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Мелян Гаяне Грантовна, к.б.н., gmggmg65@mail.ru;
Саакян Агван Джумшудович, к.с.-х.н.;
Барсегян Андраник Акопович, к.б.н.;
Варданян Араик Саджанович, к с.-х.н.
Научный Центр Агробиотехнологии, НАУА, Эчмиадзин, Армения

ВЛИЯНИЕ РЕГУЛЯТОРОВ РОСТА НА РОСТ *VITIS VINIFERA* L. СОРТА КАКАВИК В КУЛЬТУРЕ *IN VITRO*

В статье представлены результаты исследований о влиянии различных регуляторов роста на побегообразование и корнеобразование винограда сорта Какавик в условиях in vitro. Определены оптимальные концентрации регуляторов роста обеспечивающие интенсивную пролиферацию микропобегов и ризогенез. В качестве экспланта использовали пазушные почки. После стерилизации, экспланты культивировали на среде Мурасиге–Скуга (МС) с добавлением БАП в концентрациях (0.5; 0.8; 1.0 mg·L^{-1}), отдельно или в сочетании с ГК3 (0.5; 1.0 mg·L^{-1}). Наибольшее количество побегов - 2,1 шт. с максимальной длиной - 3,6 см образовалось на питательной среде МС с дополнением 1 mg·L^{-1} БАП и 1,0 mg·L^{-1}GA$_3$. Наиболее высокий процент (85,5%) корнеобразования, наибольшее количество корней (4,4) с максимальной длиной (7,6 см) наблюдалось на среде MS/2 с добавлением 1.0 mg·L^{-1} ИМК. Исследование показало, что пазушные почки винограда обладают достаточно высоким потенциалом для быстрой регенерации побегов и их последующего микроразмножения..

Ключевые слова: микроразмножение; пазушные почки; регенерация; виноградная лоза; ***in vitro***.

Melyan Gayane, Cand. Biol. Sci.;
Sahakyan Aghvan, Cand. Agric. Sci.;
Barsegyan Andranik, Cand. Biol. Sci.;
Vardanyan Arayik, Cand. Agric. Sci.
Scientific Center of Agrobiotechnology, ANAU, Echmiadzin, Armenia

EFFECTS OF GROWTH REGULATORS ON *IN VITRO* GROWTH OF *VITIS VINIFERA* L. CULTIVAR KAQAVIK

The results of the effects of different growth regulators on shoot formation and rooting of grapevine cultivar 'Kaqavik' in vitro were presented in this article. The optimal concentrations of plant growth regulators for intensive proliferation of microshoots and root formation were found out. The nodal segments were used as explants. After sterilization, explants were placed into MS (Murashige and Skoog) medium with 3 concentrations of BAP (0.5; 0.8; 1.0 mg·L^{-1}), alone or each in combination with GA$_3$ (0.5; 1.0 mg·L^{-1}). The greatest numbers of shoots with average of 2.1 and the maximum shoot length with average of 3.6 cm were produced in medium containing 1.0 mg·L^{-1}BAP and 1.0 mg·L^{-1}GA$_3$. Highest rooting percentage (85.5 %), greater number of roots (4.4) and maximum root length (7.6 cm) were observed in MS/2 supplemented with 1.0 mg·L^{-1} IBA. The present study shows that nodal segment explants of 'Kaqavik' cultivar carry a high potential for a rapid multiple shoot regeneration and a subsequent micropropagation.

Keywords: micropropagation; nodal segment; regeneration; grapevine; ***in vitro***.

Introduction. Grapevine (*Vitis vinifera* L.) is one of the economically most valuable fruit in Armenia and worldwide. Many grapevine cultivars are now endangered in Armenia and among them is cultivar 'Kaqavik'. Grapevine is commercially propagated by the classical methods of vegetative propagation, i.e. by hard wood cuttings. Today, biotechnology offers a wide range of techniques, which allow effective propagating of plant and optimizing plant genetic resource conservation. Micropropagation represents an efficient method of plant regeneration and rapid propagation through organogenesis and embryogenesis of any valuable genotype obtained by non-conventional methods (Mederos-Molina 2007).

In vitro propagation through the development of axillary buds eliminates the seasonal limitations encountered with these methods and needs a small quantity of starting material, the in vitro plants propagated in this way in many species have proved to be healthy and true to type (Shen et al., 1990).

The numerous methods of in vitro culture of grapevine have been previously described by some authors (Mhatre et al. 2000; Torregrosa et al. 2001; Read 2007; Craciunas C. 2009; Heloir et al. 1997). However, micro propagation needs a great deal of experimental work on optimization of the conditions in all its stages.

Optimal growth and morphogenesis of tissues may vary for different species of plants and even the different varieties of the same species according to their nutritional requirements. Thus, this study aims to determine the optimum balance growth regulators required to induce maximum shoot growth and rooting of grape cv. 'Kaqavik' by use of nodal segments for mass micropropagation and in vitro conservation.

Material and methods. The study was conducted at the Scientific Center of Agrobiotechnology, ANAU. 'Kaqavik' is a seedless table grapevine cultivar for eating as fresh fruit or raisins. Actively growing shoots were collected from the field grown (Ararat region, private farm) grapevines.

Explants preparation and sterilization: The nodal segments (single node) were used as explants. Initially expanded leaves were removed and single-node explants were washed thoroughly under running tap water for 15 min, followed by soaking in soapy water for 5 min, and then distilled water rinses. The surface sterilization was done with 70% Ethanol for 30 sec followed by 2.0% Calcium hypochlorite for 15 min. The explants were washed 3 times with double-distilled sterilized water to remove all traces of the sterilant.

Culture establishment: In order to standardize the most suitable culture establishment medium, the explants were cultured on Murashige and Skoog (MS) basal medium supplemented with different concentrations (0.5; 0.8; 1.0 mg·L^{-1}) of 6-benzylamino purine (BAP) either singly or each in combination with the Gibberellic acid (GA$_3$) 0.5; 1.0 mg·L^{-1}. Twelve explants were used for each treatment and experiment was repeated three times. Observations for shoot proliferation were evaluated 45 days after the beginning of the experiment, and the number of shoots per explant and length of shoots were recorded.

Root formation: Shoots longer than15 mm were used as micro cuttings and were transplanted to the half strength MS/2 agar-solidified basal medium containing various concentrations (0.2; 0.5; 1.0 mg·L^{-1}) of Indol-3-butyric acid (IBA). MS/2 medium without IBA regulators was used as a control. The rooting percentage, average number and length (cm) of roots for each rooted shoot were evaluated after 30 days of culture on rooting medium.

The pH of the media was adjusted at 5.8 before autoclaving. The test tubes were autoclaved at 121°C under 15 psi pressures for 16 minutes. The tissue culture room was maintained at 24±2°C under a light-dark cycle of 16:8h. Statistical Analysis: Correspondingly data were pooled from three independent experiments and expressed as the mean. Treatment means were compared with the standard error (SE) of the mean, the student's t-test was used to find significant

differences between the means.

Results and Discussion. It is well known fact that in vitro regeneration of an explant is effected by several factors such as hormonal composition of culture medium, species, genotype, explants and various other culture conditions.

Plant growth regulators are the most important inducing signal for shoot organogenesis. Dedifferentiation, induction and development of shoots or roots are regulated by both endogenous and exogenous growth regulators. In this respect Thomas 2000; Mukherjee et al. 2010; Kurmi U.S. 2011 mentioned that the type, concentration and combination of PGRs in culture media are key factors influencing shoots induction of grape.

Table 1 shows the effects of different PGRs and their concentrations on the in vitro establishment of nodal segments. Nodal explants were suitable for regeneration. On medium without PGRs explants did not form shoots. The presence of BAP, even at low levels, enhanced bud induction. With increases in BAP concentration (0.5-1.0 mg·L^{-1}), numbers of shoots/explant increased, while shoot length decreased with increasing BAP concentration.

Aazami (2010) also reported that BAP at 1.00 mg·L^{-1} concentration was the best for shoot proliferation in both 'Soltanin' and 'Sahebi' cultivars of grapevine.

In our experiment the interaction of BAP and GA_3 had a significant effect on the shoot length. The number of shoots per explant and shoot length were highest at 1.0 mg·L^{-1} BAP+1.0 mg·L^{-1} GA_3.

The formation of adventitious roots is a vital step in vegetative propagation of woody plant. Adventitious root formation is a complex process that is affected by multiple endogenous factors including phyto hormones and environmental factors (Xuan et al. 2008).The type of plant growth regulators and their interaction play an important role in dedifferentiation, induction and development of shoots or roots (Khanam et al., 2000).

The most important factors in rooting induction or initiation are concentration of auxin. Significant differences were observed among the treatments as showed in the Table 2 for rooting percentage of culture. Highest rooting percentage (85.5 %) was observed in 1.0 mg/l IBA followed by 0.5 mg/l IBA (74.6 %) and 61.9 % at 0.2 mg·L^{-1}IBA. Lowest rooting percentage was observed in medium without IBA. The addition of IBA had a significant effect on the number of roots and root lengths (Table 2). An IBA concentration of 1.0 mg·L^{-1} gave a greater number of roots (4.4) and maximum root length (7.6 cm) than other concentrations (0.2; 0.5 mg·L^{-1}). The least number of roots (1.5) and minimum root length (2.4 cm) were produced with MS/2 medium without growth regulators (control).

Table 1

Influence of growth regulators on shoot proliferation of grapevine

BAP concentration	GA_3 concentration mg·L^{-1}					
	00		0.5		1.0	
	number of shoots per explant (mean±SE)	length of shoots(cm) (mean ±SE)	number of shoots per explant (mean ±SE)	length of shoots(cm) (mean ±SE)	number of shoots per explant (mean±SE)	length of shoots (cm) (mean ±SE)
Medium without BAP	-	-	-	-	-	-
0.5 mg·L^{-1}	1.2±0.3	2.1±0.2	1.3±0.1	2.6±0.1	1.6±0.1	2.8±0.2
0.8 mg·L^{-1}	1.4±0.1	1.9±0.2	1.5±0.1	2.7±0.1	1.8±0.1	3.1±0.1
1.0 mg·L^{-1}	1.6±0.1	1.7±0.1	1.8±0.2	3.2±0.2	2.1±0.2	3.6±0.1

Table 2

Influence of IBA on rooting of grapevine shoots produced *in vitro*

IBA concentration	Rooting (%)	Number of roots per shoot (mean ±SE)	length of roots(cm) (mean ±SE)
Medium without IBA	50.5	1.5±0.3	2.4±0.3
0.2 mg·L^{-1}	61.9	3.1±0.2	4.0±0.4
0.5 mg·L^{-1}	74.6	3.7±0.2	6.1±0.3
1.0 mg·L^{-1}	85.5	4.4±0.4	7.6±0.3

Twelve explants and 12 microshoots were used for each treatment respectively for regeneration and rooting. The experiment was repeated three times.

Conclusions. The present study shows that the tissue culture presents a method of efficient micropropagation of grapevine cultivar 'Kaqavik'. Nodal segment explants of cultivar 'Kaqavik' carry a high potential for rapid multiple shoot regeneration and a subsequent micro propagation. These techniques can be used for propagation and conservation.

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