Influence of mineral and hormonal composition of growing medium on fruit crops and grapes explants development in vitro

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Abstract. The influence of the mineral and hormonal composition of the growing medium on the development of explants of fruit crops and grapes in vitro has been studied. As a result of the studies, it has been found that phytohormones have a positive effect on the regeneration of explants in vitro. When using standard compositions of nutrient media not adding auxins, cytokinins and gibberellins, shoot regeneration is reduced, and growth and development are inhibited. The data on the studied media for explants cultivation obtained during the research showed that agarized growing media MS are optimal for microclonal propagation in vitro. Especially this refers to their modifications with a content of 1.0 mg/l 6-BAP at the first planting; a combination of 1.0 mg/l 6-BAP with 1.0 mg/l GK3 during transplantation; 0.5 mg/L IAA at the second transplant. The study of the effect of growth regulators BAP and kinetin combinations on the reproduction factor and the average length of various explants shoots has showed that at the micropropagation stage it is advisable to use BAP and kinetin together at a concentration of 0.5 mg/l each, which ensures the maximum reproduction factor.

Key words. Grapes, fruit crops, growing medium, growth regulators, in vitro.

1 Introduction

It is known that each new variety requires an individual study of all aspects of the in vitro method, specifically, selection of optimal compositions of growing media and growth substances, safe and effective antibiotics and sterilizing substances, changing technological methods [1–3]. Currently micropropagation is the most promising, fastest and safest method for obtaining a healthy planting material [4–6]. Since the determinant in the reproduction of any plant in vitro after complying with asepsis is a growing medium, its selection and optimization for the certain culture becomes an important issue. Depending on the type and variety of agricultural plants, the growing medium optimization is required in many cases. Therefore, special approaches including those based on the methods of mathematical experiment planning have been developed [8–11]. Varying according to a certain scheme, the concentration of various components of the growing medium, primarily growth regulators and phytohormones [12] was studied in a biolaboratory.

The main objective of the study was to improve the technology of clonal micropropagation of stone and pomaceous fruit explants, strawberry and grape in vitro by selecting and optimizing growing media for cultivation. The task was to study the effect of growth regulators (auxins, cytokinins and gibberellic acid) in various concentrations and combinations on the development of crops, as well as to determine the optimal composition of growing media for growing crops in vitro, and to obtain a healthy certified planting material as a result.

2 Research methodology

After being cut into explants, apex shoots of all cultures were washed with water and disinfected with a sterilizing agent being 2% sodium hypochlorite. Standard compositions of White's and Murashige-Skoog's growing media were taken as control without adding growth substances (agar used as a medium hardener was boiled for the growing medium for about 2 hours). In the modification of White and Murashige-Skoog growing media, the following growth regulators were introduced: 6-BAP (with a concentration of 1 mg/l; 1.5 mg/l) during the first planting of meristem apexes, 6-BAP (with a concentration of 1 mg/l) in combination with GK_3 (with a concentration of 0.5 mg/l; 1 mg/l) at the stage of actual micropropagation of clones and indole acetic acid (with a concentration of 0.2 mg/l; 0.5 mg/l) at the stage of test-tube plants rooting.

The meristem was isolated using an MBS-10 microscope; planting was carried out in special test tubes 12x4 cm in size with a growing medium of 25 g each. At

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each stage, the explants were transferred to a fresh medium with new growth substances. The experiment studying the effect of the mineral composition was conducted for different crops (5 options in total). Each option had 3 replications. Each replication contained 6 tubes with microscopes. We studied the effect of modified growing media on the growth and development of the explant in vitro. The research was carried out according to generally accepted methods of biotechnology. Statistical processing was carried out by the Microsoft Excel.

3 Research results

As is known from modern scientific literature, growth hormone 6-BAP of cytokinin action shows positive results when used in growing media for the first planting of fruit and berry crops explants. The results of observation of the development of explants of the studied varieties on made on the 15th day when 6-BAP was introduced into the medium at various concentrations are presented in Table 3.

Table 1. Growth and development of explants depending on the composition of the growing medium (on the 15th day, in mm)

Culture	MS (control)	MS (concentration 6-BAP)		White (control)	White (concentration 6-BAP)	
		1	1,5		1	1,5
	Average growth of meristems, mm					
Grape	6	8	6.6	5	8.3	7
Stone and pomaceous fruit	6.5	8.5	7	6	8.5	7.9

As can be seen from the data shown in Table 1, the growth processes in different crops were not the same. Nevertheless, as expected, the increase in plant mass on modifications with the addition of 6-BAP was significantly higher than on standard media. It is not advisable to keep shoots in media with increased cytokinin concentration for a long time as this can provoke inhibition of microplants growth processes. In addition, it is known that during long-term cultivation the growing medium is depleted in nutrients and becomes unsuitable for further use. Therefore, when the medium was changed again at the next stage of microclonal reproduction, GK_3 at concentrations of 1.5 and 1 mg/l was added to its composition in addition to 6-BAP at a concentration of 1 mg/l. The results are presented in Table 2, which shows the development of test-tube plants depending on the effect of GK_3 in combination with 6-BAP. The combination of 6-BAP with gibberellin strongly stimulates stem growth by lengthening internodes and increasing their number. It is stipulated by the fact that growth regulators of cytokinin action promote cell division and differentiation, while gibberellin affects cell elongation and division.

During the next transplantation of microplants onto the growing medium, auxin indole acetic acid was included in its composition in two variants of concentration 0.2 and 0.5 mg/l. Thus, the rooting of test-tube plants was planned; the results of using indole acetic acid in the composition of both media (MS and White) are presented in Table 3.

Table 2. Development of test-tube plants depending on the effect of GK3 in combination with 6-BAP

Culture	Culture indicators	Medium MS (modification)		Medium White (modification)	
		Concentration 6-BAP+GK_3, mg/l			
		0.5 /1	1/1	0.5/1	1/1
Grape	Internodes, pcs	4.4	5	3.9	4.5
	Leaves, pcs.	8.5	10	8	10
	Stem length, cm	8.5	9.1	8.1	8.9
Stone fruit	Internodes, pcs	4.6	5.1	4.1	4.9
	Leaves, pcs.	9.4	10.3	8	11
	Stem length, cm	8.5	9.5	9	9.8
	Leaves, pcs.	11	13	10	11
	Stem length, cm	9.1	10.5	9.8	10

Table 3. Impact of indole acetic acid on the development of accessions' root system

Culture	Medium MS (modification)		Medium White (modification)	
	Concentration, mg/l			
	0,2	0,5	0,2	0,5
Grape	2,4	2,9	2,5	2,9
Stone and pomaceous fruit	3	3,7	2,8	3,2

Studies have shown that the selected concentrations are quite appropriate for the development of the root system in test tube plants. However, the varieties grown on White's medium had weak root growth and thus were retarded in comparison with their competitors.

At the stage of in vitro micropropagation of fruit and berry crops, the influence of the composition of mineral salts on rooting and shoot growth was studied. Activated carbon was excluded from the composition of the Murashige – Skoog growing medium, salts and acids were reduced by 2–4 times, and Humate+7B was added in an amount of 5–10 ml/l. Humate+7B is highly soluble in water, easily absorbed by plants, mobilizes plants' immune system, stimulates the development of a powerful root system, promotes increased nutrient intake, intensifies metabolic processes in the plant cell, reduces the content of nitrates by 2 times, increases the content of chlorophyll, vitamins, sugars and other valuable substances, stimulates the effect of all trace elements used in a mixture with humate.

Unlike the known types of humates, the proposed Humate+7B contains 60–65% of humates, minor elements (iron, copper, zinc, manganese, molybdenum, cobalt and boron). In such a combination with the advantage of boron (B), humate was not used during micro-cutting. Boron is essential for plants for normal growth and development. Boron functions are associated with metabolism, transport of sugars across membranes, synthesis of DNA, RNA and phytohormones, cell wall

formation and tissue development. Boron deficiency in plants causes various diseases. Considering the complex of micro- and macroelements used as a growing medium, Humate+7B adds valuable substances that combine a joint positive effect on the result of experiments.

Regenerant plants of 8–10 cm in size were cut into fragments that included a node with a leaf and a bud (the lower part of the internode is 1–1.5 cm longer than the upper one). The resulting micro-cuttings were planted in biological tubes 40×120 mm in size on a growing medium. The tubes were covered with foil and placed in a culture room with appropriate conditions.

The experiment was based on 5 options (see Table 4). Each option had 3 replications. Each replication contained 6 tubes with microscopes. The effect of the modified growing media on the period of growth and development of plants in vitro was studied.

Table 4 shows that growing medium No. 4 is the most optimal one and provides an increase in the number of main roots, leaves and plant height in a relatively short period.

In growing media No. 4 and 5, the amount of agar-agar, saccharose, sodium salts, magnesium, potassium and calcium, mesoinositis, cupric sulphate and nickelous chloride, nicotinic acid, pyridoxine, thiamine, ferrous sulphate, Trilon B, activated carbon are significantly reduced. Due to a significant reduction of these elements in the growing medium and the addition of Humate+7B in an amount of 5–10 ml/l, the costs of the growing medium are reduced and its efficiency is increased.

At the stage of micropropagation, the effect of the mineral composition of modified growing media and growth regulators on the reproduction rate and length of developing shoots was studied.

Regenerant plants of 8–10 cm in size were cut into fragments that included a node with a leaf and a bud (the lower part of the internode is 1–1.5 cm longer than the upper one), the resulting microscopes were planted in biological tubes 40×120 mm in size on a growing medium. The tubes were covered with foil and placed in a culture room with appropriate conditions.

The technology of cutting test-tube plants was common. The plants were observed over 41 days, daily, noting the date of roots and leaves appearance. Plant height measurements, leaf and main root counts were carried out 41 days after cuttings.

In all options, the beginning of root formation was noted on the 8th day after cutting. On the 13th day after cuttings, all plants took root. It was noted that the growth of roots in option 5 also significantly increased in the number of roots, which surpassed the control. Formation of leaves in option 5 began on the 10th day after cuttings, and on the 13th day, leaf formation began in all options of the experiment.

Further observations of the growth and development of plants showed that explants in options 2 and 3 on poor growing media lagged behind a lot in growth and development compared to options 1,4,5 although at first showed positive results in rooting.

Table 4. Composition of a nutrient medium for rooting and growth of test-tube plants (in vitro)

Mg/l, ml/l						
№	Preparation	Option 1	Option 2	Option 3	Option 4	Option 5
		Control, Modified MS Medium	Growing medium 1	Growing medium 2	Growing medium 3	Growing medium 4
1	Agar-agar	7000	7000	7000	7000	7000
2	Saccharose	15000	20000	20000	10000	10000
3	KNO_3 – potassium nitrate	950			475	475
4	NH_4NO_3 – ammonium nitrate	825			69	69
5	$MgSO_4 \times 7H_2O$ - magnesium sulfate	185		185	93	93
6	$CaCI_2 \times 2H_2O$ – calcium chloride	220		166	83	83
7	KH_2PO_4 – potassium phosphate	85			34	34
8	Mesoinositis	100		50	25	25
9	KI – potassium iodide	0.42			0.42	0.42
10	H_3BO_3 – boron hydroxide	3.1			3.1	3.1
11	$ZnSO_4 \times 2H_2O$ – zinc sulphate	4.3			4.3	4.3
12	$MnSO_4 \times 4H_2O$ – manganic sulphate	1.1			1.12	1.12
13	$CuSO_4 \times 5H_2O$ – cupric sulphate	0.025			0.013	0.013
14	$NiCI_2$ – nickelous chloride	0.025			0.013	0.013
15	Nicotinic acid	1		0,5	0.25	0.25
16	Pyridoxin B_6	1		0,5	0.1	0.1
17	Thiamine B_1	1		0,2	0.1	0.1
18	$FeSO_4 \times 7H_2O$ – ferrous sulphate	27.8			13.9	13.9
19	Trilon B Na_2ЭДТА× $2H_2O$	37.2			18.6	18.6
20	**Humate+7B (ml)**		10	10	10	5
21	Activated carbon	5000				
22	pH-media	6.6	6.6	6.6	6.6	6.6

The influence of growth regulators on rooting, growth and development of plants in vitro. It is known that success in clonal micropropagation depends on

varietal characteristics to a large extent. Most researchers used Murashige-Skoog medium or its modifications, and BAP at a concentration of 0.5-3.0 mg/l was used as growth regulators. This composition was chosen as the basis. To increase the reproduction factor, we investigated two options of growth regulators combinations being BAP with 2iP and BAP with kinetin. A modified Murashige-Skoog medium supplemented with BAP at a concentration of 0.5 mg/l and 1.0 mg/l served as a control. Micro-cuttings of strawberries, grapes, pomes, and stone fruits were planted on the experimental media. The duration of cultivation was 4 weeks, after which the reproduction factor and the average length of the shoots were determined (Table 5).

As can be seen from Table 5, the presence of 2iP in the growing medium had a negative effect on the formation of additional shoots in explants, reducing both the reproduction factor and the average length of shoots. An even greater decrease in the reproduction factor was observed in options using a combination of 2iP with BAP at a concentration of 1.0 mg/l. In the control options of explants, the reproduction factor on a medium with BAP 1.0 mg/l was more than two times higher than that in the experimental options. The presence of kinetin in the growing medium in combination with BAP had a positive effect on the development of explants (Table 5). In options with a BAP concentration of 1.0 mg/l, the presence of kinetin did not reduce the reproduction factor of explants as compared with the option without kinetin.

Table 5. Influence of growth regulators BAP and kinetin combinations on the reproduction factor and the average length of shoots of various grape varieties, pome and stone fruits

Culture	Growth regulators, mg/l		Reproduction factor	Average length of shoots, mm
	BAP	Kinetin		
Grape	0.5	-	2.7 ±0.25	27.7 ±2.55
	0.5	0.25	2.6 ± 0.25	34.5 ±3.1
	0.5	0.5	2.9 ± 0.3	20.7 ± 1.9
Stone and pomaceous fruit	1.0	-	9.0 ±0.85	19.0± 1.8
	1.0	0.25	8.0 ± 0.7	18.7 ±1.4
	1.0	0.5	7.1 ±0.6	18.0± 1.3

Thus, it is advisable to use BAP and kinetin together at a concentration of 0.5 mg/l each at the micropropagation stage, which ensures the maximum reproduction factor of explants.

4 Conclusion

As a result of the studies, it has been found that phytohormones have a positive effect on the regeneration of explants in vitro. When using standard compositions of growing media not adding auxins, cytokinins, and gibberellins, shoot regeneration decreases, and growth and development are inhibited. The data on the studied media for explants cultivation obtained during the research showed that agarized growing media MS are optimal for microclonal propagation in vitro, and especially their modifications with a content of 1.0 mg/l 6-BAP at the first planting; a combination of 1.0 mg/l 6-BAP with 1.0 mg/l GK_3 during transplantation; 0.5 mg/l indole acetic acid at the second transplant.

Based on the study of the mineral composition of the growing medium, we have noted that significant differences in the number of roots, leaves and plant height are observed in options 4 and 5. The explants placed on these media showed more intensive plant growth in height and root formation compared with the control option. The best results in terms of plant growth and development were shown by option 5 on growing medium No. 4. The introduction of a liquid concentrated organomineral preparation Humat+7B in addition to mineral salts into the composition of growing media had a significant value for the growth and development of the explant in vitro.

The study of the effect of growth regulators BAP and kinetin combinations on the reproduction factor and the average length of shoots of various explants has showed that for the micropropagation stage it is advisable to use BAP and kinetin together at a concentration of 0.5 mg/l each, which provides the maximum reproduction factor.

References

1. A.A. Batukaev, M.D. Mukailov, M.S. Batukaev, T. Minkina, S. Sushkova, Use of growth regulators in grapes grinding by in vitro method, *Int. Multidisciplinary Sci. GeoConf. SGEM*, Vol. 18, Iss. 6.2, pp. 783–790
2. A.A. Batukaev, D.O. Palaeva, M.S. Batukaev, E.A. Sobralieva, In vitro reproduction and ex vitro adaptation of complex resistant grape varieties, Adv. in Eng. Res. **151**, 895–899 (2018)
3. T.N. Bozhiday, N.V. Kukharchik, Influence of genotype and auxin on the process of ex vitro rhizogenesis of common lingonberry varieties (vacciniumvitis-ideall), *Biotechnology in fruit growing*, Mater. of the sci. conf. (Samokhvalovichi, June 13–17, 2016) (Minsk, 2016), pp. 99–101
4. A.A. Batukaev, M.S. Batukaev, D.O. Palaeva, E.A. Sobralieva, In Vitro Reproduction and Ex Vitro Adaptation of Complex Resistant Grape Varieties, Int. Conf. on Smart Solutions for Agriculture (Agro-Smart 2018), J. Adv. in Eng. Res. **151**, 895–899 (2018)
5. E.N. Besedina, E.N. Besedina, L.L. Buntsevich, M.A. Kostyuk, Study of the effectiveness of new growth stimulants of various nature in clonal micropropagation of apple rootstocks of the SK series. Fruit and berry grow. in Russ. **39**, 29–32 (2014)
6. A.A. Batukaev, I.M. Bamatov, M.A. Vinter, Studying tolerance of prune (PrunusDomestica) to the plum pox virus (PPV) by criterion “Efficiency of microshoots regeneration” in controlled in vitro conditions, J. of Pharmaceut. Sci. and Res. **1**, 59–64 (2018)

7. T.N. Bozhidai, M.S. Kastritskaya, N.V. Kukharchik, A.M. Meskhidze, M.V. Metriveli, Regenerative capacity of blueberries at the stage of introduction into culture in vitro, *Biotechnology in fruit growing*, Proc. of an Int. sci. conf. (Samokhvalovichi, 2016), pp. 105–107
8. A.A. Batukaev, D.O. Palaeva, E.A. Sobralieva, Improvement of the composition of nutrient media during grape cuttings in vitro, Sci. works of the North Cauc. Fed. Sci. Center for Horticult., Viticult. and Winemak. **18**, 76–80 (2018)
9. S.A. Kornatsky, V.A. Vysotsky, V.G. Trushechkin, Problems of clonal micropropagation of stone fruit crops, In: *Advances in fruit growing in the Non-Chernozem zone of the RSFSR*, Sat. scientific. works (Moscow, 1991), pp. 104–116
10. N.Yu. Van-Unkan, N.I. Savelyev, O.Ya. Oleinikova, Microclonal reproduction of columnar apple varieties, *Biotechnology in fruit growing*. Mater. of the sci. conf. (Samokhvalovichi, June 13–17, 2016) (Minsk, 2016), pp. 32–34
11. C. Boleriola-Lucas, M.G. Millins, Micropropogation of two French prune cultiwars (Prunus domestica L.), Agron. **4(5)**, 473–477 (1984)
12. D.W.S. Mok, M.C. Мок, Cytokinin metabolism and action, Annu. Rev. Plant Physiol and Plant Mol. Biol. **52**, 89–118 (2001)