

Conference Paper

Optimization Studies of Culture Media for In-Vitro Clonal Micropropagation of New Grape Varieties

Abdulmalik Batukaev^{1,2}, Eliza Sobralieva², and Diana Palaeva²¹Chechen State University,²Chechen Research Institute of Agriculture, Grozny, Russia**ORCID:**Abdulmalik Batukaev: <http://orcid.org/0000-0002-0868-6356>**Abstract**

This article describes the effect of Gautheret, White, Heller and Murashige & Skoog mineral salts during in-vitro clonal micropropagation of new grape varieties. The optimal mineral compositions of the culture medium that support the in-vitro regeneration of isolated grape explants were identified. The grapes that were studied were the Bart and Augustine varieties. Primary grape explants were cultivated for 30 days in a non-transplanted culture. Increased regenerative activity was observed in the Murashige & Skoog and White media. Increased haemogenesis occurred and shoots regenerated. The addition of cytokinin 6-BAP to the medium for obtaining aseptic culture led to an increase in the frequency of shoot-bud production by 5 to 6 times, depending on the type of medium. Combining 6-BAP with the auxin NAA provided an additional increase in the frequency of shoot-bud production, but to a lesser extent. Adding growth regulators to the culture medium also reduced the frequency of explant necrosis.

Keywords: grapes, mineral salts, culture medium, microclonal propagation, in-vitro, cytokinins, auxins.

Corresponding Author:

Abdulmalik Batukaev
batukaevmalik@mail.ru

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1. Introduction

At the present stage of development of the nursery, the production of improved planting material is inextricably linked with the use of biotechnological techniques, in which the real reproduction rates are hundreds or even thousands of times higher than with any of the traditional techniques [1–3]. The method of apical meristems followed by microclonal propagation of healthy clones has been widely used throughout the world. It allows getting a healthy and genetically homogeneous planting material in a short time and large quantities [1, 4–7]. However, applying this method on an industrial scale induces some difficulties. It is generally accepted that for each plant object, and sometimes for

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a variety introduced into a culture in-vitro, the individual components of the artificial culture medium are required, taking into account its genotypic features [8–11].

The aim of the studies is the development of technology for microclonal propagation of new varieties and rootstocks of fruit and berry crops based on an optimized culture medium.

The increasing of efficiency is achieved by solving the following main task: the development of improved technology for microclonal propagation of grapes. One of the main problems encountered in the industrial use of this technique is the inability to obtain regenerants from all types of explants when using a universal culture medium, since various types of explants demonstrate different efficiency in obtaining regenerants, or do not pass to organogenesis at all. Multiplication coefficients on universal media are higher than with applying traditional breeding methods but much lower than with optimized media, which ultimately affects the efficiency of nursery management. Thus, the main direction of improving the technology for microclonal propagation is to optimize the culture media for growing seedlings of various fruit crops and grapes, which takes into account the genotypic features of propagated varieties.

2. Methods and Materials

The studies were conducted based on the Joint Laboratory of Biotechnology of Agricultural Plants of the Chechen Research Institute of Agriculture (CRIA) and the Chechen State University (CSU). The main part of the material and technical base involved in the studies is a complex of specially equipped “clean” rooms. Laboratory facilities are equipped with the equipment necessary for performing microclonal propagation operations:

- preparation and sterilization of materials,
- preparation and sterilization of culture media,
- washing and heat treatment of dishes and tools.

The laboratory complex used rooms equipped for carrying out the operations on transplanting sterile cultures and placing vessels with sterile cultures. At the multiplication stage, some of the vessels with sterile cultures were placed in sterile boxes. During performing the operations, a set of equipment was used for preparing culture media, culture dishes, cultivating plant objects, etc. Separation of the meristem was performed under the microscope MBS-10, the planting was carried out in special tubes of 12x4 cm in size with a culture medium of 25 g. As each stage of development progressed, the explants of grapes were transferred to a fresh medium with new growth substances.

The research was carried out according to the methods generally accepted in viticulture biotechnology [8], the statistical processing was performed by Microsoft Excel.

The culture medium is the most important factor for obtaining the planned effect of explant development. It contains a rich composition of mineral salts (macro- and microelements), well-balanced carbohydrates, vitamins, growth regulators, and amino acids in an acidic medium. For convenience, accuracy and reduction of time for preparation of culture media, the stock solutions were prepared, which allows achieving stability over the course of several passages. Four culture media with the basic composition of Gautheret, White, Heller, and Murashige & Skoog components were studied. The stock solutions of macrosalts, microsals, phytohormones and vitamins were prepared in the laboratory. The components of the stock solutions were assorted by the selected basic formula. The studied grape varieties were Bart and Augustine.

3. Results

The most effective and frequently used culture media for fruit and berry crops are Gautheret, White, Heller, and Murashige & Skoog's [1, 12–14]. These media, differing in their mineral composition, have the same set of organic additives and physiologically active substances. Table 1 shows the composition of the listed media. In this regard, the purpose of our research was to determine the optimal mineral composition of the culture medium, which has an inducing effect on the regeneration in-vitro of isolated explants of grapes.

The first stage of the study is selection. From the four indicated culture media with the basic composition of components (Gautheret, White, Heller, Murashige & Skoog), two most effective ones are selected for their influence on the main criteria for the development of microclonal plants. Further, the assorted media will be consistently modified and evaluated again according to the main criteria adopted in plant biotechnology. The effect of mineral salts was studied according to Gautheret, White, Heller, and Murashige & Skoog (Table 1).

The selection of a single culture medium, which was then subject to the optimization, was guided by the studies of the chosen culture media, using the formulated criteria for the development of the explant. As an integral indicator of efficiency at the stage of selecting the base medium, the regenerative potential of primary explants of grapes was studied when cultivated on various culture media. The culture media included such growth regulators as BAP (0.5 mg/l) and KIN (0.5 mg/l), as well as sucrose and agar

in concentrations of 3% and 0.8%, respectively. For preliminary tests, apical shoots of grapes were used as the primary explant.

TABLE 1: The compositions of culture media

Components	Concentration in the medium for cultivating. mg/l			
	Medium Gautheret's	Medium White's	Medium Heller's	Medium Murashige & Skoog's
Ca (NO ₃) ₂	-	142	-	-
KNO ₃	1.900	81	-	38.000
NaNO ₃	-	-	600	-
NH ₄ HO ₃	1.650	-	-	33.000
NH ₄ H ₂ PO ₄	-	-	-	-
(NH ₄) ₂ SO ₄	-	-	-	-
MgSO ₄ ·7H ₂ O	370	74	250	7.400
CaCl ₂ ·2H ₂ O	440	-	75	8.800
KCl	-	65	750	-
KH ₂ PO ₄	170	12	-	3.400
NaH ₂ PO ₄ ·H ₂ O	-	-	125	-
MnSO ₄ ·7H ₂ O	-	-	-	4.460
MnSO ₄ ·4H ₂ O	22.3	-	0.1	-
KI	0.83	-	0.01	166
H ₃ BO ₃	6.2	-	1	1.240
ZnSO ₄ ·7H ₂ O	8.6	-	1	1.720
CuSO ₄	-	-	-	-
CuSO ₄ ·5H ₂ O	0.025	-	0.03	5
Na ₂ Mo O ₄ ·2H ₂ O	0.25	-	-	50
CoCl ₂ ·6H ₂ O	0.025	-	-	5
AlCl ₃	-	-	0.03	-
NiCl ₂ ·6H ₂ O	-	-	0.03	-
FeCl ₂ ·6H ₂ O	-	-	1	-
FeSO ₄ ·7H ₂ O	27.86	-	-	5.560
Fe ₂ (SO ₄) ₃	-	2.46	-	-
Sequestrene 330 Fe	-	-	-	-
Na ₂ EDTA	37.26	-	-	7.460
Mesoinosite	100	-	-	20.000
Thiamine-HCl	0.4	-	-	100
Nicotinic acid	-	-	-	100
Pyridoxine-HCl	-	-	-	100
Yeast extract	-	100	-	-
Sucrose	30.000	20.000	-	30.000
pH	5.8	-	-	-

The cultivation results of primary grapes explants for 30 days in a non-transplanted culture showed that the increased regenerative activity on their basis was observed in the media of Murashige & Skoog and White. The process of explants cultivation was accompanied by the increased haemogenesis and regeneration of shoots.

The results of the experiment presented in Table 2 showed that the effectiveness of obtaining an aseptic culture was largely determined by the type of explant and to a lesser extent by the mineral and hormonal (in the case of the apical part of the shoots) composition of the culture media. When using lateral buds as explants, the frequency of their inducing was slightly higher on the Murashige & Skoog medium, in comparison with other culture media (White, Gautheret, Hiller).

When using the upper part of young shoots as explants, a similar pattern was observed. The addition of growth regulators to the culture medium was a critical factor in the case of lateral buds. Thus, the addition of cytokinin 6-BAP to the medium for obtaining aseptic culture contributed to an increase in the frequency of shoot-bud inducing by 5 to 6 times, depending on the type of medium. Combining 6-BAP with the auxin NAA provided an additional increase in the frequency of shoot-bud inducing, but not as marked as in the previous case. Adding growth regulators to the culture medium also reduced the frequency of explant necrosis. All these observations are mainly related to the fact that the apical meristems, from which future shoots develop, are in the resting buds in an inactive state and the absence of hormonal pressure, these meristems died after a strong stress effect (sterilization).

Following the data presented above, it can be concluded that it is preferable to use lateral buds for obtaining aseptic grape culture, which, when placed on a culture medium with a combination of 6-BAP and IAA, provide a high frequency of sterile inducing buds.

The potential of grape propagation was also studied as an integral indicator of efficiency at the stage of selecting the base medium. The average reproduction coefficient from one test tube was analyzed for 3 passages. The results of preliminary tests of standard culture media indicate their different potential when used in the biotechnological system of microclonal plant propagation.

For further modification and testing, two basic culture media were assorted - White and Murashige & Skoog (MS), since the average multiplication coefficient chosen as an integral indicator of suitability assessment demonstrated greater efficiency on them. Thus, the coefficient of propagation of grapes in the media of White and Murashige & Skoog was 12.5-83.3% higher than in the Gautheret and Heller's media.

TABLE 2: The influence of the mineral composition of culture media on the effectiveness of obtaining aseptic grape culture (3 weeks after sterilization), n=30

Culture media options	Grapes (varieties Bart. Augustine)			
	Lateral buds		Top of the shoot	
	DP*. %	DN**. %	DP. %	DN. %
Murashige & Skoog's				
Without hormones	19.7 ±4.6	59.9 ±19.3	24.6 ±3.9	66.2 ±16.0
6-BAP (1 mg/l)	32.3 ±11.5	30.7 ±18.1	19.0 ±6.2	51.2±12.6
6-BAP (1 mg/l) + IAA (0.25 mg/l)	42.6 ±11.1	20.1 ±14.1	16.9 ±5.9	65.6 ±14.2
Gautheret's				
Without hormones	15.9 ±2.2	60.5 ±11.7	14.3 ±4.2	69.3 ±20.1
6-BAP (1 mg/l)	30.4 ±17.3	42.8 ±12.0	15.0 ±4.9	57.6 ±18.6
6-BAP (1 mg/l) + IAA (0.25 mg/l)	39.5 ±19.4	32.2 ±10.8	25.1 ±3.0	81.2 ±16.4
White's				
Without hormones	12.9 ±1.2	65.5 ±11.7	11.3 ±4.7	62.3 ±20.1
6-BAP (1 mg/l)	27.4 ±11.3	38.8 ±10.1	13.0 ±4.4	66.6 ±17.6
6-BAP (1 mg/l) + IAA (0.25 mg/l)	27.5 ±16.4	26.2 ±9.8	22.1 ±3.1	71.6 ±14.4
Hiller's				
Without hormones	4.9 ±2.2	60.5 ±11.7	14.3 ±4.2	69.3 ±20.1
6-BAP (1 mg/l)	31.4 ±17.3	32.8 ±12.0	15.0 ±4.9	57.6 ±18.6
6-BAP (1 mg/l) + IAA (0.25 mg/l)	39.5 ±19.4	32.2 ±10.8	25.1 ±3.0	81.2 ±16.4

* - Percentage of explants with the marked growth, in terms of the number of uninfected explants

** - Percentage of necrotic explants, in terms of the number of uninfected explants

Taking into account the relatively equal values in the difference in the multiplication coefficient between the compared culture media, the further evaluation of grape varieties during modification is carried out within one general group – grape varieties.

4. Conclusion

The conducted studies are mainly related to the fact that the apical meristems, from which future shoots develop, are inactive in the resting buds and the absence of hormonal pressure, these meristems died after severe stress (sterilization).

Under the data presented above, it can be concluded that it is preferable to use lateral buds for obtaining aseptic grape culture, which, when placed on a culture medium with a combination of 6-BAP and IAA, provide a high frequency of sterile inducing buds. The basic culture media - White and Murashige & Skoog's (MS) were optimum since the average multiplication coefficient chosen as an integral indicator of suitability

assessment showed greater efficiency on them. Thus, the coefficient of propagation of grapes in the media of White and Murashige & Skoog was 12.5-83.3% higher than in the Gautheret and Heller's media.

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