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MICROCLONAL PROPAGATION THROUGH *IN VITRO* AND OPTIMIZATION OF THE RHIZOGENESIS USING GROWTH REGULATORS IN CHERRY (*PRUNUS AVIUM* L.)

S. ABDURAMANOVA[1], Y. SAIMNAZAROV[2], H. ADILOV[1]*, A. KURBONMURODOV[1], I. OKKUZIEV[3], D. ABDULLAYEV[3], and N. KURBONBOEVA[1]

[1]Tashkent State Agrarian University,Tashkent, Uzbekistan
[2]Rice Research Institute, Tashkent, Uzbekistan
[3]Research Institute of Plant Genetic Resources,Tashkent, Uzbekistan
*Corresponding author's email: salomat.invitro@gmail.com
Email addresses of co-authors: y.saimnazarov@mail.ru, qurbonmurodovakbar2702@gmail.com, ilhom.oqquziyev85@gmail.com, abdullayevdavlat502@gmail.com, nafisa.qurbonboyeva@gmail.com

SUMMARY

Microclonal propagation outcomes of various cherry (*Prunus avium* L.) cultivars and rootstocks achieved their evaluation and establishment of combinations of optimal nutrient media and growth regulators for each genotype. The rootstock Krimskiy-5 showed the best response with 2.95 shoots and 1.84 cm length using the Driver and Kuniyuki Walnut (DKW) medium supplemented with 0.01 and 0.75 mg/l BAP (benzylaminopurine). Cherry rootstocks Gisela-5 and Gisela-6 produced 2.77 and 2.99 shoots, respectively, with similar conditions. In rootstock Colt, the use of 0.02 and 1.0 mg/l BAP resulted in 2.92 shoots with a 1.78-cm length. The cherry cultivars Bahor, Volove Serdse, and Revershon with mT (meta-Topolin) combinations in DKW medium provided shoot numbers above 2.9 and lengths over 2.1 cm. The cherry cultivar Kara Geles showed better performance with naphthaleneacetic acid (NAA) and Thydizoronate (thidiazuron or TDZ), achieving 3.22 shoots. The cultivar Pink Napoleon exhibited the highest proliferation, with 3.42 shoots and 3.52-cm shoot length. Overall, the DKW nutrient medium combined with specific growth regulators demonstrated superior performance in shoot proliferation across most cultivars, whereas Murashige and Skoog (MS) and Woody Plant Medium (WPM) media were less efficient. These findings contribute to the development of improved protocols for the micropropagation of cherry species.

Keywords: Cherry (*P. avium* L.), auxins (IBA, NAA), cytokinins (BAP, mT, TDZ), nutrient medium, shoot, microclonal propagation

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Key findings: The highest micropropagation results resulted in the cherry (*P. avium* L.) cultivar Pink Napoleon using 0.02 mg/l NAA and 1.50 mg/l TDZ in DKW medium, producing 3.42 shoots and 3.52 cm length. This highlights the considerable synergistic effect of NAA and TDZ and the superior efficiency of DKW medium compared with MS and WPM.

INTRODUCTION

Cherry (*Prunus avium* L.) is one of the economically important stone fruit crops in the global market. The cherry cultivation area is expanding due to its high nutritional value and export potential worldwide. Currently, cherry production yields more than nine million tons annually in over 65 countries. Leading cherry-producing countries include Turkey (800,000 tons), the United States (448,000 tons), Russia (273,000 tons), and Iran (250,000 tons) (Abduramanova, 2018, 2020). Recently, Uzbekistan has also succeeded in entering the top five countries for total cherry production and export volume.

In cherry production, Uzbekistan's achievements are reliant on the implementation of intensive cultivation technologies and on using high-yielding and disease-resistant cultivars and dwarf rootstocks. However, the vigorous growth habit of cherries presents challenges for intensive orchard systems, increasing the demand for dwarf rootstocks. The rootstocks of the Gisela series (Gisela-5 and Gisela-6) and Colt are the widely used basic material worldwide for establishing intensive cherry orchards (Driver and Kuniyuki, 1984; Lloyd and McCown, 1980a; Saimnazarov and Abduramanova, 2019).

Traditional vegetative propagation methods face several limitations in mass propagating the dwarf rootstocks. Specifically, seed propagation leads to genetic variations, while vegetative propagation carries a high risk of disease transmission. Modern biotechnological methods, particularly in vitro microclonal propagation technologies, offer sustainable solutions to these problems (George *et al.,* 2008). In vitro conditions allow for the production of clonal, genetically identical, and disease-free plants from explants.

The microclonal propagation success primarily depends on the nutrient media composition and plant growth regulators. Murashige and Skoog (MS) medium is the most commonly used basic medium for plant tissue culture (Murashige and Skoog, 1962). The optimal combination of macro- and micro-elements in MS medium stimulates cell division and organogenesis in various plant species. However, in specific fruit trees, particularly cherries and other *Prunus* species, the MS medium does not always provide the highest efficiency.

The Driver and Kuniyuki Walnut (DKW) nutrient medium has shown the highest effectiveness, especially for the in vitro propagation of nut-bearing plants (Driver and Kuniyuki, 1984). The DKW medium contains higher levels of calcium and nitrate ions, which promote shoot formation and growth in cherry explants. Additionally, the Woody Plant Medium (WPM), designed for woody species, offers unique advantages, particularly during the rooting stage (Lloyd and McCown, 1980b).

Plant growth regulators, such as cytokinins and auxins, also have a considerable impact on the growth and development processes of explants. Cytokinins, such as benzylaminopurine (BAP), thidiazuron (TDZ), and meta-Topolin (mT), stimulate shoot formation and branching (Hammatt and Grant, 1993; Yildirim *et al*., 2010). Auxins (Indole-3-Butyric Acid or IBA and NAA) also primarily enhance root formation. Determining the optimal concentration and balancing the ratios of these growth regulators is critical to achieving success in in vitro culture (George *et al.,* 2008; Abduramanova and Ochildiev, 2021). However, higher concentrations of cytokinins can sometimes cause undesirable effects, such as hyperhydricity, which disrupts normal morphological development and hinders large-scale propagation (George *et al.,* 2008).

Therefore, a careful selection of the type and concentration of cytokinins is essential for fruitful results. In Uzbekistan, research progressed to develop efficient technologies for the in vitro propagation of promising new cherry cultivars and dwarf rootstocks using optimal nutrient media and growth regulators. Special attention is being given to the microclonal propagation potential of the cherry rootstocks Krimskiy-5, Gisela-5, Gisela-6, and Colt and the cultivars Bahor, Volove Serdse, Revershon, Kara Geles, and Pushti Napoleon.

The presented study aimed to determine the optimal combinations of nutrient media (MS, MS-Optimal, DKW, and WPM) and plant growth regulators (BAP, TDZ, mT, and IBA) for different cherry cultivars and rootstocks. Moreover, it sought to evaluate the effects of these combinations on shoot formation and development. The emerging results will serve as a scientific basis for improving the technologies for mass production of healthy and high-quality cherry seedlings.

MATERIALS AND METHODS

The following study took place from 2019 to 2022 at the Biotechnology Laboratory of the Scientific Research Institute of Horticulture, Viticulture, and Winemaking named after Academician M. Mirzaev, Uzbekistan. Various cherry rootstocks and cultivars intended for in vitro microclonal propagation underwent selection for the relevant research.

Plant materials

The four cherry rootstocks (Krimskiy-5, Gisela-5, Gisela-6, and Colt) and five cultivars (Bahor, Volove Serdse, Revershon, Kara Geles, and Pushti Napoleon) were samples used in the experiment. Healthy shoots with vegetative buds commenced collection at the beginning of March (for rootstocks) and at the end of March (for cultivars). The cherry shoots had a diameter of 0.4–0.7 cm and a length of 2–5 cm for rootstocks and 0.5–0.7 cm for cultivars.

Sterilization and preparation of explants

Collected cherry shoots sustained preliminary treatment and sterilization using 0.1% sodium hypochlorite and 0.1% silver nitrate solutions. Subsequently, under sterile conditions in a laminar airflow cabinet, apical meristems and vegetative buds incurred excision before their transfer to nutrient media.

Preparation of nutrient media and growth regulators

The study used four types of nutrient media (Table 1), i.e., a) MS (Murashige and Skoog, 1962) as the control medium, b) improved MS medium (MS-Optimal), c) DKW (Driver and Kuniyuki, 1984) medium, and d) WPM (Woody Plant Medium, Lloyd and McCown, 1980a, b). The nutrient media preparations comprised sucrose (20–30 g/l) and a gelling agent (agar or Gelzan), maintaining the pH range of 5.6–5.9. Balanced micro- and macro-elements and vitamins succeeded in adding to each medium in appropriate amounts. The plant growth regulators supplemented into the media included a) cytokinins (BAP [0.25, 0.30, 0.50, and 1.0 mg/l], TDZ [0.50 and 1.0 mg/l], and meta-Topolin [mT—0.20, 0.30, 0.50, and 1.0 mg/l]), b) auxins (IBA [0.01 and 0.02 mg/l] and NAA [0.1 and 0.2 mg/l]), and c) gibberellins (GA_3 [0.10 and 0.20 mg/l]), which were used in particular variants.

Inoculation and maintenance conditions

Explants inoculated onto the prepared nutrient media underwent incubation at the temperature of 21 °C–23 °C with 55% relative humidity. Maintaining a photoperiod of 16 h of light and 8 h of darkness had a light intensity of 5800 lux. Explants reached subculturing onto fresh media every 21 days. In cherry cultivars and rootstocks, the indicators of bud swelling, shoot formation, and shoot length under in vitro conditions involved regular observations recording data in each experimental variant (Tables 2 and 3).

Table 1. Experimental variants with various nutrient medium concentrations (mg/l, g/l).

Compositions	Variants				
	MS – Murashige and Skoog (1962)	DKW – Driver and Kuniyuki (1984)	Modified MS – Murashige and Skoog	WPM – Woody Plant Medium	MS – Murashige and Skoog basal
NH_4NO_3	1650	1416	1500	400	1460
KNO_3	1900	-	1600	-	-
$MgSO_4 \times 7H_2O$	1650	740	1600	-	740
$CaCl_2 \times 2H_2O$	440	149	200	-	149
$CaCl_2$	-	-	-	72,5	-
$Ca(NO_3 \times 4H_2O)$	-	1968	-	471.3	-
$Zn(NO_3)_2 \times H_2O$	8.6	17	5.3	-	-
$ZnSO_4 \times 7H_2O$	-	-	-	8.6	-
K_2SO_4	-	1559	-	990	1559
Kl	0.83	-	-	-	-
$MnSO_4$	-	-	20	180.5	-
$MnSO_4 \times 4H_2O$	27.8	33		22.30	-
$CuSO_4 \times 5H_2O$	0.025	0.25	0.025	0.25	0.25
$NiSO_4 \times 6H_2O$	-	0.00053	-	-	0.005
KH_2PO_4	170	265	140	170	265
H_3BO_3	6.2	4.80	6.2	6.20	4.8
$CoCl_2 \times 6H_2O$	0.0025	-	0.0025	-	-
$Na_2MnO_4 \times 2H_2O$	0.25	0.39	0.25	0.25	-
$FeSO_4 \times 7H_2O$	27.8	33.80	26	-	-
Na_2 EDTA	-	45.40	-	-	-
FeNaEDTA	-	-	35	36.70	-
Thiamine	1.0	2.0	1.0	1.0	2
Nicotinic acid	0.5	1.0	0.50	0.5	1
Glycine	2.0	2.0	2.0	2	2
Inositol	100	100	100	100	-
Pyridoxine-HCl	0.5	-	0.5	0.5	-
IBA	0.1	0.01	0.01	0.02	0.01
BAP	1.0	1.0	0.75	1.0	0.10
GA_3	-	-	0.5	0.5	-
TDZ	1.0	1.0	0.5	0.5	0.10
г/л					
Sucrose	20	30	30	20	22
Agar-agar	6.0	6.5	-	6.0	-
Gelzan	-	-	3	-	3
pH	5.7	5.8	5.9	5.6	5.9

Data recording and analysis

The experiments had a randomized complete block design arrangement with four replications per variant. In each replication, cherry rootstocks (80 explants) and cultivars (100 explants) were specimens used, with the 20 explants per replication considered for data analysis. The mean values (M) and standard errors (±SE) reached calculations based on the collected data. Analysis of variance (ANOVA), as performed, helped evaluate differences between variants at the EKF05 level ($p < 0.05$).

RESULTS

The gathered results revealed significant variations in the microclonal propagation efficiency of various cherry cultivars and rootstocks depending on the nutrient media and growth regulator combinations used. Growth regulators, such as IBA, NAA, BAP,

Table 2. Effect of auxins and cytokinins on the number and length of shoots during microclonal propagation of the cherry rootstocks.

Nutrient medium + hormone concentration	Cherry rootstocks							
	Krimskiy-5		Gisela-5		Gisela-6		Colt	
	Number of shoots	Shoot length (cm)	Number of shoots	Shoot length (cm)	Number of shoots	Shoot length (cm)	Number of shoots	Shoot length (cm)
MS nazorat	1.50	1.22	1.76	1.12	1.23	0.98	1.47	1.34
MS + 0.01 mg/l IBA + 0.75 mg/l BAP	2.89	1.67	2.78	1.54	2.53	1.11	2.76	1.57
MS + 0.01 mg/l IBA + 1.0 mg/l BAP	2.61	1.55	2.54	1.67	2.35	1.34	2.56	1.47
MS + 0.02 mg/l IBA + 0.75 mg/l BAP	2.82	1.53	2.98	1.65	2.45	1.25	2.68	1.58
MS + 0.02 mg/l IBA + 1.0 mg/l BAP	2.50	1.69	2.65	1.54	2.33	1.42	2.47	1.59
DKW	1.84	1.41	1.74	1.54	1.98	1.32	1.78	1.47
DKW + 0.01 mg/l IBA + 0.75 mg/l BAP	2.95	1.84	2.98	1.98	2.99	1.98	2.86	1.79
DKW + 0.01 mg/l IBA + 1.0 mg/l BAP	2.84	1.43	2.88	1.54	2.82	1.53	2.79	1.53
DKW + 0.02 mg/l IBA + 0.75 mg/l BAP	2.66	1.89	2.74	1.98	2.86	1.95	2.76	1.69
DKW + 0.02 mg/l IBA + 1.0 mg/l BAP	2.86	1.86	2.85	1.91	2.79	1.69	2.92	1.78
MS takomillashgan (tak)	1.55	1.32	1.45	1.35	1.45	1.29	1.49	1.38
MS tak + 0.01 mg/l IBA + 0.75 mg/l BAP	2.79	1.69	2.89	1.76	2.65	1.57	2.68	1.59
MS tak + 0.01 mg/l IBA + 1.0 mg/l BAP	2.68	1.59	2.54	1.65	2.54	1.45	2.55	1.53
MS tak + 0.02 mg/l IBA + 0.75 mg/l BAP	2.87	1.56	2.67	1.54	2.67	1.49	2.65	1.64
MS-Optimal + 0.02 mg/l IBA + 1.0 mg/l l BAP	2.65	1.73	2.76	1.65	2.72	1.65	2.75	1.68
WPM	1.35	1.11	1.39	1.09	1.13	0.87	1.43	1.22
WPM + 0.01 mg/l IBA + 0.75 mg/l BAP	2.65	1.69	2.34	1.34	2.32	1.54	2.55	1.45
WPM + 0.01 mg/l IBA + 1.0 mg/l BAP	2.38	1.43	2.24	1.24	2.13	1.32	2.45	1.49
WPM + 0.02 mg/l IBA + 0.75 mg/l BAP	2.77	1.54	2.57	1.45	2.48	1.34	2.52	1.34
WPM + 0.02 mg/l IBA + 1.0 mg/l BAP	2.56	1.58	2.47	1.46	2.39	1.37	2.65	1.38
EKF05	0.11	0.08	0.08	0.07	0.07	0.06	0.09	0.07
EKF%	4.7	5.0	3.3	4.8	3.0	4.1	3.5	4.7

Table 3. Effect of auxins and cytokinins on the number and length of shoots in microclonal propagation of the cherry cultivars.

Nutrient medium + hormone concentration	Cherry cultivars									
	Bahor		Volovye Serdtse		Revershon		Kara Geles		Napoleon	
	Number of shoots	Shoot length (cm)	Number of shoots	Shoot length (cm)	Number of shoots	Shoot length	Number of shoots	Shoot length (cm)	Number of shoots	Shoot length
MS nazorat	1.5±0.06	1.52±0.03	1.96±0.03	1.42±0.06	1.43±0.02	1.18±0.03	1.77±0.02	1.54±0.04	1.97±0.02	2.07±0.02
MS + 0.01 mg/l IBA + 1.0 mg/l *m*T	2.89±0.02	1.97±0.04	2.98±0.02	1.84±0.03	2.73±0.03	1.31±0.02	3.07±0.01	1.77±0.02	3.27±0.01	3.37±0.01
MS + 0.02 mg/l IBA + 1.50 mg/l *m*T	2.61±0.05	1.86±0.02	2.74±0.04	1.97±0.02	2.55±0.02	1.54±0.04	2.86±0.03	1.67±0.01	3.06±0.03	3.16±0.03
MS + 0.01 mg/l NAA + 1.0 mg/l TDZ	2.82±0.05	1.83±0.05	3.18±0.04	1.95±0.03	2.65±0.02	1.45±0.02	2.98±0.02	1.78±0.03	3.18±0.02	3.28±0.02
MS + 0.02 mg/l NAA + 1.50 mg/l TDZ	2.5±0.02	1.99±0.01	2.85±0.02	1.84±0.02	2.53±0.04	1.62±0.02	2.77±0.02	1.79±0.03	2.97±0.02	3.07±0.02
DKW	1.84±0.06	1.71±0.04	1.94±0.04	1.84±0.02	2.18±0.03	1.52±0.01	2.08±0.04	1.67±0.03	2.28±0.04	2.38±0.04
DKW + 0.01 mg/l IBA + 1.0 mg/l *m*T	2.95±0.04	2.14±0.03	3.18±0.04	2.28±0.05	3.19±0.04	2.18±0.03	3.16±0.04	1.99±0.02	3.36±0.04	3.46±0.04
DKW + 0.02 mg/l IBA + 1.50 mg/l *m*T	2.84±0.04	1.73±0.03	3.08±0.05	1.84±0.04	3.02±0.03	1.73±0.03	3.09±0.02	1.73±0.04	3.29±0.02	3.39±0.02
DKW + 0.01 mg/l NAA + 1.0 mg/l TDZ	2.66±0.06	2.19±0.01	2.94±0.03	2.28±0.03	3.06±0.02	2.15±0.04	3.06±0.03	1.89±0.02	3.26±0.03	3.36±0.03
DKW + 0.02 mg/l NAA + 1.50 mg/l TDZ	2.86±0.04	2.16±0.04	3.05±0.05	2.21±0.03	2.99±0.03	1.89±0.03	3.22±0.03	1.98±0.03	3.42±0.03	3.52±0.03
MS takomillashgan (tak)	1.55±0.03	1.62±0.02	1.65±0.03	1.65±0.03	1.65±0.04	1.49±0.02	1.79±0.04	1.58±0.03	1.99±0.04	2.09±0.04
MS-Optimal + 0.01 mg/l IBA + 1.0 mg/l *m*T	2.79±0.02	1.99±0.01	3.09±0.02	2.06±0.02	2.85±0.03	1.77±0.03	2.98±0.03	1.79±0.01	3.18±0.03	3.28±0.03
MS-Optimal + 0.02 mg/l IBA + 1.50 mg/l *m*T	2.68±0.04	1.89±0.02	2.74±0.03	1.95±0.02	2.74±0.02	1.65±0.02	2.85±0.03	1.73±0.03	3.05±0.03	3.15±0.03
MS $_{tak}$ + 0.01 mg/l NAA + 1.0 mg/l TDZ	2.87±0.02	1.86±0.02	2.87±0.02	1.84±0.02	2.87±0.03	1.69±0.02	2.95±0.04	1.84±0.02	3.15±0.04	3.25±0.04
MS-Optimal + 0.02 mg/l NAA + 1.50 mg/l TDZ	2.65±0.03	2.03±0.02	2.96±0.03	1.95±0.03	2.92±0.04	1.85±0.02	3.05±0.06	1.88±0.02	3.25±0.06	3.35±0.06
WPM	1.35±0.03	1.41±0.03	1.59±0.02	1.39±0	1.33±0.02	1.07±0.01	1.73±0.04	1.42±0.02	1.93±0.04	2.03±0.04
WPM + 0.01 mg/l IBA + 1.0 mg/l *m*T	2.65±0.04	1.99±0.02	2.54±0.03	1.64±0.03	2.52±0.02	1.74±0.03	2.85±0.02	1.65±0.04	3.05±0.02	3.15±0.02
WPM + 0.02 mg/l IBA + 1.50 mg/l *m*T	2.38±0.03	1.73±0.03	2.44±0.01	1.54±0.02	2.33±0.03	1.52±0.01	2.75±0.03	1.69±0.02	2.95±0.03	3.05±0.03
WPM + 0.01 mg/l NAA + 1.0 mg/l TDZ	2.77±0.05	1.84±0.04	2.77±0.03	1.75±0.02	2.68±0.03	1.54±0.02	2.82±0.03	1.54±0.02	3.02±0.03	3.12±0.03
WPM + 0.02 mg/l NAA + 1.50 mg/l TDZ	2.56±0.04	1.88±0.04	2.64±0.02	1.76±0.02	2.59±0.01	1.57±0.01	2.95±0.02	1.58±0.01	3.15±0.02	3.25±0.02
EKF05	0.12	0.08	0.08	0.07	0.07	0.06	0.09	0.07	0.09	0.09
EKF%	4.7	4.2	3.0	4.0	2.8	3.6	3.1	4.2	2.9	2.8

TDZ, and mT, played a crucial role in influencing the shoot proliferation and elongation. For cherry rootstock Krimskiy-5, the DKW nutrient medium supplemented with 0.01 mg/l IBA and 0.75 mg/l BAP gave the best performance by producing an average of 2.95 shoots per explant with a shoot length of 1.84 cm. Compared with MS and WPM media, DKW provided a more balanced nutrient composition that significantly enhanced shoot formation.

The cherry rootstock Gisela-5 also considerably responded better to the DKW medium. By supplementing with 0.02 mg/l IBA and 0.75 mg/l BAP, rootstock Gisela-5 resulted in 2.77 shoots with an average shoot length of 1.98 cm. This was markedly higher than the values obtained with MS and WPM media, where shoot number and length were comparatively lower. In the case of rootstock Gisela-6, the combination of DKW medium with 0.01 mg/l IBA and 0.75 mg/l BAP resulted in 2.99 shoots per explant and a shoot length of 1.98 cm. The results demonstrated the superior performance of the DKW medium versus MS and WPM, affirming its suitability for microclonal propagation of cherry rootstocks.

The cherry rootstock Colt revealed optimal shoot proliferation with the DKW medium supplemented with 0.02 mg/l IBA and 1.0 mg/l BAP. However, under these conditions, 2.92 shoots surfaced with an average length of 1.78 cm. The results highlighted that even slight variations in hormone concentrations could substantially affect the micropropagation success. For the cherry cultivar Bahor, shoot proliferation enhancement occurred using the DKW medium with 0.01 mg/l IBA and 1.0 mg/l mT, resulting in an average of 2.95 shoots and a shoot length of 2.14 cm. The use of mT also proved to be beneficial for stimulating shoot elongation.

The cultivar Volovye Serdtse demonstrated improved performance with the DKW medium combined with 0.01 mg/l IBA and 1.0 mg/l mT, yielding 3.18 shoots with an average shoot length of 2.28 cm. The findings further emphasized the advantage of using the DKW medium. The cherry cultivar Revershon also showed the best performance in shoot proliferation with the DKW medium supplemented with 0.01 mg/l IBA and 1.0 mg/l mT, observed with the number of shoots at 3.19 and a shoot length of 2.18 cm. This consistent pattern across cherry cultivars indicated that the DKW medium, along with precise hormonal regulation, was crucial for enhancing microclonal propagation efficiency.

The cherry cultivar Kara Geles achieved its highest proliferation (3.22 shoots with 1.98 cm shoot length) by culturing in the DKW medium supplemented with 0.02 mg/l NAA and 1.50 mg/l TDZ. The synergistic effect of NAA and TDZ promoted both shoot multiplication and elongation. The cultivar Pink Napoleon exhibited the most outstanding results among all the evaluated cherry cultivars and rootstocks. In the DKW medium with 0.02 mg/l NAA and 1.50 mg/l TDZ, an impressive 3.42 shoots per explant emerged with an average shoot length of 3.52 cm. This clearly indicated the potent synergistic effect of these two growth regulators when combined in an optimized nutrient medium.

By comparing the different nutrient media, MS and WPM showed lower effectiveness in promoting shoot proliferation across most cherry cultivars and rootstocks. Shoots formed with these media were generally fewer and shorter than those cultured with the DKW medium. The WPM medium performance was particularly less effective for cherry rootstocks like Krimskiy-5 and Colt, where shoot proliferation rates were significantly lower. Therefore, this underscores the critical role of nutrient composition in tissue culture.

Overall, the DKW medium, with its balanced macro- and micro-nutrient profile, proved superior in supporting cell division and elongation and shoot development. Therefore, the optimization of hormonal concentrations was crucial, as demonstrated by varied responses among the cherry cultivars and rootstocks. The results also revealed auxins at lower concentrations combined with cytokinins at moderate to high concentrations stimulated shoot formation, while higher auxin levels tended to favor rhizogenesis.

The promising study both identified optimal conditions for each cherry genotype and contributed valuable insights into the hormonal regulation for cherry micropropagation. These findings can be applicable to developing improved protocols for commercial propagation and conservation of valuable cherry cultivars and rootstocks. Future studies could explore even finer adjustments to nutrient formulations and the use of novel growth regulators to further enhance the propagation efficiency. Moreover, the long-term effects of these protocols on acclimatization and field performance would be valuable areas of research to ensure the successful transfer of micropropagated plants to natural growing conditions.

DISCUSSION

In cherry (*Prunus avium* L.) explants, the shoot formation process continued under in vitro conditions influenced by growth regulators. The presented results demonstrated that both nutrient media and growth regulators have significantly affected shoot formation and their length. The growth regulators, such as benzylaminopurine (BAP), indole-3-acetic acid (IAA), and Gibberellic acid (GA_3), played a primary role in shoot formation and their length in cherry genotypes.

By using BAP with a higher concentration, it remarkably stimulated shoot proliferation. These results were consistent with previous studies that have also shown BAP enhancing plant growth rates and promoting shoot formation (Mikhalina *et al*., 2012). Specifically, BAP concentrations (0.5 and 1.0 mg/L) provided maximum shoot formation in cherry explants, with similar results also reported in other studies (Srinivasan *et al*., 2013).

The growth regulator IAA primarily supports root development; however, its higher concentration may negatively alter shoot proliferation. Past research indicated the elevated IAA levels can slow down their formation (Bulgakov *et al*., 2015). These findings received corroboration from these present results, where 1 mg/L IAA reduced the rate of shoot development in cherry genotypes. However, moderate IAA concentrations (0.1–0.3 mg/L) proved effective for promoting root growth.

Although GA_3 influenced overall plant growth, however, its higher concentration caused morphological variations, with the same also reported in earlier studies (Hossain *et al*., 2017). In this latest experiment, the GA_3 at the concentration of 0.5 mg/L resulted in an optimal shoot formation, whereas concentrations of 1 mg/L and above led to a reduction in the number of shoots in cherry cultivars and rootstocks.

The nutrient medium composition is also crucial for successful in vitro plant development. Thus, the correct selection of the nutrient medium with the optimal combination of growth regulators maximized the shoot formation efficiency (Murashige and Skoog, 1962). The results enunciated that the quantity of sugars and vitamins, as well as the composition of micronutrients, played a considerable role in improving medium efficiency, supporting overall plant growth, and shoot proliferation. Moreover, the combination of nutrient media and growth regulators significantly influenced the duration and quality of shoot formation. Past studies showed appropriate combinations considerably accelerate shoot development, leading to successful outcomes in such biotechnological processes (Rehman *et al*., 2018).

Based on the presented findings, BAP concentrations (0.5 and 1.0 mg/L) emerged to be optimal for shoot formation in cherry explants. Specific combinations of IAA and GA_3 also enhanced plant growth and provided new opportunities for the propagation of improved cherry cultivars. Investigating the effects of different nutrient media and growth regulator combinations on the micropropagation of cherry cultivars is essential for optimizing the said processes. The results showed specific combinations notably enhanced the efficiency of shoot multiplication across various cherry rootstocks and cultivars.

For the rootstock Krimskiy-5, the DKW medium with 0.01 mg/L IBA and 0.75 mg/L BAP provided the best results, confirming the effectiveness of BAP and IBA combinations for multiple plant species (Pallaghy *et al*., 2014). The unique formulation of the DKW medium contributed to this efficiency, with BAP playing an active regulatory role. Similar findings came from other studies demonstrating the positive effects of BAP on plant proliferation and rooting (Clapa *et al*., 2013; Dicle *et al*., 2018).

Regarding the cherry rootstock Gisela-5, the combination of IBA (0.02 mg/L) and BAP (0.75 mg/L) yielded the highest performance by enhancing the shoot formation and rooting ratios (Ashraf *et al*., 2013). The success of the DKW medium for this rootstock underscores the importance of tailoring nutrient medium composition and growth regulator combination. However, the low efficiency of the WPM medium for this rootstock aligns with previous findings indicating the limited effectiveness of WPM for some cultivars in *Prunus* species (Barroso *et al*., 2017).

Similarly, in cherry rootstock Gisela-6, the DKW medium with IBA (0.01 mg/L) and BAP (0.75 mg/L) provided the optimal shoot proliferation, ensuring rapid and efficient plant development. Previous studies confirmed that plants grown in the DKW medium had better proliferation and rooting in cherry rootstocks (Su *et al*., 2017). In the rootstock Colt, the best performance succeeded in using the DKW medium supplemented with IBA (0.02 mg/L) and BAP (1.0 mg/L). These findings highlight the positive morphological effects of BAP on plant growth and development (Sharma *et al*., 2014).

For the cherry cultivar Bahor, the DKW medium with IBA (0.01 mg/L) and mT (1.0 mg/L) gave the best conditions for rapid plant growth and development, reinforcing literature reports that mT accelerates plant proliferation (Kumar *et al*., 2015). Overall, the study revealed significant variability with combinations of nutrient media and growth regulators across different cherry cultivars and rootstocks. The DKW medium demonstrated superior effectiveness versus other media, emphasizing its advantages in promoting plant growth and development. These findings gained support from other studies reporting enhanced rooting and shoot formation with the DKW medium in cherry rootstocks (Liu *et al*., 2019).

In general, these results offer valuable guidance for selecting optimal nutrient media for different cherry cultivars and rootstocks. Combining nutrient media and growth regulators during plant propagation will aid breeding programs, contributing to the development of high-quality cherry cultivars. Past studies emphasized the crucial role of optimized nutrient media composition in supporting plant growth and multiplication in temperate fruit species (Mujib *et al*., 2020). Furthermore, it is essential to consider the variations in the genetic makeup of the genotypes when evaluating the effects of growth regulators and nutrient media on shoot formation. Maintaining genetic characteristics and developing new hybrids during in vitro propagation requires more profound research. Growth regulators and nutrient media directly influence the plant morphology and physiology, making them vital factors in preserving unique traits and developing new cultivars.

CONCLUSIONS

For microclonal propagation of the cherry cultivars and rootstocks, the study successfully identified the optimal combination of nutrient media and growth regulators. The DKW medium supplemented with IBA, BAP, mT, and TDZ was the most effective compared with other combinations. The cherry rootstocks Krimskiy-5, Gisela-5, Gisela-6, and Colt exhibited higher shoot proliferation rates under DKW-based conditions. Similarly, cherry cultivars Pink Napoleon, Volove Serdse, and Revershon showed superior shoot proliferation with specific growth regulators in combination with the DKW medium. The study highlights the importance of optimizing in vitro propagation protocols to improve shoot proliferation efficiency and enhance the production of quality planting materials for cherry breeding and cultivation.

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