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THE USE OF 24-EPIBRASSINOLIDE TO INITIATE IN VITRO CULTURES OF HIGHBUSH AND LOWBUSH BLUEBERRY CULTIVARS

O.A. KUDRYASHOVA¹, P.I. SUPRUNYUK¹, A.A. VOLOTOVICH¹, V.A. KHRYPACH²

¹Polessky State University,

Pinsk, Republic of Belarus

²Institute of Bioorganic Chemistry of NAS of Belarus,

Minsk, Republic of Belarus

Introduction. Highbush blueberry (*Vaccinium corymbosum* L.) is perspective and economically significant variety for industrial cultivation under southern climatic zone of Belarus [1]. Micropropagation of *Vaccinium* species is economically sound [2, 3] and is considered as one of the basic intermediate stages of planting stock production in industrial volumes [4]. Micropropagation of plants *in vitro* is possible only after explants sterilization and initiation of new axillary shoot growth *in vitro*. Brassinosteroids are perspective [5] and least studied group of natural hormonal regulators of Ericaceae plant growth. The results of increase of shoot growth activity of highbush and lowbush blueberry cultivar explants *in vitro* in presence of 24-epibrassinolide are presented in the article.

Materials and methods. Researches spent at biotechnological research laboratory of Plant Cell Technologies of Polessky state university from September till December, 2010. As object of researches used explants of 5-year-old 'Blue crop', 'Patriot', 'Jersey', 'Early blue' *in vivo* highbush blueberry and 'Northland' lowbush blueberry plants were taken in September 2010. Stem sections were cut into 3-5 cm pieces, disinfected with 7.5% sodium hypochlorite with 3 ml Tween 20/liter and 20 mg ascorbic acid/liter added, shaken on a rotary shaker for 25 min, and rinsed twice in sterile distilled water for 30 min each time. Stem sections then were cut into 1.0-1.5-cm single node pieces and planted in 224100-mm glass tubes on 5 ml of woody plant medium [6] containing of 15.00 mg N6-(2-isopentenil)adenine (2iP)/liter and 4.00 mg indolil-3-acetic acid/liter with 24-epibrassinolide added in concentration of 0.25, 0.50 or 0.75 mg/liter. As the control were used woody plant medium [6] containing of 15.00 mg/l of N6-(2-isopentenil) adenine (2iP) and 4.00 mg/l of indolil-3-acetic acid without of 24-epibrassinolide. The medium was adjusted to pH 4.8, solidified with 8 g agar/liter, and the tubes were capped with foil caps and autoclaved. The effects of 24-epibrassinolide on 5 genotypes of *V. corymbosum* and *V. angustifolium* were tested under 16-h days, 25°C, 6000 lux illumination and relative humidity of air of 70%. During two months after initial culture, the number of sterile explants with new axillary shoot growth were recorded and new shoots were transferred to woody plant medium containing either of 5.00 mg N6-(2-isopentenil)adenine (2iP) /liter and 1.00 mg indolil-3-acetic acid /liter or 0.5 mg zeatin/liter for continued growth in glass flask with foil caps.

The general mathematical analysis of the data spent on standard methods of variation statistics [7], using STATISTICA 6.0.

Results and discussion. According to the received data (Table), the presence of 0.25, 0.50, 0.75 mg 24-epibrassinolide per liter of woody plant medium containing of 15.00 mg N6-(2-isopentenil)adenine (2iP)/liter and 4.00 mg indolil-3-acetic acid/liter increase the number of new axillary shoots *in vitro* of all investigated cultivars by 3.8-65.0 %.

Table – Number of explants with new axillary shoot growth in 8 weeks after disinfecting and planting on woody plant medium (WPM) containing hormones

Cultivar	24-epibrassinolide concentration, mg/liter of WPM ^Z	Total explants	Number of sterile explants,%	Number of sterile explants with new axillary shoot growth,%
Patriot	0.00 (control)	161	58.0±16.0 ^Y	13.5±4.3
	0.50	132	74.7±18.2 ^X	25.8±0.1 ^X
Northland	0.00 (control)	48	68.0	17.0
	0.50	28	100.0	82.0
Jersey	0.00 (control)	48	87.5	12.5
	0.50	104	90.4	24.0
Early blue	0.00 (control)	43	88.4	11.6
	0.50	67	82.1	17.9
Blue crop	0.00 (control)	57	33.4	15.7
	0.25	43	25.0	16.3
	0.50	44	25.6	20.5
	0.75	58	46.6	34.5

Notes.

^Z WPM containing of 15.00 mg N6-(2-isopentenil)adenine (2iP)/liter and 4.00 mg indolil-3-acetic acid/liter with 24-epibrassinolide added (nor added).

^Y Data presented as mean ± standard error.

^X Data are significant at P<0.05

Thus in the presence of 0.50 mg 24-epibrassinolide/liter the number of explants with new axillary shoots growth were above in 4.80; 1.92; 1.90; 1.54 and 1.31 times for ‘Northland’, ‘Jersey’, ‘Patriot’, ‘Early blue’ and ‘Blue crop’ cultivars, correspondingly.

The effects of brassinosteroids on acceleration of plant growth are noted, but the mechanism of its action is differing in comparison with other phytohormones. Brassinosteroids act on the growth and development of plants like a synergist with respect to other phytohormones, for example to auxins [5]. Brassinosteroids strengthen the reaction of geotropism, promote the xylem differentiation, detain of leaves aging, and raise the stability of plants to stress.

It is interesting the fact of sterile explants number increase in the presence of 24-epibrassinolide consisting of woody plant medium for *in vitro* initiation of plant culture (Table). At the same time the separate researches with *Oidiodendron* ssp. (an endophyte of ericoid mycorrhiza in *Vaccinium* ssp.) isolated on WPM from cultivars under consideration have not established material positive or negative effect of 24-epibrassinolide in analyzed concentrations on growth and development of single microorganism on WPM without any plants (the data are not published).

It were established increasing of number of ‘Blue crop’ sterile explants with new axillary shoot growth with increase of 24-epibrassinolide concentration from 0.25 mg/liter to 0.75 mg/liter consisting of woody plant medium for *in vitro* initiation of plant culture (Table). Thus the increasing of number of ‘Blue crop’ sterile explants with new axillary shoot growth were by 0.6% (in 1.03 times above), 4.8% (in 1.31 times above) and 18.8% (in 2.20 times above), accordingly to raised 24-epibrassinolide concentration.

Conclusions. It is established the law of essential increasing of number of sterile explants with new axillary shoot growth up to in 4.8 times above in the presence of 24-epibrassinolide consisting of nutrient woody plant medium for *in vitro* initiation of high- and lowbush blueberry plant culture.

The revealed effect has applied value, because of reduced terms of *in vitro* culture initiation of any *V. corymbosum* cultivars as well as of reduced terms of its *in vitro* propagation. Besides the amount of WPM components of is essentially reduced that leads to some depreciation of planting stock.

LITERATURE

1. Rupasova, Zh.A. 2007. Highbush blueberry: evaluation of adaptive potential at introduction under conditions of Belarus / Zh.A. Rupasova. – Minsk. – 442 p. (In russian)
2. Sidorovich, E.A. 1996. Clonal micropropagation of fruit-berry plants / E.A. Sidorovich, E.N. Kutas. – Minsk. – 246 p. (In russian)

3. Reshetnikov, V.N. 2007. Some aspects of highbush blueberry and lingonberry micropropagation / V.N. Reshetnikov, T.V. Antipova, V. Philippenya. – Pomiculture. 19:209-216. (In russian)
4. Volotovitch, A.A. 2010. Development and adoption of new technology of accelerated production of *Ericaceae* planting stock on the basis of Poleski State University: IV Sci. Conf. Mater. / A.A Volotovitch [and other]. – Pinsk. I I: 163-165. (In russian)
5. Khripach, V.A.1999. Brassinosteroids. A new class of plant hormones. / V.A. Khripach, V.N. Zhabinskii, A.E. Groot. – San Diego : Academic Press. 450 p.
6. Trigiano, R.N. 2000. Plant tissue culture concepts and laboratory exercises / R.N. Trigiano, D.J. Gray // US/MA, CRC Press LLC. 454 p.
7. Dospheov, B.A. 1985. Field experiment technique. / B.A. Dospheov. – Moscow : Agropromizdat. 351 p. (In russian)

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Summary

Presence of 24-epibrassinolide in concentration of 0.50 mg/ l consisting of agarous, modified WPM medium containing of 15.00 mg N⁶-(2-isopentenil) adenine (2iP) /liter and 4.00 mg indolil-3-acetic acid/liter allows to actively initiate of new axillary shoot growth in vitro by 3.80-65.00% depending on a cultivar genotype. It has been established the increase of number of new initiated 'Blue crop' axillary shoots in vitro with concentration increase of 24-epibrassinolide from 0.25 mg/l till 0.75 mg/l. Thus there was an increase in quantity of new initiated 'Blue crop' shoots in vitro from 0.6% till 18.8%.

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