Improvement of the hormonal and mineral composition of nutrient media used for in vitro regeneration of grape plants

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Abstract. It was established that phytohormones have a positive effect on in vitro regeneration of grape varieties. When using standard nutrient media lacking auxins, cytokinins and gibberellins, shoot regeneration was reduced, and growth and development were inhibited. For in vitro microclonal propagation, MS agarized nutrient media, especially their modifications with 6-BAP (1.0 mg/l) for the first planting, 6-BAP (1.0 mg/l) combined with GK3 (1.0 mg/l) for the transplantation, and IAA (0.5 mg/l) for the second transplantation are optimal.

1. Introduction
It is known that for each new variety, the study of all aspects of the in vitro method is required: the selection of optimal compositions of media and growth substances, safe and effective antibiotics and sterilizing substances, and changes in technological methods [1, 2, 6].

Currently, microclonal propagation is the most promising, fastest and safest method for producing the healthy planting material. And since the decisive factor in the in vitro reproduction of any plant is a nutrient medium, its selection and optimization become an important issue [1, 4, 10–12].

Depending on the type and variety of agricultural plants, optimization of the nutrient medium is required; therefore, special approaches have been developed. By varying concentrations of various components of the nutrient medium (growth regulators, phytohormones), the best option can be found [5–9].

The main purpose is to improve the method of clonal micropropagation of grapes by selecting and optimizing the nutrient medium.

The task is to study the influence of growth regulators (auxins, cytokinins and gibberellic acid) of various concentrations and combinations on the development of grapes, as well as to determine the optimal composition of the nutrient media for growing invitro grapes and and producing the healthy certified grape planting material.

2. Methods and materials
The apical shoots of all four grape varieties, after they were cut into one-eyed explants, were washed with water and disinfected with a 2 \% sodium hypochlorite. For control, standard compositions of white and Merasige-Skoog media without growth substances (agar was used as a hardener of the medium) were used. In the modified nutrient media of White and Merasige-Skoog, growth regulators were introduced: 6-BAP (at a concentration of 1 mg/l; 1.5 mg/l) – for the first planting of meristem apexes, 6-BAP (at a concentration of 1 mg/l) in combination with GK3 (at a concentration of 0.5;
1 mg/l) – at the stage of actual micropropagation of clones, and IAA (at a concentration of 0.2; 0.5 mg/l) – at the stage of plant rooting.

Separation of the meristem was carried out using the MBS-10 microscope; meristem apexes were planted into special test tubes 12x4 cm in size with a nutrient medium of 25 g. At each stage, grape explants were transferred into the fresh environment with new growth substances. The study was conducted according to generally accepted methods [5]. Statistical processing was carried out in Microsoft Excel.

3. Results

The growth hormone 6-BAP cytokinin shows good results when used as part of the nutrient medium during the planting of fruit plant explants [1, 13–16]. The results of observation on the 15th day of the development of explants when applying 6-BAP at different concentrations are presented in Table 1.

Table 1 shows that the growth processes in different varieties are not similar. As expected, when applying 6-BAP, an increase in the plant mass is higher than in the standard media. In the modified Murashige-Skoog medium, the growth of Arkadia variety was 11.9 mm at a concentration of 1 mg/l and 9.5 mm at a concentration of 1.5 mg/l, in the White medium, it was 9.5 mm at a concentration of 1 ml/l and 9 mm at a concentration of 1.5 mg/l. For other varieties, the values are as follows: Moldova variety 8 and 6.6 mm; 8.3 and 7 mm; Magarach’s gift variety 8.5 and 7 mm, 8.5 and 7.9 mm; Kodryanka variety – 10.7 and 8 mm; 9.3 and 8.5 mm.

| Variety samples | MS (control) | MS (6-BAP concentration) | White (control) | White (6-BAP concentration) |
|----------------|--------------|---------------------------|-----------------|-----------------------------|
| Arkadia        | 7            | 11.9                      | 6               | 9.5                         |
| Moldova        | 6            | 8                         | 5               | 8.3                         |
| Magarych’s Gift| 6.5          | 8.5                       | 6               | 8.5                         |
| Kodryanka      | 6.5          | 10.7                      | 7               | 9.3                         |

It is not advisable to keep shoots in environments at an increased cytokinin concentration, as this can cause inhibition of growth processes. In addition, it is known that the nutrient medium becomes unsuitable for further use [9, 14, 17, 18]. Therefore, when the medium was changed at the next stage of microclonal propagation of grapes, in addition to 6-BAP at a concentration of 1 ml/l, GK3 at concentrations of 1.5 and 1ml/l was added (Table 2).

The combination of 6-BAP with gibberellin stimulates the growth of stems, by lengthening the internodes, and increasing their number, since the growth regulators of the cytokinin action promotes cell division and differentiation, and gibberellin affects cell extension and division. Significant differences in the development of shoots showed varieties Arkadia – 5.5 internodes, 11 leaves and 11.7 cm shoot length; and Kodryanka – 6; 13; 10.5, respectively. The largest number of internodes, leaves and stems were observed at concentrations of 1 mg/l for 6-BPA and 1 mg/l for GK.

During the next transplantation of microplants onto the nutrient medium, auxin IAA at two types of concentration (0.2 and 0.5 mg/L) was added. Thus, it was planned to root vitro grape plants. The results of the use of IAA in both media (MS and White) are presented in Table 3.

Studies have shown that the concentrations are acceptable for the development of the root system. However, samples grown in the White medium had a weak root growth and lagged behind their competitors. The best result was shown by Magarych’s Gift variety, the length of its roots was 3.7 cm and they filled the bottom of the tube.
Table 2. Development of test plants depending on the effect of GK in combination with 6-BAP

| Varieties    | Internodes, pcs. | Leaves, pcs. | Длина стебля, см | Leaves, pcs. | Stem length, cm | Leaves, pcs. | Stem length, cm | Leaves, pcs. | Stem length, cm |
|--------------|------------------|--------------|------------------|--------------|-----------------|--------------|-----------------|--------------|----------------|
| Arkadia     | 4.8              | 9            | 10.6             | 8.5          | 8.5             | 9.4          | 8.5             | 11           | 9.1            |
| Moldova     | 5.5              | 11           | 11.7             | 10           | 9.1             | 10.3         | 8.5             | 13           | 10.5           |
| Magarych’s gift | 4.4            | 8.5          | 3.9              | 10           | 4.1             | 8.1          | 9.5             | 10           | 8.9            |
| Kodryanka   | 4.5              | 8.5          | 9.9              | 10.5         | 8.1             | 10.3         | 9.8             | 10           | 9.8            |

Table 3. Impact of iuk on development of root system of grape varieties

| Variety sample | Medium MS (modification) | Medium White (modification) |
|----------------|--------------------------|-----------------------------|
|                | Concentration, mg/L      |                             |
|                | 0.2                      | 0.5                         | 0.2 | 0.5 |
| Arkadia        | 2.7                      | 3.1                         | 2.4 | 2.8 |
| Moldova        | 2.4                      | 2.9                         | 2.5 | 2.9 |
| Magarych’s gift| 3                        | 3.7                         | 2.8 | 3.2 |
| Kodryanka      | 3                        | 3.5                         | 2.7 | 3   |

At the stage of intrinsic in vitro micropropagation of grapes, the effects of the composition of mineral salts on rooting and shoot growth were studied. Activated carbon is excluded from the composition of the Murashige-Skoog nutrient medium, salt and acid content was reduced 2–4 times, and Humate + 7B in an amount of 5–10 ml/l was added. Humate + 7B is soluble in water, easily absorbed, mobilizes the immune system, stimulates the development of a powerful root system, promotes enhanced nutrient intake, intensifies metabolic processes, reduces nitrate content 2 times, increases the content of chlorophyll, vitamins, sugars and other valuable substances, stimulates the effect of all trace elements used in mixtures with humate.

In contrast to the known types of humates, Humate + 7B contains 60–65 % of humates and trace elements (iron, copper, zinc, manganese, molybdenum, kibalt and boron) [10, 11]. In the combination with boron (B), humate has not been used for grape micropropagation. Boron is required for normal growth and development. Its functions are associated with metabolism, transportation of sugars through membranes, synthesis of DNA, RNA and phytohormones, formation of cell walls and tissue development. The lack of boron causes various diseases. Given the complex of micro- and macronutrients used as a nutrient medium, Gumat + 7B supplements valuable substances combining joint positive effects on the result of experiments.

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

The experiment was conducted on Augustin grape variety (Table 1). The effect of modified nutrient media on the in vitro growth and development of a grape plant was studied.

These results show (Table 4) that the nutrient medium No. 4 is the most optimal. It ensures an increase in the number of main roots, leaves and plant height for a short period.

In nutrient media No. 4 and No. 5 (Table 4), the amount of agar agar, sucrose, sodium, magnesium, potassium and calcium salts, mesoinositol, copper sulfate and nickel chloride, nicotinic acid, pyridoxine, thiamine, iron sulfate, trilon B and activated carbon is significantly reduced. Due to the
significant reduction of these elements in the nutrient medium and the introduction of Humate + 7B in the amount of 5–10 ml/l, the cost of the nutrient medium is reduced and its effectiveness increases.

**Table 4.** The composition of the nutrient medium for rooting and growth of test tube grape plants (in vitro method)

| no. | Name of medicines | Option 1 | Option 2 | Option 3 | Option 4 | Option 5 |
|-----|-------------------|----------|----------|----------|----------|----------|
|     |                   | Control, modified MS medium | Nutrient medium No. 1 | Nutrient medium No. 2 | Nutrient medium No. 3 | Nutrient medium No. 4 |
| 1   | Agar agar         | 7000     | 7000     | 7000     | 7000     | 7000     |
| 2   | Sucrose           | 15000    | 20000    | 20000    | 10000    | 10000    |
| 3   | KNO₃              | 950      | 475      | 93       | 83       | 83       |
| 4   | NH₄NO₃           | 825      | 69       | 93       | 83       | 83       |
| 5   | MgSO₄×7H₂O        | 185      | 185      | 93       | 83       | 83       |
| 6   | CaCl₂×2H₂O       | 220      | 166      | 34       | 34       | 34       |
| 7   | KH₂PO₄           | 85       | 50       | 25       | 25       | 25       |
| 8   | Mesoinositis      | 100      | 50       | 25       | 25       | 25       |
| 9   | KI                | 0.42     | 0.42     | 0.42     | 0.42     | 0.42     |
| 10  | H₂BO₃            | 3.1      | 3.1      | 3.1      | 3.1      | 3.1      |
| 11  | ZnSO₄×2H₂O       | 4.3      | 4.3      | 4.3      | 4.3      | 4.3      |
| 12  | MnSO₄×4H₂O       | 1.1      | 1.12     | 1.12     | 1.12     | 1.12     |
| 13  | CuSO₄×5H₂O       | 0.025    | 0.013    | 0.013    | 0.013    | 0.013    |
| 14  | NiCl₂             | 0.025    | 0.013    | 0.013    | 0.013    | 0.013    |
| 15  | Nicotinic acid    | 1        | 0.5      | 0.25     | 0.25     | 0.25     |
| 16  | B₆                | 1        | 0.5      | 0.1      | 0.1      | 0.1      |
| 17  | B₁                | 1        | 0.2      | 0.1      | 0.1      | 0.1      |
| 18  | FeSO₄×7H₂O       | 27.8     | 13.9     | 13.9     | 13.9     | 13.9     |
| 19  | Trilon B Na₂ЭДГА×2H₂O | 37.2   | 18.6     | 18.6     | 18.6     | 18.6     |
| 20  | **Humate + 7B (ml.)** | 10       | 10       | 10       | 5        | 5        |
| 21  | Activated carbon | 5000     |          |          |          |          |
| 22  | pH                | 6.6      | 6.6      | 6.6      | 6.6      | 6.6      |

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

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

The experiment was conducted on Augustin grape variety (Table 4). The effect of modified nutrient media on the in vitro growth and development of a grape plant was studied.

The cutting technology was traditional. Daily observations were carried out for 41 days, marking the date of emergence of roots and leaves. Measurements of plant height, leaves and roots were carried out on the 41st day after cutting.

In all the option, the root development began on the 8th day after cutting. On the 13th day after cutting, all the plants had roots. In option 5, the number of roots exceeded the control number. In option 5, leaf development began on the tenth day after cutting, and on the 13th day, leaf development began in all options of the experiment.

Further observations showed that the explants in options 2 and 3 had the worst development results compared to those in options 1, 4, 5 (Fig. 1).

Significant differences in the number of roots, leaves and plant height were observed in options 4 and 5. The more intensive plant growth and root formation were observed (table).
The best results were shown in option 5 in the nutrient medium No. 4. Introduction of liquid concentrated organomineral preparation Gumat + 7B was essential for the in vitro growth and development of the explant.

![Figure 1. The effect of various nutrient media on the growth and development of grape plants during micro-cutting (in vitro method), Augustine variety (41st day after cutting)](image)

### 4. Conclusion

It has been established that phytohormones have a positive effect on the in vitro regeneration of grape varieties. When using standard compositions of media, without auxins, cytokinins and gibberellins, shoot regeneration was reduced, and growth and development were inhibited. IAA has a favorable effect on the rooting.

The data obtained showed that for in vitro microclonal propagation, MS agarized nutrient media are optimal (1.0 mg/l 6-BAP during the first planting; a combination of 1.0 mg/l 6-BAP with 1.0 mg/l GK3 during the transplantation; and 0.5 mg/l IAA during the second transplantation). It was also possible to observe that Augustin, Kodryanka and Arkadia had better development results, and Magarych’s Gift had the most strongly developed root system.

There are significant differences in the number of roots, leaves and plant height in Options 4 and 5. Explants were characterized by a more more intensive growth.

The best results were observed in option 5 in the nutrient medium No. 4. In addition to mineral salts, the liquid concentrated organomineral preparation Humat + 7B introduced into the composition of nutrient media was essential for the in vitro growth and development of the explant.

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