



The influence of hormone additions on regenerative potential of *Rhododendron luteum* Sweet, introduced varieties of *Vaccinium corymbosum* L., *Vaccinium vitis-idaea* L. in sterile culture

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Abstract

The paper presents data on the regenerative capacity of *Rhododendron luteum*, introduced varieties of *Vaccinium corymbosum* and *Vaccinium vitis-idaea* in sterile culture. It was shown the dependence of the regenerative potential of the examined plants on the content of hormone additions in the nutrient medium and plant genotype. Variety 'Red Pearl' of *V. vitis-idaea* has maximum regenerative potential (7.70 shoots per explant) on the medium Anderson, containing 4 mg L⁻¹ IAA and 15 mg L⁻¹ 2iP. Variety 'Elizabeth' of *V. corymbosum* has minimum regenerative potential (1.40 shoots per explant) on the medium WPM. And variety 'Ammerland' of *V. vitis-idaea* has minimum regenerative potential (1.20 shoots per explant) on the medium Anderson (A) at the content of the same amount of hormones in the media (1 mg L⁻¹ IAA and 5 mg L⁻¹ 2iP).

Keywords: sterile culture, regulators of growth, regenerative capacity, *Rhododendron luteum*, *Vaccinium corymbosum*, *Vaccinium vitis-idaea*.

Introduction

Plant regeneration is the nodal point in the whole methodology of culture cells and tissues. There is no sense of research in culture in vitro without plant regeneration, because the final stage of this work is ultimately the regeneration of plants. That's why this issue is devoted to a huge number of publications in which the authors present the results of studies obtained in the investigation of factors that influence on this process.

Issue of depending on the banana regeneration from ratio of hormones in the nutrient medium, and the effect of genotype on this process is devoted to work Bernerjee and Sharma (1988). The authors conclude

that the addition of the nutrient medium 0,2 mg L⁻¹ IAA (indole acetic acid) and 0,2 mg L⁻¹ benzylaminopurine (BAP) promotes regeneration of shoots banana, while replacing by BAP kinetin reduced the rate of regeneration.

Influence of hormonal additions on the regeneration of sugar beet in culture in vitro is devoted to work Las and Lasa (1987). It is shown that the best growth of explants was observed on the Murashige-Skoog medium containing 4,4mkM BA (benzyladenine), 0,5 mkM and 0,3 mkM GA₃ (gibberellic acid).

A detailed study on the regeneration of forest species (*Pinus sylvestris* L., *P. Zheffreya*, *P. strobes* L., *Pseudotsuga menziesii* (Mirbel) Franco, *Robinia pseudoacacia* L.) was held Bara Magdalena (1986). Explants were top and the median parts of the 5-7-week-old seedlings. According to the author, the active shoot formation occurred on medium containing at benzyladenine concentration 2-2,5 mg L⁻¹, 2- dimethylaminopurine– 0,5-0,9 mg L⁻¹naphthylene acetic acid – 0,02-0,04 mg L⁻¹.

Soroka (2004) studied the regeneration processes of the two hybrid genotypes of flax on nutrient media N₆ and LMA-1 at various concentrations of BAP. It was shown that the growth and development of better callus occur in the medium at a concentration of BAP 2 mg L⁻¹ compared to 4 and 6 mg L⁻¹. Regeneration of shoots and roots was observed in only genotype F₁ 6-8 clustered × M22 and did not depend on the concentration of BAP in the medium and on the medium itself.

Kurenina et al. (2001) conducted studies of the process of regeneration of red clover *Trifolium pratense* L. to obtain regenerated plants. The authors found, that the optimal combinations of phytohormones in the regeneration process were (mg L⁻¹): BAP – 4.0; NAA – 0.1, kinetin – 2.0 and BAP – 4.0; NAA – 0.05; kinetin – 1.0 for a number of varieties (VIC –7, Early–2, Arlington, Altyn, C7 –11, RP150).

Issue of plant regeneration in cell and tissue culture is devoted to extensive literature (Smirnov et al., 1986; Tuskan, 1990; Kalyaeva et al., 2000; Seetharam et al., 2002; Sharad et al., 2004; Ghanti et al., 2004; Lemeshet al., 2006; Duong et al., 2007; Orlovskaya et al., 2008; Sarmast et al., 2009; Lebedev and Shestibratov 2010; Concioiu et al., 2010; Byadovsky, 2011; Muhametvafina and Akhmetov, 2011; Ruži et al., 2012; Cüce et al., 2013; Kunyi et al., 2013; Kakarla et al., 2014, Nqobile et al., 2015). However, for each species or plant cultivars, this problem is solved experimentally.

Based on this, we have conducted research aimed at studying the regeneration potential of *R. luteum*, introduced varieties of *V.corymbosum*, *V. vitis-idaea*, depending on the content of hormonal additions in the medium.

Materials and Methods

Objects of study were introduced varieties of *V. corymbosum* ('Elizabeth'), *V. vitis-idaea* ('Ammerland', 'Red Pearl') and *R. luteum*. Experiments were put on two nutrient media - Anderson (Anderson, 1975) and WPM (Lloyd and McCown, 1981)–with different ratios of hormone additions in them. The level of pH of the medium was to 4.8 before autoclaving at 1.06 kg/cm² pressure for 20 min at 121°C.

As explants we used sterile micrografts of introduced varieties of *V. corymbosum*, *V. vitis-idaea* and *R. luteum* and placed into sterile culture. Explants of 20 pieces for each variety and type of plants were planted on an agar medium containing IAA and izopenteniladenin (IAA:2iP) in the following ratios 1:5, 2:4, 4:15 (mg L⁻¹). Flasks with planted explants were placed on the shelves in a culture room where the illumination is 4000 lux, temperature 24°C, 16 h photoperiod.

Counting the number of regenerants per explant (regenerative potential) morphometric parameters of regenerants (height of shoot, cm; leaf length, mm) were performed after 2 months from the beginning of the experiment. Experimental data are statistically processed and presented in the Table. The figures in the Table are arithmetic means with their standard errors.

Results, Discussion and Conclusion

As follows from the Table, the maximum number of *R. luteum* shoots (5 pieces per explants) was recovered by Anderson medium, containing 4 mg L⁻¹ IAA and 15 mg L⁻¹ 2iP. At the medium WPM, containing the same amount of hormones, in this variety (*R.luteum*) this parameter is lower (4.50 pieces), but on insignificant amount.

Maximum number of regenerants per explants for variety 'Red Pearl' of *V. vitis-idaea* was noted on the medium Anderson and WPM, containing 4 and 15 mg L⁻¹ IAA and 2iP, respectively, and was 7.70 and 6.30 pieces. A similar pattern is characteristic of variety 'Ammerland' of *V. vitis-idaea* and variety 'Elizabeth' of *V.corymbosum*. In variety 'Ammerland' of *V. vitis-idaea* number of shoots per explant was 3.90 on the medium of Anderson and 1.80 on the medium WPM, the variety 'Elizabeth' of *V. corymbosum* - 3,80 and 3.05, respectively, at a ratio of IAA: 2iP = 4:15.

able. Regenerative potential and morphometric characteristics of yellow *Rhododendron luteum*, introduced varieties *Vaccinium corymbosum*, *Vaccinium vitis-idaea*, depending on the content of hormonal additions in the culture medium

Species, variety	Medium	Hormones, mg L ⁻¹		Quantity of shoots per 1 explant, pieces	Shoot height, m	Leaf length, mm
		IAA	2iP			
1	2	3	4	5	6	7
<i>Rhododendron luteum</i>		1	5	2,75 ± 1,19	2,03 ± 0,16	4,00 ± 0,52
		2	4	3,06 ± 1,11	2,10 ± 0,20	2,72 ± 0,41
		4	15	5,00 ± 1,50	1,49 ± 0,19	2,60 ± 0,38
	WPM	1	5	3,15 ± 1,05	6,00 ± 0,09	8,00 ± 0,45
		2	4	2,70 ± 1,19	5,10 ± 0,17	5,21 ± 0,18
		4	15	4,50 ± 1,21	3,29 ± 0,11	4,37 ± 0,13
'Red earl'		1	5	3,50 ± 1,10	1,10 ± 0,14	2,82 ± 0,14
		2	4	3,10 ± 1,12	1,41 ± 0,13	2,47 ± 0,23
		4	15	7,70 ± 1,15	1,05 ± 0,03	1,97 ± 0,21
	W M	1	5	3,00 ± 1,17	1,95 ± 0,13	3,95 ± 0,17
		2	4	3,90 ± 1,06	2,00 ± 0,11	3,51 ± 0, 29
		4	15	6,30 ± 1,29	1,95 ± 0,17	2,71 ± 0,17

1	2	3	4	5	6	7
'Ammerland'		1	5	1,20 ± 0,07	1,20 ± 0,24	3,44 ± 0,61
		2	4	1,39 ± 0,21	1,25 ± 0,12	4,83 ± 0,59
		4	15	3,90 ± 1,14	0,69 ± 0,07	2,91 ± 0,43
	WPM	1	5	1,30 ± 0,12	5,00 ± 0,28	6,50 ± 0,25
		2	4	1,49 ± 0,20	4,11 ± 0,61	6,22 ± 0,34
		4	15	1,80 ± 1,16	3,90 ± 0,30	4,76 ± 0,21
'Elizabeth'		1	5	1,80 ± 0,60	2,00 ± 0,13	2,33 ± 0,16
		2	4	1,95 ± 0,50	2,10 ± 0,15	2,90 ± 0,27
		4	15	3,80 ± 1,09	1,75 ± 0,15	2,54 ± 0,19
	WPM	1	5	1,40 ± 0,10	2,50 ± 0,29	4,90 ± 0,52
		2	4	1,60 ± 0,25	3,05 ± 0,12	4,30 ± 0,46
		4	15	3,05 ± 1,11	1,98 ± 0,16	3,01 ± 0,31

Minimum number of shoots per explant was formed for *R.luteum* on the medium Anderson containing 1 and 5 mg L⁻¹ (IAA:2iP) and WPM medium with 2 and 4 mg L⁻¹ (IAA:2iP) and was 2,75 and 2,70 regenerant per explant respectively. In variety 'Red Pearl' of *V. vitis-idaea* minimum number of shoots per explant (3, 50 pieces) was recovered on the medium Anderson at a ratio of IAA:2iP = 1:5 mg L⁻¹ and on the medium WPM (3,00) at the same ratio of hormones.

For *V.vitis-idaea* (variety 'Ammerland') and *V.corymbosum* (variety 'Elizabeth') the minimum number of regenerants per explant 1.20, 1.30 and 1.80, 1.40, respectively, observed on both types of nutrient media (Anderson and WPM) at the content of hormonal additions when the ratio 1:5 mg L⁻¹.

As the analysis of figured material, presented in the Table, the greatest potential for the regeneration of the investigated varieties of *V. corymbosum* two varieties of *V. vitis-idaea* and *R. luteum* was marked on both nutrient media (WPM and Anderson) containing 4 mg L⁻¹ IAA and 15 mg L⁻¹ 2iP, the lowest - on the same mediums, containing 1 mg L⁻¹ IAA and 5 mg L⁻¹ 2iP.

On the regenerative potential of the investigated plants affected not only the amount of hormonal additions, contained in the medium, but also plant genotype, influenced on this indicator. Thus, variety 'Red Pearl' of *V. vitis-idaea* has a maximum regenerative potential (7,70 shoots per explant), variety 'Elizabeth' of *V. corymbosum* and variety 'Ammerland' of *V.vitis-idaea* have a minimum regenerative potential, when the content in both mediums of the same amount of hormones (4 mg L⁻¹ IAA and 15 mg L⁻¹ 2iP) (see Table).

Further analysis of figured material, presented in the Table, allowed to come to the conclusion that there are differences not only in relation to the regenerative capacity of the plants examined, but also in relations to morphometric parameters: the height of shoot and leaf length. Maximum height of shoot (6cm) was observed in *R. luteum* on the medium WPM, containing 1 mg L⁻¹ IAA and 5 mg L⁻¹ 2iP, this parameter is somewhat lower (5cm) in variety 'Ammerland' of *V.vitis-idaea*, well below as for variety 'Elizabeth' of *V.corymbosum* - 2,5 cm and variety 'Red Pearl' of *V.vitis-idaea* - 1,95cm.

It is the fact, that variety 'Red Pearl' of *V. vitis-idaea*, which regenerated a maximum number of shoots per explant (7,70 medium Anderson IAA:2iP=4:15), has a height of shoot is minimal (1,05 cm). Logical to

assume, that with fewer shoots per explant shoot height would be greater. For example, for variety 'Ammerland' of *V. vitis-idaea* number of shoots per explant was 1,80 at the height of shoot 3,90 cm.

This can probably be explained by the proportional using up of the nutrients, which is reflected, on the one hand, to increase the number of shoots per explant and decrease in their height, and the other, to reduce the number of shoots and increase their height.

With regard to the leaf length of regenerants, it depends on the composition of the nutrient medium and taxonomic affiliation of plant. Leaves of *Rhododendron luteum* had the greatest length (8 mm) on the medium WPM, containing 1 mg L⁻¹ IAA and 5 mg L⁻¹ 2iP, leaves of variety 'Red Pearl' of *V. vitis-idaea* had the smallest length (1.97 mm) on the medium Anderson, containing 4 mg L⁻¹ IAA and 15 mg L⁻¹ 2iP.

Thus, the regenerative capacity of the studied introduced varieties of *V.corymbosum*, *V. vitis-idaea* and *Rhododendron luteum* and their morphometric parameters are closely dependent on the content of hormonal additions in the nutrient medium, the composition of the nutrient medium and associated with the genotype of the plant.

Maximum regenerative potential (7,70 shoots per explant) has variety 'Red Pearl' of *V. vitis-idaea* on the medium Anderson, containing 4 mg L⁻¹ IAA and 15 mg L⁻¹ 2iP; minimum regenerative potentials have variety 'Elizabeth' of *V.corymbosum* (1,40 shoots per explant) on the medium WPM and variety 'Ammerland' of *V.vitis-idaea* (1,20 shoots per explant) on the medium Anderson (A) when the content in the media of the same amount of hormones (1 mg L⁻¹ IAA and 5 mg L⁻¹ 2iP).

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