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Regeneration potential of the introduced varieties of *Vaccinium corymbosum* L. on the different modifications of nutrient media

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Abstract

The results of experimental studies of the influence of 18 different modifications of nutrient media on the regeneration potential of 14 introduced varieties of highbush blueberry are presented. It is shown that the highest regeneration potential for all 14 varieties, without exception, is characteristic for media of two modifications (8th for WPM and 9th for Anderson). These two modifications of nutrient medium are used for clonal micropropagation of introduced varieties of highbush blueberry

Keywords: regeneration potential, nutrient media, highbush blueberry, aseptic culture.

Introduction

The nutrient medium is that substrate on which all morphogenetic and regenerative processes proceed, that characteristic for the explant, which was introduced into the culture *in vitro*. A large number of publications have been devoted to the selection and optimization of the composition of nutrient media. Their analysis allows us to conclude that the success of clonal micropropagation of plants dependson the presence in the nutrient medium of components capable of causing the regeneration of plants from the explant tissue.

In the first stage of micropropagation, in order to preserve the viability of explants, one of the prerequisites is the addition of antioxidants to the nutrient medium, which help prevent the activation of hydrolytic enzymes and the death of explants.

After survival of explants on nutrient medium, the researcher faces the task of causing certain morphogenetic processes, for example, callusogenesis (Soroka, 2004; Flinn and Webb, 1986;Duangsee and Bunnag, 2014), formation of somatic embryoids(Rugini, 1984; NealandTopoleski, 1983;Kakarlaet al.,2014; Pranita and Pandhure, 2016), regeneration of shoots directly from explant tissue (Lin et al., 1997;Khurana-Kaulet al., 2010; Verma et al., 2011; Afrasiab et al., 2017), differentiation of buds in callus or explant tissues and other processes.

In this connection, it is necessary to introduce hormonal compounds, nitrogen salts, non-hormonal substances (vitamins, amino acids, casein hydrolyzate) into the nutrient medium.

The necessity of explant for these or other factors, capable of induce of shoot regeneration for each plant species is established experimentally (Hildebrandt et al., 1946; Goodwin, 1966;Anderson, 1975; Chu et al., 1975; Delfel and Smith, 1980;Albrecht, 1986; Drew, 1987; Singhaet al., 1987; Freytaget al., 1988; Gertsson, 1988; Pasqua et al., 2002; Sharadet al., 2004; Romano, 2005; Parthibhan, 2012; Noreldaim, 2012; Wiszniewska et al., 2013; Aruna et al., 2012; Shirin et al., 2015;Mohammadiet al., 2017).

Materials and Methods

We conducted experimental investigations on the study of the regeneration potential of 14 introduced highbush blueberry varieties ('Bluecrop', 'Blueray, 'Dixi', 'Herbert', 'Rancocas', 'Covill', 'Earlyblue', 'Scammel', 'Atlantic', 'Concord', 'Tifblue', 'Woodart', 'Delite', 'Stanley') on 4 types of culture media: Murashige-Skooga (MS), WPM (medium for woody plants), Anderson, Lyrene, represented by 18 modifications (Table 1).

As primary explants we used buds of young, newly blossoming shoots. The material was sterilized in 0.1% silver nitrate solution. The calculation of the number of regenerants per explant was carried out after the second subcultivation. The results of the experimental data are presented in Table 2. The figures in the table are from two replications (for each replication of 10-15 explants).

Results and Discussion

Analysis of the experimental material showed that the regeneration potential of the introduced species of highbush blueberry is depending on the modification of the medium, in other words, on its composition, on the components, which are presented in it.

Regeneration was absent in all studied varieties on medium of the 12th modification containing macro- and microelements, Lyrene vitamins. Similarly, varieties behave on nutrient media of the 3rd, 6th, 7th modification. Of the 5 modifications with Lyrene salts (6th, 7th, 12th, 15th, 16th), the most favorable was the medium of the 15th modification (Table 1), since regeneration was observed in all varieties without exception (Table 2).

Of the three modifications of nutrient media (1st, 3rd, 10th), based on the Murashige-Skooga medium, on the medium of the 1st and the 10th modification the vast majority of highbush blueberry varieties regenerated the shoots.

Investigated by us 4 modifications of nutrient media (5th, 9th, 14th, 18th) on the base of Anderson medium give grounds to believe that the medium of the 9th modification is optimal for regeneration of the introduced varieties of highbush blueberry, since the regeneration potential amounted to 2 from the 'Woodart' variety to 7 from the 'Dixi' variety of regenerants per explant.

From the investigated 6 modifications of nutrient media according to WPM (2nd, 4th, 8th, 11th, 13th, 17th) for successful shoot regeneration, by optimal occured the medium of the 8th modification, on which the regeneration potential is equal an average of 4 regenerants per explant.

Conclusion

Thus, from the investigated 18 different modifications of nutrient media, only on media of two modifications (8th in WPM and 9th in Anderson), was noted the highest regeneration potential for all 14 varieties without exception. Consequently, these two modifications of nutrient media (WPM 8th and Anderson 9th modification) are useful for clonal micropropagation of introduced highbush blueberry varieties.

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Table 1. Composition of nutrient media used for investigation of the regeneration capacity of introduced varieties*Vaccinium corymbosum* L.

Component, mg/l	Modification of the media																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Salts and vitamins for MS	+	-	1/2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Salts and vitamins for WPM	-	+	-	-	-	-	-	+	-	-	+	-	+	-	-	-	+	-
Salts and vitamins for Anderson	-	-	-	-	+	-	-	-	+	-	-	-	-	+	-	-	-	+
Salts and vitamins for Lyrene	-	-	-	-	-	+	+	-	-	-	-	+	-	-	+	+	-	-
Mesoinositol	100	100	100	100	100	100	100	100	100	80	80	80	80	80	100	80	100	100
Adenine sulfate	-	80	80	80	80	40	60	80	80	60	60	40	60	-	80	60	-	-
Indolylacetic acid	1,0	5,0	-	2,0	2,0	1,5	2,5	4,0	4,0	5,0	5,5	5,0	-	-	4,0	1	1	1
Gibberelic acid	-	4,0	-	-	-	-	-	-	-	-	-	1,5	2,0	-	-	-	-	-
Naphtylacetic acid	-	-	-	-	-	-	-	-	-	-	-	2,0	1,0	2,0	-	-	-	-
Benzylaminopurine	-	-	-	-	-	2,0	-	-	-	-	-	-	5,0	4,0	-	-	-	-
Isopenteniladenine	10	10	2,0	5,0	4,0	-	10	15	15	20	25	20	-	-	15	5	5	5
Saccharose, g/l	20	20	20	30	30	20	20	30	30	20	25	25	25	25	30	20	30	30
Agar, g/l	9	9	9	9	9	9	9	9	9	9	9	9	99	9	9	9	9	9
pH	4,8	4,8	4,8	4,8	4,8	4,8	4,8	4,8	4,8	4,8	4,8	5,8	5,8	4,8	4,8	4,8	4,8	4,8

Note: << + >> - the component is present in the medium; << - >> - the component is absent in the medium; ½ is the half dose of the component in the medium.

Table 2.Regeneration potential of introduced *Vaccinium corymbosum* L. varieties on media of various modifications.

Variety	Modification of the media																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
	Quantity of regenerants per explant																	
'Bluecrop'	1±0	2±0	-	1±0	1±0	-	1±0	3±0	4±1	1±0	-	-	-	1±0	2±0	1±0	1±0	1±0
'Blueray'	1±0	2±0	-	1±0	1±0	-	-	3±1	3.5±1	1±0	-	-	-	1±0	2±0	1±0	1±0	1±0
'Dixi'	1±0	3±0	-	1±0	1±0	-	1±0	6±2	7±1	3±1	1±0	-	-	1±0	2±0	1±0	2±0	2±0
'Herbert'	1±0	2,5±0	1±0	1±0	1±0	-	-	4±1	5±1	1±0	1±0	-	1±0	1±0	1±0	1±0	2±0	1±0
'Rancocas'	1±0	1,5±0	-	1±0	1±0	-	-	5±1	4±1	1±0	1±0	-	-	-	1±0	1±0	2±0	2±0
'Covill'	1±0	2±0	-	1±0	1±0	-	-	4±1	4±1	1±0	1±0	-	1±0	-	1±0	1±0	1±0	1±0
'Earlyblue'	1±0	1±0	-	1±0	1±0	1±0	-	3±1	4±1	1±0	-	-	1±0	-	2±0	1±0	1±0	1±0
'Scammel'	1±0	1±0	-	1±0	1±0	-	-	3±1	4±1	1±0	1±0	-	-	-	1±0	1±0	1±0	1±0
'Atlantic'	1±0	3±0	-	1±0	1±0	-	-	4±1	3±1	-	-	-	1±0	-	1±0	-	1±0	1±0
'Concord'	1±0	3,5±1	1±0	1±0	1±0	-	-	5±1	5±1	-	-	-	-	-	1±0	-	1±0	1±0
'Tifblue'	-	1±0	-	1±0	1±0	-	1±0	3±1	4±1	-	-	-	-	-	1±0	-	2±0	1±0
'Woodart'	-	1±0	-	1±0	1±0	-	-	3±1	4±1	1±0	1±0	-	-	1±0	1±0	-	1±0	2±0
'Delite'	-	1±0	-	1±0	1±0	-	-	4±1	4±1	-	-	-	1±0	-	1±0	-	1±0	2±0
'Stanley'	-	1±0	-	1±0	1±0	-	-	3±1	3±1	-	1±0	-	-	-	1±0	-	1±0	1±0

Note: << - >>– no regeneration.

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