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USING CYTOKININS IN BERRIES CLONAL MICROPROPAGATION

Встановили, що для розмноження *in vitro Rubus fruticosus L.* (сорт Рубен), *Ríbes nígrum L.* (сорт Ювілейна Копаня), *Ríbes rubrum L. (*сорт Йонкер Ван Тетс), *Ríbes Grossularia* (сорт Черномор) доцільно використовувати метод із утворенням у вихідних рослин конгломерату розеток шляхом додавання у живильне середовище БАП (0,5–1,0 мг/л). Концентрація БАП 2,0 мг/л призвела до збільшення кількості вітрифікованих рослин (*R. grossularia* – 69 %). За подальшого субкультивування рослин на середовищі із високим вмістом БАП (2,0 мг/л) негативний вплив посилювався. Збільшення віку вихідних рослин зменшувало фітотоксичність надлишкової концентрації БАП у *Rubus fruticosus L.* та *Ríbes Grossularia L.*

Ключові слова: вітрифікація, експлант, мікроклональне розмноження, пагін, цитокінін, *in vitro.*

Problem statement. Using plants clonal micropropagation, particularly in berries, allows solving a lot of problems in plants cultivation: propagation coefficient increase, getting healthy planting material etc. However, this technology needs some improvement depending on the plants species and their varietal characteristics.

Analysis of recent research and publications. Propagation in vitro is possible with direct and indirect morphogenesis. Regenerant plants formation from a callus is the characteristic of indirect morphogenesis. Direct morphogenesis is characterized by the activation of existing meristem, the formation of buds. Axillary buds growth activation and using axillary sprouts is the most common type of the clonal micropropagation of plants. Plant axillary buds growth in the native conditions is overwhelmed by apical dominance and stimulated by removing the stem top or its processing with cytokinins [1]. only Two types of sprouts are generated with the “direct morphogenesis” cloning technology (fig. 1).

The first type is characterized with the formation of one sprout with clear-cut dominance. Engraftments having a part of stem with the leaf and the bud in the axil are formed under such division sprout. The sprout of the future regenerant forms from this bud, adventitious roots form the basal part of the stem. This way of the propagation in aseptic conditions is typical for of potatoes, carnation, chrysanthemum [2, 3].

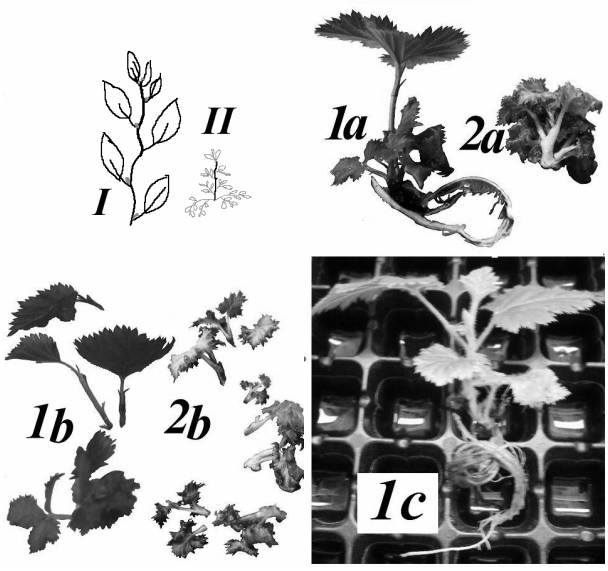

Figure 1. **Exogens cytokinin benzylaminopurine concentration influence on the *Rubus fruticosus* 'Reubenregenerants development**
I, II Different development types of regenerants sprout under direct morphogenesis:
I – with the marked apical dominance; II – with rosettes sprouts formation. 1 – benzylaminopurine 0,5 mg/l;2 – benzylaminopurine 1,0 mg/l; **a** – regenerant; **b** – regenerat division by cuttings; **c** – the regenerant grown on hydroponics for 15 days is suitable for the planting in the soil.

However, this method is unproductive or unacceptable for some types of grassy crops like gerbera and hosta which have shorter sprouts [4, 5]. Therefore, technological methods, which provide the formation of so-called rosettes of the sprouts, are used for these crops.

Cytokinins introduction into nutrient culture medium evokes lateral buds and involves the development of numerous new buds, from which the rosettes are formed [6 - 8]. At the same time, high concentrations of cytokinins promote the development of axillary buds which results in abnormal and degenerated forms. The explants could even die because of the of cytokinins high concentrations affect.

The study purpose and objectives. The purpose of the research is testing different varieties of berry crops reactions on exogenous cytokinins *in vitro*.

Material and methods. The cultivation methods is common for aseptic growth [5]. A nutrient medium is used according to the Murashige-Scoog prescription. *Rubus fruticosus L.* (Ruben sort), *Ríbes nígrum L.* (Jubilee Kopanya sort), *Ríbes rubrum L.* (Jonker Van Tets sort), *Ríbes Grossularia* (Chernomor sort) species were grown under this methods: Benzylaminopurine (BAP) was used as a cytokinin with 0,25 mg/l indolyl oil acid as the backdrop. Hormones produced by "Sigma-Aldrich Chemie GmbH" (Germany) were used in the research.

Results and discussion. It has been found out that the first way of the studied crops microclonal propagation is next to unacceptable since *R. Grossularia* growth buds are formed on the apical side of the lateral and basal sprouts [9]. Under these plants dividing into one nodded sprouts-cuttings, some of them (except for apical ones) died. The most acceptable method for the studied cultures is the one of clonal micropropagation with the formation of sprouts rosettes (table 1) though some of the plants, like blackberry, can reproduce itself with the two cutting types. Yet, a smaller number of cuttings is formed in the sprout formation with the apical dominance. Such cuttings need longer time for axillary buds awakening, while the explants of the second type already have formed microsprouts. The reaction of a plant organism on the cytokinins (BAP) depended of the sort of the plant.

Table 1 – **The plants response to exogenous cytokinin depending on the plants species**[1]

Plants species	BAP concentration, mg/l	Regenerants height, mm	Shoots in a rosette, number	Vitrificated plants, %
Rubus fruticosus L.	0	67,9 ±0,9	1,0 ±0,2	-
	0,5	75,3 ±0,8	1,4 ±0,3	-
	1,0	56,7 ±0,7	5,2 ±0,4	3 ±0,3
	2,0	43,1 ±0,9	3,8 ±0,3	28 ±0,4
Ríbes nígrum L.	0	82,3 ±1,2	1,0 ±0,1	-
	0,5	87,7 ±1,1	1,1 ±0,2	-
	1,0	64, 0 ±0,9	4,1 ±0,2	1 ±0,2
	2,0	52,7 ±0,6	3,1± 0,3	16 ±0,5
Ríbes rubrum L.	0	71,07 ±1,2	2,7 ±0,2	-
	0,5	52,4 ±1,3	4,9±0,3	-
	1,0	46,8 ±1,0	5,7 ±0,1	9 ±0,4
	2,0	32,1 ±1,3	1,2 ±0,2	40 ±1,9
Ríbes Grossularia L.	0	64,3 ±0,8	1,1 ±0,1	-
	0,5	56,1 ±0,7	4,7 ±0,3	2 ±0,3
	1,0	48,7 ±1,2	2,1 ±0,3	47 ±2,1
	2,0	25,1 ±0,5	1,8 ±0,2	69 ±2,4

[1]The age of donor plants of explants are 45 days

A concentration of 1,0 mg/l is required to activate the growth of axillary buds in Ruben sort blackberry The formed sprouts cluster grows quickly and is further divided into smaller fasciles.

Under low concentrations of cytokinins the regenerants have a developed root system, which make them more suitable morphologically for postaseptic cultivation. Plant organism reaction on cytokinin depended on the plant type. Sprouts with the marked apical dominance were formed in *R. fruticosus* and *R. nígrum* (Fig. 2) under the lowest concentration in the experiment (0,5 mg/l).

The quantity of sprouts per one regenerat in these sorts was close to one: *R. fruticosus* – 1,4 and *R. nígrum* – 1,1. Under the same conditions apical dominance in *R. rubrum* and *R. Grossularia* was not observed and they formed respectively 4,9 and 4,7 sprout rosettes. The concentration increase to 1,0 mg /l enlarged the number of sprouts in the rosettes. At the same time, there was a small part of sprouts with vitrification signs. The largest amount of vitrificated plants (47%) was observed in *R. Grossularia*. In other studied sorts the plants number was 1 - 3%. Further increase in BAP content to

2,0 mg/l enlarged the number of vitrificated plants significantly. The largest amount of vitrificated organisms was observed in *R. Grossularia* - 69%, and the smallest one - in *R. nígrum*.

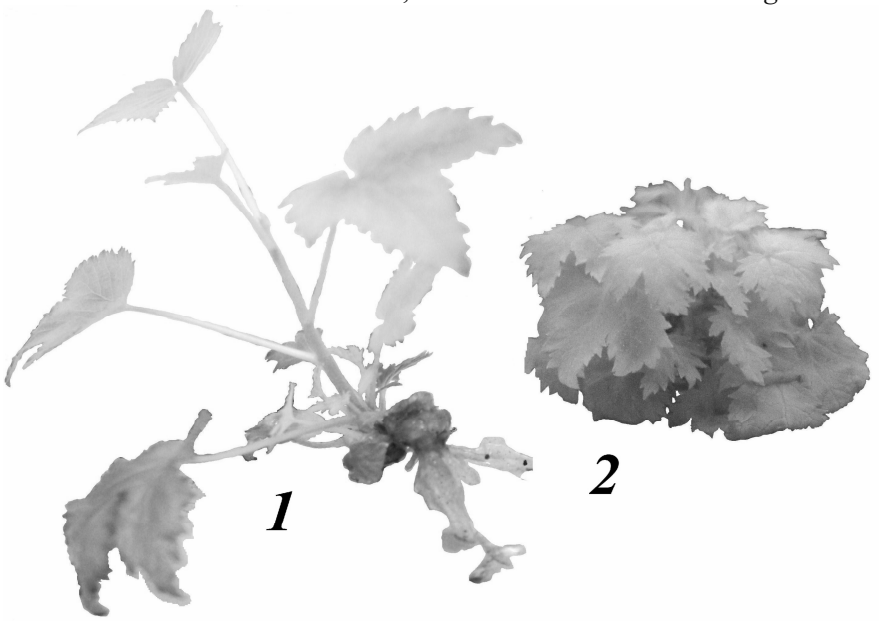

Figure 2. P**lants response to exogenous cytokinin (BAP 0,5 mg/l) depending on the plants species:**
1. *Ríbes nígrum. L.* apical dominance sprout; *2. Ríbes rubrum L.* sprouts rosettes.

Under plants cultivating in the culture medium with a high BAP content (2,0 mg/l) the negative impact was amplified. Thus, *R. fruticosus* regenerantes were of smaller size and formed smaller numbers of sprouts in the rosettes (Fig. 3). Under these conditions *R. Grossularia* regenerats got chlorotic and died within 10-15 days.

Figure 3. E**xogenous cytokinin excessive concentration (BAP 2,0 mg/l) influence underur subcultivation:** 1. *Rubus fruticosus L.*, first passage; 2. *Rubus fruticosus L.*, second passage; 3. *Ríbes Grossularia L.,* second passage.

The negative effect of cytokinins depended on the explants donor mother plants age as well (table 2).

Table 2 – **The regenerants response to excessive[2] exogenous cytokinin depending on the age of explants donor plants**

Donor plant age, days	Vitrificated regenerants, %	
	Rubus fruticosus L.	*Ríbes Grossularia L.*
15	61	98
30	43	74
45	28	69
60	13	27
LSD_{05}	3	7

[2]BAP 2,0 mg/l

Conclusions. Method of rosettes conglomerate formation in basic plants is advisable to use for the propagation of the studied species *in vitro*. Their inductor of their formation is adding BAP in the amounts of 1.0 – 2.0 mg/l in the nutrient medium. Higher concentrations are toxic. Increasing the explants donor age reduced the toxicity.

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Применение цитокининов при клональном микроразмножении ягодных культур

В.В. Мацкевич, Л.Н. Филиппова

Установили, что для размножения *in vitro Rubus fruticosus L.* (сорт Рубен), *Ríbes nígrum L.* (сорт Юбилейная Копаня), *Ríbes rubrum L.* (сорт Йонкер Ван Тетс), *Ríbes Grossularia* (сорт Черномор) целесообразно использовать метод с образованием в исходных растений конгломерата розеток путем добавления в питательную среду БАП (0,5–1,0 мг/л). Концентрация БАП 2,0 мг/л привела к увеличению количества витрифицированных растений (R. grossularia – 69 %). При последующем субкультивировании растений на среде с высоким содержанием БАП (2,0 мг/л) негативное влияние усиливалось. Увеличение возраста исходных растений уменьшало фитотоксичность избыточной концентрации БАП у *Rubus fruticosus L.* и *Ríbes Grossularia L.*

Ключевые слова: витрификация, эксплант, микроклональное размножение, побег, цитокинин, *in vitro.*

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