Biotechnology methods to produce planting material of the genus *Rubus* L.

E N Raeva-Bogoslovskaya¹, O I Molkanova¹, I L Krakhmaleva¹, and E V Soboleva¹

¹Tsitsin Main Botanical Garden of Russian Academy of Sciences, 127276 Botanicheskaya street 4, Moscow, Russian Federation

E-mail: katyaraeva@rambler.ru

Abstract. The work is devoted to improving clonal micropropagation methods of the genus *Rubus* representatives. When cultivating *R. arcticus* L. cultivars, the optimal concentration of 6-benzylaminopurine (6-BAP) was 0.3 mg L⁻¹. Significant effect of FeEDTA concentration in the nutrient medium on multiplication of *R. idaeus* L. cultivars was established. The interaction of auxin type and plant genotype was revealed during rooting of blackberry cultivars and raspberry-blackberry hybrids. The nutrient medium supplemented with 0.5 mg L⁻¹ indoleacetic acid contributed to the highest percentage of rhizogenesis in *R. arcticus* representatives (82%). The optimal growth regulator for conservation of raspberry explants was 6-BAP at a concentration of 0.3 mg L⁻¹. One of the most representative *in vitro* collections of *Rubus* cultivars has been created.

1. Introduction

The cultivation of berry plants is one of the most important areas of the agricultural industry worldwide. Berries are a rich source of various biologically active compounds which makes them popular both in the food and pharmaceutical industries. [1-4]. *Rubus* is recognized as one of the largest genera in the family Rosaceae Juss., it is widespread in the Northern hemisphere [5]. Raspberry, blackberry and arctic raspberry are some representatives of this genus. *R. idaeus* is the most popular berry crop in Russia: today the country occupies a leading position in production of raspberries [6]. On the contrary, blackberry has only recently become in demand. Active breeding in the USA forced agricultural holdings from other countries to pay attention to the crop which potential yield can exceed raspberry by 3-4 times [7-8]. The arctic raspberry is a wildberry plant but cultivated representatives of the species have already been bred. The main suppliers of arctic raspberry fruits are northern countries such as Finland, Russia, etc. [9-10]. *R. arcticus* is the most expensive of all exported forest berries [11].

To expand the range, as well as to increase the production of *Rubus* fruits, it is necessary to solve a number of problems. So, for example, many new bred raspberry cultivars characterized by keeping quality, disease resistance and high productivity are widely used in agricultural industry. At the same time cultivars characterized by other valuable qualities may be uncultivated for a long time and then lost [12]. The issue of reproduction and assessment of adaptive potential of abroad cultivars is relevant for blackberry and arctic raspberry. [7, 10].

Biotechnological methods of plant propagation can solve these problems. The creation of *in vitro* collection provides access to a large amount of plant material at any time of the year [13], whereas
clonal micropropagation complete realizes morphogenetic potential of the plant. The latter method contributes to the maximum multiplication rate of the culture and, as a consequence, a larger amount of planting material [14]. Researchers interest in clonal micropropagation of the genus *Rubus* does not fade away [15-20]. The choice of the optimal cultivation model is closely related to the biological characteristics of the plant and requires an individual approach for each taxon.

Thus, the purpose of our work was to create *in vitro* collection of *Rubus* representatives and to optimize the conditions for cultivation and preservation of this genus.

2. Materials and methods

Various representatives of the species *R. idaeus*, *R. fruticosus*, *R. arcticus* and raspberry-blackberry hybrids from *in vitro* collection of the Laboratory of Plant Biotechnology of Tsitsin Main Botanical Garden of Russian Academy of Sciences (MBG RAS) were used as objects of the research.

Various concentrations of 6-BAP (0.0 mg L\(^{-1}\), 0.3 mg L\(^{-1}\), 0.5 mg L\(^{-1}\), 1.0 mg L\(^{-1}\)) were compared for *R. arcticus* at the multiplication stage. The experiment of the influence of FeEDTA concentrations (37.0 and 74.0 mg L\(^{-1}\)) in a nutrient medium on raspberry propagation was carried out. Murashige-Skoog (MS) (1962) [20] medium was used in these experiments. After 30 days of cultivation the multiplication rate was calculated.

The rooting stage was considered at arctic raspberry, blackberry and raspberry-blackberry hybrids. The halfstrength MS medium supplemented with β-indole butyric acid (IBA) or β-indole acetic acid (IAA) at the concentrations of 0.3; 0.5; 1.0 mg L\(^{-1}\) was tasted. After 28 days of cultivation the percentage of rooted explants was calculated.

The following conditions for multiplication and rooting stages were maintained in the culture room: 2000 lux light intensity, 16/8 h photoperiod, 23-25 °C temperature.

MS nutrient medium containing 6-BAP and 2-isopentadenine (2-IP) at the concentration of 0.3 mg L\(^{-1}\) was used for conservation of *R. idaeus* explants. The assessment of the explants viability was carried out after 6 and 12 months of storage. Culture conditions of the climatic chamber were: 16/8 h photoperiod and 15-17 °C temperature.

All experiments were carried out in triplicate each with 10 samples. Statistical data processing was performed by the ANOVA method using Microsoft Office Excel 2010 software.

3. Results and Discussions

Nowadays, MBG RAS’s *in vitro* *Rubus* collection is one of the most representative in Russia and includes 55 raspberry cultivars, 16 blackberry cultivars, 2 raspberry-blackberry hybrids and 6 arctic raspberry cultivars.

*In vitro* plant organogenesis is influenced by many factors. The correct choice of growth regulator and its concentration is very important for obtaining actively proliferating *in vitro* culture. Comparative data analysis did not show significant differences between the multiplication rates of the *R. arcticus* cultured at various 6-BAP concentrations (Fig.1).
Figure 1. Effect of 6-BAP concentration on multiplication rate of *R. arcticus* (P<0.05).

Makarov S.S. successfully cultivated *R. arcticus* explants on a medium containing 6-BAP at a concentration of 0.5-1.0 mg L\(^{-1}\) [10]. Since our study found no differences between 6-BAP concentrations, a medium containing low concentration (0.3 mg L\(^{-1}\)) of growth regulator could be used for arctic raspberry propagation. It would increase profitability of planting material production. It should be noted that all the studied cultivars were spontaneously rooted after 35-50 days of cultivation on MS medium supplemented with 0.3 mg L\(^{-1}\) 6-BAP (Fig. 2). That allows accelerating the process of clonal micropropagation by 30%.

Figure 2. Spontaneous rooting of *R. arcticus*.

The mineral medium composition is no less important for organogenesis than growth regulators. Iron is one of the main components of the nutrient medium, as it takes part in the biosynthesis of chlorophyll and regulatory processes [15]. We found a significant difference in the multiplication rate for explants cultivated on nutrient media with different concentrations of FeEDTA. (Fig. 3)
Figure 3. Influence of FeEDTA concentration on the multiplication rate of R. idaeus cultivars (P<0.05).

Double FeEDTA content (74 mg L\(^{-1}\)) in the medium showed the increase of the multiplication rate and better development of raspberry microshoots. The multiplication rate averaged 14.9 ± 0.4. Some researchers also noted an increase of growth processes with an increase of iron concentration in the nutrient medium [15, 18].

The next step of clonal micropropagation is rooting. Various types of auxins and their concentrations can have a significant effect on the rhizogenesis of microshoots. We established the interaction of genotype and auxin on the rooting percentage of blackberry and raspberry-blackberry hybrids (Fig. 4).

Figure 4. Influence of various types of auxin on rhizogenesis of raspberry cultivars and raspberry-blackberry hybrids (P<0.05).

The blackberry cultivar 'Cacanska Bestrna' was characterized by the highest percentage of rooted microshoots (100%) on the nutrient medium containing 1.0 mg L\(^{-1}\) IAA. At the same time, 'Gazda' showed the lowest percentage of rhizogenesis on IAA (73%), and the highest on IBA (97%). The rooting of the raspberry-blackberry hybrids also significantly differed depending on the type of auxin.
Boysenberry rooted better on the medium with IAA (90%), while Tayberry rooted better on the medium with IBA (89%).

The rhizogenesis of *R. arcticus* plants was also influenced by the type and concentration of growth regulators in the nutrient medium. (Fig. 5).

![Figure 5. Influence of the hormonal composition of the nutrient medium on the rhizogenesis of *R. arcticus* cultivars (P<0.05).](image)

During the study, statistically significant differences were found. Culture media containing IAA were found to be optimal for the rooting of *R. arcticus* explants. The highest rooting percentage was observed on the medium containing 0.5 mg L\(^{-1}\) IAA (82.2%).

Plant conservation is a separate area in biotechnological methods of obtaining planting material. The essence of this stage is the maximum reduction of the growth processes to maintain explants viability for long period of time. During the study of the influence of the hormonal medium composition on the explant viability, we revealed significant differences between different sources of cytokinin (Fig. 6).

![Figure 6. Influence of the hormonal medium composition on the percentage of viable explants of *R. idaeus* cultivars (P<0.05)](image)

Conservation at the temperature of 15-17 ° C on the MS medium containing 0.3 mg L\(^{-1}\) 6-BAP promoted preservation 100% of raspberry explants for 6 months and 93% of explants for 12 months. The viability of explants cultivated on the medium containing 0.3 mg L\(^{-1}\) 2-IP decreased by 30% after...
12 months. It should be noted that microshoots cultured on the medium with 6-BAP were characterized by a lower height than the medium with 2-IP (Fig. 7).

![Figure 7](image_url)

Figure 7. The influence of growth regulators on the height of microshoots during conservation: a - 6-BAP; b - 2-IP.

4. Conclusions
Thus, the methods of clonal micropropagation of the genus *Rubus* representatives were optimized. It was found that the concentrations of the growth regulator and the genotype do not have a significant effect on the organogenesis of *R. arcticus* cultivars. The recommended 6-BAP concentration for clonal micropropagation of arctic raspberry was 0.3 mg L\(^{-1}\). The optimal FeEDTA concentration for *R. idaeus* cultivars was 74 mg L\(^{-1}\). The type of auxin significantly affected the rhizogenesis of blackberry cultivars and blackberry-raspberry hybrids at the stage of rooting. IAA was the appropriate auxin for 'Cacanska Bestrna' and Boysenberry, and IBA was the appropriate auxin for 'Gazda' and Tayberry. The medium containing IAA at the concentration of 0.5 mg L\(^{-1}\) was optimal for rooting of *R. arcticus* cultivars. The deposition of raspberry cultivars was successful at the temperature of 15-17 °C on the MS nutrient medium with 0.3 mg L\(^{-1}\) 6-BAP. After 12 months of storage the viability of the explants was 93%. One of the most representative in Russia *in vitro* collections of the genus *Rubus* was created.

Acknowledgments
The work was carried out in accordance to Institutional research project № 18-118021490111-5.

References
[1] Badjakov I, Georgiev V, Georgieva M, Dincheva I, Vrancheva R, Ivanov I, Georgiev D, Hristova D, Kondakova V and Pavlov A Bioreactor Technology for In Vitro Berry Plant Cultivation (*Plant Cell and Tissue Differentiation and Secondary Metabolites*) pp 383–431
[2] Débora P M, Josiane F C, Juliano S B, Márcia V, Carla A F, Cristiano A B and Milene T B 2021 Influence of the cultivar on the composition of blackberry (Rubus spp) minerals (*Journal of Food Composition and Analysis* vol 100)
[3] Makarkina M A, Pavel A R, and Vetrova O A 2020 Study of the biochemical composition of fruits at VNIISPK *Breeding and variety cultivation of fruit and berry crops* 7 99–102
[4] Patell A V, Rojas-Vera J and Dacke C G 2004 Therapeutic Constituents and Actions of Rubus Species (*Current Medicinal Chemistry* vol 11) pp 1501–1512
[5] Graham J and Woodhead M 2010 *Wild Crop Relatives: Genomic and Breeding Resources* pp 179–196
[6] Ulaszewska M, Garcia-Aloy M, Vázquez-Manjarrez N, Soria-Florido M T, Llorach R, Mattivi F and Manach C 2020 Food intake biomarkers for berries and grapes (*Genes & Nutrition* vol 15) p 35
[7] Gruner L A and Kornilov B B 2020 Priority trends and prospects of blackberry breeding in conditions of Central Russia (Vavilov journal of genetics and breeding vol 24(5)) pp 489–500

[8] Ladyzhenskaya O V, Kryuchkova V A and Samoschenkov E G 2021 Reproduction and cultivation of large-fruited varieties of blackberries ‘Karaka Black’ and ‘Black Butte’ under various conditions (AgroEkoInfo: Electronic scientific and production journal vol 2) pp 1–12

[9] Kostamo K, Toljamo A, Kokko H, Kärenlampi S O and Rita H 2018 Reasons for large annual yield fluctuations in wild arctic bramble (Rubus arcticus ssp arcticus) in Finland (Botany vol 96) pp 1–29

[10] Makarov C C, Kuznetsova I B and Smirnov 2018 Improving Technology of Clonal Micropropagation of Arctic Bramble (Rubus arcticus L) (Forastry information vol 4) pp 91–97

[11] Kurlovich E L 2009 Prospects for the use of food and medicinal forest resources of the Russian Federation: The state of the forests of the Far East and topical problems of forest management (Khabarovsk: FGU "DalNIILH") pp 138–140

[12] Evdokimenko S N and Podgaetsky M A 2019 State of the raspberry assortment in Russia and problems of its improvement (Pomiculture and small fruits culture in Russia vol 59) pp 294–300

[13] Lebedev V G, Vidyagina E O, Evdokimenko S N, Sorokopudov V N and Shestibratov 2019 In vitro collections of perspective raspberry and strawberry lines for breeding new cultivars Study and conservation of biodiversity in botanical gardens and other introduction centers (Donetsk) pp 232–236

[14] Molkanova O I, Egorova D A and Meleschuk E A 2018 The use of biotechnological methods in conservation and accelerated proliferation of berry crops (Breeding and variety cultivation of fruit and berry crops vol 5 (1)) pp 73–76

[15] Khlebova L P, Titova A M and Pirogova A V 2019 Biotechnological approaches to the reproduction of remontant forms of red raspberry (Ukrainian Journal of Ecology vol 9 (3)) pp 402–405

[16] Welander M 1985 In vitro culture of raspberry (Rubus ideaus) for mass propagation (Journal of Horticultural Science vol 60 (4)) pp 493–499

[17] Martinussen I, Nilsen G, Svenson L, Juntila O and Rapp K 2004 In vitro Propagation of Cloudberry (Rubus chamaemorus) (Plant Cell, Tissue and Organ Culture vol 78) pp 43–79

[18] Muratova S A, Solovykh N V and Terekhova V I 2011 Induction of morphogenesis from isolated somatic plant tissues p 107

[19] Vysotsky V A 1998 Biotechnological method in the system of production of healthy planting material and selection of fruit and berry plants p 44

[20] Murashige T and Skoog F 1962 A revised medium for rapid growth and bioassays with tobacco tissue cultures (Physiol Plant vol 15) pp 473–497