

НАЦИОНАЛЬНАЯ АКАДЕМИЯ НАУК БЕЛАРУСИ

ЦЕНТРАЛЬНЫЙ БОТАНИЧЕСКИЙ САД



КУЛЬТУРА БРУСНИЧНЫХ ЯГОДНИКОВ: ИТОГИ И ПЕРСПЕКТИВЫ

*Материалы Международной научной конференции
Минск, 15-19 августа 2005 года*

М и н с к 2 0 0 5

УДК 581.522.4:634.739.3:631.5

ББК

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Культура Брусничных ягодников: итоги и перспективы: Материалы Международной научной конференции. Минск, 15-19 августа 2005 г. – Минск: 2005. – ... с.

Представлены результаты исследований учёных Беларуси, России, Украины, Эстонии, Польши, Словакии, Чехии. В них отражена экологическая проблематика и перспективы развития нетрадиционного ягодоводства, интродукции и селекции, биотехнологии и переработки ягодных растений сем. *Брусничные* в Беларуси и странах ближнего и дальнего зарубежья.

Материалы конференции изданы при финансовой поддержке Белорусского республиканского фонда фундаментальных исследований.

MORPHOGENESIS OF FOUR INTRODUCED VARIETIES OF *VACCINIUM VITIS-IDAEA* L. IN ACEPTIC CULTURE

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Abstract

A thorough studying of morphogenesis has been conducted by us aiming at development of clonal micropropagation technology of introduced varieties of *Vaccinium vitis-idaea*. The research was made on four its varieties (Koralle, Masovia, Ermedank, Erntecröne) using three types of nutrient culture of various modifications.

Key words: Morphogenesis, *Vaccinium vitis-idaea*, aseptical culture.

Introduction

Voluminous literature is dedicated to the tissue of morphogenesis in cells and tissues culture. Its analysis permits to come to a conclusion that morphogenesis is a complicated and multifaceted process depending on type and physiological conditions of an explant (growth, culture composition) i.e. components inherent in growth culture (macronutrients, vitamins, carbohydrates, hormone additives) as well as medium's pH, cultivating conditions and a number of other factors. Numerous experimental researches confirm this (Butenko, 1975).

Material and methods

Different types of explants of above mentioned varieties were used as research objects. As explants served epicotyl, hypocotyl, cotyledons root, leaves of juvenile plantlets which were grown by us earlier in aseptical conditions on Andersons on Anderson's modified nutrient culture (Sidorovich, Kutas, 1991) as well as young shoot buds of adult mother plant.

Dipped in 70-degree ethyl alcohol and irrigated in three changes of sterile distilled water (15 minutes each), buds with 3-4 mm long stem pieces were sterilized in 0,1 per cent solution of diacid for 10 minutes. Sterile materials (buds, epicotyl, hypocotyl, cotyledons, leaves, roots, were transplanted into retorts of equal volume (containing 15 ml of medium each) on three nutrient cultures: of Murashige-Skoog, WPM, and of Anderson. Each medium was represented by several modifications differed in concentration of microsalts and macrosalts, combination of hormone additives and other components (Table 1). The explants transplanted were cultivated under the following conditions: temperature 26°C, air humidity 56%, photoperiod 16 h, illumination degree 4000 lx.

Results and discussion

After 5 weeks vegetation shoots developed out of all varieties of *Vaccinium vitis-idaea*. On replanting on the fresh nutrient culture proliferation of new shoots of 3rd-4th order was observed. In four cultivating weeks 5-10 microshoots, on the average, developed out of each micrograft, depending on nutrient medium composition (Table 2).

Table 1. Composition of Modified Nutrient Mediums Used for Studying of Morphogenesis of Introduced varieties of *Vaccinium vitis-idaea*

Component of Nutrient Mediums (in mg/l)	Medium Modification Number									
	1	2	3	4	5	6	7	8	9	10
Macrosalts according to MS	CR*	-	0,5	-	-	0,5	0,5	-	-	-
			MS			MS	MS			
Microsalts according to MS	CR	-	0,1	-	-	0,5	0,1	-	-	-
			MS			MS	MS			
Macrosalts according to WPM	-	CR	-	-	-	-	-	CR	-	-
Microsalts according to WPM	-	CR	-	CR	-	-	-	CR	-	-
Macrosalts according to Anderson	-	-	-	-	CR	-	-	-	-	CR
Microsalts according to Anderson	-	-	-	-	CR	-	-	-	-	CR
Mesoinosit	100	100	100	100	100	100	100	100	100	100
Sulfat adenin	-	80	80	80	80	40	40	80	80	80
Tiamin (B ₁)	0,4	-	-	0,4	-	0,1	0,1	0,4	0,1	0,1
Pyridoxin (B ₆)	-	-	-	-	0,4	-	-	-	-	-
Indolilacetic acid (IAA)	1,0	5	-	2	1	1,5	2,5	4	4	4
Benzilaminopurin (BAP)	-	-	-	-	-	2	-	-	-	-
Gibberellic acid (GA)	-	4	-	-	-	-	-	-	-	-
Isopenteniladenin (2-iPA)	10	10	2	5	2	-	10	15	15	15
Sucrose, g/l	20	20	20	20	20	20	20	30	30	30
Agar, g/l	6	6	6	6	6	6	6	6	6	7
pH	4,0	4,0	4,0	4,0	4,0	4,0	4,0	4,0	4,0	4,0

Note: *CR – Complete Rate

Table 2. Shoot formation of *Vaccinium vitis-idaea* as subject to nutrient medium composition

Medium Modification nr.	Amount of shoots per one plant				Note
	Koralle	Masovia	Erntedank	Erntekrone	
1	8,5±1,2	7,9±2,0	8,0±1,0	7,6±1,5	
2	7,5±1,5	7,0±2,0	7,8±1,4	7,4±1,3	
3	2,0±1,0	2,5±1,5	2,9±0,0	2,4±0,0	Shoots with prolonged internodiums
4	3,3±1,5	5,0±1,0	4,5±1,2	5,0±2,0	Big leaves shoots
5	5,5±1,0	5,0±1,2	5,4±2,0	4,1±1,1	
6	1,0±1,0	0,9±0,2	1,1±0,5	1,7±1,2	
7	1,5±1,9	1,8±1,3	1,0±0,0	1,9±1,0	
8	15±2,0	14±1,3	15,2±2,7	41,7±1,9	
9	16±2,5	15±3,2	16,3±2,3	15,5±2,7	

Out of all a medium types investigated the most active sprout shooting was observed in WPM (No.8) and Anderson (No.9, Table 2) mediums containing the composition of macro and microsalts with the following additives (in mg/l): mesoinosit – 100, sulphat adenin – 80, tiamin – 0,4; indolilacetic acid – 4, isopenteniladenin – 4, sucrose – 30 g/l, agar, - 6 g/l, pH medium 4,0 (Table 1). This fact testifies that one can achieve a high morphogenesis level by changing quantity and proportion of components in nutrient medium. In the case given it was a success to

activizise the development of meristems axillaris by concellation of apical dominations and to have regenerants.

After 4-5 passages the rhyso-genesis was observed with all micrografts transplanted sprout shooting in No.8 and No.9 mediums. It was not observed in mediums of other modifications and proves universality of these mediums for both morphogenetical processes: sprout shooting and rhyso-genesis.

Despite of the fact that rhyso-genesis is mostly induced with regenerants after their replanting on rhyso-genesis-enabling medium, in this case rootage of regenerants of introduced varietis of *Vaccinium vitis-idaea* on the sprout shooting medium allows us to suppose that regenerants of these varieties contain enough internal auxin able to promote rooting without replantation on a specific rhyso-genesis medium supplemented with exogenous auxin with other explants (epicotyl, hypocotyl cotyledons, root, leaves) the organogenic callus developed after 5-6 week of cultivation followed by regenerating of vegetation shoot out of it.

It should be pointed out that the development of organogenetic callus and shoot regeneration followed are feature of explants (epicotyl, hypocotyl, cotyledons, root, leaves) grown out of freshlycollected seeds. Sprouts shooting with the explants grown out of seeds stratified occurred immediately out of explant tissue, without callusing. It can be assumed that could connect to a different proceeding of physiological, biochemical, cytological etc., processes with the explants of freshly collected and stratified seeds, as well as to a different content of internal phytohormones.

Conclusions

Thus the principal was shown to regenerate *Vaccinium vitis-idaea* by two methods: meristem axillaris activation, though callus proiferation and shoot formation followed, obtained by the study of morphogenesis proceeding with its explants and cells in tissue culture on various nutrient mediums (9 modifications). It should be pointed out it conclusion that the research results are used by us in clonal micropropagation technology development, obtained during the study of morphogenesis of various explants of introduced varieties of *Vaccinium vitis-idaea* on modified nutrient mediums.

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МОРФОГЕНЕЗ ЧЕТЫРЕХ ИНТРОДУЦИРОВАННЫХ СОРТОВ *VACCINIUM VITIS-IDAEA* L. В АСЕПТИЧЕСКОЙ КУЛЬТУРЕ

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Резюме

Приведены результаты экспериментальных исследований морфогенеза четырех сортов брусники обыкновенной (Koralle, Masovia, Erntedank, Erntekönig) в культуре *in vitro* на трех типах питательных сред девяти модификаций.

Показано, что из всех исследованных типов сред наиболее активное побегообразование и ризогенез наблюдали на среде WPM (№ 8) и Андерсона (№ 9), содержащей полный состав макро- и микросолей со следующими добавками (в мг/л): мезоинозит – 100, аденин сульфат – 80, тиамин – 0,4, индолуксусная кислота – 4,0, изопентениладенин – 15, сахароза – 30 г/л, агар – 6г/л, pH среды – 4,0. Это служит доказательством универсальности этих типов сред для обоих морфогенетических процессов: побегообразования и ризогенеза. На основании изучения морфогенеза, протекающего у эксплантов *Vaccinium vitis-idaea* в асептической культуре, на различных типах питательных сред, показана принципиальная возможность регенерировать ее двумя методами: 1) путем активации пазушных меристем, 2) через пролиферацию каллуса и последующее образование из него побегов.

Результаты исследований, полученные при изучении морфогенеза у различных типов эксплантов четырех интродуцированных сортов брусники обыкновенной на модифицированных питательных средах, использованы нами при разработке технологии клонального микроразмножения данных сортов.