IJSRM
INTERNATIONAL JOURNAL OF SCIENCE AND RESEARCH METHODOLOGY
An Official Publication of Human Journals

Human Journals
Research Article
October 2020 Vol.:16, Issue:4

Outcomes of Introducing Some Species of the Genus Rhododendron L. to In Vitro Culture

IJSRM
INTERNATIONAL JOURNAL OF SCIENCE AND RESEARCH METHODOLOGY
An Official Publication of Human Journals

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Submission: 23 September 2020
Accepted: 30 September 2020
Published: 30 October 2020

Keywords: Rhododendron, microclonal, *in vitro*, explants, sterile, aseptic

ABSTRACT

The article represents the primary outcomes of introducing highly decorative species of the genus Rhododendron to *in vitro* culture, such as *Rhododendron delavayi* Franch., *Rhododendron japonicum* A. Gray., *Rhododendron brachycarpum* D. Don., *Rhododendron arborescens* (Pursh.) Torr., containing bioactive substances and introduced in the Batumi Botanical Garden. These species are very difficult to propagate in generative and vegetative ways; their propagation is almost impossible and only single units are represented in the garden. Among the attempts of introducing them to *in vitro* culture, at this stage, we have positive outcomes for 2 species of Rhododendrons: *Rhododendron delavayi*, *Rhododendron japonicum*. The research aim was to optimize sterilization conditions and identify morphogenetic reactions on various growth regulators on *in vitro* culture, in the explants of the *Rhododendron* species and create the method for *in vitro* culture in order to get aseptic cultures of these taxa considering the age factor of the primary plants. At this stage of the research, it was identified, that in order to get sterile plant materials of research Rhododendrons, and they must be preliminarily processed by fundazol, for example, 0, 4 % Ditan M-45. Regarding deciduous species - *Rhododendron japonicum*, in order to get aseptic culture, there is WPM feeding area containing 5 zeatin or 5 mg/l2 izopentiladenine and 1 mg/l indolilaceticacid and the evergreen species - *Rhododendron delavayi* needs feeding area with WPM 5 mg/l zeatin and 0,5 mg/l of thidiazuron or 5 mg/l 2 izopentyladenine and 0,5 mg/l thidiasuron. On the basis of conducted works, there are received aseptic cultures of the shoots of *Rhododendron japonicum* and *Rhododendron delavayi.*

INTRODUCTION

The species of the genus Rhododendron l. are distinguished by a number of useful qualities, first of all, they are highly decorative and then the content of biologically active compounds arouse pharmaceutical, medicinal and other interests toward them.

The species of the genus Rhododendron are found in the Northern zone, mainly spread in East Asian countries.

It is noted in the literature, that up to 500 species of 41 genus of Ericaceae family are used in decorative gardening, half of them are represented by Rhododendrons. Moreover, 500-600 species of Rhododendron exist in the collections of the world botanical gardens. Therefore, botanists, gardener-decorators, introducers and biotechnicians have to try their best to take unique Rhododendrons, unknown for cultures, out of the collections of the botanical gardens and put them into practice. None of decorative plants among bushy plants can compete to them in terms of forms diversity, beauty and color of leaves and flowers and decorativeness in all the seasons of a year [5].

Various species and breeds in open ground start flowering in different periods. If selected successfully, it is possible to create a group of nice blooming Rhododendrons from April including July.

In parallel with decorative values, they bear some medicinal qualities containing essential oils, tanner and other substances. Therefore, they have farming importance and of course, there is a necessity of their rapid and efficient propagation.

Certain organs of the Rhododendrons contain physiologically active compounds including highly active glycosides and P-vitamins. The Rhododendron leaves contain Andromedotoxin, Rhododendrin, Ericolin, Arbutin, essential oils, tanning substances and a number of organic acids. Medicinal treatments obtained from the Rhododendrons are widely used against cardiovascular diseases. They are characterized with sedative and hypotensive activity. According to existed data, they are extremely efficient against mercury poisoning and mucous membrane diseases. Moreover, they have positive affect on kidneys. It is known, that alcohol

exhausts from the Rhododendron leaves have bactericidic activity, inhibit the development of streptococcus, staphylococcus and pathogenic intestinal microflora.[5-6]

Diversity of bioactive substances in different parts of the Rhododendrons and their importance is endless in terms of using the Rhododendrons for various purposes.

The propagation of the research species of Rhododendrons introduced in the Batumi Botanical Garden is an extremely difficult issue, especially certain examples. That's why after lots of attempts of propagating them by cuttings, we applied for microclonal propagation.

METHODOLOGY

Research objects were the species of the genus Rhododendron growing in the Batumi Botanical garden: *Rhododendron delavayi* Franch., *Rhododendron japonicum* A. Gray., *Rhododendron brachycarpum* D. Don., *Rhododendron arborescens* (Pursh.) Torr. *In vitro* propagation method elaborated for Rhododendron was preferred among other research methods.[1-2; 7-8]

RESULTS AND DISCUSSION

Rhododendron japonicum - naturally spread in Central and North Japan, 1-2 meters tall deciduous shrub. The branch peel is grey. Young shoots are covered with colorless or silverish trichomes. Its bud is egg-shaped, sharp-edged and greyish-brown; some particles in the edges are covered with white trichomes. Annual growth of the shoot reaches 7-8 cm; leaves are narrow, long-lancet-type, 4-10 cm long, 2-4 cm wide, sharp-edged with cuneiform endings; Both sides are green when fully grown; lower veins are covered with thin trichomes. The stem is big, 0.5-1.0 cm long; 6-12 flowers, clustered in catkins, developed before leafing or while leafing. Its gynoecium is large funnel-shaped with wide snout and usually shorter than side parts; orange-yellowish in color, with big orange 6-7 cm long diameter spot. Cup leaves are covered with small, greyish fuzz. 5 stamens, shorten than its gynoecium. The dark brown lower part of its pollen-bearing thread is trichomy; blooming can last for a month. The fruit is a box with numerous too thin seeds.

Rhododendron dalavayi spread in Tibet, India, Thailand and Vietnam – in broad-leaved or mixed forests, on cliffy slopes 3200 m above the sea level. It is an evergreen shrub or a tree, 1-7 m tall with a grey cortex. Young shoots have greenish-whitish color; trichomy leaves are 7-15 cm long

and 1-5 cm wide, upper side is dark green and lower side – light green; 10-20 scarlet or red flowers are clustered and create catkins; crown petals are 5; the number of stamen is 10, with varied, oblong 2-8 mm long pollen-bearing threads; the fruit is 8 mm long box with too thin black seeds, gets ripen in late Autumn.

Rhododendron brachycarpum D.Don - straight-standing, evergreen tree, 2-4 m tall. Leaves are lancet-shaped, 8-20 cm long and 3-5 cm wide, upper surface of the leaf is light green, glossy, lower surface – grayish with gray trichomes. Its stem is 1-3 cm long. White-pinkish 10-20 flowers are clustered in round catkins 10-12 cm in diameter. Fruit box is 8-10 mm long. Spread in Korea, Japan (Honshu, Hokkaido).

Rhododendron arborescens (Pursh.) Torr.- Deciduous, straight-standing shrub, 2-3 m tall. Leaves are narrow, upside-down egg-shaped, oblong and lancet-shaped, 4-8 cm long and 1.5-3 cm wide, upper surface of the leaf is bright green, lower surface – pale green with a stem is 5-7 cm long. White-pinkish 3-6 flowers are clustered in catkins, too aromatic. Fruit box is 11-12 mm long. Naturally grow in North America.

After getting in touch with our colleagues from the Department of Biotechnology of the Minsk Botanical Garden of the Academy of Science of Belorussia with a great experience of working on Rhododendrons, the experiment was started.

Together with Belorussian colleagues, once more we analyzed the topicality of studying the Rhododendrons, which is very important for them too as they work on this topic and create the unique collection of Rhododendrons.

The alternative method of traditional propagation methods (vegetative and generative) is clonal micropropagation having lots of advantages, in particular, getting planting materials in a short period of time, any seasons and unlimited amount.

Clonal propagation is based on the unique quality of a plant cell – giving rise to an entire organism of a plant as a result of experimental influence. Using this method is important for decorative gardening, forestry, agriculture, medicine, especially for the propagation of single and endangered plants.

Although this method is quite progressive, clonal micropropagation technology is not perfectly elaborated for many plants. Rhododendrons belong to this kind of plants, especially their introduced species. Even not to count single examples, their propagation will bring economic benefits as the planting material of this valuable and rare decorative plant does not exist for realization. They are almost not used in green constructions of parks, gardens, squares and boulevards of our country and Belorussia too.

The main topic in our research is that, the only method for the preservation and restoration of single and endangered species and taxon existing in the collections of the Batumi Botanical garden is elaboration of their clonal micropropagation method and creation of *in vitro* plant bank. While creating this type of banks, it is necessary to ensure the vitality of the samples from collections, genetic integrity and the quality of collection samples giving us the opportunity for their further scientific and economical usage.

In order to get *in vitro* culture and achieve efficient plant propagation, morphogenetic potential of cultivated tissues are studied and the factors are revealed, which react on their realization (physical conditions of cultivation, mineral and hormonal composition of feeding areas, etc.) and also optimal conditions for cloning and adventive root production are determined *in vitro*. [3-4; 9]

Picture №. 1. The branches of Rhododendron prepared for the experiment

After numerous repetitions of the experiment, at this stage, we have achieved certain outcomes for 2 species: evergreen *Rhododendron delavayi* and deciduous *Rhododendron japonicum.*

As recognized, for microclonal propagation of the genotype of valuable wood plants, it is necessary to use primary meristems as the first materials and seeds can be applied for the propagation of species. Besides that, as less is the absolute age of the plant as intensively meristems are developed and shoots are grown. At the stage of getting *in vitro* culture, optimal ratio of cytokinins and auxines in the area gives an opportunity for induction of direct regeneration of shoots from the primary meristems. The said ratio has a nature of species-specificity (often breed-specificity).

Efficient technologies for micropropagation are elaborated for certain species of Rhododendrons and foreign selection breeds. [1-2; 7-8]

These types of technologies are based on comprehensive studies of regeneration peculiarities and shoots morphogenesis in various types of explants. Traditionally, for the purpose of getting microclones, a feeding area with 2-izopentiladenine and indolylacetic acid are used *in vitro* culture. Zeatin is used as a regulator for the growth of cytokinin activity and in recent years thidiazuron has been also applied to, which is a strong inductor of morphogenetic reactions for hardwood plants *in vitro* culture.[4; 9]

Our research goal was to optimize sterilization conditions and identify morphogenetic reactions on various growth regulators on *in vitro* culture, in the explants of *Rhododendron delavayi* Franch. and *Rhododendron japonicum* and create the method for *in vitro* culture in order to get aseptic cultures of these taxa considering the age factor of the primary plants.

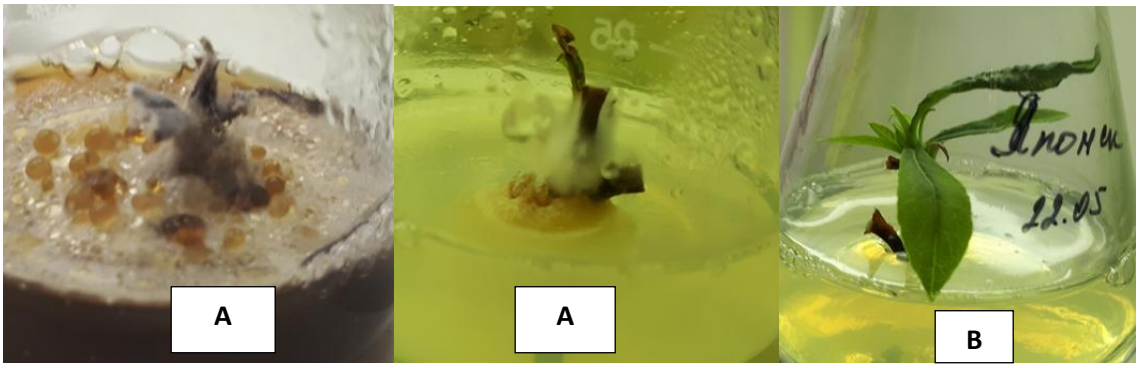
Explants were vegetative buds taken from 50-60 years old single examples. Moreover, cuttings with two bosoms from ontogenetically young and actively growing shoots were taken from these plants and delayed under room conditions. While cultivating the shoots, we used the areas in accordance with WPM (Woody plant medium) by adding plant growth regulators – auxines and cytokinins.[7-8] Before starting autoclaving, pH of all the feeding areas were reached to 4,8 - 5,0 importance. Passing of aseptic cultures on new feeding areas was the following: 0 passage – for the 12th week, from the first passage 1 – to every 8th week. Cultivation of plant materials was

carried out in the climatic chamber at 25±2˚C, during 16 hours photoperiod and 50% relative humidity of air.

For getting aseptic cultures of research plants, the following scheme for the sterilization of plant materials was applied to: the shoots were washed by brush with detergent through running water. Sterilization was carried out by putting the plant materials consecutively in Ditan M-45 and fungicide 0,4% solution for 60 minutes and 9% solution of $Ca(ClO)_2$ (Calcium Hypochlorite) for 30 minutes. In order to saturate the surface of the explant, sterilization compound was added a droplet of tween 80. Then washed with sterile distilled water three times for 5-5- minutes. Prepared explants were placed in the feeding areas by tested growth regulators.

The following growth regulators were added to the feeding areas on relative: zeatin with the concentration 5 mg/l; 5 mg/l zeatin and 0,1 mg/l thidiazuron; 5 mg/l 2 - isopentiladenine and 0,1 mg/l thidiazuron; 5 mg/l zeatin and 0,5 mg/l thidiazuron; 5 mg/l 2 – isopentiladenine and 0,5 mg/l thidiazuron; 15 mg/l 2 isopentiladenine და 4 mg/l indolylacetic acid.

Big infection of primary explants in case of both Rhododendrons are indicated by us. 100% fungus infection was revealed. Applying to Ditan M-45 gave us an opportunity to decrease fungi pathogens and receive sterile primary shoots, which means that while sterilizing, without primary treatment by fungicide solution, the material was infected in 100%. After using Ditan-45, infection with fungi pathogens was decreased and sterile primary shoots were received (Pic. №. 2).



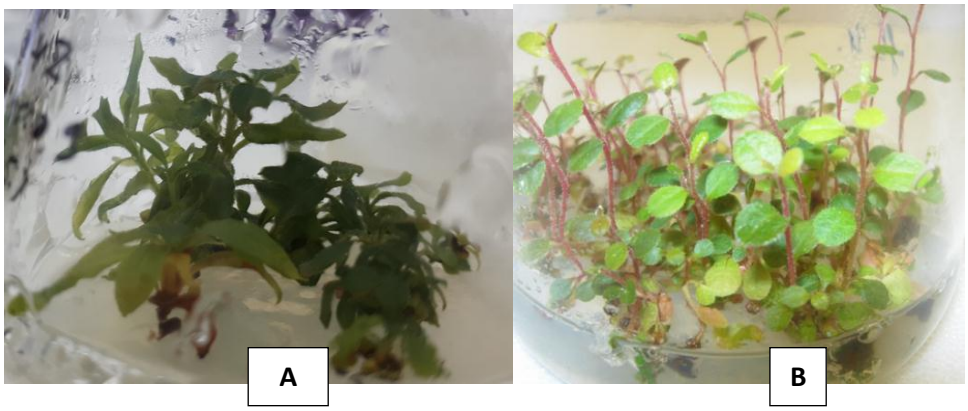
Picture № 2: A) Infected explants of research Rhododendrons (sterilization without Ditan M-45); B) sterile primary explants.

Table No. 1: Received sterile materials in accordance with the sterilization scheme, %

	9,0 % $Ca(ClO)_2$	0,4 % Ditan M-45 + 9% Ca(ClO)2
Rhododendron japonicum	0	50,0
Rhododendron delavayi	0	35,3

Morphogenic response in the explants of *Rhododendron delavayi* was received on the area containing traditional phytohormones for the propagation of rhododendrons: 2 isopentyladenine and zeatin with the concentration of 5 mg/l added to 0,5 mg/l thidiazuron. The growth of buds in size and iniciacion of meristems were visible in the sterile shoots on the 7th day. Although on the 8th week, further development of shoots was detected only in 50% of explants. Areas containing 15 mg/l 2 isopentyladenine and 4 mg/l indolylaceticacid, also thidiazuron with limited concentration to 0,1 mg/l in combination with the other growth regulators were found inefficient. For *Rhododendron japonicum* was detected as follows: the growth of buds for this species was faster than *Rhododendron delavayi*. The development of shoots from the primary meristems was detected on the 4th day of cultivation on the feeding area containing zeatin. On the 14th day of the beginning of the experiment, the growings of young shoots on these areas reached 0,8-1,0 cm on average (Picture 3). Activation and development of primary meristems on the area containing 15 mg/l 2 isopentiladenine and 4 mg/l indolylaceticacid, are almost the same as the feeding area with Zeatin.

Picture №. 3: Growings of young shoots of explants of *Rhododendron japonicum*



Picture №. 4: Aseptic cultures of the shoots of *Rhododendron japonicum (A)* and *Rhododendron delavayi* (Б)

CONCLUSION

Thus, it is identified, that in order to get sterile plant materials of research Rhododendrons, they must be preliminarily processed by fundazol, for example, 0,4 % Ditan M-45. Regarding deciduous species - *Rhododendron japonicum*, in order to get aseptic culture, there is WPM feeding area containing 5 zeatin or 5 mg/l2 izopentyladenine and 1 mg/l indolylaceticacid and the evergreen species - *Rhododendron delavayi* needs feeding area WPM 5 mg/l zeatin and added 0,5 mg/l of thidiazuron or 5 mg/l 2 izopentyladenine and 0,5 mg/l thidiasuron.

On the basis of conducted works, there are received aseptic cultures of the shoots of *Rhododendron japonicum* and *Rhododendron delavayi* (pic.4).

Further research works for the optimization of rooting and cloning stages are planned.

ACKNOWLEDGMENT

For active participation in the experiment and preparation of the article, we express our gratitude to the scientists from the Central Botanical Garden of National Academy of Sciences of Belarus, (Minsk, Belarus): Veranika Filipenia, Ivan Valodzka, Liudmila Goncharova; Employees of the Batumi Botanical Garden (Batumi, Georgia): Mariam Kandelaki, Julieta Jayeli; To the chief researchers of the Institute of Phytopathology and Biodiversity of the Batumi Shota Rustaveli State University (Batumi, Georgia): Mariam Metreveli, Avtandil Meskhidze.

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