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The influence of retardants on the reduction of growth rate and the preservation of viability of sterile cultures

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Abstract

The paper presents the results of experimental studies on the effect of different concentrations of chlorcholinchloride and abscisic acid on the activation of the meristem, the growth rate and viability of regenerants of sterile cultures of 6 varieties of highbush blueberry and 3 varieties of cranberry ordinary. It was shown, that activation of meristems, the growth rate and viability of regenerants of examined varieties dependent on retardants, contained in the nutrient medium, their concentrations and origin of plants.

Keywords: highbush blueberry, cranberry ordinary, retardants, viability, sterile culture.

Introduction

In world practice for many years to maintain a collection of sterile cultures there were used constant growing of them under optimal conditions for these plants. However, it occurred, that such cultivation has some significant disadvantages: firstly, the possibility of somaclonal variation (due to violations of the genetic stability of often transplanted material); secondly, the risk of contamination with foreign genetic material and its random loss of own genetic material; Third, the complexity of the processes (the need of regular transplantation on the fresh medium); fourthly, the high cost of components of nutrient medium, required for frequent transfers.

Taking into account the duration of conservation of sterile cultures, Engelmann and Engels (2002) isolated two main categories of their storage: slow growth and cryopreservation (storage in liquid nitrogen).

Deceleration of growth of the culture can be accomplished by lowering of temperature of cultivation (Dale, 1980; Henshaw et al., 1985; Ruredzo& Hanson, 1991; Dorion et al., 1994; Hae-Boong et al., 1996; Samarina et al., 2014; Ali et al., 2016), in combination with low illumination or darkness (Mullin and Schlegel, 1976), changes in the concentration of carbonhydrate and mineral composition of the nutrient medium (Ng and Ng, 1991; Samsonova, 1991; Vysotskaya, 1994; Homes et al., 1982; Rubluo and Kartha, 1985; Rolland et al., 2006), a decrease in oxygen in culture vessels (Brindgen and Staby, 1981; Muromtsev et al., 1990; Winthers, 1979; Brindgen and Staby, 1983; Dorion et al., 1994), the induction of osmotic stress (Henshaw et al., 1980; Ng and Ng, 1991; Ruredzo and Hanson, 1991; Tukai et al., 1988; Codaccioni and Vescovi, 1987; Schilde-Renther et al., 1982; Wanas et al., 1986; Pua and Chong, 1985; Rahman et al., 2010; Ciobaniui and Constantinovici, 2012), the use of retardants (Westcott, 1981; Cathey, 1964; Zadontsev et al., 1973; Chailakhyan, 1967; Kende et al., 1963).

The main obstacle in storage of regenerants of introduced varieties of cranberries, blueberries, rhododendrons is their rapid aging, leading ultimately to the death of plants. To prevent this process, you should regularly, every 2-3 weeks, transplant regenerated on fresh nutrient media.

In our opinion, one way of solving this problem is the deceleration of growth of regenerants with the help of retardants.

As the review of the literature on the subject showed, the information relating to the influence of retardants on viability of regenerants viable in culture in vitro, are single. (Vysotsky, 1994; Salmatova et al., 2009; San José et al., 2010).For introduced varieties of high bush blueberry, cranberry ordinary they are absent at all.

The aim of research was to study of the effect of different concentrations chlorcholinchloride (CCC) and abscisic acid (ABA) on the viability of introduced varieties of highbush blueberry and cranberry ordinary in the culture *in vitro*.

Materials and Methods

As objects of study we used six introduced varieties *Vaccinium corymbosum* L. highbush blueberry ('Bluecrop', Blueray, 'Dixi', 'Herbert', 'Rancocas', 'Scammel') and introduced three introduced varieties of *Vaccinium vitis-idaea* L. cranberry ordinary ('Koralle', 'Masovia', 'Erntedank'), regenerated in culture *in vitro* by method of activation of axillary meristems. Experiments were performed with chlorcholinchloride (CCC) (concentration of 2 mg $\cdot L^{-1}$) and abscisic acid (ABA) (concentration of 8, 10 mg $\cdot L^{-1}$) in a nutrient medium WPM (Lloyd and McCown, 1981) at 16 h photoperiod, illumination 4000 lux ,at a temperature 25°C.

During the experiments, the plants were cultivated without transplantation on fresh nutrient medium for 12 months. Viability (the term "viability" is used by us as a concept characterizing the potential duration of the life of plants in sterile culture in long-term cultivation them without renovation of culture media) of the material was evaluated by our developed method, comprising the five-point scale, according to which 0 points - the death of the plant, 1-3 - an intermediate state, 4 - maximum viability, as well as took into account the activation of meristems and rate of shoot growth. Data are taken after 2, 4 and 12 months of cultivation. Experimental data are shown in Table 1-2. The figures in the tables is the average values for the 10 plants of each variety.

Results and Discussion

An analysis of the material presented in the Table 1, during the first 2 months of cultivation of regenerants on a nutrient medium containing in one case 2mg $\cdot L^{-1}$CCC(medium № 1) in the other case − 8 mg $\cdot L^{-1}$ABA (medium № 2), the third case − 10 mg $\cdot L^{-1}$ABA (medium№ 3), the viability of all varieties, without exception, was high and composed 4 points on all media.

On medium, containing CCC plants had an intense green color of leaves and shortened internodes compared to control plants, that were grown on medium containing ABA and CCC. By the end of the first month of cultivation they ended their growth, and by the end of the fourth month − they died (control plants), because they were not transplanted on fresh medium.

Further observations of the plants, cultivated on medium, containing retardants, showed that after 4 months of cultivation, growth of regenerants is not stopped, since shoot length was increased, slightly was decreased index of viability(an average of 0,5 points). Maximum viability was preserved by 2 blueberry varieties ('Blueray' - 3,49 points, 'Scammel' - 3,95) and by all varieties of cranberry (Table 1). On the medium number 1, containing 2 mg $\cdot L^{-1}$CCC; by 2 blueberry varieties ('Dixi' - 3,64 points, 'Herbert' - 3,59) in a medium № 2, containing 8 mg $\cdot L^{-1}$ABA, and by 2 varieties of blueberry ('Bluecrop' - 3,51, 'Rancocas' - 3,81) in a medium № 3, containing 10 mg $\cdot L^{-1}$of ABA.

After 12 months of cultivation viability index decreased significantly. However, plants are not died, they had an intermediate index of viability of 1,15-3,21 points.

Relatively high point of viability numerically for varieties 'Bluecrop' (1,24 points), 'Scammel' (1,91) and for 2 varieties of cranberry ('Koralle' - 3,21 and 'Erntedank' - 3,16 points) was observed in the medium№ 2, containing 8 mg $\cdot L^{-1}$ABA, and for other varieties ('Blueray' - 1,37, 'Dixi' - 1,39, 'Herbert' - 1,45, 'Rancocas' - 1,51, 'Masovia' - 3,18 points) - to the medium№ 3, containing 10 mg $\cdot L^{-1}$ABA (Table 2).

Table 1.Effect of content of chlorcholinchloride (CCC) and abscisic acid (ABA) on the growth and viability (V) of grades *Vaccinium corymbosum* L. and *Vaccinium vitis-idaea* L. in culture *in vitro*

Variety	2 months of cultivation					
	medium № 1 (CCC, 2 mg · L^{-1})		medium № 2 (ABA, 8 mg · L^{-1})		medium № 3 (ABA, 10 mg · L^{-1})	
	shoot lengthon 1 plant, cm	V, point	shoot length on 1 plant, cm	V, point	shoot lengthon 1 plant, cm	V, point
Vaccinium corymbosum						
'Bluecrop'	0,39±0,17	4,00±0,00	0,24±0,03	4,00±0,00	0,45±0,27	4,00±0,00
'Blueray'	0,47±0,11	4,00±0,00	0,41±0,21	4,00±0,00	0,56±0,29	4,00±0,00
'Dixi'	0,34±0,19	4,00±0,00	0,31±0,17	4,00±0,00	0,41±0,21	4,00±0,00
'Herbert'	0,41±0,21	4,00±0,00	0,36±0,16	4,00±0,00	0,39±0,17	4,00±0,00
'Rancocas'	0,46±0,24	4,00±0,00	0,42±0,24	4,00±0,00	0,63±0,31	4,00±0,00
'Scammel'	0,39±0,07	4,00±0,00	0,37±0,19	4,00±0,00	0,49±0,16	4,00±0,00
Vaccinium vitis-idaea						
'Koralle'	1,01±0,36	4,00±0,00	0,96±0,27	4,00±0,00	1,17±0,41	4,00±0,00
'Erntedank'	1,19±0,74	4,00±0,00	1,01±0,21	4,00±0,00	1,29±0,38	4,00±0,00
'Masovia'	1,24±0,39	4,00±0,00	1,03±0,53	4,00±0,00	1,37±0,31	4,00±0,00
Variety	4 months of cultivation					
	medium № 1 (CCC, 2 mg · L^{-1})		medium № 2 (ABA, 8 mg · L^{-1})		medium № 3 (ABA, 10 mg · L^{-1})	
	Shoot lengthon 1 plant, cm	V, point	Shoot lengthon 1 plant, cm	V, point	shoot length on 1 plant, cm	V, point
Vaccinium corymbosum						
'Bluecrop'	1,07±0,51	3,47±0,51	1,01±0,30	3,41±0,29	1,24±0,25	3,51±0,29
'Blueray'	1,29±0,54	3,49±0,65	1,17±0,29	3,37±0,16	1,37±0,39	3,29±0,31
'Dixi'	1,34±0,36	3,41±0,57	1,26±0,35	3,64±0,51	1,51±0,57	3,44±0,67
'Herbert'	1,37±0,44	3,29±0,56	1,29±0,35	3,59±0,63	1,44±0,65	3,47±0,86
'Rancocas'	1,53±0,47	3,50±0,05	1,41±0,47	3,71±0,66	1,66±0,64	3,81±0,51
'Scammel'	1,35±0,57	3,95±0,07	1,35±0,29	3,39±0,44	1,43±0,66	3,55±0,55
Vaccinium vitis-idaea						
'Koralle'	1,89±0,27	3,97±0,91	1,74±0,37	3,85±0,45	2,01±0,55	3,91±0,84
'Erntedank'	1,94±0,71	3,84±0,67	1,81±0,44	3,74±0,17	2,17±0,57	3,80±031
'Masovia'	1,93±0,58	3,89±0,56	1,88±0,65	3,71±0,75	2,21±0,79	3,85±0,26

Table 2.The viability of introduced varieties of *Vaccinium corymbosum* L. and *Vaccinium vitis-idaea* L. on various nutrient media after 12 months of cultivation

Variety	medium № 1 (CCC, 2 mg · L^{-1})		medium № 2 (ABA, 8 mg · L^{-1})		medium № 3 (ABA, 10 mg · L^{-1})	
	shoot lengthon 1 plant, cm	V, point	Shoot lengthon 1 plant, cm	V, point	Shoot lengthon 1 plant, cm	V, point
Vaccinium corymbosum						
'Bluecrop'	3,15±0,51	1,15±0,29	3,01±0,28	1,24±0,25	3,27±0,28	1,22±0,51
'Blueray'	3,74±0,63	1,21±0,30	3,52±0,19	1,35±0,38	3,91±0,33	1,37±0,57
'Dixi'	2,97±0,52	1,19±0,37	2,01±0,10	1,19±0,54	3,41±0,56	1,39±0,36
'Herbert'	3,08±0,56	1,26±0,40	2,84±0,15	1,39±0,34	3,94±0,50	1,45±0,51
'Rancocas'	3,51±0,50	1,34±0,46	3,45±0,66	1,47±0,40	3,51±0,47	1,51±0,60
'Scammel'	3,24±0,40	1,51±0,57	3,15±0,51	1,91±0,52	3,75±0,56	1,84=0,63
Vaccinium vitis-idaea						
'Koralle'	4,29±0,84	3,15±0,50	4,15±0,71	3,21±0,57	4,81±0,87	2,98±0,45
'Erntedank'	4,75±0,67	2,74±0,44	4,63±0,63	3,16±0,50	4,29±0,75	2,84±0,31
'Masovia'	4,79±0,70	3,01±0,60	4,81±0,72	3,07±0,49	4,67±0,79	3,18±0,38

The highest viability as a result of direct cultivation of material on the fresh nutrient media for 12 months was marked for variety of cranberries 'Koralle' (3,21 points) on a medium № 2, containing 8 mg · L^{-1}ABA (Table 2) and the lowest viability (1,15 points) for a variety of blueberry 'Bluecrop' № 1 on the mediun № 1, containing 2 mg · L^{-1} CCC. The other varieties have taken an intermediate position on this index.

This is an evidence of depending of viability both, variety accessory of material, and the inhibitor, contained in the nutrient medium.

The optimal variant for long-term cultivation in the 12 months of studied varieties of highbush blueberry ('Bluecrop', 'Blueray', 'Dixi', 'Herbert', 'Rancocas', 'Scammel') should be regarded as media№ 2 and №3, containing ABA (8 and 10 mg· L^{-1}). In this case, the ABA had an inhibitory effect on the growth rate of highbush blueberry, thereby preventing aging, which resulted in a high index of viability.

For long-term cultivation of studied varieties of cranberry ordinary are optimal media № 1-3, comprising CCC (2 mg· L^{-1}) and ABA (8 and 10 mg· L^{-1}) (Table2).

Comparative analysis of the viability of highbush blueberry and cranberry ordinary varieties showed, that it is higher in cranberries varieties regardless of inhibitor present, containing in the nutrient medium. This is probably due to the functional features, inherent to the evergreen shrubs, to which cranberry belongs.

Conclusion

The experimental results suggest to conclude, that the addition of inhibitors of growth (chlorcholinchloride and abscisic acid) into the nutrient medium, helps to reduce the rate of growth, prevention of aging and provision of the viability of the studied introduced varieties of highbush blueberry ('Bluecrop', 'Blueray', 'Dixi', 'Herbert', 'Rancocas', 'Scammel') and introduced varieties cranberry ordinary ('Koralle', 'Masovia', 'Erntedank') for 12 months without transplantations them on fresh nutrient medium. It is necessary to take into account the variety and species specificity of the studied plants, clearly manifested by the action of inhibitors and also selective action of inhibitors themselves.

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