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Rooting and acclimatization of *in vitro* propagated microshoots of the Ericaceae

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Abstract

The effective methods of rooting and acclimatization in the sphagnum moss of *in vitro* propagated microshoots of commercially promising plants: *Rhododendron ledebourii, R. hybridum cv.* 'Cunningham's White' and *Vaccinium uliginosum cv.* 'Golubaya rossyp' were developed for the first time. Two methods were studied: 1) rooting *in vitro* and acclimatization in the substrate and 2) rooting and acclimatization *ex vitro* in the substrate. Taking into account two factors (rooting of microshoots only *in vitro* conditions and only from already rooted *in vitro* regenerants), we have achieved 100 % of rooting of bog blueberry *cv.* 'Golubaya rossyp' and 73 % of plants adapted to *ex vitro* conditions. The method of *ex vitro* rooting in the substrate has been proved as effective for *R. ledebourii*, rooting of it's shoots reached 80-90 % with 87 % viable plants. *R. hybridum cv.* 'Cunningham's White' showed the greatest plasticity among the studied species and cultivars on ability to rooting of microshoots. All tested methods gave up to 90-100 % rooted and adapted plants of this cultivar. It was revealed that *in vitro* rooting microshoots, followed by acclimatization in the substrate was optimal for *V. uliginosum cv.* 'Golubaya rossyp'; for *R. ledebourii* – the rooting and acclimatization in the substrate; and for *R. hybridum cv.* 'Cunningham's White' - both methods were effective. From a commercial point of view, the proposed methods decrease the costs of plant production significantly through a reduction in the time and labour needed to obtain well-rooted and acclimatized Ericaceae microplants. Therefore, it could make the micropropagation of commercially promising Ericacea plants in the nursery industry both possible and profitable.

Key words: *Rhododendron hybridum cv.* 'Cunningham's White'*, Rhododendron ledebourii*, *Vaccinium uliginosum cv.* 'Golubaya rossyp', *in vitro*, *ex vitro,* rooting, acclimatization

Introduction

The success of micropropagation systems can be assessed by their ability to produce plants that have been successfully acclimatized (Zimmerman, 1988; Kirdmanee *et al.*, 1995; Hartmann *et al.*, 2002; Choudhary *et al.*, 2017). Plantlets and shoots that have been grown *in vitro* were continuously exposed to a special conditions of microenvironment that has been selected to provide minimal stress and optimal parameters for multiplication of plants. Regenerants were propagated within the culture vessels under low intensity of light, in aseptic conditions, on a medium containing ample amount of sugar and nutrients to allow the heterotrophic growth, and in humid atmosphere. These conditions produce a culture-induced phenotype of plants that cannot survive in external environment when directly placed in a greenhouse or field (Gigolashvili *et al.*, 1997; Hazarika, 2003). Since the stomata are not functional (Sallanon *et al.*, 1991), the juvenile plants are placed under the conditions of potentially water stress and photoinhibition (Van Huylenbroeck *et al.*, 1995). Therefore, the main purpose of acclimatization is the elimination of "weaknesses" of regenerates.

Rooting of microcuttings can be achieved *in vitro* or *ex vitro* (McCown, 1988). Normally, agar-based media into which bases of shoots are placed for both root initiation and root elongation is used for *in vitro* rooting. The roots growing in agar-based media are brittle, they are often dysfunctional, lacking root hairs, and transplantation of such plants are complicated, consequently they may die after transfer to soil. Improving of micropropagation efficiency means the minimizing losses in laboratory and during acclimatization. *Ex vitro* rooted plants have better developed root system, compared to the plants rooted *in vitro* (Bozena, 2001; Leva, 2011). Besides, the *ex vitro* technique is economical, saves time, and requires less labor, chemicals, and equipments. Moreover, *ex vitro* rooted plantlets did not require any additional acclimatization prior to transplanting them to the regular greenhouse conditions (Pruski *et al*., 2000; Shekhawat *et al*., 2017).

R. ledebourii Pojark is a highly decorative and officinal species and it has the potential for landscape gardening (Karakulov, 2014). In the Altai Territory (Russia) it is included in the Red Book as a threatened species because of mass collection for bouquets (The Red book…, 2006). Widespread use in the cultural events is helpful in conservation of this species. Shoot cultures can be established *in vitro* but the rooting is difficult in this condition (Erst *et al.*, 2014). *R. hybridum* hort. *cv.* 'Cunningham's White' is an attractive horticulture plant widely used in mass urban landscape plantings. It is well rooted in *in vitro* culture, but often forms the roots over the culture medium, so plants lack nutrients (Erst *et al.*, 2012).

Vaccinium uliginosum L. *cv.* 'Golubaya rossyp' is a promising fruit crop for the Siberian region. However, unsuccessful traditional methods of vegetative propagation has hindered extension of area under this new berry cultivation. Green grafting technique allows sufficient rooting in shoots of *V. uliginosum* with plant

growth regulators (120 mg L^{-1} IBA) and without it, respectively (Gorbunov *et al.*, 2010; Gorbunov and Snakina, 2013). It grows well in *in vitro* shoot culture, but rooting can vary on agar medium (Erst and Vechernina, 2010).

Plants of Ericaceae family are important commercial crops that are widely used as ornamentals and food. Methods of *in vitro* propagation are often used for mass reproduction of these species and varieties (Mao *et al.*, 2011; Litwińczuk, 2012; Zaytseva *et al.*, 2015, 2016, 2018). However, the more widespread use of the plants is often restricted by the high percentage of plants die or damaged after transferring to *ex vitro* conditions. Depending on the biological peculiarities and genetic potential of the species, the methods which work for *in vitro* environment of one species are not necessarly satisfactory to ensure the survival of another (Ahuja, 1993).

Therefore, considering the commercial importance of the family Ericaceae representatives, the present experiment was initiated to study the different methods of rooting and acclimatization of *in vitro* propagated microshoots of *R. ledebourii*, *R. hybridum cv.* 'Cunningham's White' and *V. uliginosum cv.* 'Golubaya rossyp'.

Materials and methods

Plant material: All studied species and cultivars are recommended for growing in Siberia region of Russia.

Microcutting preparation: Tip cuttings of *R.hybridum cv.* 'Cunningham's White', *R. ledebourii* (approximately 1-2 cm long) and *Vaccinium uliginosum cv.* 'Golubaya rossyp' (approximately 3-4 cm long) were prepared from 4-weeks-old cultures growing on shoot multiplication medium (see the section "Media composition and treatments").

Culture conditions: Explants were cultivated at temperature of 25±1°C under cool white fluorescent lights (35 µmol m^{-2} s^{-1}) at 16 h light photoperiod, and subcultured on fresh media every 4 weeks.

Media composition and treatments: The shoot multiplication media for *R. hybridum cv.* 'Cunningham's White', *R. ledebourii* and *V. uliginosum cv.* 'Golubaya rossyp' contained macronutrients and micronutrients (Anderson, 1975), vitamins MS (Murashige and Skoog, 1962), and was supplemented with 5 µM 2-isopentyl adenine (2-iP). Shoots were rooted with the use of *in vitro* and *ex vitro* methods. All of the nutritional media were supplemented with 30 g L^{-1} sucrose and solidified with 6 g L^{-1} agar. The pH of the media was adjusted to 5.4.

Two methods were tested to study *in vitro* rooting. The first method - the shoots were cultured in half-diluted final concentration of A macronutrients and micronutrients (½ A), containing 3 or 10 µM of indole-3-butyric acid (IBA). The second method - the basal ends of the shoots were dipped into 30 mg L^{-1} of IBA for 4 hours, then cultured on ½ A auxin-free medium.

For *ex vitro* rooting the shoots were dipped into 30 mg L^{-1} of IBA for 24 hours, then transferred into plastic container with a lid filled with sphagnum moss. The regenerants were under a plastic cover for 7 to 10 days to maintain high humidity. Acclimatization period was 4-5 weeks.

Two methods of rhizogenesis and acclimatization were tested.

Experiment 1: Rooting *in vitro*, acclimatization in the substrate (sphagnum moss).

Experiment 2: Rooting and acclimatization *ex vitro* in the substrate (sphagnum moss).

After acclimatization, the plants were transplanted into containers with nutritive soil mixture (Azalea®, Russia) and grown under greenhouse conditions. The plants were fed every two weeks (top-dressing for azaleas, Bona Forte, Russia) at the rate of 3.3 mL L^{-1} starting 1 month after planting.

30 plants were used per treatment and the experiment was conducted twice. The statistical differences between treatments were analyzed based on a confidence interval method at $\alpha = 0.05$, using the Microsoft Exel. The data are presented as mean values and confidence intervals ($P \leq 0.05$)

Results

Experiment 1 (Rooting *in vitro*, acclimatization in the substrate (sphagnum moss)): A high percentage of *R. hybridum cv.* 'Cunningham's White' and *V. uliginosum* 'Golubaya rossyp' rooting was achieved *in vitro*, while the shoots of *R. ledebourii* were unable to root in this experiment. An influence of auxin IBA was tested by two application methods: supplementing the culture medium with auxin and soaking the shoots in solution of IBA for 4 hrs and then culturing on a nutrient medium without plant growth regulators. Both methods were effective, however we often observed that root formation in *V. uliginosum* starts in *in vitro* culture, but the further root growth was inhibited in a medium containing auxins. *In vitro* rooted regenerants of *R. hybridum cv.* 'Cunningham's White' and *V. uliginosum* 'Golubaya rossyp' were transferred on special substrate for acclimatization. The results of experiments are shown in the Table 1 and on Fig. 1.

During the period of acclimatization in sphagnum moss, the plants (*V. uliginosum cv.* 'Golubaya rossyp') made notable growth. Increase in the length of shoot and number of leaves was 2.8 and 2 times, respectively. It should be noted that *V. uliginosum* is a typical plant of winter dormancy that was observed even for *in vitro* propagated plants: in late October, the plants stopped growing, and partially exfoliated. Plant growth was resumed only in late March, mainly due to the development of axillary buds. Plant measurements were made after 6 months of acclimatization in the substrate at the early release of plants from the dormancy, however, some growth and development parameters (height of shoots, number of leaves per stem) were lower than after the acclimatization of plants cultured *in vitro*.

Experiment 2: Rooting and acclimatization *ex vitro* in the substrate (sphagnum moss): We failed to root the shoots of *R. ledebourii* in tissue culture. We were able to provoke the formation of roots in *R. ledebourii* shoots only in *ex vitro* conditions. Shoots were soaked for 24 hrs in 30 mg L^{-1} IBA solution, and then planted into sphagnum moss with increased air humidity. The first roots appeared after 10-12 days of culture. In 45 days, an output of rooted shoots was 80-90 %.

The technique of *ex vitro* rooting of bog blueberry microshoots was inefficient also due to a long rooting period. The microshoots

Table 1. Growth parameters of *R. hybridum cv.* 'Cunningham's White', *R. ledebourii* and *V. uliginosum* 'Golubaya rossyp' with different methods rooting and acclimatization *ex vitro* conditions

Parameter	*R. hybridum*. 'Cunningham's White' Experiment 1	*R. hybridum* 'Cunningham's White' Experiment 2	*R. ledebourii* Experiment 2	*V. uliginosum* 'Golubaya rossyp' Experiment 1
Before acclimatization				
Height of shoot (cm)	2.5±0.4	2.3±0.6	1.5±0.4	5.1±0.4
Number of leaves	7.3±0.7	5.6±0.5	5.3±1.2	10.2±1.2
Number of roots	5.9±1.8	0	0	3.5±0.3
After acclimatization				
% viable plants	100	92	87	73
Height of shoot (cm)	3.7±0.3	4.0±0.3	3.8±0.4	14.5±1.6
Number of leaves	9.1±0.8	11.0±1.8	9.8±1.2	21.2±3.0
Number of roots	8.7±1.3	6.8±1.2	3.2±1.7	3.5±1.3
6 month after acclimatization				
% viable plants	100	100	90	87
Height of shoot (cm)	7.4±0.4	11.3±1.3	9.0±0.7	7.4±2.3
Number of leaves	11.2±1.1	18.8±1.6	13.7±1.7	6.1±1.8
Number of developed shoots	1	1	5.9±1.2	3.2±1.7

Mean separation within columns by a confidence interval method at $\alpha = 0.05$ (n=30)

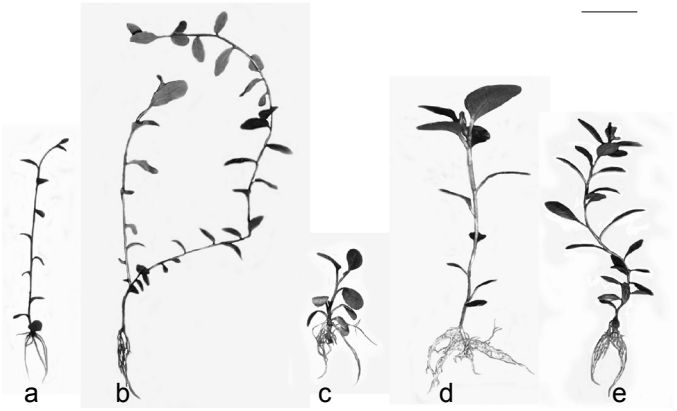

Fig. 1.. The regenerants of *V. uliginosum cv.* 'Golubaya rossyp', rooting *in vitro* on the ½ A (a), *V. uliginosum cv.* 'Golubaya rossyp' after acclimatization in the substrate (b); *R. hybridum cv.* 'Cunningham's White', rooting *in vitro* on the ½ A+IBA 3 μM (c), *R. hybridum cv.* Cunningham's White' after acclimatization in the substrate (d); *R. ledebourii*, after rooting and acclimatization *ex vitro* in the substrate (e). Bar: 1cm.

of *V. uliginosum* were treated with solution of auxin, and then transplanted into containers with sphagnum moss. After four weeks of cultivation, only single shoots were rooted and capable to further growth, the most of microshoots had perished.

Microshoots of *R. hybridum cv.* 'Cunningham's White' demonstrated good ability to rooting and acclimatization under all tested approaches (Table 1, Fig. 1).

Discussion

There are two basic methods of rooting of microshoots: the treating of microcuttings with IBA solution (30mg L^{-1}) for 4 hours followed by transplantation either into *ex vitro* substrate or *in vitro* culture without plant growth regulators; the rooting of microshoots *in vitro* with relatively low concentration of auxin in medium. It should be noted that the main difference between these techniques is (i) soaking in auxin has less impact on microshoots, thus relatively stable auxins as IBA are preferred here. (ii) use of *in vitro* rooting, auxin penetrates through a microcut during a long period of time and can eventually inhibit the development of root primordia and can lead to the formation of callus at the base of shoots (De Klerk *et al.*, 1995; Gurung and Singh, 2010).

Microshoots of *V. uliginosum cv.* 'Golubaya rossyp' showed the ability to form the roots (76 %) only *in vitro* conditions after 3-4 weeks of cultivation. It was often noticed that start of root formation took place in *in vitro* culture, but the further root growth was inhibited. It is known that process of root development consists of two general stages: root initiation and root elongation (Blazich, 1988). Root initiation involves differentiation of specific cells of sprouts to form the root meristems. Then, the meristem develops and elongates. Root initiation and root elongation have different optima for plant growth regulators, particularly auxin; and for conditions of cultivation. Therefore, for many plant species, *in vitro* use of various nutrient media and culture conditions for root initiation and root development has been shown effective. Rooted *in vitro* shoots of *V. uliginosum* including the shoots with root primordium were transferred into sphagnum moss for acclimatization that caused the growth and elongation of root system. Another feature of *V. uliginosum* of microshoots from already rooted *in vitro* regenerants results better rooting (Erst and Vechernina, 2010). Taking into account these two factors, we achieved 100 % of rooting of bog blueberry *cv.* 'Golubaya rossyp' and 87 % of plants adapted to *ex vitro* conditions.

Some studies (Lin *et al.*, 1995; Newell *et al.*, 2003, 2006) have shown that rooting of shoots depends on the intensity of aeration, which can be insufficient on agar nutrient media. To eliminate this negative effect, different methods are used, including *ex vitro* rooting of shoots. This method has been proved effective for *R. ledebourii* as rooting of it's shoots reached 80-90 %. These results are in agreement with previously reported results for other Rhododendrons which were successfully rooted *ex vitro* (Douglas, 1984; Briggs *et al.*, 1994). Furthermore, in commercial practice the micropropagated shoots of *Rhododendron* are rooted *ex vitro* since it is more cost effective (Hazarika, 2003).

R. hybridum cv. 'Cunningham's White' has shown the greatest plasticity among the studied species and cultivars on ability of rooting of microshoots. All tested methods gave up to 90-100 % rooted and adapted plants.

Thus, we have found the optimal mode of rooting and

acclimatization of *in vitro* propagated microshoots of the Ericacea plants. It allows high yield of the planting material, including the propagation of hard-to-root plants, for further wide usage for cultivation.

This is the first report of a successful application of different methods of rooting and acclimatization of *in vitro* propagated microshoots of commercially promising plants: *R. ledebourii*, *R. hybridum cv.* 'Cunningham's White' and *V. uliginosum cv.* 'Golubaya rossyp'. This is important owing to the economic importance of Ericaceae, and the need to establish successful and cheap methods of micropropagation.

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References

Ahuja, M.R. 1993. *Micropropagation of Woody Plants*. Khwer Academic Publishers, Dordrecht.

Anderson, W.C. 1975. Propagation of rhododendrons by tissue culture. I. Development of a culture medium for multiplication of shoots. *Proceedings of International Plant Propagators Society*, 25: 129-135.

Blazich, F.A. and J.R. Acedo, 1988. Micropropagation of flame azalea. *J. Environ. Hort.*, 6: 45-47.

Bozena, B. 2001. Morphological and physiological characteristics of micropropagated strawberry plants rooted *in vitro* or *ex vitro*. *Scientia Hort*., 89: 195-206.

Briggs, B.A., S.M. McCulloch and, L.A. Caton, 1994. *In vitro* propagation of *Rhododendron*. *Acta Hort.*, 364: 21-26.

Cent.Eur.J.Biol., 6: 352-358.

Choudhary, S.K., A.K. Patel, S. Shekhawat and N.S. Shekhawat, 2017. An improved micropropagation system, *ex vitro* rooting and validation of genetic homogeneity in wild female *Momordica dioica*: an underutilized nutraceutical vegetable crop. *Physiol. Mol. Biol. Plants*, 23: 713-722.

De Klerk, G.J., M. Keppel, J. Ter Brugge and H. Meekes, 1995. Timing of the phases in adventitious root formation in apple microcuttings. *J. Expt. Bot.*, 46: 965-972.

Douglas, G.C. 1984. Propagation of eight cultivars of *Rhododendron in vitro* using agar-solidified and liquid media and direct rooting of shoots *in vivo*. *Scientia Hort.*, 24: 337-347.

Erst, A.A. and N.A. Vechernina, 2010. Micropropagation of promising new varieties of *Vaccinium uliginosum* L. *Bull. Kharkov Natl. Agr. Univ. Ser. Biol.*, No 2.

Erst, A.A., A.V. Karakulov and A.S. Erst, 2014. Propagation and Conservation *in vitro* Unique Genotypes of *Rhododendron ledebourii* Pojark. (Ericaceae DC). *Adv. Environ. Biol.*, 8(10): 276-282.

Erst, A.A., T.I. Novikova and Y.G. Zaytseva, 2012. Adaptation of regenerants of *Rhododendron hybridum* to *ex vitro* conditions. *Sci. Bull. BSU*, 9(128): 44-48.

Gigolashvili, T.S., O.N. Rodkin and V.G. Reutsky, 1997. *In vitro* conditions are formed a specific culture phenotype. *The Biology of plant cells in vitro, biotechnology and preservation of the gene pool: Proceeding of the VII International Conference*, Moscow, 1997, p. 413.

Gorbunov, A.B. and T.I. Snakina, 2013. Blueberries. In: *Introduction of non-traditional fruit, berry and vegetable plants in West Siberia,* I.Yu. Koropachinskiy and A.B. Gorbunov (eds.). Publishing House Geo, Novosibirsk. p. 109-127.

Gorbunov, A.B., V.E. Volchkov and T.I. Snakina, 2010. Green cuttings of the blueberry bog. *Forest Science of the XXI century: Proceeding of the International Scientific Conference*, Gomel, 2010, p. 520-524.

Gurung, B. and K.K. Singh, 2010. *In vitro* mass propagation of Sikkim Himalayan Rhododendron (*R. dalhousiae* Hook. f.) from nodal segment. *J. Appl. Hort*., 12(1): 42-45.

Hartmann, H.T., D.E. Kester and F.T. Davies, Jr. 2002. *Plant Propagation: Principles and Practice.* Seventh Edition. Prentice-Hall, Englewood Cliffs, NJ.

Hazarika, B.N. 2003. Acclimatization of tissue-cultured plants. *Current Sci.*, 85 (12): 1704-1712.

Karakulov, A.V. 2014. Flower Shape and colour Variation in *Rhododendron ledebourii* L. from Mountains of Southern Siberia (Russia). *J. Amer. Rhododendron Soc.*, 1: 39-41.

Kirdmanee, C., Y. Kitaya and T. Kozai, 1995. Effect of CO_2 enrichment and supporting material *in vitro* on photoautotrophic growth of *Eucalyptus* plantlets *in vitro* and *ex vitro*. *In vitro Cell. Dev.Biol.-Plant*, 31: 144-149.

Leva, A. 2011. Innovative protocol for "*ex vitro* rooting" on olive micropropagation.

Lin, X., B.A. Bergann and A. Stomp, 1995. Effect of medium physical support, shoot length and genotype on *in vitro* rooting and plantlet morphology of *Sweetgum*. *J. Environ. Hort.*, 13: 117-121.

Litwińczuk, W. 2012. Micropropagation of Vaccinium sp. by *In vitro* Axillary Shoot Proliferation. In: *Protocols for Micropropagation of Selected Economically-Important Horticultural Plants. Methods in Molecular Biology (Methods and Protocols)*, Volume 994, M. Lambardi, E. Ozudogru and S. Jain (eds). Humana Press, Totowa, NJ. p. 63-76.

Mao, A.A., S. Kaliamoorthy, Ranyaphi, Jayashankar Das, Sanjoy Gupta, James Athili, J. Y. Yumnam and L.I. Chanu, 2011. *In vitro* micropropagation of three rare, endangered, and endemic rhododendron species of Northeast India. *In vitro Cell.Dev.Biol.-Plant*, 47: 674-681.

McCown, B.H. 1988. Adventitious rooting of tissue cultured plants. In: *Adventitious Root Formation in Cuttings*: T.D. Davis, B.E. Hassing and N. Sankhla (eds.). Discorides Press, Portland, OR, USA. p. 287-302.

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plant.*, 15(2): 473-497.

Newell, C., D. Growns and J. McComb, 2003. The influence of medium aeration on *in vitro* rooting of Australian plant microcutting. *Plant Cell Tiss. Org.*, 75: 131-142.

Newell, C., D. Growns and J. McComb, 2006. Aeration is more important than shoot orientation when rooting Lentil (*Lens culinaris* Medik.) *cv.* «Digger» microcuttings *in vitro*. *In vitro Cell Dev.Biol.-Plant*, 42: 197-200.

Pruski, K.W., T. Lewis, T. Astatkie, J. Nowak, 2000. Micropropagation of Chokecherry and Pincherry cultivars. *Plant Cell Tiss. Org.*, 63: 93-100.

Sallanon, H., D. Laffray and A. Coudret, 1991. Ultrastructure and functioning of guard cells of *in vitro* cultured rose plants. *Plant Physiol. Biochem*., 29: 333-339.

Shekhawat, M.S., M. Manokari and J. Revathi, 2017. *In vitro* propagation and *ex vitro* rooting of *Aerva lanata* (L.) Juss. ex Schult.: a rare medicinal plant. *Indian J. Plant Physiol*., 22: 40-47.

The Red Book of the Altai Territory. Rare and endangered species of plants, 2006. Edited R.V. Kamelina and A.I. Shmakov (eds.). PPC "Altai", Barnaul.

Van Huylenbroeck, J.M., H. Huygens and E.C. Debergh, 1995. Photoinhibition during aeclimatization of micropropagated spathiphyllum "petite" plantlets. *In vitro Cell. Dev. Biol.-Plant*, 89: 360-365.

Zaytseva, Y.G. and T.I. Novikova, 2015. Conservation and propagation of Rhododendron schlippenbachii using biotechnological methods. *Rastitel'nyj Mir Aziatskoj Rossii* (Plant Life of Asian Russia), 4: 79-85.

Zaytseva, Y.G., T.V. Poluboyarova and T.I. Novikova, 2016. Effects of thidiazuron on *in vitro* morphogenic response of Rhododendron sichotense Pojark. and Rhododendron catawbiense *cv.* Grandiflorum leaf explants. *In vitro Cell. Dev. Biol.Plant*, 52: 56-63.

Zaytseva, Y.G., E.V. Ambros and T.I. Novikova, 2018. Rooting and acclimatization to *ex vitro* conditions of regenerants of frost-resistant members of Rhododendron. *Turczaninowia*, 21(1): 144-152.

Zimmerman, R.H. 1988. Micropropagation of woody plants: Post tissue culture aspects. *Acta Hort*., 227: 489-499.

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