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Evaluation of the Effect of BAP, Kinetin, *Meta*-Topolin Growth Regulators on Grape Cultivars and Molecular Analysis of SSR Markers

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Abstract

Preserving genetic similarity is one of the key points to enhance resilience, functionality, and unique adaptation. Micropropagation is the most reliable approach to preserving genetic similarity. In this work, we compared the efficiencies of the three modified growth culture media – Murashige and Skoog (MS), Woody Plant Medium (WPM), and Driver-Kuniyuki Wallnut (DKW) on shooting number and length. The efficiency of BAP, kinetin, and *meta*-topolin in the above culture media has been established for four local Uzbek grape (*Vitis vinifera* L.) cultivars. MS was found to be the most efficient culture media among the studied ones to grow Odqum kishmish, Rizamat, Toyfi, Sugdiyona kishmish grape cultivars, belonging to highly productive ones in Uzbekistan. The numb er of shoots in the studied cultivars increased by 8–10 when grown *In vitro* in the modified MS culture media compared to the unmodified one. Significant improvements were observed in the shoot length in all grape cultivars when grown in modified MS media. Using eight Simple Sequence Repeat markers, we determined the preservation of genetically identical material of *in vitro* grown Oqdum kishmish explants with the highest parameters. In this work, grape microplants were *in vitro* grown in modified culture media and were widely used in research. MS was established to be optimum for all four cultivars. We also determined that culture-grown Oqdum kishmish micropropagations were genetically similar to mother plant.

Keywords: *Vitis vinifera* L; Kinetin; BAP; *m*T; SSR markers

Introduction

Grape (*Vitis vinifera* L.), a perennial plant grown worldwide, is the subject of numerous experiments to preserve its genetic diversity. Findings from plant selection, genetics, genomics, and genome editing help better understand molecular markers (Nadeem *et al*. 2018). Of the various methods for identifying grape cultivars, DNA methods and PCR analysis based on simple sequence repeat (SSR) markers are the fastest and most practical (Migliaro *et al*. 2013). Emanuelli *et al*. (2013) used specific SSR markers to analyze the genomes of grape cultivars from special collections. Abdel-Hameed *et al*. (2020) used molecular markers based on random amplified polymorphic DNA (RAPD) to study the genetic diversity of three grape cultivars.

Genetic analysis of 49 Turkish grape cultivars using SSR markers revealed 196 alleles from 22 SSR loci, with an average of 8.91 alleles per locus (Arslan *et al*. 2023). DNA editing research identified 521 unique genotypes in the oldest vineyards in Spain, of which 476 were genetically compatible and 45 were novel. Several synonymies and homonymies were also identified, with all alleles accounting for about 13% of total crossbreeding (Cretazzo *et al*. 2022).

Allelic diversity is another important criterion for choosing SSR markers. Roshni *et al*. (2016) found that the SSR marker VVMD7 had the highest allelic diversity (0.82). The level of genetic variability is another significant point to consider. Meneghetti *et al*. (2011) used SSR loci with 14 nucleotide sequences to study the genetic variability and distribution of 53 cultivars from different countries.

In the study of Kwon *et al*. (2019) molecular analyses of grapevine were carried out using (ISSR) markers. No genetic changes were observed when grape varieties were *in vitro* grown in MS medium holding 5 μM benzyladenine. The modified MS culture was found optimum among the studied variants in another study, five grape varieties were

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propagated using the *in vitro* method. The MS culture medium holding 0.5 mg L^{-1} BAP was reported as another effective means to grow grapes from meristem. As a result, virus-free plants were obtained (Mahmoudzadeh 2019). Modified Gamburg B5 grape explants also showed efficacy. BAP, kinetin (Kin), and GA3 in 0.6: 0.2: 0.5 mg L^{-1} ratio in the culture medium developed highest level of regeneration of seedless grape explant. In this case, the average number of micropropagations in each explant was 4.5 pieces. The highest rate of rhizogenesis of regenerating microshoots was recorded in MS nutrient medium (Melyan *et al.* 2015). Sajid *et al.* (2006) carried out a comparative study in which the efficacies of various growth regulators were researched. The modified MS culture medium enriched with 0.3 mg L^{-1} BAP improved both the number and length of explants.

The current research was conducted in order to propagate local Uzbek varieties of *V. vinifera* plants in various nutrient media, and to ensure genetic unity with the mother plant.

Materials and Methods

Grape cultivars

A collection of grape cultivars was planted in a 2.5 × 3 m field at the Center of Genomics and Bioinformatics of the Uzbekistan Academy of Sciences. Oqdum kishmish, Rizamat, Toyfi and Sugdiyona kishmish cultivars were selected as mother material from the existing collection. Samples were collected from these cultivars and transported to the laboratory in specialized containers.

Plant materials and sterilization

The explants were isolated from the meristem tissues of the terminal and lateral shoots of the Oqdum-kishmish cultivar grown in the experimental field. Explants were kept in fungicide solution (fundazol 0.02%) for 1 min and in running water for 10 min. Explant tissues were treated with 70% ethanol (10 sec), and 5% sodium hypochlorite (5 min) for disinfection. In total, 50 explants that survived the surface sterilization step were chosen to obtain explants from the meristem.

Autoclave sterilization

Filter paper, tweezers, and other necessary tools were sterilized with moist steam under high pressure at a temperature of 121°C for 60 min. The pH of the nutrient media was slightly reduced to 5.6 using HCl and sterilized at 115–121°C and 0.7–0.8 atmosphere for 15–20 min.

Surface sterilization

Under aseptic conditions, surface sterilization was performed in a laminar box *HFsafe LC* (China). The effectiveness of several chemical compounds for explant sterilization at different time intervals was studied (Table 1). For each variant, 50 explants were selected from the samples. The optimal duration of surface sterilization was observed in option 2 of unsterile explants (Table 1).

Conditions for explants

Explants were planted on modified MS, Woody Plant Medium (WPM), and DKW nutrient media and kept in special conditions: temperature, 23±1°C; light, 39.5±4.18 W/m^2 (16/8 light/dark); humidity, 50–55%. The chemical compositions of the modified nutrient media are given in Table 2.

DNA isolation

Doyle JJ, JL Doyle (1987) method was used to extract DNA. Young leaf tissues were homogenized in liquid nitrogen to extract genomic DNA (gDNA) from Oqdum kishmish cultivar. Next, gDNA was isolated using GeneJet^{MT} Kit.

PCR condition

PCR reactions were performed with 10×PCR buffer containing 0.3 µM $(NH_4)_2SO_4$, 2.5 mM $MgCl_2$, 10 µM dNTP, 0.2 µM F and R primers, 0.5 µM Taq polymerase, 0.3 µM of each (**VVMD**5, -7, -25, -27, -28, -32 and **VrZAG**62, -79) primers, 5.02 mg L^{-1} H_2O and 40 ng DNA extracted from Oqdum kishmish cultivar.

Analysis of SSR markers using PCR was carried out in three conditions separately. The following profile was used for **VVMD**5 and -7: initial denaturation at 94°C for 2 min; next on 94°C for 30 sec; 58°C for 30 sec; and at the end of 72°C for 10 min for a total of 35 cycles. We used similar temperature cycle and duration for **VVMD**25, -27, -28, -32 markers. The difference belonged to annealing temperature: 58°C for **VVMD**5 and -7; 61°C for **VVMD**25, -27, -28, -32; 65°C for VrZAG62 and -79. The temperature cooled down to 4°C after PCR ended (Table 3).

Gel electrophoresis

DNA samples for PCR were run on a 3.5% high-resolution agarose gel in 0.5 × TVE buffer under an electric voltage of 6 v/cm. Gels were stained with ethidium bromide and examined using Alpha Imager 300 (Innotech Inc., USA). Their concentration was adjusted to a working concentration (25 mg) and stored frozen (in a freezer at -20°C) until studies.

Statistical analysis

Statistical analyses were performed in the ANOVA program using the *t*-test criterion. The analysis was carried out in three biological replicates, each consisting of 30 samples.

Table 1: Means of surface sterilization and its duration

Variants	Time/sterilizing agent		
Unsterile explants 1	2 min, H_2O_2 (3%)	10 sec, C_2H_5OH (70%)	3 min, 2% NaClO
Unsterile explants 2	4 min, H_2O_2 (3%)	15 sec, C_2H_5OH (70%)	6 min, 2% NaClO
Unsterile explants 3	6 min, H_2O_2 (3%)	20 sec, C_2H_5OH (70%)	9 min, 2% NaClO

*Control and surface sterilization optimal concentrations are shown in gray

Table 2: Chemical compositions and the concentration of reagents used for the preparation of culture media

Components	Dose (mg L^{-1})					
	MS_1 (A_1)	MS_2 (A_2)	WPM_1 (B_1)	WPM_2 (B_2)	DKW_1 (C_1)	DKW_2 (C_2)
KNO_3	1900	1900	-	-	-	-
K_2SO_4	-	-	990	990	1559.00	1559.00
NH_4NO_3	1650	1650	400	400	1416.00	1416.00
$Ca(NO_3)_2$	386	386	386	386	1367.47	1367.47
$MgSO_4$	370	370	-	-	361.38	361.38
$CaCl_2{\cdot}2H_2O$	331	331	72.5	72.5	149.00	149.00
KH_2PO_4	170	170	170	170	265.00	265.00
$MnSO_4{\cdot}5H_2O$	24.1	24.1	180.7	180.7	33.50	33.50
$ZnSO_4{\cdot}7H_2O$	8.6	8.6	8.6	8.6	-	-
H_3BO_3	6.2	6.2	6.2	6.2	4.80	4.80
$Zn(NO_3)_2{\cdot}6H_2O$	-	-	-	-	17.00	17.00
$CuSO_4{\cdot}5H_2O$	0.25	0.25	0.25	0.25	0.25	0.25
$FeSO_4{\cdot}7H_2O$	27.8	27.8	27.85	27.85	33.80	33.80
$Na_2EDTA{\cdot}2H_2O$	37.3	37.3	37.3	37.3	45.40	45.40
KJ	0.83	0.83	-	-	-	-
$Na_2MoO_4{\cdot}2H_2O$	0.25	0.25	0.25	0.25	0.39	0.39
$CoCl_2{\cdot}6H_2O$	0.025	0.025	-	-	-	-
Myo-inositol	-	-	100	100	100	100
Meso-inositol	75.0	75.0	-	-	-	-
Thiamine·HCl	-	-	1	1	2.00	2.00
Nicotinic acid	-	-	0.5	0.5	1.00	1.00
Pyridoxine·HCl	5.0	5.0	0.5	0.5	-	-
Glycine	10	10	2	2	2.00	2.00
Glutamine	50.0	50.0	-	-	-	-
Sucrose	30.0	30.0	30.0	30.0	30.0	30.0
Agar-agar	700	700	700	700	700	700
6-Benzylaminopurine	1.0	1.0	1.0	1.0	1.0	1.0
Kinetin	0.5	0.5	0.5	0.5	0.5	0.5
Meta-Topolin	-	0.5	-	0.5	-	0.5

*Growth regulators in various nutrient media are highlighted in grey

MS_1, MS_2, WPM_1, WPM_2, DKW_1 and DKW_2 culture media are given as A_1, A_2, B_1, B_2, C_1 and C_2 further in histograms

Results

Effects of growth regulators on meristem explants

In our earlier investigations, we determined the effects of a growth regulating preparation in combination with two culture media on root length and root numbers of *V. vinifera* (Ubaydullaeva *et al*. 2023b). In this work, we researched the effects of various culture on shooting number and length.

We planted grape explants in modified nutrient media, and the effect on the number and length of shoots was studied to establish the optimal concentrations of growth regulators. The surviving meristem tissues from the surface sterilization step were initially introduced into the MS nutrient medium containing BAP, Kin, and *m*T (Fig. 1a). The starting and lateral shooting began to form within 3–4 weeks (Fig. 1b). We observed an increased number and length of shooting after five weeks (Fig. 1c). In the 6th and 7th weeks of the study, the number and length of the shooting reached the maximum level (Fig. 1d).

Effect of growth regulators on the proliferation of explants

Growth, reproduction, and development of plants depend on the totipotency feature and genotype. Finding the optimal composition of culture media is one of the essential factors affecting plant growth. The optimum concentration of salts ions and growth regulators provided a good condition for plant development. In this work, we compared the effects of MS_1, WPM_1 and DKW_1 nutrient media (not holding *m*T) with MS_2, WPM_2 and DKW_2 nutrients containing *m*T. In MS_2, WPM_2 and DKW_2 media, there was a significant increase in shoot number and length.

The shoot number of Oqdum kishmish and Rizamat cultivars were compared in media containing *m*T (MS_2) and without (MS_1). Explants grown in MS_1 nutrient media (Fig. 2b and 2d) had 5, 6 buds, while explants grown in MS_2 nutrient media (Fig. 2a and Fig. 2c) had an average increase of 8, 10 buds.

Compared to MS_1 (A_1) medium, MS_2 (A_2) nutrient

Table 3: Forward & Reverse nucleotide sequences of DNA fragments used as SSR markers

Primer name	Annealing temperature (°C)	Basepair (bp)	Primer sequence (5' – 3') Forward & Reverse
VVMD5	58	245–250	F-CTAGAGCTACGCCAATCCAA
			R-TATACCAAAAATCATATTCCTAAA
VVMD7		255–265	F-AGAGTTGCGGAGAACAGGAT
			R-CGAACCTTCACACGCTTGAT
VVMD25	61	244–250	F-TTCCGTTAAAGCAAAAGAAAAAGG
			R-TTGGATTTGAAATTTATTGAGGGG
VVMD27		188–195	F-GTACCAGATCTGAATACATCCGTAAGT
			R-ACGGGTATAGAGCAAACGGTGT
VVMD28		245–255	F-AACAATTCAATGAAAAGAGAGAGAGAGA
			R-TCATCAATTTCGTATCTCTATTTGCTG
VVMD32		248–255	F-TATGATTTTTTAGGGGGGTGAGG
			R-GGAAAGATGGGATGACTCGC
VrZAG62	65	216–220	F-GGTGAAATGGGCACCGAACACACGC
			R-CCATGTCTCTCCTCAGCTTCTCAGC
VrZAG79		266–270	F-AGATTGTGGAGGAGGGAACAAACCG
			R-TGCCCCCATTTTCAAACTCCCTTCC

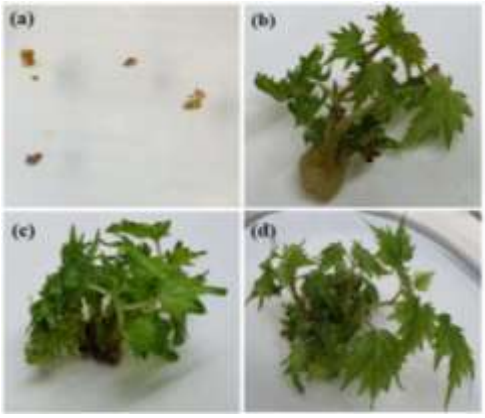

Fig. 1: Shooting stages of Oqdum kishmish explant in MS culture media containing 1.0 mg L^{-1} BAP, 0.5 mg L^{-1} Kin, and 0.5 mg L^{-1} *m*T. a – Initial meristem in MS culture media; b – shooting for 3–4 weeks; c – shooting for 5 weeks; d – shooting for 6–7 weeks

medium showed a reduction in the day of shooting of Oqdum kishmish and Rizamat cultivars (Fig. 3). No significant difference was found in the shooting days of Toyfi and Sugdiyona kishmish cultivars. Significant differences were observed in the shoot number and length of two cultivars grown in the A_2 media. Greater differences belonged to Oqdum kishmish over Rizamat. There were no significant differences in the number of shoots of Toyfi and Sugdiyona kishmish cultivars grown in the A_1 and A_2 samples; the difference was found only in the shoot length (Fig. 3). Among the four cultivars, Oqdum kishmish was the best indicator. The average number of shoots was 8–9, and the length increased by 5–6 cm (Fig. 3). The research results show that growing plants in artificial nutrient media does not always ensure normal growth; it depends on the genotype of the plants as well.

Explants grown in WPM_1 (B_1) and WPM_2 (B_2) media under the influence of 0.5 mg L^{-1} *m*T showed a difference in shoot number in Oqdum kishmish cultivar. A clear difference in the shoot length was shown in Toyfi cultivar. In Rizamat cultivar, the number of shoots increased in those grown in B_2 media. In the Sugdiyona kishmish cultivar, the shoot number and length increased in B_2, which is attributed to *m*T (Fig. 4). Under the influence of *m*T, the number of shoots of all cultivars increased. Different growth of the reign length is a process depending on the genotype of the plants.

In Oqdum kishmish and Rizamat cultivars, B_2 had a shorter shooting day compared to B_1. No significant difference was found in the shooting day of Toyfi and Sugdiyona kishmish cultivars. A significant difference was in the shooting number of Oqdum kishmish cultivar grown in *m*T holding B_2 medium. There were no significant differences in the shooting number in Toyfi, Rizamat, and Sugdiyona kishmish cultivars. In all cultivars, significant differences were observed by adding *m*T (Fig. 5).

Significant differences were observed in the shooting days of Oqdum kishmish and Rizamat cultivars when grown in DKW media that holds *m*T; no significant differences were found in Toyfi and Sugdiyona kishmish cultivars. Significant differences in the shooting numbers were observed in C_2 in Oqdum kishmish, Rizamat, and Sugdiyona kishmish cultivars; we defined no differences in Toyfi cultivar. There were significant differences in the shooting length of Oqdum kishmish, Rizamat, and Sugdiyona kishmish cultivars under the influence of *m*T (Fig. 6).

Molecular analysis of Okdum kishmish cultivars

Three samples of the Oqdum kishmish cultivar, one from the mother plant and two from microplants grown *in vitro*, were selected, and their identification was carried out to further determine the genetic aspect of the genotype. Based on the obtained results, molecular genetic analysis was performed. We found no genetic cultivars in the explants, which explained that the plants grown *in vitro* had the same genetic parameters as the mother plant. In the study, the degree of similarity to the mother plant was investigated

Fig. 2: Shooting of explants of Rizamat and Oqdum kishmish varieties in MS media
a – Rizamat explants grown in MS_2 media – 1.0 mg L^{-1} BAP, 0.5 mg L^{-1} Kin, and 0.5 mg L^{-1} *m*T; b – Rizamat explants grown in MS_1 media – 1.0 mg L^{-1} BAP and 0.5 mg L^{-1} Kin; c – Oqdum kishmish explants grown in MS_2 – 1.0 mg L^{-1} BAP, 0.5 mg L^{-1} Kin, and 0.5 mg L^{-1} *m*T; d – Oqdum kishmish explants grown in MS_1 – 1.0 mg L^{-1} BAP and 0.5 mg L^{-1} Kin

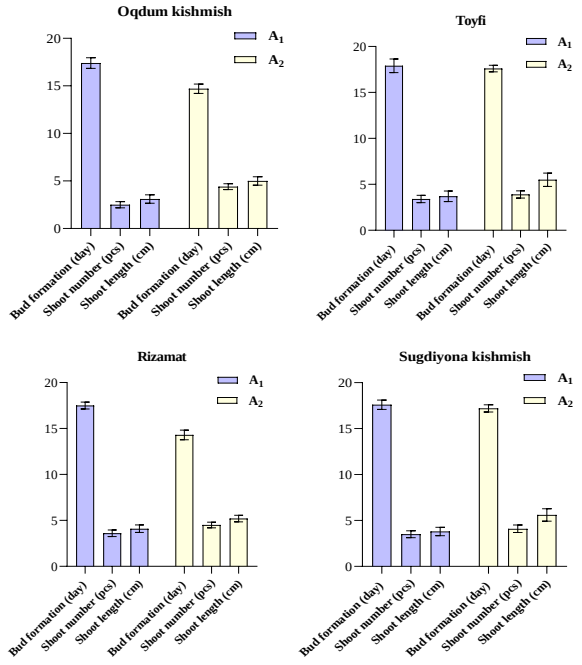

Fig. 3: Bud formation, shooting number, and length of explants of various grape cultivars in MS culture media
A_1 belongs to MS_1 culture media that holds 1.0 mg L^{-1} BAP and 0.5 mg L^{-1} Kin
A_2 belongs to MS_2 culture media that holds 1.0 mg L^{-1} BAP, 0.5 mg L^{-1} Kin, and 0.5 mg L^{-1} *m*T
The chemical compositions of MS_1 and MS_2 culture media are given in Table 3

using eight SSR markers: **VVMD**5, -7, -25, -27, -28, -32, **VrZAG**62 and -79. All molecular indicators showed belonging to the mother plant (Fig. 7).

Analysis of the amplification results showed that the samples taken from the mother plant in the field were not genetically different from the explants grown *in vitro*. No

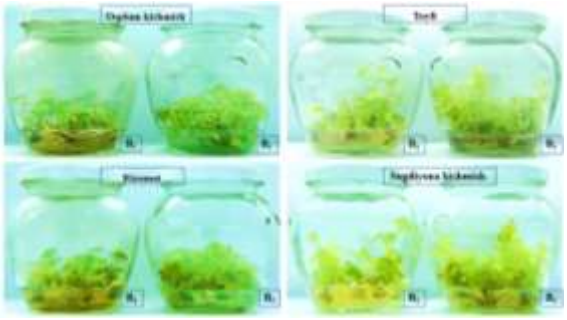

Fig. 4: Shooting of various grape cultivars in WPM media
B_1 – explants grown in WPM_1; B_2 – explants grown in WPM_2
WPM_1 contained 1.0 mg L^{-1} BAP + 0.5 mg L^{-1} Kin. WPM_2 contained 1.0 mg L^{-1} BAP, 0.5 mg L^{-1} Kin, and 0.5 mg L^{-1} *m*T. The chemical compositions of WPM_1 and WPM_2 culture media are given in Table 2

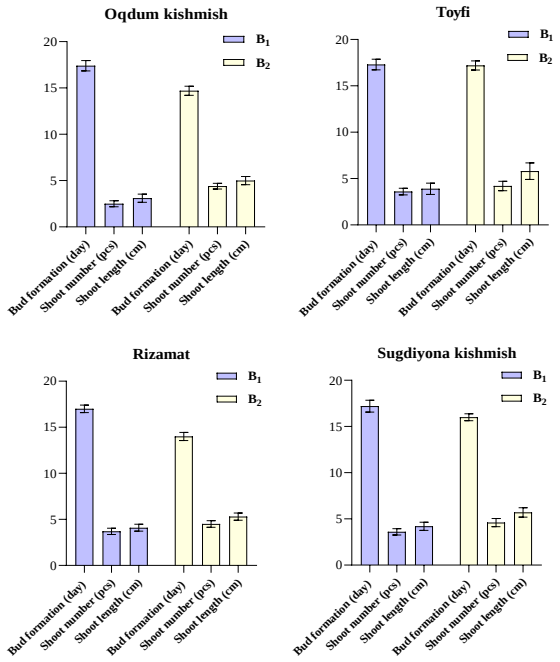

Fig. 5: Bud formation, shooting number, and length of explants of various grape cultivars in WPM culture media
B_1 belongs to WPM_1 culture media that holds 1.0 mg L^{-1} BAP and 0.5 mg L^{-1} Kin
B_2 belongs to the WPM_2 culture media that holds 1.0 mg L^{-1} BAP, 0.5 mg L^{-1} Kin, and 0.5 mg L^{-1} *m*T
The chemical composition of WPM_1 and WPM_2 culture media are given in Table 3

genetic variability was detected between the samples studied by PCR analysis (Fig. 7). In our study, molecular analysis of local grape cultivars was carried out using standard SSR markers **VVMD**5, -7, -25, -27, -28, -32, **VrZAG62** and -79. The reliability of these markers has been shown in previous studies.

Discussion

In our research, three different nutrient media were used, and the number and length of the shooting of four grape cultivars were compared by using growth regulators (Table 2).

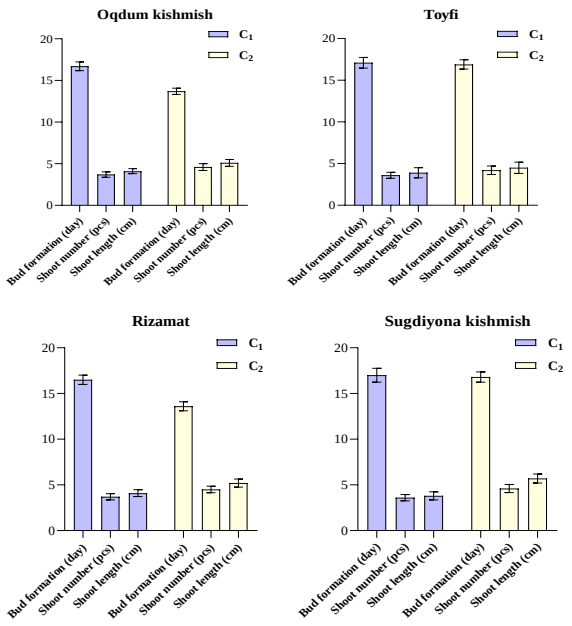

Fig. 6: Bud formation, shooting number, and length of explants of various grape cultivars in DKW culture media

C_1 belongs to DKW_1 culture media that holds 1.0 mg L^{-1} BAP and 0.5 mg L^{-1} Kin

C_2 belongs to DKW_2 culture media that holds 1.0 mg L^{-1} BAP, 0.5 mg L^{-1} Kin, and 0.5 mg L^{-1} *m*T

The chemical composition of DKW_1 and DKW_2 culture media are given in Table 2

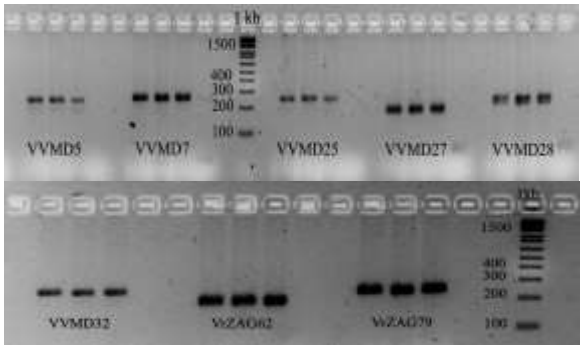

Fig. 7: Molecular analysis of Oqdum kishmish cultivar based on eight SSR markers (DNK fragments in 3.5% agarose gel). The left band belongs to an explant grown in MS culture media; the middle one was grown in WPM media; the right band is from an explant grown in DKW culture media

Following the results of former research works, we tested nutrients and growth regulators in different concentrations and achieved positive results in our work. In the research of Salami *et al*. (2005), virus-free growth meristems of grapevine were grown in an MS nutrient medium. As a result, the shooting of the grape was extended by 20–25 mm in the MS nutrient medium containing 2.0 mg L^{-1} BAP. In another study of *in vitro* propagation of grapevine, it was observed that the number of shoots increased in MS medium containing the growth regulator BAP, and the authors established the optimum BAP concentration (Tehrim *et al*. 2013). Similarly, Yerbolova *et al*. (2013) determined an optimum concentration of growth regulator. The highest index of root yield of the Riesling cultivar (133 ± 1.6 mm) grown in an IM nutrient medium was observed in the one containing 4.4 mg L^{-1} BAP and 0.1 mg L^{-1} NAA.

Bud formation was observed in all experimental explants in MS nutrient medium containing Kin, BAP and *m*T 1.0; 0.5 and 0.5 mg L^{-1}. This nutrient medium was selected as the optimal variant (Fig. 1). In a similar study when sterilized explants were grown in MS medium supplemented with 0.5, 1.0 and 2.0 mg L^{-1} BAP and 0.1 mg L^{-1} naphthalene acetic acid (NAA), the optimum MS medium was found to be the one containing 2.0 mg L^{-1} BAP and 0.1 mg L^{-1} NAA in which shoot length was significantly increased (Abido *et al*. 2013). In another study, evaluating the effect of growth regulators *in vitro* propagation, growth regulators showed the best results in the MS medium containing 1.0 mg L^{-1} BAP + 0.1 mg L^{-1} NAA (Arifuzzaman *et al*. 2016). *In vitro* propagation of grapes requires the development of optimal nutrient media. Authors often establish the most efficient one among the used ones. Nookaraju *et al*. (2008) compared the effects of various nutrient media such as MS, Eriksson, Hamburg, Nitsch and Nitsch, and WPM. The maximum shoot growth rate (90.0%) was observed in the MS nutrient medium. Experiments were conducted on modified MS-1, MS and WPM nutrient media in another study. As a result, in the MS-1 nutrient medium containing 1.0 mg L^{-1} BAP, the growth rate of shoots increased significantly, and 4.75 new growth shoots were formed in six weeks (Mukherjee *et al.* 2010).

In the *in vitro* propagation of explants brought from non-sterile conditions, the doubled growth of explants in the MS nutrient medium containing 2.0 mg L^{-1} BAP and 1.0 mg L^{-1} NAA was observed. Thus, the authors developed a protocol that allowed an increase in the number of plants from 85,000 to 119,000 per year (Singh *et al*. 2004). Similarly, virus-free meristems of grapes grown in non-sterile field conditions were obtained by Lazo-Javalera *et al*. (2016). The authors developed a methodology for *in vitro* growing and multiplying plants. In another study, grape explants were grown *in vitro* in an MS medium containing BAP, Kin, glycine, and gibberellic acid (GA_3). As a result, the maximum shoot length was 2.75 cm, and the number of shoots was 5.33 (Khan *et al*. 2015). In the study of Mhatre *et al.* (2000), three grape cultivars were propagated *in vitro*. Shoot explants were grown in MS medium containing BAP, NAA and IBA. As a result, several shoots appeared between the 2nd and the 3rd weeks. In another study, Fráguas *et al*. (2004) developed a protocol for growing grape explants *in vitro*. When grapes were grown in a WPM nutrient medium containing 0.5 mg L^{-1} Kin, shoots with an average length of 4.8 cm were observed. Among the various topolins, *meta*-Topolin (*m*T) is becoming the most popular. In recent years, *m*T has been observed to be a better cytokinin in plant micropropagation (Naseem and Miroslav 2021). Yildirim and Ozdemir (2018) investigated several nutrient media and

growth regulators. The best result for shooting was observed in a nutrient medium containing 1 mg L^{-1} BAP. In our study, growth regulators and nutrient media were optimized.

The growth regulators utilized in our study were also used for other plants. In our early trial, regeneration processes were studied using growth regulators in Desiree and Sarnav potato cultivars. Callus formation and development in nutrient media containing 1.0 mg L^{-1} NAA and 1.5 mg L^{-1} BAP growth regulators were almost maximum effective in both varieties (Babadjanova *et al*. 2023). Naseem and Miroslav (2021) investigated the effects of the growth regulator *m*T on Arabidopsis flowering and root length. They determined that root elongation doubled nutrient uptake; two or three times more biomass was obtained due to the increase in the root length. Yildirim and Ozdemir (2018) selected MS media as the most efficient among the MS, DKW, QL and WPM nutrient media that were tested on many artificial nutrient media. The MS resulted in the highest shoot number (6.28) and the length (1.41 cm).

One of the methods of vegetative reproduction of plants based on the achievements in the cell and tissue culture field – asexual reproduction of plants *in vitro* allows growing seedlings genetically identical to the original copy. The basis of the maximum use of plant cells by the *in vitro* method is their unique totipotency; a plant organism appears under the influence of exogenous factors. There are several advantages of using this method in plant propagation: obtaining genetically identical materials, making plants virus-free by using meristem culture, high reproduction coefficient (10^5–10^6 for herbaceous and flowering plants, 10^4–10^5 for shrus) (Ubaydullaeva *et al*. 2023a), acceleration of plant growth phases, the possibility of working throughout the year, the economy of areas for growing planting materials and the possibility of automating the growing process, the economic efficiency of growth regulators contained in nutrient media, etc. (Ubaydullaeva *et al*. 2023b).

Tympakianakis *et al*. (2023) conducted a phylogenetic analysis of the most widely used molecular markers in *V. vinifera* grape cultivars and developed a protocol for the use of primers to address the challenges of propagation and exploitation of these cultivars. Another research was conducted on the renewal and identification of varietal diversity in aging vineyards using the microsatellites VVS2, **VVMD**5, -7, -27, **VrZAG**62 and -79. The research served to preserve 23 grape cultivars that were under threat of extinction (Casanova *et al*. 2011). Atak *et al*. (2012) determined the ampelographic and molecular characteristics of 23 grape hybrids using SSR markers with 20 nucleotide sequences. Moravcova *et al*. (2006) determined the SSR profiles of 6 grape cultivars, with an average of 13.2 alleles detected per locus. Heterozygosity ranged from 0.745 (VrZAG79) to 0.922 (VVMD7). Heuertz *et al*. (2008) used the SSR markers VVS2, **VVMD**5, -7, -27, **VrZAG**62, and -79 to study the diversity of grape cultivars and their relationships with each other. The developed method allowed the identification of two clusters.

In this study, using SSR markers, we determined matching bp in grape samples brought from the mother plant that was similar to explants propagated *in vitro* (Fig. 7). Grape explants grown *in vitro* and samples of grapes brought from the mother plant show that they have not changed genetically under the influence of growth regulators in culture media (Fig. 7). Kwon *et al*. (2019) carried out molecular analysis of mother plants compared to those after *in vitro* propagation grown in modified culture media. They found no genetic variability between the mother plant and *in vitro* explants when ISSR markers were used. Casanova *et al*. analyzed genetic diversity using VVS2, **VVMD**5, -7, -27, **VrZAG**62, and -79 markers. Several ancient genotypes were identified on their basis; grape seedlings were propagated and preserved so that the corresponding genotypes would not disappear (Casanova *et al*. 2011).

Conclusion

In this work, we established that BAP, Kin, and *m*T in 1.0: 0.5: 0.5 mg L^{-1} doses significantly increased the number and length of shoots of four cultivars of *Vitis vinifera.* We found that growth regulators increased and prolonged the number of shoots. SSR markers were used to compare the obtained explants with the mother plant in order to determine the purity of the cultivar and genetic variability. As a result, it was found that the explants are genetically similar to the mother plant. The modified MS_2 nutrient medium was found to be the most effective nutrient for the studied grape cultivars.

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Author Contributions

ANA: Formal analysis, Data curation, investigation, methodology, visualization, resources, validation, writing – original draft. ZTB: Writing – data curation. AMA: Writing – review, editing. JBE, AAB, SAA, MMD, FIB, DEU: Data curation, investigation, resources. KhAU: Conceptualization, Investigation, Methodology, Project administration.

Conflicts of Interest

The authors declare no conflict of interest.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable to this paper.

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