since sodium hypochlorite treatment was not sufficient for surface sterilization, $HgCl_2$ was used in surface sterilization and successful results were obtained. Although positive results were not obtained from subculturing practices to prevent browning, results were obtained that ascorbic acid and citric acid applications could positively affect the success. It was determined that the best results in Kaman 1 walnut variety in terms of shoot development were obtained from the medium containing 0.5 mg/l BAP + 1.0 mg/l IBA. In Kaman 5 walnut genotype, a low rate of shoots was obtained only in one environment, while no shoot development was observed in other environments. It shows that there is a significant difference between the ability of these varieties to grow in tissue culture. In order to increase shoot development, pretreatment with 10 mg/l BAP was applied, but positive results were obtained only in Kaman 1 walnut variety. It was concluded that both methods used for rooting were not successful in Kaman 1 walnut variety and Kaman 5 walnut genotype. As a result, it has been shown that the Kaman 1 walnut variety has the potential to be propagated by tissue culture, but it has been concluded that more detailed studies need to be carried out to determine a protocol that can be applied in a practical sense.

Author Contributions

The percentages of the authors' contributions are presented below. All authors reviewed and approved the final version of the manuscript.

	E.Ş.	S.S.Y.	K.Y.
C	30	40	30
D	30	40	30
S	20	40	40
DCP	40	40	20
DAI	50	30	20
L	50	30	20
W	100		
CR	20	60	20
SR	50	30	20
PM	40	50	10
FA	40	20	40

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Since no studies involving humans or animals were conducted, ethical committee approval was not required for this study.

Acknowledgements

This study is derived from the master's thesis of Ebru Şirin and was supported by the Scientific Research Projects Commission of Gaziosmanpaşa University (Project No: 2013/35).

References

Akça Y. 2009. Ceviz yetiştiriciliği. 3. baskı, Anıt Matbaası, Ankara, Türkiye, pp: 329-335.

Babaoğlu M, Gürel E, Özcan S. 2002. Bitki biyoteknolojisi-I, doku kültürü ve uygulamaları. Selçuk Üniversitesi Basımevi, Konya, Türkiye, pp: 162.

Çördük N, Akı C. 2011. Inhibition of browning problem during micropropagation of sideritis trojana bornm., an endemic medicinal herb of Turkey. Romanian Biotechnol Lett, 16(6): 6760-6765.

D'Amato F 1977. Cytogenetics of differentiation in tissue and cell cultures. In: Reinert J, Bajaj YPS (Eds), Plant Cell, Tissue and Organ Culture, Narosa Publ House, New Delhi, pp: 343-357.

Dirlik C, Kandemir H, Çetin N, Şen S, Güler B, Gürel A. 2022. Effects of different culture media compositions on in vitro micropropagation from paradox walnut rootstock nodes. GU J

Sci Part A, 9(4): 500-515.

Ellialtıoğlu Ş. 1999. Doku kültürü yoluyla vegetatif çoğaltmada doku kararmasi sorunu, nedenleri ve çözüm yollari. Biyotek Kükem Derg, 24(1): 37-47.

Emiroğlu Ü, Gürel A. 2005. Bitki ıslahında doku kültürü tekniklerinin kullanımı. Tohum Bilimi ve Teknolojisi, Cilt 1, s. 91-155, EÜ Tohum Teknolojisi ve Araştırma Merkezi, Bornova, İzmir, Türkiye, pp:52-64.

Gruselle R, Badia N, Boxus P. 1987. Walnut micropropagation: first results. Acta Hort, 212: 511-516.

Güleryüz M. 1982. Bahçe ziraatında büyütücü ve engelleyici maddelerin kullanılması ve önemi. Atatürk Üniversitesi Yayınları, No: 279.

Kepenek K, Kolağası Z, 2016. Micropropagation of walnut (Juglans regia L.). Acta Phys Pol A, 130(1): 150-156.

Leslie C, McGranahan G. 1992. Micropropagation of Persian walnuts (Juglans regia L). In: Bajaj YPS (Ed), Biotechnology in Agriculture and Forestry, Vol. 18, High-Tech and Micropropagation, Springer, Berlin, pp: 136-150.

Murashige G, Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol Plant, 15: 473-497.

Myrselaj M, Sota V, Kongjika E. 2020. Reducing oxidative stress on zygotic embryos of walnut (Juglans regia L.) under in vitro conditions by their pretreatment with ascorbic acid. Eur J Biotechnol Gen Eng, 7(1).

Navatel J, Bourrain L. 2001. Production of walnut (Juglans regia L.) by in vitro multiplication. Acta Hort, 544: 465-471.

Penuela R, Gravito C, Sanchez-Tames R, Rodriquez R. 1988. Multiple shoot-bud stimulation and rhizogenesis induction of embryonic and juvenile explants of walnut. Acta Hort, 227: 457-459.

Revilla MA, Majada I, Rodriquez R. 1989. Walnut (Juglans regia L) micropropagation. Ann Sci For (Paris), 46(Suppl): 149-151.

Rios-Leal D, Sanchez-Olate M, Aviles F, Materan ME, Uribe M, Hasbun R, Rodriquez R. 2007. Micropropagation of Juglans regia L. In: Jain SM, Hüggman H (Eds), Protocols for Micropropagation of Woody Trees and Fruits, pp: 381-390

Rodriquez R, Lopez C, Diaz-Sala C, Berros B. 1993. Simultaneous shoot-bud development on walnut tissues of different ages; macromorphological and histological analyses. Acta Hort, 311: 141-152.

Rout GR, Samantaray S, Mottley J, Das P. 1999. Biotechnology of the rose: a review of recent progress. Sci Hortic, 81: 201-228

Saadat YA, Hennerty MJ. 2002. Factors affecting the shoot multiplication of Persian walnut (Juglans regia L.). Scientia Hortic, 95: 251-260.

Şen SM. 2011. Ceviz Yetiştiriciliği, besin değeri folklörü. İstanbul, Yürkiye, pp:45-96.

Tekinsoy B, Gülşen Y. 1993. Cevizin (Juglans nigra L.) doku kültürü tekniği ile çoğaltılmasında kahverengileşme sorununun çözüm yolları. 8. Kükem Kongresi, Kükem Dergisi, 16(2), Ankara, Türkiye, pp:25-41.

Tuan PN, Meier-Dinkel A, Höltken AM, Wenzlitschke I, Winkelmann T. 2016. Paving the way for large-scale micropropagation of Juglans × intermedia using genetically identified hybrid seed. Plant Cell Tiss Organ Cult, 126: 153-166.

Türen T, Savalan Ş, Pehlivan EC. 2024. FeSO4.7H2O, indol-3-bütirik asit ve farklı besin ortamlarının in vitro ceviz (Juglans regia L.) fidanı çoğaltımı ve mikroaşılma üzerine etkisi. Tekirdağ Ziraat Fak Derg, 21(3): 748-758.

Vahdati K, Leslie CA, Zamani Z, McGranahan G. 2004. Rooting and acclimation of in-vitro grown shoots from mature tree of three Persian walnut cultivars. HortScience, 39: 324-327.

Vahdati K, Najafianashrafi E, Ebrahimzadeh H, Mirmasoumi M. 2009. Improved micropropagation of walnut (Juglans regia L.) on media. Acta Hort, 839: 117-124.

Yıldırım HT. 2018. Bazı ceviz çeşitlerinde in vitro mikroçoğaltim. Yüksek Lisans Tezi, Tekirdağ Namık Kemal Üniversitesi, Fen Bilimleri Enstitüsü, Tekirdağ, Türkiye, pp:54-84.

Zamir R, Shah ST, Ali N, Khattak GSS, Muhammad T. 2004. Studies on in vitro surface sterilization and antioxidants on guava shoot tips and nodal explants. Pak J Biotechnol, 1(2): 12-16.