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The Acceptable Concentration of Cytokinin and Auxin in The Regeneration of Grape Varieties "*In Vitro"* In The Content of Ms and DKW Nutritional Condition

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Abstract: In this article **,** the difference in the growth indicators of grape varieties "Toyfi rozovi", "Rizamat", "Pobeda (Mers)", "Husayne beli" in "*in vitro*" conditions in Murashige and Skoog and Driver and Kuniyuki nutritional condition, the effect of cytokinin and auxin on them and the optimal concentration were determined. In the study, the concentration of auxin NAA (naphthylacetic acid) was 0.01 and 0.5 mg/l, cytokinin BAP (6-benzylaminopurine) was used 0.1; 0.5; 1.0; 2.0 mg/l. Explants branching length, number of branches, number of leaves, root length, growth indicators were determined. According to the results of Murashige and Skoog nutrient condition, the branching number of explants is high, NAA (naphthylacetic acid) at two concentrations of 0.01 and 0.5 mg/l, BAP (6- benzylaminopurine) concentration of 0.5; 1.0 mg/l was recommended. In Driver and Kuniyuki nutrient condition, the growth and rooting of explants are high, NAA (naphthylacetic acid) in concentration of 0.01 and 0.5 mg/l, BAP (6- benzylaminopurine) 0.1; 0.5; 1.0 mg/l was recommended.

Keywords: nutrient condition, in vitro, variety, concentration, auxin, cytokinin, regeneration, explant, branch, root, leaf.

1. Introduction

The method of plant reproduction using the technique of isolated tissues and organs has attracted the attention of physiologists, virologists, breeders, as well as practitioners and, first of all, nurserymen. Currently, this trend in tissue culture is developing rapidly and is considered extremely promising [1; 2; 9; 12; 25; 26; 27]. In recent decades, many scientific results have been published on the culture of organs, tissues and cells of grapes [1; 3; 5; 6; 10; 15; 19; 20; 21].

The next step is the actual micropropagation process. At this stage, it is emphasized that factors such as variety and species characteristics, plant structure, origin, composition of the nutrient condition, and physical conditions of cultivation are the decisive process [22; 18; 24].

Scientists are divided onto the influence of cultivar characteristics on in vitro micropropagation. Some of them [4; 6; 15] considered that cultivar characteristics of plants significantly influence the regenerative capacity of the meristem tip, inter-varietal differences may have a greater effect than inter-species differences [17].

Primary explants in in vitro propagation of grapes are leaf primordia, buds' tops, meristems with green one-eyed nodes [13; 14; 21].

Three nutrient conditions developed on the basis of Murashige and Skoog condition are recommended for propagation of grapes in isolated tissue culture, and for axillary bud growth, they use liquid nutrient condition A.B.Burgutin [9] noted that when introduced into in vitro culture, it is written that it is preferable to carry out the first subcultures in a solid condition.

B.A.Vysotsky [11] pointed out that culture conditions according to the recipe of Murashige and Skoog are the most common for the stage of plant reproduction, paying attention to the fact that it is favorable for the growth of callus tissue.

Cytokinins played the main role in this and caused the activation of all axillary meristems as a result of loss of apical dominance of the bud apex grown in vitro. Among substances with cytokinin activity, kinetin and β-benzylaminopurine (6-BAP) were noted to be the most intensively affected [1; 4; 12].

A.A.Batukaev [3] noted that the growth of adventitious buds from the tops of grape stems 6-benzylaminopurine, P.Ya. Holodriga et al [13] confirmed a stronger effect of 6-benzylaminopurine, with almost no effect on the growth and development of grapevine plants in vitro, emphasizing its significant stimulation.

The next important step in the micropropagation process is root induction in the propagating vine shoots. Before

proceeding to this stage, it is necessary to prepare prickle of buds, for which they need to be elongated, which can be achieved by enriching the nutrient condition with gibberlic acid or reducing the content of 6-BAP, according to the recommendations of the aforementioned authors [11].
A number of authors reported the positive effect of naphthylacetic acid (NAA) in grapevine micropropagation with stem apices, the best survival (>80%), buds and root development was obtained by modifying the Murashige and Skoog condition to which NAA was added together with 6-BAP noted [32].
I.R.Li, G.V.Eaton pretreatment of bud tips for 15 min at a concentration of 3.94×10-3 M with 6-BAP NAA (5.7×10-6 m) increased the dry weight of developing buds [29]. R.Chee, M.R.Pool found that a decrease in succharose concentration of up to 1% and an increase in NAA concentration of 3.2 mm promoted taking root [33].
From this literature review, attention should be paid to obtaining aseptic culture, selecting and optimizing the nutrient condition composition, selecting and sterilizing explants to address tissue rooting issues, and adapting the resulting plants to non-sterile conditions.
However, based on the morphological and physiological characteristics of local grape varieties of Uzbekistan, new nutritional composition has not been developed, and scientific publications have not been published. Therefore, it is important to determine the optimal nutrient condition concentrations for the development of local grape varieties of Uzbekistan *in vitro culture.*

2. Materials and Methods

Necessary equipment: petri dish, tweezers, scissors, sterile gauze, sterile paper, 0.5 l jar, 70% alcohol.
Procedure: The study was conducted in 2021-2022 at the De Nova Agro *In Vitro* Laboratory based on the recommendations given in J.Driver's methodical manual "Cultivation of tissues and cells in artificial (test tube) under laboratory conditions" [16]. The local grape varieties Toyfi Rozovy, Rizamat, Pobeda (Mers), Husayne Beli were selected as objects for the experiment.
In the study, MS (Murashige and Skoog 1962) and DKW (Driver and Kuniyuki 1984) nutrient condition were used and it was enriched with concentrations of auxin NAA 0.01 and 0.5 mg/l and concentration of cytokinin BAP 0.1; 0.5; 1.0; 2.0 mg/l. According to the concentration of BAP in MS and DKW nutrient condition, the amount of 1.0 mg/l was taken as a control. In the study, 2000 explants were monitored for a period of 30 days, and the growth parameters of the explants in terms of branching length, branch number, leaf number, and root length were determined (see Table 1).

3. Results and Discussion

According to the results of the study, the best results were obtained when the type of grape Type Rozovy had a concentration of NAA of 0.01 mg/l in the MS nutrient condition. At the amount of BAP 0.5 mg/l, the branch length of the explants was 2 cm, the number of branches was 2 pieces, the number of leaves was 5 pieces, and the root length was 2 cm. When the BAP was 1.0 mg/l, the branch length was 1 cm, the number of branches was 5 pieces, the number of leaves was 5, and rooting was not observed. DKW nutrient condition when NAA is 0.01 mg/l and BAP was 0.5 mg/l, the branch length was 3 cm, the number of branches was 2 pieces, the number of leaves was 5 pieces, and the root length was 5 cm. When BAP was 1.0 mg/l, the branch length was 1 cm, the number of branches was 3, the number of leaves was 4, and no rooting was observed.
When BAP was used 0.1 and 2.0 mg/l in MS and DKW nutrient condition, middle results were returned in the development of explants.
Optimum concentration for Rizamat cultivar, in MS nutrient condition BAP content was recorded at 0.5 mg/l. According to it, the length of the branch was 1 cm, the number of branches was 4 pieces, the number of leaves was 7 pieces, and the root length was 1 cm. At BAP concentrations of 1.0 and 2.0 mg/l, the number of branches was 3, and explant growth and rooting were not observed.
Intermediate results in the development of explants were returned when MS and DKW nutrient condition contained BAP at 0.1 and 2.0 mg/l.
Optimum concentration for Rizamat cultivar, in MS nutrient condition It was returned when the concentration of BAP was 0.5 mg/l. According to it, the length of the branch was 1 cm, the number of branches was 4 pieces, the number of leaves was 7 pieces, the length of the root was 1 cm. At BAP concentrations of 1.0 and 2.0 mg/l, the number of branches was 3, and explant growth and rooting were not observed.
when BAP was 0.1 mg in DKW nutrient condition, branching was not observed, the branch length was 5 cm and the root length was 5 cm. Good results on branching parameters were returned at BAP concentration of 1.0 mg/l, with 3 branching per explant. Results on explant growth performance at BAP 2.0 mg/l concentration were not

replicated.
No rooting was observed in the explants of Pobeda (Mers) cultivar MS when NAA was 0.01 mg/l. The best result was observed at BAP concentration of 0.5 mg/l, branch length was 1 cm, branch number was 5 pieces, leaf number was 5 pieces. Medium results on branching were returned at other BAP concentrations.
DKW is in the nutritional environment When the concentration of BAP was 0.1 mg/l, the highest results were obtained by growing the branch length to 4 cm and the root length to 5 cm. At BAP concentrations of 1.0 and 2.0 mg/l, branching was 3 in each explant, and rooting was not observed.
Rooting of the explants was not observed when Husayne beli cultivar MS was exposed to 0.01 mg/l of NAA. The best branching was returned at a BAP concentration of 1.0 mg/l, which was 4 per explant. At BAP levels of 0.5 and 2.0 mg/l, almost the same results were observed, and branching was 2 units.
No rooting was observed at any concentration in DKW nutrient condition. The best amount for branching was 3 units at 1.0 mg/L BAP and 2 units at 2.0 mg/L BAP (see Table 1).

Table 1. Development of explants of vine cultivars in different nutrient conditions and grower substances *in vitro*

Varieties name	BAP, mg/l	MS nutrient condition				DKW nutrient condition			
		Branch length, cm	Number of branches, pcs	Number of leaves, pcs	Root length, cm	Branch length, cm	Number of branches, pcs	Number of leaves, pcs	Root length, cm
NAA (0.01 mg/l)									
Tayfi Rozovy	0.1	1	1	4	1	2	-	3	3
	0.5	2	2	5	2	3	2	5	5
	1.0 (con.)	1	5	5	-	1	3	4	-
	2.0	-	3	6	-	-	2	5	-
Rizamat	0.1	2	-	5	1.5	5	-	5	5
	0.5	1	4	6	1	2	2	5	1.5
	1.0 (con.)	-	3	5	-	2	3	4	1
	2.0	-		5	-	-	-	3	-
Pobeda (Mers)	0.1	2	2	4	-	4	-	6	5
	0.5	1	5	5	-	1	1	3	3
	1.0 (con.)	-	3	5	-	-	3	5	-
	2.0	-	2	5	-	-	3	6	-
Husayne beli	0.1	1	-	2	-	2	-	4	-
	0.5	1	2	4	-	1	-	2	-
	1.0 (con.)	-	4	6	-	1	3	5	-
	2.0	-	2	4	-	-	2	4	-
NAA – (0.5 mg/l)									
Tayfi Rozovy	0.1	2	-	3	2	2	-	4	3
	0.5	2	2	4	3	4	2	6	6
	1.0 (con.)	2	4	5	-	1	2	4	-
	2.0	1	2	4	-	1	2	5	-
Rizamat	0.1	2	-	4	2	3	-	4	5
	0.5	2	4	6	3	2	1	4	3

Varieties name	BAP, mg/l	MS nutrient condition				DKW nutrient condition			
		Branch length, cm	Number of branches, pcs	Number of leaves, pcs	Root length, cm	Branch length, cm	Number of branches, pcs	Number of leaves, pcs	Root length, cm
	1.0 (con.)	1	3	5	1	2	3	5	2
	2.0	-	2	5	-	1	2	5	1
Pobeda (Mers)	0.1	2	-	3	2	3	-	4	3
	0.5	2	2	4	-	4	2	6	3
	1.0 (con.)	2	3	5	-	2	2	4	-
	2.0	1	2	4	-	1	2	4	-
Husayne beli	0.1	3	-	3	3	3	-	3	3
	0.5	2	2	4	2	2	2	6	2
	1.0 (con.)	2	4	6	1	1	3	5	1
	2.0	1	2	4	-	1	2	4	-

Different results were obtained for regeneration of grape varieties even when NAA 0.5 mg/l was used in MS and DKW nutrient condition. Type rozovy variety in MS nutrient condition When the concentration of BAP was 0.1 mg/l, the branch length was 2 cm, branching was not observed, the number of leaves was 3, and the root length was 2 cm. At a concentration of BAP 0.5 mg/l, the length of the branch reached 2 cm, the number of branches was 2, the number of leaves was 4, and the root length was 3 cm. The best branching was observed at BAP 1.0 mg/l, which was 4 per explant.

In the DKW nutrient condition, the best result was obtained when the amount of BAP was 0.5 mg/l, the branch length was 4 cm, the number of branches was 2 cm, the number of leaves was 6 pieces, and the root length was 6 cm. When BAP was 1.0 and 2.0 mg/l, the results were almost no different, and the branch length was 1 cm, the number of branches was 2 cm, the number of leaves was 4.5, and no rooting was observed.

when planting Rizamat variety in the MS nutrient condition, the best result was observed when BAP was 0.5 mg/l. According to it, the length of the branch was 2 cm, the number of branches was 4 cm, the number of leaves was 6, and the length of the root was 3 cm. When the BAP concentration was 0.1 mg/l, the branch length was 2 cm, no branching was observed, the number of leaves was 4, and the root length was 2 cm. When BAP was 1.0 mg/l, the branch length was 1 cm, the number of branches was 3 pieces, the number of leaves was 5 pieces, and the root length was 1 cm. When BAP concentration was 2.0 mg/l, branch length and rooting were not observed, the number of branches was 2 pieces.

Good results were also obtained when the Rizamat variety was planted in DKW nutrient condition. At BAP concentration of 0.1 mg/l, the highest values of shoot length of 3 cm and taking root of 5 cm were returned, while good values for all standards were observed at BAP of 1.0 mg/l. According to it, the length of the branch was 2 cm, the number of branches was 3 cm, the number of leaves was 5, and the length of the root was 2 cm. When BAP was 2.0 mg/l, branch length and taking root were not observed, branching was 3 units, number of leaves was 5 units.

When the Pobeda (Mers) variety was planted in the MS nutrient condition, good results for all parameters were returned when the BAP concentration was 0.5 mg/l, according to it, the length of the branch was 2 cm, the number of branches was 2 cm, the number of leaves was 5 pieces, and the length of the root was 2 cm. Almost identical results were observed at BAP concentrations of 1.0 and 2.0 mg/l, and taking rooting was not observed. When BAP was 0.1 mg/l, branching was not observed, the branch length was 2 cm, the number of leaves was 3 pieces, and the root length was 2 cm.

When the Pobeda (Mers) variety was planted in DKW nutrient condition, the best results were observed with a BAP concentration of 0.5 mg/l, the length of the branch was 4 cm, the number of branches was 2 pieces, the number of leaves was 6 pieces, and the root length was 3 cm. When BAP content was 1.0 and 2.0 mg/l, branch length was 1.2 cm, number of branches was 2 cm, number of leaves was 4.5, and taking root was not observed at both concentrations.

Figure 1: Standard growth for all parameters in MS+NAA(0.5mg/l)+BAP(0.5mg/l) nutrient condition of Tayfi rozovy variety.

Figure 2: Growth and root taking parameters of explant in nutrient condition of Pobeda (Mers) DKW+NAA(0.01mg/l)+BAP (0.1 mg/l).

When Husayne beli variety was planted in MS and DKW nutrient condition, almost the same values were noted in terms of BAP concentrations. The best result was observed when BAP concentration was 1.0 mg/l, in MS nutrient condition, branch length was 2 cm, branch number was 4 cm, leaf number was 6 pieces, root length was 1 cm, and in DKW nutrient condition, this indicator were: branch length was 1 cm, branch number was 3 cm, number of leaves were 5 pieces, root length was 1 cm. When BAP was 2.0 mg/l, root taking was not observed in both nutrients, the length of the branch was 1 cm, the number of branches was 2 cm, and the number of leaves was 4. Middle results were observed for the remaining BAP concentrations of 0.1 and 0.5 mg/l (see Table 1)

4. Conclusion

The results obtained from the experiment showed that grape varieties with complex genotypes have different effects on regeneration in the same nutrient condition, depending on the concentration of auxin and cytokinin. For example, BAP and NAA according to the concentration ratio, it was found that branching was higher in MS nutrient condition, while branch length and root taking of explants were strong in DKW nutrient condition.
In the literature, the authors also concluded that BAP 6 should be adapted for each culture or growing condition. D.V.Bobilev [8] studied the concentration of 6-BAP and found that the amount of 2.0 and 5.0 mm in the nutrient condition was optimal for the seedless Cereal variety and provides the highest number of plants with more than two leaves.
In grape propagation, the goal is to achieve a large number of buds of sufficient size (3-4 nodes, 1.5 cm) and quality in a short time, for which were buds development (5 mm), elongated buds (2 mm) and maximum expansion of explant internodes (1 mm) were stated that the optimal concentration of 6-BAP [14; 23; 30]. In 1986, L.Reisoft also found a weak effect of kinetics, stating that grape seedlings grew better in nutrient condition containing 5-7 mm 6-BAP [28].
The best root taking results at 0 to 0.05 mg/L NAA/IBA auxin combinations were obtained with 0.05 mg/L IBA combined with 0.01 mg/L NAA [31].
We have the type MS and DKW in the nutrient condition of Tayfi rozovy when the NAA is 0.01 mg/l for standard growth and best branch length and root length for all parameters, BAP concentrations were set at 0.5 and 1.0 mg/l.
In Rizamat variety, the optimal concentration of BAP for standard growth of all indicators in MS nutrient condition was determined as 0.5 mg/l. In the DKW nutrient condition, the optimal concentration of BAP for the best branch length and root length was found to be 0.1 mg/l. Standard growth for all indicators was determined when the concentration of BAP was equal to 1.0 mg/l.
Pobeda (Mers) variety for regeneration, in MS nutrient condition, the best branching was determined at a BAP concentration of 0.5 mg/l. According to the best branch length and root length indicators, the BAP concentration in DKW nutrient condition was determined as 0.1 mg/l, the best branching BAP concentration was 2.0 mg/l, and the standard growth BAP concentration was 0.5 mg/l for all parameters found.
In Husayne beli variety, the same concentration of BAP in MS and DKW nutrient condition was determined as 1.0 mg/l for the best branching. By all indicators, standard growth and root taking were not observed at all.
When NAA was 0.5 mg/l in MS and DKW nutrient condition for Tayfi rozovy variety, in MS nutrient condition, the BAP concentration for best branching was found to be 1.0 mg/l. Standard growth for all parameters was observed when BAP was 0.5 mg/l. In DKW nutrient condition, the optimal concentration to all indicators by the standard growth BAP concentration was set at 0.5 mg/l.
in MS nutrient condition, standard growth for all parameters was determined as acceptable when the BAP concentration was 0.5 mg/l for Rizamat variety. In DKW nutrient condition, the best branch and root length was observed at BAP 0.5 mg/l, while the standard growth concentration for all parameters was found to be BAP 1.0 mg/l.
Pobeda (Mers) variety in MS nutrient condition, the optimum concentration of BAP for standard growth was found to be 0.5 mg/l for the best branching. The optimal concentration of BAP for standard growth according to all indicators was found to be 0.5 mg/l. BAP was set at 0.5 mg/l for standard growth in all parameters also in DKW nutrient condition.
The standard growth of Husayne beli variety for all indicators was determined as acceptable when the concentration of BAP was 1.0 mg/l in both MS and DKW nutrient condition. For best branch length and root length, BAP concentration of 0.1 mg/l was determined in DKW nutrient condition, while standard growth BAP was found to be 0.5 mg/l for all parameters.

Recommendation
According to the obtained results and conclusions, optimal nutrient condition and concentration for in vitro regeneration of local varieties of grapes were determined and recommended for use (see Table 2).

Table 2. Optimum nutrient condition and concentrations recommended for grape varieties of Tayfi rozovy, Rizamat, Pobeda (Mers), Husayne beli

No	Variety	Nutrient condition and concentration			
		MS +NAA (0,01мг/л)	MS+NAA (0,5 мг/л)	DKW+NAA (0,01мг/л)	DKW+NAA (0,5мг/л)
1	Tayfi rozovy	BAP (mg/l)			

		0,5; 1,0	0,5; 1,0	0,5	0,5
2	Rizamat	0,5; 1,0	0,5; 1,0	0,1; 1,0	0,1; 1,0
3	Pobeda (Mers)	0,5; 1,0	0,5; 1,0	0,1; 0,5	0,5; 1,0
4	Husayne beli	1,0	0,1; 0,5;1,0	0,1; 1,0	0,1; 0,5; 1,0

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