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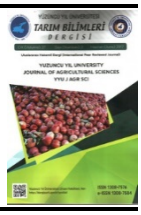

Research Article

Optimization of Meristem Culture to Obtain Virus-Free Clonal Basic Material of Grape Cultivars

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Abstract: The main purpose of this study was to obtain a clone free from viruses and virus-like diseases to rapidly reproduce these clones. Meristems were extracted and cultured for the production of the base material for the Kalecik Karası number 4 and 23-2 clones. After the explants formed shoots, the effects of 12 dissimilar auxin (IBA) and cytokine (2İP and BAP) concentrations on the growth of the root and plant were investigated. In the meristem stage clones showed a 60 % and 80 % viability rate. In the rooting stage, it was determined that shoot formation and leaf numbers were higher in the Kalecik Karası clone number 23-2 and the 1.0 mg. L^{-1} IBA+0.5 mg. L^{-1} BAP / 2 IP concentration showed the highest shoot formation and leaf number value. The highest callus levels were determined as 0.43 cm for clone number 4 and 0.72 cm for clone number 23-2. M S (Murashige & Skoog) with 2mg. L^{-1} IBA showed the highest rooting value. The longest root values were 3.47 cm with 1mg. L^{-1} IBA for clone number 4, and 3.90 cm with 0.5mg L^{-1} IBA for clone number 23-2.

Virüsten Ari Klonal Üzüm Çeşitlerine Ait Baz Materyal Eldesinde Mersitem Kültürünün Optimizasyonu

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Anahtar Kelimeler
Baz Materyal,
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Mikroçoğaltım,
Vitis vinifera, L.,
Optimizasyon.

Öz: Bu çalışmanın temel amacı, ülkemiz ve dünya bağcılığı için en önemli etmenlerden biri olan klon kökenli sertifikalı asma fidanı üretimi için, klona dayalı kalem damızlıkların kurulabilmesinde kullanılacak, virüs ve virüs benzeri hastalıklardan ari klonal çoğaltma materyali eldesi ve bunların hızlı bir şekilde çoğaltılmasıdır. Kalecik Karası 4 ve 23-2 kodlu klonlarına ait baz materyal üretimi için meristemlerin çıkartılması, kültüre alınması ve sürgün aşamasında sabit olan ortam konsantrasyonlarının ardından köklenme aşamasında 12 değişik oksin (IBA) ve sitokinin (2 iP ve BAP) konsantrasyonunun, kök gelişimine ve bitkiye dönüşümüne etkileri incelenmiştir. Meristem aşamasında, Kalecik Karası 4 kodlu klonunda % 60, 23-2 kodlu klonunda ise % 80'lik bir yaşama oranı tespit edilmiştir. Meristem ve sürgün aşamasında kallus oluşmamıştır. Sürgün aşamasında 2.0 mg/L BAP+0.5mg/L IBA konsantrasyonu ile ortalama sürgün sayısı bakımından sırasıyla 3.67 ve 3.83 adet; ortalama sürgün uzunluğu bakımından ise 2.42 cm ve 2.04 cm uzunluk belirlenmiştir. Köklendirme aşamasında ise Kalecik Karası 23-2'de sürgün oluşumu ve yaprak sayısının daha fazla olduğu gözlemlenmiştir. 1.0 mg/L IBA+0.5 mg/L BAP/2 İP konsantrasyonunda ise en yüksek sürgün oluşumu ve yaprak sayısı değeri tespit edilmiştir. Söz konusu aşamada kallus oluşumu oksin

ve stokinin düzeylerinin artmasına paralellik göstermiştir. Kallus düzeyleri Kalecik Karası 4 kodlu klon için 0.43 cm ve 23-2 kodlu klon için 0.72 cm olarak tespit edilmiştir. En yüksek köklenme değerini 2 mg/L IBA eklenmiş olan yetiştirme ortamı vermiştir. En uzun kök değerleri ise 4 kodlu klon için 1 mg/L IBA ile 3.47 cm, 23-2 kodlu klon için 0.5 mg/L IBA ile 3.90 cm olarak gerçekleşmiştir.

1. Introduction

As in many vegetative propagating plants, significant problems arise in grapevines that are exposed to virus and virus-like diseases all over the world. This has encouraged extensive researches on an international scale for many years, providing for the continuation of such researches.

International Council for the Study of Viruses and Virus-like Diseases of the Grapevine (ICVG) recognizes that more than 75 infectious organisms (viruses, viroids and phytoplasms) recorded in grapevine may have a negative impact on plant viability and longevity, quality and amount of yield and may be very harmful for the yield. Infected propagation material is largely responsible for the spread of diseases between countries and in vineyards. Therefore, all opportunities should be mobilized to improve health conditions (Martelli, 2014).

The only way to obtain a regular, stable and healthy product in vineyards is to provide material for propagation only from clean breeding plants by removing material that has been tested for viruses and virus-like diseases and which carries the disease agent. Base material production and certification is a powerful and effective strategy to control these infectious factors and promote the quality, profitability and sustainability of the production.

Base material production, free from diseases and pests, tested and proved to be clean and provided by clonal selection, is important for world viticulture.

The propagation of grapevines with traditional methods is a slow-working system for new varieties or elite types, and *in vitro* techniques, which are complementary to traditional methods, are widely used in genetic progress programs, in obtaining and propagating healthy varieties. Thus, it provides great convenience to the plant breeders with the shortening of the time in grapevine breeding (Lavee, 2000, Mhatre et al., 2000, Torrerosa et al., 2001, Thomas and Schiefelbein, 2004).

In order to obtain virus-free certified seedlings from clones 4 and 23-2 of Kalecik Karası, which is the ‘base material’ to be used in the establishment of clone-based pen breeding parcels, which is absolutely necessary for the production of certified grapevine seedlings of clone origin that is very important for the future of viticulture, the main purpose of this study is to use *in vitro* meristem culture technique and to optimize the method in a practical and economical way.

For this purpose, it was tried to determine the most suitable combinations for rooting with various applications made at the rooting stage of the shoots obtained *in vitro* conditions after the meristem stage.

2. Material and Method

2.1. Material

As the plant material, the stocks infected with ArMV and GLRaV-3, among the clones 4 and 23-2 of Kalecik Karası grapevine variety found in the 'Clone Collection Vineyard' of the Department of Horticulture, Faculty of Agriculture, and Ankara University, were used.

2.2. Acquisition of plant material and shoot tip disinfection

Grapevine cuttings were taken from the collection vineyard during pruning and disinfected until planting medium was prepared, and stored in cold storage at 90-95% relative humidity at +4 °C in polyethylene bags. Meristems were taken from the shoot tips of cuttings planted in pots no. 7, which contain a mixture of cocopeat, perlite and peat in the greenhouse at the volumetric ratio of 1:1:1 into stratification containers and tubes in growing chamber. Shoot tip disinfection was carried out in a 20% sodium hypochlorite solution for 15 minutes and then washed 3 times with distilled water for 5 minutes.

2.3. Meristem stage tissue culture applications

Tissue culture applications were performed under aseptic conditions, and for this purpose, sterilization was performed by keeping the nutrient media in autoclave under 1.2 atmospheric pressure for 20 minutes at 121 °C. MS (Murashige and Skoog, 1962) basic place composition was used as the nutrient media. 3 % sucrose and 0.7 % agar were added to the MS medium and applications were made by adjusting the pH to 5.7.

With the removal of 0.2-0.4 mm shoot tip meristems in the sterile cabinet, disinfected shoot tips were planted in full-strength M S initial place (pH 5.7) containing 0.5 mg. L^{-1} GA_3, 2.5 mg. L^{-1} BAP with 4 explants in each petri and cultured in the climate room for approximately 4-5 weeks (Figure 1).

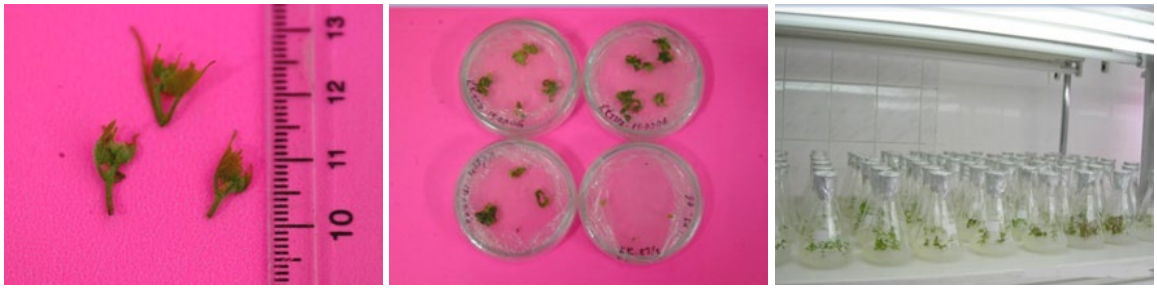

Figure 1. Views from meristem culture and shoot stages.

2.4. Shoot propagation and rooting stage

Developed in *in vitro* conditions approximately 4-5 weeks after the meristem stage, the meristems were transferred to a full-strength M S place containing '2 mg. L^{-1} BAP, 0.5 mg L^{-1} IBA' in the sterile cabinet, developed for 4 weeks (Figure 1).

The meristems developed in the shoot medium were transferred to the full-strength MS medium (pH 5.7), which was modified with the different combinations of auxin and cytokine specified in Table 1, for the rooting stage.

Table 1. Combinations of auxin and cytokine used in the medium

No	Combination	No	Combination
1	0.5 mg. L^{-1} IBA+0.0 mg. L^{-1} BAP	7	0.5 mg. L^{-1} IBA+0.0 mg. L^{-1} İP
2	0.5 mg. L^{-1} IBA+0.5 mg. L^{-1} BAP	8	0.5 mg. L^{-1} IBA+0.5 mg. L^{-1} İP
3	1.0 mg. L^{-1} IBA+0.0 mg. L^{-1} BAP	9	1.0 mg. L^{-1} IBA+0.0 mg. L^{-1} İP
4	0 mg. L^{-1} IBA+0.5 mg. L^{-1} BAP	10	1.0 mg. L^{-1} IBA+0.5 mg. L^{-1} İP
5	0 mg. L^{-1} IBA+0.0 mg. L^{-1} BAP	11	2.0 mg. L^{-1} IBA+0.5 mg. L^{-1} İP
6	0 mg. L^{-1} IBA+0.5 mg. L^{-1} BAP	12	2.0 mg. L^{-1} IBA+0.5 mg. L^{-1} İP

2.5. Culture conditions and acclimatization

Petri dishes in the initial stage, flasks in the shoot stage and cultures in the tubes at the rooting stage were grown in a climate room with a temperature of 25±1 °C, a day length of 16 hours and a light intensity of 3200-3500 lux.

The plants in the flasks were removed without any damage to the roots with the help of a forceps, washed in three separate containers filled with distilled water until there was no artificial nutrient medium in the roots, then the root part was immersed in 0.1 % benomyl-added solution and placed on blotting paper. After root pruning was done, it was transferred to the growing chamber by suturing in plastic containers with sterilized peat+perlite medium. Regularly closed containers were gradually opened and the plants were gradually acclimatized to external conditions. Plants left in plastic container were transferred to pots in the fourth week (Figure 2).

Figure 2. View from rooting stage and acclimatization stage.

2.6. Evaluation of the results

The evaluation stage took place in three stages as given in Table 2.

Table 2. Evaluation stages of the results

Initial Stage	Shoot Stage	Rooting Stage
Macro and micro elements and vitamins of MS, pH: 5.8 GA_3: 0.5 mg. L^{-1} BAP: 2.5 mg. L^{-1}	Macro and micro elements and vitamins of MS, pH:5.8 BAP: 2.0 mg. L^{-1} IBA:1 mg / l	Macro and micro elements and vitamins of MS, pH :5.7 Different hormone applications
Survival rate of meristems (%)	Shooting rate (%)	Shooting rate (%)
Development level of meristems (cm) (Width+Height/2)	Number of Shoots/Explant (n)	Number of shoots (n)
Callus formation rate and level of meristems	Shoot length (cm)	Shoot length (cm)
Vitrification	Callus creation rate (%) and level (cm)	Callus creation rate (%) and level (cm) Rooting (Transformation into complete plant) rate (%) Number of roots (n) and length

2.7. Evaluation of Data

SPSS (Version 19.0) software program was used to evaluate the data obtained from the experiments (SPSS, 2011). Duncan's multiple comparison test was used to differentiate the means of the treatments.

3. Results and Discussion

One of the most important stages is the rooting of *in vitro* shoots obtained for the production of base material at a high rate and transferring them to external conditions. Therefore, in our study that we carried out to determine the most suitable growth regulator combination for rooting *in vitro* shoots, rooting was achieved in both clones and a complete plant was obtained. In terms of the features mentioned, the study was established and evaluated according to the 'Random Parcel Trial Pattern' and in a Factorial pattern. In statistical analysis, there are two levels in the genotype factor, including Kalecik Karası clones 4 and 23-2, and 12 levels in the application factor (Table 1). As a result of the calculations regarding the variance analysis technique, the 'genotype X application' interaction was found to be statistically significant in point of average number of shoots, shoot length, callus value, longest root, root length, average number of roots ($P<0.01$). Duncan test was performed for multiple comparisons. In the calculations made in terms of average number of leaves, 'genotype x application' interaction was not statistically significant. Only the difference between the averages of Kalecik Karası clones 4 and 23-2 was statistically significant ($P<0.01$).

3.1. Initial Stage

Findings related to the survival rate (%), development levels (cm), callus formation rate (%) and levels (mm) of the grapevine shoot tip meristems cultured in MS with '0.5 mg. L^{-1} GA_3, 2.5 mg. L^{-1} BAP' as the initial stage and vitrification are given in Table 3-5, respectively.

3.2. Survival rate of meristems (%)

Z test was used to compare survival rates of shoot tip meristems of the grapevine genotypes studied. There was no statistically significant difference between Kalecik Karası clones 4 and 23-2 in terms of survival rates of the meristems cultured (Table 3).

Table 3. Survival rates of two different Kalecik Karası (K.K.) clones (c.) (%)

Genotype	Survival rates (%)
K.K. c. 4	65
K.K. c. 23 / 2	80

The fact that this difference is not statistically significant shows that our findings are compatible with the previous research results.

There was no callus formation and vitrification in Kalecik Karası clones 4 and 23-2.

3.3. Development levels of meristems (cm)

Variance analysis technique was used to compare genotypes in terms of width, height and development levels of meristems. As a result of the calculations, the width and height measurements and the development levels obtained from them are shown in Table 4. There was no statistical difference between the values obtained after measuring the width and height and their average, that is, in terms of development levels between Kalecik Karası clones (Table 4).

Table 4. Development levels of meristems and shoots of two different Kalecik Karası (K.K.) clones (c.)

Genotype	Width (cm)	Height (cm)	Development Levels (Width+Height/2)
K.K. c. 4	0.25±0.09	0.69±0.30	0.22±0.01
K.K. c. 23 / 2	0.22±0.08	0.71±0.25	0.25±0.01

On the other hand, Roubelakis-Angelakis (1990), Dalloul et al. (1990) reported that *in vitro* growth and development of meristems varied depending on the genotype, and that the development levels of the shoot tip meristems in the initial medium differed statistically. It is possible to say that the two genotypes used in our study did not exhibit different growth strengths *in vitro* conditions because they came from the same origins in terms of many features and they were different clones of the same variety. It is normal to expect that if such a study is repeated with different grapevine genotypes, different rates of development will occur among the genotypes.

3.4. Shoot propagation stage

After being developed in the initial medium for 4 weeks, as the shooting stage, findings related to the number of shoots (n), shoot length (cm), callus formation rate (%) and level (cm) were explained in the genotypes cultured in MS with '2.0 mg. L^{-1} BAP+0.5 mg. L^{-1} IBA' were explained in the sub-titles below and shown in Table 5, respectively. For Kalecik Karası clone 4, the number of shoots and the shoot length were 3.67 and 2.42 cm respectively, while they were 3.83 and 2.04 cm for the clone 23-2.

Table 5. Number and length of shoots of two different Kalecik Karası (K.K.) clones (c.)

Genotype	**Number of shoots (n)**
K.K. c. 4	3.67±0.5
K.K. c. 23 / 2	3.83±0.6
	Shoot length (cm)
K.K. c. 4	2.42±1.2
K.K. c. 23 / 2	2.04±0.3
	Callus Formation (%)
K.K. c. 4	0
K.K. c. 23 / 2	0

While comparing the number and length of shoots, the present observations were subjected to square root transformation and then examined by t test. As a result of the t test performed in terms of the number and length of the shoots, the difference between the averages of Kalecik Karası clones 4 and 23-2 was not found to be statistically significant. In terms of the number of shoots, the highest values obtained from previous studies in the literature were reported to be obtained from MS compositions in which cytokine and auxins are used together (Sudarsono and Goldy, 1991). As a result of the study carried out by Baydar (2000), it was determined that the most suitable nutrient place for adventitious shoot formation was the medium with '2mg. L^{-1} BAP' for Kalecik Karası, Çavuş, Sultani seedless and Kober 5BB.

The shoot lengths, which were obtained from meristem culture and grown *in vitro* conditions, in Çavuş, Müşküle, Hafızali, Razakı, Sultani seedless and Çal karası varieties varied between 3.783-0.858 cm (Kara, 1992). In this case, it is thought that differences in genotypes may occur in terms of shoot length, but the location and time factor where tissue culture is carried out may also have an effect on this difference. These results may be due to the development strength that will result from the genotype, as well as the variables such as different laboratory conditions, the ecology of the plant material and the nutritional status of the donor plant, and the structure of the nutrient media used. However, the values that we obtained are among the reference values given by Kara (1992) and reflect a healthy development strength. No callus formation was observed in the clones in our study.

3.5. Rooting stage

Number of shoots (n), shoot length (cm), number of leaves in shoots, callus formation rate (%) and level (mm) rooting rate (rate of transformation into complete plant) (%), number of roots (n) and length (width) in the shoots developed in the composition of 12 different nutrient media (Table 1) used to determine the most suitable rooting medium for the shoots obtained at the shooting stage were examined.

3.6. The number of shoots formed at the rooting stage (n)

As a result of the calculations regarding the analysis of variance technique, evaluations were made regarding the number of shoots formed in the shoots at the rooting stage. Genotype X application interaction was found to be statistically significant in terms of the average number of shoots. As seen in Table 7, the difference between genotypes is statistically significant in the 2nd, 4th and 6th applications, and shoot formation is higher in clone 23-2. The highest number of shoots for clone 4 was obtained from the 10th application using the doses of '1.0 mg. L^{-1} IBA+0.5 mg. L^{-1} 2İP' (2.11 pieces), while the 4th application combinations for clone 23-2 provided the highest number of axillary shoot formation. The number of shoots formed in these combinations is 2.11 in both clones (Table 6).

To report a general observation, it is possible to say that the tendency to form shoots in clone 4 is lower than in clone 23-2. However, 2 iP affected the shoot formation in combination with low doses of auxin in clone 4 in a significantly positive manner. It would not be wrong to say that in media where

clone 23-2 has a higher tendency to form shoots and BAP is added, if the auxin pressure is low, the tendency to form shoots becomes more evident.

Table 6. Number of shoots of two different Kalecik Karası (K.K.) clones (c.) at the rooting stage (pieces)

Applications	K.K. c. 4	K.K. c. 23 / 2
1.	1.0±0.000 c* A**	1.30±0.15 bc A
2.	1.1±0.1 b B	1.9±0.35 ab A
3.	1.25±0.16b A	1.00±0.0 c A
4.	1.10±0.10 b B	2.11±0.42 a A
5.	1.25±0.16 b A	1.33±0.21 bc A
6.	1.0±0.0 b B	1.9±0.25 ab A
7.	1.0±0.0 b A	1.22±0.14 c A
8.	1.7±0.33 ab A	1.43±0.3 bc A
9.	1.3±0.15 b A	1.0±0.0 c A
10.	2.11±0.45 a A	1.0±0.0 c A
11.	1.0±0.0 b A	1.2±0.22 c A
12.	1.32±0.22 b A	1.12±0.12 c A

*According to the Duncan multiple comparison test, the differences between the averages shown with different letters are significant ($p<0.01$).
* Lowercase letters show the differences between applications.
** Uppercase letters show the differences between genotypes.

3.7. Length of the shoots formed (cm)

In our study, the highest shoot length values obtained were 2.46 cm with the 6th application for clone 4, and 3.25 cm with the 9th application for clone 23-2. We see the difference between the two genotypes in the 1st, 3rd and 5th applications (Table 7). There is no statistically significant difference between the applications for clone 4 in terms of shoot length formed at the rooting stage, whereas the difference between applications for clone 23-2 is statistically significant. Kara (1992) obtained the number of nodes between 5.958 and 1.417 and a shoot length between 3.783 and 0.858 cm in a study on some clones of Çavuş, Müşküle, Hafızali, Razakı, Sultani seedless and Çal Karası.

Table 7. Shoot lengths of two different Kalecik Karası (K.K.) clones (c.) formed at the rooting stage

Applications	K.K. c. 4	K.K. c. 23 / 2
1.	1.37±0.11 a* B **	3.02±0.5 ab A
2.	1.87±0.26 a A	1.71±0.22 ba A
3.	1.29±0.09 a B	3.25±0.73 a A
4.	1.74±0.33 a A	1.9±0.4 bc A
5.	1.69±0.23 a B	2.67±0.53 abc A
6.	2.46±0.7 a A	2.07±0.23 abc A
7.	2.21±0.39 a A	1.53±0.2 c A
8.	1.93±0.14 a A	2.04± 0.4 abc A
9.	2.35±0.30 a A	2.42±0.52 abc A
10.	2.07±0.30 a A	1.71±0.23 bc A
11.	2.17±0.71 a A	2.13±0.45 abc A
12.	2.06±0.30 a A	1.2±0.42 abc A

*According to the Duncan multiple comparison test, the differences between the averages shown with different letters are significant ($p<0.01$).
*Lowercase letters show the differences between applications.
**Uppercase letters show the differences between genotypes.

3.8. Number of leaves (n)

In the calculations made in terms of average number of leaves, genotype X application interaction was not found to be statistically significant. The highest value in terms of the number of

leaves on a shoot was obtained from the 10th application (4.1±0.41 pieces) for clone 4, and the 9th application (5.13±1.23 pieces) for clone 23-2 (Table 8).

Table 8. Number of leaves of two different Kalecik Karası (K.K.) clones at the rooting stage (pieces)

Applications	K.K. c. 4	K.K. c. 23 / 2
1.	2.11±0.35	4.3±0.79
2.	2.67±0.50	3.30±0.30
3.	2.31±0.69	5.13±1.23
4.	1.70±0.33	3.45±0.39
5.	1.75±0.52	4.16±0.95
6.	3.22±0.1	3.8± 0.36
7.	3.0±1.26	2.56±0.35
8.	3.85±0.85	3.45±0.84
9.	3.60±0.6	4.0±0.84
10.	4.1±0.41	2.8±0.74
11.	2.67±1.37	3.44±0.67
12.	2.57±0.5	2.70±0.76

3.9. Callus formation rate (%) and level (cm)

While callus formation was not observed in the shoot stage, callus formation appeared in the basal parts of the explants at the rooting stage and the difference between these callus levels was found to be statistically significant. The highest callus levels are 0.43 cm in the 6th application combination for Kalecik Karası clone 4 and 0.72 cm in the 12th application combination for clone 23-2 (Table 9).

Table 9. Callus levels of two different Kalecik Karası (K.K.) clones (c.) at the rooting stage

Applications	K.K. c. 4	K.K. c. 23 / 2
1.	0.00±0.00 e* A**	0.00±0.00 d A
2.	0.13±0.05 de A	0.06±0.04 cd A
3.	0.02±0.02 e A	0.05±0.03 d A
4.	0.27±0.04 bc A	0.12±0.07 bcd B
5.	0.07±0.04 de A	0.00±0.00 d A
6.	0.43±0.04 a A	0.24±0.06 b B
7.	0.00±0.00 e A	0.05±0.02 cd A
8.	0.00±0.00 e B	0.21±0.12 bc A
9.	0.00±0.00 e A	0.02±0.02 d A
10.	0.19±0.01 cd A	0.22±0.05 b A
11.	0.08±0.06 de B	0.23±0.06 b A
12.	0.38±0.06 ab B	0.71±0.01 a A

*According to the Duncan multiple comparison test, the differences between the averages shown with different letters are significant ($p<0.01$).
* Lowercase letters show the differences between applications.
**Uppercase letters show the differences between genotypes.

3.10. Rooting (transformation into complete plant) rate (%)

In the shoots grown in rooting medium for four weeks, the rooting rate for both genotypes was found to be 100 %.

3.11. Longest root values (cm)

Genotype X application interaction was found to be statistically significant in terms of the longest root value. As can be seen in Table 10, the longest root value was obtained from the 9th application (3.47 cm) for Kalecik Karası clone 4, and from the 1st application (3.9 cm) for clone 23-2 (Table 10).

Table 10. The longest root value of two different Kalecik Karası (K.K.) clones (c.) at the rooting stage

Applications	K.K. c. 4	K.K. c. 23 / 2
1.	0.0±0.0 b* B**	3.9±0.70 a *A
2.	0.21±0.21 b A	0.4±0.33 cd A
3.	0.11±0.11 b B	3.2±0.79 ab A
4.	0.07±0.07 b B	2.84±1.63 abcd A
5.	0.48±0.28 b B	2.66±0.66 abcd A
6.	1.64±0.83 ab A	2.4±0.90 abcd A
7.	0.86±0.86 b A	0.72±0.27 bcd A
8.	0.84±0.58 b A	1.26±0.93 bcd A
9.	3.47±1.28 a A	2.74±0.59 abcd A
10.	2.10±1.11 ab A	0.30±0.21 d B
11.	2.54±1.07 ab A	2.02±0.38 abcd A
12.	0.37±0.27 b B	2.97±1.17 abcd A

*According to the Duncan multiple comparison test, the differences between the averages shown with different letters are significant ($p<0.01$).
*Lowercase letters show the differences between applications.
**Uppercase letters show the differences between genotypes.

In the study of Kara (1992), root lengths between 6.333 and 0.33 cm were obtained in Çavuş, Müşküle, Hafızali, Razakı, Sultani seedless and Çal Karası varieties.

3.12. Average length of roots (cm)

The average root lengths obtained from the evaluation of the average of the lengths of all the roots formed in each of the *in vitro* shoots of the two different Kalecik Karası clones at the rooting stage are given in Table 11. Accordingly, it was determined that the highest value was 3.34±1.27 cm in the 9th application combination for Kalecik Karası clone 4, and 2.33±1.38 cm in the 4th application combination for clone 23-2.

Table 11. Root lengths of different Kalecik Karası (K.K.) clone (c.) at the rooting stage

Applications	K.K. c. 4	K.K. c. 23 / 2
1.	0.0±0.0 b*B**	2.29±0.43 a A
2.	0.17±0.16 b A	0.41±0.33 ab A
3.	0.10±0.09 b B	1.95±0.41 ab A
4.	0.08±0.08 b B	2.33±1.38 a A
5.	0.27±0.14 b A	1.63±0.29 ab A
6.	1.24±0.69 b A	1.71±0.60 ab A
7.	0.69±0.68 b A	0.59±0.21 ab A
8.	0.61±0.40 b A	0.96±0.69 ab A
9.	3.34±1.27 a A	1.93±0.44 ab A
10.	1.40±0.78 b A	0.23±0.16 b A
11.	1.53±0.69 b A	1.50±0.25 ab A
12.	0.24±0.17 b A	1.31±0.45 ab A

*According to the Duncan multiple comparison test, the differences between the averages shown with different letters are significant ($p<0.01$).
*Lowercase letters show the differences between applications.
**Uppercase letters show the differences between genotypes.

3.13. Average number of roots (n)

The difference between the data of both combinations was statistically significant in terms of the number of roots ($p<0.01$), while the highest value was determined as 7.6±2.4 in the 11th application combination for clone 4 and 6.83±1.4 in the 5th application combination for clone 23-2 (Table 12).

Table 12. Average number of roots of grapevine genotypes at the rooting stage

Applications	K.K. c. 4	K.K. c. 23 / 2
1.	0.00±0.00 b* B**	3.65±0.6 bc A
2.	0.44±0.44 b A	0.40±0.3 d A
3.	0.38±0.38 b B	5.45±1.4 ab A
4.	0.10±0.1 b A	0.89±0.51 d A
5.	2.25±1.21 b B	6.83±1.4 a A
6.	2.78±1.19 b A	1.33±0.50 cd A
7.	0.40±0.4 b A	1.22±0.45 cd A
8.	0.60±0.34 b A	1.29±0.84 cd A
9.	2.7±0.95 b B	5.22±1.10 ab A
10.	1.00±0.52 b A	0.40±0.30 d A
11.	7.60±2.42 a A	5.33±0.96 ab A
12.	0.30±0.20 b B	3.75±0.75 bc A

*According to the Duncan multiple comparison test, the differences between the averages shown with different letters are significant ($p<0.01$).
*Lowercase letters show the differences between applications.
**Uppercase letters show the differences between genotypes.

In their study, Ergönül and Öztürk (2016), have eliminated the viruses identified in the certification system of the European Union countries and our country and the grapevine pathogen Agrobacterium vitis with thermotherapy and meristem culture methods by using clones of 14 grapevine varieties and 6 rootstocks, which are of economic importance for our country. Yepes et al. (2019) obtained clean material in the *V. vinifera* cv. Riesling variety, infected with Agrobacterium vitis and grown in the greenhouse, by means of the meristem culture method and propagated it through tissue culture. Most of the plants can be successfully protected from viral contamination with tissue culture-virus elimination programs, and the criteria for the use and propagation of clonal, high quality and healthy (certified) grapevine seedlings free from viruses and similar are included in the European Union directives, which are binding in the candidate countries of the European Union (Mullins, 1990).

4. Conclusions

This study, which we have conducted for the production of base material for Kalecik Karası clones 4 and 23-2, consists of extracting and culturing meristems, forming shoots from explants, rooting, transforming them into complete plants and acclimatizing them to external conditions. In addition, the number and lengths of the shoots formed by the explants during the shoot formation and rooting stages were also examined. During the meristem and shoot formation, callus formation was observed, and the survival rate in clones 4 and 23-2 was determined as 60% and 80%, respectively. In addition to the full-strength MS medium during the shoot formation stage of the explants, 2.0mg. L^{-1} BAP+0.5 mg. L^{-1} IBA was used, and the average number of shoots was 3.67 and 3.83, and the average shoot length was 2.42 cm and 2.04 cm, respectively. In addition to MS nutrient media, 12 different auxin (IBA) and cytokine (2iP and BAP) concentrations were used at the rooting stage, and the effects of these concentrations on explant root development, number of shoots, shoot length and transformation into complete plant were examined.

At the rooting stage, it was determined that the formation of shoots and the number of leaves were higher in Kalecik Karası clone 23-2, and that 1.0 mg. L^{-1} IBA+0.5 mg. L^{-1} BAP/2 iP concentration gave the highest shoot formation and number of leaves. At this stage, callus formation was in parallel with the increase of auxin and cytokine levels. The highest callus levels were determined as 0.43 cm for Kalecik Karası clone 4 and 0.72 cm for clone 23-2. MS media with 2mg. L^{-1} IBA gave the highest rooting value. The longest root values were 3.47 cm with 1mg. L^{-1} IBA for clone 4, and 3.90 cm with 0.5mg. L^{-1} IBA for clone 23-2.

One of the most important problems of grapevine cultivation in our country is that the seedlings produced are infected with viruses and these seedlings are distributed to different parts of the country. What needs to be done is to work with clean plant material, certified grapevine seedlings, that is, genotype/clone and rootstock breeds that do not contain any disease agents.

Plants taken out of controlled conditions are very sensitive to environmental conditions, so losses can occur. In our study, the rate of acclimatization to external conditions in both clones is 90%. There was no difference between clones in terms of rate of acclimatization to external conditions.

Consequently, meristem culture studies with clones 4 and 23-2 belonging to Kalecik Karası grapevine variety revealed in our conditions that this method can be used practically for the grapevine and is applicable during the production of seedlings by commercial firms.

The general feature of the tissue culture also manifested itself during the meristem culture in the grapevine, and it was observed that different results can be obtained even if all conditions are kept constant between different clones and they belong to the same variety. This difference was significant in terms of features such as the development of meristems, the number of shoots and rooting rates and rooting capacities. Therefore, when it is desired to use meristem culture technique to obtain base material in the grapevine seedling production, it should not be overlooked that the method may need to be optimized for each genotype due to the genotype effect.

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References

Baydar, N. G. (2000). Study of adventitious shoot formation in leaves of grape (*Vitis* spp.). *Turkish Journal of Biology, 24*(3), 645-656.

Dalloul, A., Misik, S., & Olah, L. (1990). İn viiro micropropagation of grape varieties. *Vitis Special Issue* 463-464. https://doi.org/10.5073/vitis.1990.29

Ergönül, O., & Öztürk, L. (2016). Purification of some grape cultivar (*Vitis vinifera* L.) and rootstock clones eliminated from viruses with thermotherapy and meristem culture. *Trakya University Journal of Natural Sciences, 16*(2), 57-6. ISSN 2147-0294

Kara, S. (1992). *Researches on the reproduction of vines through meristem culture* (Ph.D. Thesis). Ege university graduate school of natural and applied sciences, Department of horticulture, Izmir.

Lavee, S. (2000). *Grapevine (Vitis vinfera) growth and performance in warm ciimates*. Ammon Erez (ed) Temperate fruit crops in warm climates, Netherlands. 343-366.

Martelli, G. P. (2014). Dırectory of vırus and vırus-lıke dıseases of the grapevıne and theır agents. *Journal of Plant Pathology, 96* (1), 1-4. http://dx.doi.org/10.4454/JPP.V96I1SUP

Mhatre, M., Salunkhe, C. K., & Raoe,. P. S. (2000). Micropropagation of *Vitis vinifera* L. towards an improved protocol. *Scientia Horticulturae, 84*(4), 357-363. https://doi.org/10.1016/S0304-4238(99)00109-0

Murashige, T., Skoog, & F. A. (1962). A revised medium for rapid growth and bio-qqassays with tobacco tissue cultures. *Physiologia Plantarum 15*, 473-497.

Mullins, M. G. (1990). Application of tissue culture to the genetic ımprovement of grapevines, *Vitis Special lssue*., 399-401. https://doi.org/10.5073/vitis..29.special-issue.

Roubelakis Angelakis, K. A., & Katsirdakis, K. C. (1990). *In vitro* micromultiplication of grapevine: effect of age, genotype and culture conditions on induction of callus in vitis spp. leaf segments. *Plant Aging Basic and Applied Approaches,* 89-95. https://doi.org/10.1007/978-1-4684-5760-5_11.

SPSS. (2011). IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM.

Sudarsono, S., & Goldy, R. G. (1991). Growth regulator and axillary bud position effects on *in vitro* establishment of *Vitis rotundifolia*. *Hortscıence 26*(3), 304-307. https://doi.org/10.21273/HORTSCI.26.3.304

Torregrosa, L., Bouguet, A., & Goussard, P. G. (2001). *In vitro* culture and propagation of grapevine, K. A. Roubelakis-Angelakis (Eds.), *Molecular Biology & Biotechnology of the Grapevine*, Netherlands, 281-326. https://doi.org/10.1007/978-94-017-2308-4_12

Thomas, P., & Schiefelbein, J. W. (2004). Roles of leaf in regulation of root and shoot growth from ingle node softwood cuttings of grape (*Vitis vinifera*), *Ann. Appl. Biol., 144,* 27-37.

Yepes, L. M., Burr, T., Reid, C., & Fuchs, M. (2019). Elimination of the crown gall pathogen, agrobacterium vitis, from systemically ınfected grapevines by tissue culture. *Am. J. Enol. Vitic, 70*:(3) https://doi.org/ 10.5344/ajev.2019.18083