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Shoot tip culture of Bilecik İrikarası, Sarı Üzüm, Kartal Çavuş and Razakı grape varieties grown in Bilecik province

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Abstract
The aim of this study was to determine the propagation potential of some local varieties using the in vitro shoot-tip culture method. Bilecik İrikarası, Sarı Üzüm, Kartal Çavuş and Razakı were used as materials. As a result of the study, the values of rooting rate, number of roots, length of roots, number of shoots, length of shoots, number of leaves and number of nodes of the grape varieties were determined. When the cultivars were compared in terms of rooting characteristics, the highest rooting rate was 54.7%, the highest root number was 3.71 per plant, and the highest average root length value was 17.93 cm from Bilecik İrikarası. When the cultivars were evaluated in terms of shoot length, the highest shoot length value was determined in Razaki variety with 5.72 cm. Similarly, considering the number of leaves and nodes in the shoots were determined, with the highest leaf number value was of 8.71, the highest node number value was of 6.71 in Razakı variety. As a result of the study, local varieties that used as material in this study showed positive results in in vitro propagation.

Keywords: Bilecik, Grape, Tissue Culture, Local Variety, Shoot Tip Culture

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INTRODUCTION

Local grape varieties are the most important grapevine genetic resource and has an important role in breeding studies (Sağlam and Çalkan Sağlam, 2018; Ekbiç and Yılmaz, 2018). In addition to using these varieties for breeding purposes, their local production is also in demand (Sağlam and Çalkan Sağlam 2017; Ergönül and Çelik, 2018).

When it comes to the production of local varieties for different purposes, insufficient production material is often the most important problem. For this reason, production methods that can produce as much as possible from this scarce material are needed. Tissue culture is one of these methods. Tissue culture is also used for the critical materials protected under controlled conditions. On the other hands, with tissue culture, it is possible to carry out breeding and production activities that would take years in a much shorter time. It also enables large quantities of production to be carried out in a small area.

With the tissue culture method, it is possible to obtain a new plant under in vitro conditions by taking parts from various parts of the plant (meristem, shoot tip, embryo, anther, etc.) (Ergül et al., 2017). There are many studies on tissue culture practices and meristem culture in viticulture (Stamp et al., 1990; Adıyaman, 1998; Péros et al., 1998; Baydar, 2000; Park et al., 2001; Karaca, 2006; Jaskani et al., 2008; Ekbiç et al., 2015; Sağlam et al., 2016; Ergönül and Çelik, 2018; Balı et al., 2020).

According to studies conducted on grapevine; It has been determined that the regeneration rate in grape varieties varies depending on the variety (Miljani´c et al., 2022). On the other hand, in studies conducted on different grape varieties, it has been determined that the contents of the growing medium used are important in terms of development criteria (Skadia et al., 2010; Ayman et al., 2011; Batukaev et al., 2021).

In addition, the concentration of growth regulators added to the growth medium significantly affects the success of tissue cultures (Gomes et al., 2004; Skadia et al., 2010; Ayman et al., 2011; Khan et al., 2015; Kumsa, 2016; Kavitha and Surakhshitha, 2023). As a matter of fact, while there was no callus development in the Cabernet Franc grape variety in the medium containing no Benzyl adenine purine (BA), callus development occurred in the medium containing 10mg BA (Garcia et al., 2023).

In this study, the suitability of Bilecik İrikarası, Sarı Üzüm, Kartal Çavuş and Razakı grape varieties, which are genetic resource of Bilecik, Turkey, for clonal propagation by shoot tip culture, which is one of the tissue culture techniques, was determined.

MATERIALS AND METHODS

This study was carried out in the Tissue Culture and Plant Breeding laboratory within Bilecik Şeyh Edebali University Biotechnology Application and Research Center in 2019-2021.

Materials

Bilecik İrikarası, Kartal Çavuş, Sarı Üzüm and Razakı varieties were used as material in the research.

The long term of climatically data for Bilecik were found in table 1.

Table 1. Bilecik province long-term climate data averages (1991-2020) (Anonymus, 2024)

BILECIK	January	February	March	April	May	June	July	August	September	October	November	December	Yearly average
Average temperature (°C)	2.7	4.1	7.2	11.6	16.5	20.4	22.9	23.0	19.1	14.4	8.9	4.5	12.9
Average maximum temperature (°C)	6.2	8.5	12.4	17.5	22.7	26.8	29.6	30.0	25.9	20.2	13.7	7.9	18.4
Average minimum temperature (°C)	0.0	0.8	3.2	6.9	11.3	14.8	17.0	17.4	13.9	10.2	5.5	1.9	8.6
Average sunshine duration (h)	3.1	3.8	4.8	6.4	8.0	9.6	10.6	10.0	8.1	5.5	4.2	2.8	6.4
Average number of rainy days	13.63	13.20	12.70	10.77	10.27	8.67	3.77	3.77	5.60	8.67	9.57	13.43	114.0
Monthly total rainfall average (mm)	48.0	44.4	48.7	48.6	43.9	50.6	16.4	11.2	27.0	50.1	37.1	56.1	482.1
Maximum temperature (°C)	22.0	24.6	30.2	33.3	35.8	38.2	41.0	40.6	38.4	34.3	27.4	25.0	41.0
Minimum temperature (°C)	-16.0	-14.3	-11.6	-6.0	1.0	6.0	7.7	8.2	3.2	-0.8	-9.2	-14.5	-16.0

Methods

During the resting period, cuttings were taken from the grape varieties used as material and planted with 3-4 buds in pots containing a mixture of 10-liter peat-perlite. The pots were left to develop in a climate chamber at 25±1 °C, 16 hours of light, 8 hours of darkness, and 4000 lux illumination. After shoots were formed from these shoots, the shoot tips taken from these shoots were transferred to the nutrient medium.

Figure 1. Cuttings planted in pots

Figure 2. Shoot formation in cuttings

MS (Murashige and Skoog) medium was used in the study. The medium was prepared manually. While preparing the medium, the pH of the medium was adjusted to 5.7-5.8. Then, 1 mgL^{-1} BAP, (Benzyl Amino Purine) was added to this solution (Fotini et al., 2010; Ekbiç ve Yılmaz, 2018; Beza, 2010; Baydar, 2000) to encourage shoot formation and 2 mgL^{-1} IBA (Indole-3-Butyric Acid) (Ekbiç vd., 2015; Jaskani vd., 2008) was added to encourage root formation. The prepared medium was sterilized in an autoclave at 121 ^{o}C at 1 atm pressure for 20 minutes. Then, this medium was transferred to sterilized 150 ml jars, 20 ml per jar, before it solidified.

Shoot tips, which served as study material, were collected from the shoots developed in the climate chamber and subjected to sterilization. For sterilization, 10% sodium hypochlorite solution was used. For this purpose, the material was kept in a shaker in sodium hypochlorite solution for 10 minutes, then washed with pure water in a shaker three times for five minutes each. After sterilization, these shoot tips were planted in MS medium and left to develop in a climate chamber with a temperature of 25±1 ^{o}C, relative humidity of 50-60% and a photoperiod of 16 hours light and 8 hours dark with 4000 lux illumination.

Figure 3. Development in in vitro climate chamber

Data were taken by measurements 4-6 weeks after planting, depending on the development status. In the study, data on the number of rooted plants, number of roots, root length (cm), number of shoots, shoot length (cm), number of nodes and number of leaves were collected and evaluated. The study was designed in a Randomized Parcels Trial design with 3 replications, and 25 shoot tips were planted in each replication. All data obtained were subjected to statistical evaluation with the help of JMP 16.0 package program.

RESULTS AND DISCUSSION

When the rooting status of the varieties was evaluated statistically, it was found to be significant within the 95% confidence interval. Data regarding rooting rate are given in Table 2. When Table 2 is examined; the highest rooting value was in the Bilecik İrikarası variety with 54.7%.

Table 2. Number of roots, root length, number of shoots, shoot length and number of nodes values obtained from the study

Variety	Root ratio (%)	Root number/plant (n)	Average Root Length (cm)	Average Shoot Length (cm)	Average Number of Leaves(n)	Average Number of Nodes(n)
Bilecik İrikarası	54,7 a	3,71 a	17,93 a	4,71 ab	6,29 ab	5,55 ab
Razakı	18,7 b	3,50 a	17,60 ab	5,72 a	8,71 a	6,71 a
Kartal Çavuş	26,7 ab	1,55 b	12,32 bc	3,01 b	2,48 c	2,48 c
Sarı Üzüm	20,0 ab	3,53 a	9,14 c	4,93 ab	4,40 bc	4,67 b

In the study, when the lengths of the shoots formed from the planted shoot tips were evaluated statistically, the differences were found to be significant within the 95% confidence interval. If the varieties were compared in terms of shoot lengths, the highest shoot length value was obtained from the Razakı variety with 5.72 cm. Razakı variety was followed by Sarı Üzüm with 4.93 cm and Bilecik İrikarası with 4.71 cm, and the length value of the Kartal Çavuş variety was 3.01cm (Table 2).

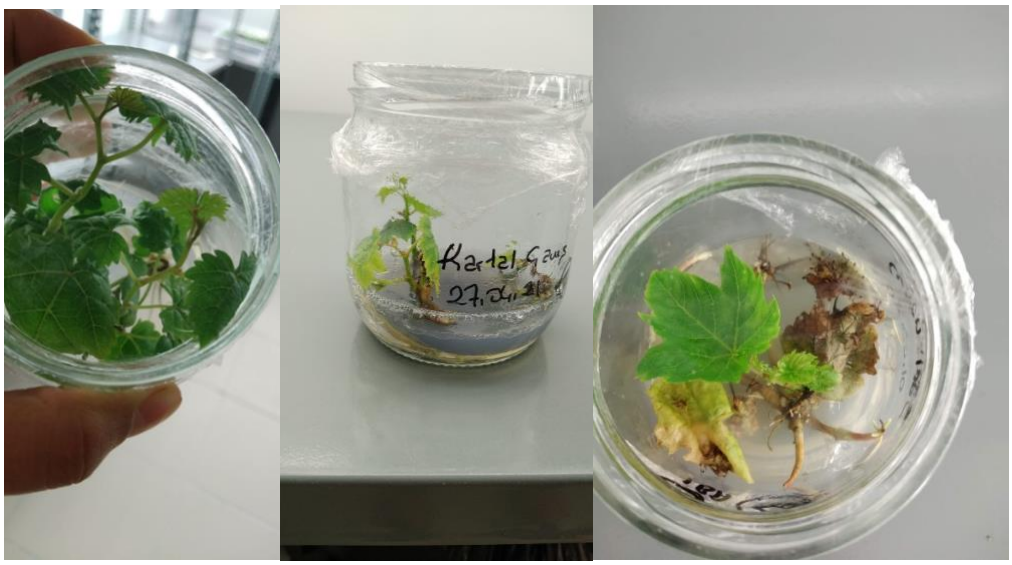

Figure 4. In vitro shoot development

When the root numbers are examined; It is seen that the highest number of roots was obtained from Bilecik İrikarası variety with 3.71 per plant. Sarı Üzüm ranked second with 3.53, and Razakı ranked third with 3.50 (Table 2). Differences in the number of roots between varieties were found to be statistically significant at a 95% confidence interval.

Figure 5. Root formation

Considering the length values of the roots formed from the planted shoot tips; The highest average root length value was obtained from Bilecik İrikarası variety with 17.93 cm (Table 2). As a result of the statistical analysis,

the differences between varieties in terms of root length were found to be statistically significant at a 95% confidence interval.

In terms of the number of leaves on the shoots, the highest leaf number value was obtained from the Razakı variety with 8.71, while the lowest leaf number value was obtained from the Kartal Çavuş variety with 2.48 (Table 2). If the leaf number data were subjected to statistical evaluation, the differences between the varieties were found to be significant within the 95% confidence interval.

Considering the varieties were compared in terms of number of nodes, the highest number of nodes was obtained from the Razakı variety with 6.71, and the lowest value was obtained from the Kartal Çavuş variety with 2.48 (Table 2). When the number of nodes values were evaluated statistically, the differences between the varieties were found to be significant within the 95% confidence interval.

As a result of the study, it was determined that there were differences between the varieties in terms of root and shoot formation. The Bilecik İrikarası variety had higher values compared to other varieties in terms of rooting rate, number of roots and root length. The Razaki variety came to the fore in terms of shoot length, number of leaves and nodes. The differences between the varieties considering rooting, number of roots, root length, shoot length, number of leaves and number of nodes are similar to the results obtained from previous studies. Previous studies have found that there are differences between varieties within a species as well as between species (Karoğlan et al., 1990; Péros et al., 1998; Adıyaman, 1998; Baydar, 2000; Ekbiç and Yılmaz, 2018). It has been determined that different BAP concentrations produce different results in shoot development and callus formation (Gomes et al, 2004; Skadia et al., 2010; Ayman et al., 2011; Kumsa, 2016; Kavitha and Surakhshitha, 2023).

According to the results of the study, the rooting rate of the Bilecik İrikarası variety is higher than the others. Although different IBA doses were not tested in the study, it is thought that more positive results can be obtained if different doses are used (especially at doses higher than $2mgL^{-1}$). Supporting this, different IBA doses were used in some previous studies. In the Balıkçı Siyahı grape genotype, IBA was used at concentrations of 0-4 mg/L for rooting and the best results were obtained in the medium containing 4 mgL^{-1} IBA (Ekbiç and Yılmaz, 2018), while the most suitable IBA concentration for rooting in Sultani Çekirdeksiz and Cheema Sahabi grape varieties is 1 mgL^{-1}(Aazami, 2010). In another study, the most appropriate IBA dose for different grape varieties was found to be 0.1 mgL^{-1} (Mozafari et al., 2016).

As a result of the study, it was determined that Bilecik İrikarası, Razakı, Sarı Üzüm and Kartal Çavuş varieties could be propagated by the shoot tip culture method. According to Ekbiç et al. (2015), although the highest rooting rate for the Isabella grape variety was obtained from MS medium supplemented with 2 mgL^{-1} IBA, 2 mgL^{-1} IBA was not found sufficient for the varieties used in this study. Another studies conducted previously, the best result was given at a BAP concentration of 1 mgl^{-1}, similar to this study (Beza, 2010; Jaskani vd., 2008).

CONCLUSION

With this study, the in vitro propagation possibilities of four local grape varieties, which are of economic importance for the Bilecik region in Türkiye was determined and the basis was laid for future studies such as determining the resistance of these varieties to biotic and abiotic stress conditions and obtaining virus-free plants.

Considering the data obtained from the study, it is thought that different IBA concentrations should be used. Similarly, it is recommended to use different BAP concentrations in these varieties in future studies.

Because of the different BAP and IBA concentrations produce different results in shoot development and callus formation, it is thought that studies should be carried out to determine the appropriate IBA and BAP doses for each variety, and different plant growth regulators should also be investigated to promote rooting.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

Thre are not any conflict of interest

Author contribution

This article was prepared from a part of Seda Özdemir Memiş's master's thesis. Hayri Sağlam is the thesis advisor.

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