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THE USEFULNESS OF DIKEGULAC IN PROPAGATION OF HIGHBUSH BLUEBERRY (*Vaccinium corymbosum* L.) 'HERBERT'

Wojciech Litwińczuk* and Agata Prokop

Department of Plant Physiology and Biotechnology
Faculty of Biology and Agriculture
University of Rzeszów
Ćwiklińskiej 2, 35-601 Rzeszów, POLAND
*Corresponding author: e-mail: wlitw@univ.rzeszow.pl

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A B S T R A C T

The experiments were carried out on highbush blueberry 'Herbert' both on *in vitro* cultures and plants *in vivo*. In the case of the *in vitro* study, the modified Zimmerman and Broome (1980) medium was used. For the first subculture dikegulac was tested at a 0.1-10 mg l^{-1} concentration together with 2iP (5 mg·l^{-1}). For the second subculture, dikegulac (1-4 mg l^{-1}) was added both into medium supplemented with 2iP (10 mg·l^{-1}), and into medium without 2iP. In the case of the *in vivo* study, dikegulac (100-1000 mg·l^{-1}) was applied as a foliar spray on four-month old plantlets. Dikegulac (0.1-5 mg·l^{-1}) gradually slowed down the elongation of axillary shoots *in vitro,* in the presence of 2iP at a lower (5 mg·l^{-1}) concentration. It also retarded development of adventitious shoots, while proliferation of axillary shoots was unaffected. Cultures grew very slowly when 2iP was omitted regardless of the concentration of the retardant. Plants sprayed with dikegulac (1000 mg·l^{-1}) solution *in vivo* developed more lateral shoots which were shorter, and the plants had reduced leaf blades. Cuttings collected from plants treated with retardant rooted better, compared with the control. Dikegulac may be useful to keep the germplasm bank and in propagation of highbush blueberry, both *in vitro* and through cuttings.

Key words: growth retardant, micropropagation, axillary shoots, adventitious shoots

INTRODUCTION

Dikegulac is one of the growth retardants. Information concerning the influence of such growth retardants on ericaceous fruit crops is scarce. There are reasons to suppose that growth retardants could be useful in propagation of this genus. Growth retardants may be used to control growth of shoots of ericaceous ornamentals (Banko and Stefani, 1995; Marosz and Matysiak, 2005). Nevertheless, there are few reports about the reaction of ericaceous plants to dikegulac. Banko and Stefani (1995) wrote that dikegulac could reduce shoot elongation with simultaneous stimulation of additional shoot production of several species (not ericaceous). They also observed such an effect on rhododendron but it was not proved statistically. On the other hand, Nowak and Grzesik (1997) reported that azaleas treated with dikegulac produced more shoots compared to the control. If such an effect, like producing more shoots, is achieved in highbush blueberries, it could increase the efficiency of plants as a source of cuttings in traditional propagation. Such an effect could also lower costs of micropropagation thanks to the replacement of expensive cytokinins with the much cheaper dikegulac. The aim of the present study was to determine the influence of dikegulac on *in vitro* cultures and plants *in vivo*, and to assess its usefulness in propagation of highbush blueberry.

MATERIAL AND METHODS

Plant material

The experiments were carried out on highbush blueberry (*Vaccinium corymbosum* L.) 'Herbert', both on *in vitro* cultures, and four-month old plantlets *in vivo*. *In vitro* cultures were established and multiplied through axillary shoots on the modified Zimmerman and Broome (1980) medium supplemented with N^6-[γ,γ-dimethylallyl]adenosine (2iP, 5-10 mg·l^{-1}), adenine sulfate (AS, 80 mg·l^{-1}), indole-3-butyric acid (IBA, 1 mg·l^{-1}), L-cysteine (5 mg·l^{-1}), sucrose (30 g·l^{-1}), pH 5.0, and solidified with Bacto-Difco agar (8.0 g·l^{-1}). Cultures were grown at a temperature of 26 ºC (±1 ºC). Light was provided by cool white fluorescent lamps (OSRAM) at approximately 23 µmol·m^{-2}·s^{-1} with a 16/8 hr photoperiod.

In vitro study

The direct influence of dikegulac (2,3:4,6-Di-O-isopropylidene-2-keto-L-gulonic acid) on *in vitro* cultures was investigated in two subcultures. Dikegulac was dissolved/sterilized in 70 % ethanol and added to the autoclaved medium before its solidification. In the previous experiments, it was found that ethanol in a concentration necessary to sterilize dikegulac did not influence culture development. For the first subculture dikegulac was tested at a 0.1-10 mg l^{-1} concentration together with 2iP (5 mg·l^{-1}). For the second subculture dikegulac (1-4 mg l^{-1}) was added both into the medium supplemented with 2iP (10 mg·l^{-1}), and into me-

dium without 2iP. Ten nodal explants (about 6 mm long and having 2-3 nodes) of axillary origin were placed in a jar. Cultures had been grown for two months *in vitro,* in glass jars (350 ml) with ventilated polypropylene twist lids, filled with 50 ml of the medium.

In vivo study

Both direct influence of dikegulac and its after-effect were examined. The experiments were conducted on four-month old plantlets. They were obtained by rooting of microcuttings (ca 3 cm long) *in vivo*. Lower parts of the microcuttings were dipped in a 50 % ethanol-water solution of IBA (3.0 $g \cdot l^{-1}$). Then, they were rooted in mist chambers in a peat and sand mixture (2:1 v/v; pH = 4.0), watered with the fertilizer "SCOTTS Peters Plant Starter" solution (0.8 $g\ l^{-1}$), and sprayed with the solution of the fungicides Previcur 607 SL (0.15 %) and Rovral Flo 255 (0.15 %). Fully acclimatized plantlets were transplanted to ca 0.2 l pots filled with the same substrate. Plantlets were then sprayed twice (after 4 and 8 weeks) with a dikegulac solution (0, 100, 500, 1000 $mg \cdot l^{-1}$). The solution also contained ethanol (2 $ml \cdot l^{-1}$) and a few drops of Sandovit detergent. Ten days after the second treatment the plants were measured. Then the cuttings were collected and rooted following the same method used with the microcuttings. The plants were grown in a 16h/8h day/night photoperiod under sodium light at 60-100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD and at a temperature of 22-25 °C.

Measurements and statistical analyses

A randomised block design was used in all the experiments. Each treatment consisted of 40 (4 × 10) cultures *in vitro,* and 18 (6 × 3) plantlets, or 50 (2 × 25) cuttings *in vivo*. In the case of the *in vitro* study, the length of the longest axillary shoot from each culture was measured. The number of axillary (AX) and adventitious (AD) shoots was also determined in the *in vitro* study. Next, the ratio of AD shoots was calculated according to following formula: 100 % × number of AD shoots/total number of shoots (both AX and AD ones). In the case of the *in vivo* study the number and length of shoots, length of the biggest leaf blade, and number of rooted cuttings were recorded. Collected data were subjected to an ANOVA, LSD mean separation test at $p = 0.05$ and analysis of regression (more appropriate for quantitative treatments) using Statistica 8.0 computer software. Data presented as percentages (the number of rooted cuttings) were submitted to the test of the difference between two proportions (Statistica 8.0).

RESULTS

In vitro study

All explants placed onto medium supplemented with dikegulac 10 $mg \cdot l^{-1}$ died. Retardant applied in lower doses (0.1-5 $mg \cdot l^{-1}$) did not influence proliferation of axillary shoots (Tab. 1, 2). Dikegulac slowed down the elongation of axillary shoots when cytokinin 2iP was present in a 5 $mg \cdot l^{-1}$

Table 1. The influence of dikegulac on the development of blueberry 'Herbert' *in vitro* cultures on the medium supplemented with 2iP 5 mg l^{-1}

Analysed traits (y)	Concentration of dikegulac [mg·l^{-1}] (x)						Analyses of regression ($y = a + b\ x^{SL}$)
	0	0.1	0.5	1	2	5	
Number of axillary shoots (3-14 mm)	0.7 a[a]	0.5 a	0.4 a	0.7 a	0.7 a	1.0 a	$y = 0.5 + 0.1\ x^{**}$
Number of axillary shoots (> 15mm)	1.0 ab	1.1 b	1.3 b	1.3 b	1.0 ab	0.7 a	$y = 1.2 - 0.1\ x^{**}$
Total number of axillary shoots (> 3 mm)	1.7 a	1.6 a	1.7 a	1.9 a	1.7 a	1.7 a	$y = 1.7 - 7.5 \cdot 10^{-4}\ x^{ns}$
Mean length of axillary shoot [cm]	2.4 a	2.4 a	2.4 a	2.2 a	2.6 a	1.8 a	$y = 2.5 - 0.1\ x^{*}$
Number of adventi-tious shoots	1.5 d	0.9 cd	0.4 abc	0.7 bc	0.2 ab	0.1 a	$y = 0.9 - 0.21\ x^{***}$
Ratio of adventitious shoots in culture[b] [%]	33.7 c	19.9 b	13.4 ab	13.2 ab	6.1 a	3.4 a	$y = 20.9 - 4.25\ x^{***}$
Total number of shoots	3.2 c	2.6 abc	2.1 ab	2.6 bc	1.8 ab	1.8 a	$y = 2.6 - 0.21\ x^{**}$

[SL]level of significance
[a]different letters in the rows indicate significant differences among means for $p < 0.05$
[b]with regard to total number of shoots

Table 2. The influence of dikegulac on the development of blueberry 'Herbert' *in vitro* cultures on the medium supplemented with 2iP 10 mg l^{-1}

Analysed traits (y)	Concentration of dikegulac [mg·l^{-1}] (x)				Analyses of regression ($y = a + b\ x^{SL}$)
	0	1	2	4	
Number of axillary shoots (3-14 mm)	0.8 a	0.6 a	0.6 a	0.9 a	$y = 0.6 + 0.04\ x^{ns}$
Number of axillary shoots (> 15mm)	2.9 a	2.9 a	2.4 a	2.7 a	$y = 2.9 - 0.08\ x^{ns}$
Total number of axillary shoots (> 3 mm)	3.7 a	3.5 a	3.1 a	3.5 a	$y = 3.5 - 0.04\ x^{ns}$
Mean length of axillary shoot [cm]	2.9 a	3.2 a	2.6 a	3.0 a	$y = 2.9 - 0.01\ x^{ns}$
Number of adventitious shoots	2.1 b	1.2 ab	0.1 a	0.3 a	$y = 1.6 - 0.42\ x^{*}$
Ratio of adventitious shoots in culture [%]	17.4 b	6.8 a	3.6 a	6.8 a	$y = 11.9 - 2.06\ x^{*}$
Total number of shoots	5.8 a	4.7 a	3.2 a	3.9 a	$y = 5.1 - 0.46\ x^{ns}$

concentration (Tab. 1). Such a relationship was not proved when 2iP was used in a higher dose (Tab. 2). *In vitro* cultures treated with dikegulac (> 1 mg·l^{-1}) had visibly shortened internodes and more leaves, which were bigger and greener compared to the control (0 mg·l^{-1}). As the concentration of dikegulac increased the development of adventitious shoots decreased. This decrease refers particularly to the shoots which emerged from a small callus at the explant base. This effect was recorded both for media supplemented with lower doses of 2iP and for media supplemented with higher doses (Tab. 1,2). Thanks to this effect, the ratio of adventitious shoots in cultures dropped while the concentration of retardant increased. However, the total productivity of cultures (total number of shoots) was also lowered. Cultures grew very slowly when 2iP was omitted regardless of the dikegulac concentration (data not presented).

In vivo study

Dikegulac applied as a foliar spray also influenced the plants *in vivo*. The higher the dose of retardant was used the treated plants developed more lateral shoots (Tab. 3). New shoots were shorter and had reduced leaf blades. Such differences were especially visible in plants sprayed with the dikegulac (1000 mg·l^{-1}) solution compared to the control. An after-effect of the plant treatment was also found. The cuttings, collected from plants treated with a higher dose of dikegulac, rooted better than control ones (Tab. 4). The relationship between origin of cuttings and branching of obtained plants was unclear. However, the higher the dose of dikegulac applied on mother plants, the longer shoots were observed on newly obtained plants (Tab. 4).

DISCUSSION

The reports on the effect of the application of different growth retardants on ericaceous fruit crops are scarce. To our best knowledge, the influence of dikegulac was not defined. Only Mendoza et al. (2008) described the growth of woody plant *in vitro* cultures treated with dikegulac (5-40 mg·l^{-1}) to the medium. Dikegulac stimulated shoot multiplication of 3 olive cultivars when used with zeatin (1 mg·l^{-1}). An optimal result in number of shoots and nodes was obtained while retardant was applied in 20 mg·l^{-1} concentration. Dikegulac in higher doses did not stimulate additional shoot and node formation. Instead, it resulted in a drastic reduction in shoot height. A similar influence of dikegulac on elongation of shoots and internodes of highbush blueberry was observed in the current study. However, it was more distinct when 2iP was used in a lower dose (5 mg·l^{-1}). Dikegulac also retarded blueberry shoot multiplication. Surprisingly, this result was observed on adventitious shoots, while proliferation of axillary shoots was unaffected. It seems to be a valuable effect as the routine method of multiplication of blueberries and cranberries *in vitro* causes propagation through highly habituated

Table 3. The influence of dikegulac on the growth of blueberry 'Herbert' plants

Analysed traits (y)	Concentration of dikegulac [mg·l^{-1}] (x)				Analyses of regression ($y = a + b\ x^{SL}$)
	0	100	500	1000	
Number of lateral shoots	5.0 a	4.8 a	5.5 a	7.1 b	$y = 4.8 + 2.1 \cdot 10^{-3}\ x^{***}$
Length of lateral shoot [cm]	21.1 b	21.5 b	20.3 b	17.2 a	$y = 21.5 - 3.9 \cdot 10^{-3}\ x^{***}$
Length of leaf [cm]	4.3 b	4.6 b	4.3 b	3.7 a	$y = 4.5 - 6.3 \cdot 10^{-4}\ x^{**}$

Table 4. The relationship between the dikegulac treatment of mother plants, rooting of cuttings and growth of obtained blueberry 'Herbert' plants

Analysed traits (y)	Concentration of dikegulac [mg·l^{-1}] (x)				Analyses of regression ($y = a + b\ x^{SL}$)
	0	100	500	1000	
Number of rooted cuttings [%]	86 a	86 a	88 ab	96 b	$y = 85 + 0.01\ x^{*}$
Number of shoots after one month	1.3 ab	1.5 b	1.5 b	1.2 a	$y = 1.3 - 9.9 \cdot 10^{-5}\ x^{ns}$
Length of shoot [cm]	8.4 a	9.0 a	9.2 ab	10.0 b	$y = 8.3 + 1.5 \cdot 10^{-3}\ x^{**}$

adventitious shoots (madshoots) (Litwińczuk and Wadas 2008, Litwińczuk and Wadas-Boroń 2009). Some reports describing differences in bush growth, flowering and yield among cutting-derived and micropropagated blueberries, might be explained by the adventitious, not the axillary origin of *in vitro* obtained plants. Methods of long storage *in vitro,* or an efficient propagation through axillary shoots exclusively, might be elaborated reducing the risk of somaclonal variation. In the current study, it was found that dikegulac did not work without 2iP thus, unfortunately dikegulac cannot substitute for 2iP during micropropagation of blueberries.

The reactions of highbush blueberry plants to dikegulac were reduction of shoot elongation and stimulation of branching. These reactions seem to be typical and support previous reports on the use of growth retardants in the nursing of ericaceous ornamentals (Banko and Stefani, 1995; Nowak and Grzesik, 1997; Marosz and Matysiak, 2005). The aforementioned authors did not mention whether they studied the rooting of cuttings collected from retardant-treated plants and the growth of obtained plants. Mendoza

et al. (2008) did not find significant differences in rooting among dikegulac-derived olive microshoots and the control ones. The phenotypic variation was also not observed. In the current study, the cuttings gathered from blueberry plants treated with dikegulac, rooted even better and developed longer shoots while compared to the control. Similar effects were noted for chrysanthemum treated with paclobutrazol (Kucharska and Orlikowska, 2008). Such effects may be connected with the retardant-gibberelin antagonism.

To sum up, the described facts support the statement that dikegulac may be useful to keep for the germplasm bank of cultivars. Dikegulac may also be useful for the propagation of highbush blueberry, both *in vitro* and through cuttings. It is also possible that application of retardant could facilitate the combination of biotechnological and conventional methods of propagation as it takes place in the case of strawberry. The well-known facts that retardants enhance flowering of plants and their tolerance to abiotic stresses allow, us to suppose that field performance of plants obtained through the application of dikegulac, might be improved. However, it should be emphasised that the current study was performed only on one cultivar while different reactions of various blueberry genotypes to growth regulators is common. Therefore, the obtained results should be considered as preliminary. The possible application of dikegulac, in practice, demands more detailed and complex studies.

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PRZYDATNOŚĆ DIKEGULAKU W ROZMNAŻANIU BORÓWKI WYSOKIEJ (*Vaccinium corymbosum* L.) 'HERBERT'

Wojciech Litwińczuk i Agata Prokop

S T R E S Z C Z E N I E

Badania prowadzono na kulturach *in vitro* i roślinach borówki wysokiej 'Herbert'. W kulturach *in vitro* zastosowano zmodyfikowaną pożywkę Zimmermana i Broome (1980). W pierwszym pasażu dikegulak był użyty w stężeniu 0.1-10 mg l^{-1} razem z 2iP (5 mg·l^{-1}). W drugim pasażu dikegulak (1-4 mg l^{-1}) był dodany zarówno do pożywki pozbawionej, jak i zawierającej 2iP (0 lub 10 mg·l^{-1}). Dikegulak (100-1000 mg·l^{-1}) w postaci oprysku dolistnego stosowano też *in vivo* na czteromiesięczne rośliny. Dikegulak (0,1-5 mg·l^{-1}) stopniowo spowalniał wydłużanie pędów kątowych *in vitro*, gdy 2iP była obecna w niższym (5 mg·l^{-1}) stężeniu. Hamował także rozwój pędów przybyszowych, podczas gdy proliferacja pędów kątowych pozostała niezmieniona. Kultury rosły bardzo wolno przy braku 2iP niezależnie od stężenia retardantu. Rośliny mateczne opryskane roztworem dikegulaku (1000 mg·l^{-1}) *in vivo* wytworzyły więcej pędów bocznych, które w porównaniu z kontrolą były krótsze i miały zmniejszone blaszki liściowe. Sadzonki pobrane z roślin traktowanych retardantem ukorzeniały się lepiej. Wydaje się, że dikegulak może być pomocny w prowadzeniu kolekcji *in vitro* odmian i rozmnażaniu borówki wysokiej zarówno w kulturach *in vitro*, jak i przez sadzonki.

Słowa kluczowe: retardant, mikrorozmnażanie, pędy kątowe, pędy przybyszowe