ISSN 1995-4255, Contemporary Problems of Ecology, 2019, Vol. 12, No. 4, pp. 368–376. © Pleiades Publishing, Ltd., 2019.
Russian Text © The Author(s), 2019, published in Sibirskii Ekologicheskii Zhurnal, 2019, No. 4, pp. 455–465.

Regenerative In Vitro Capacity of Rare Species *Rhodiola rosea* L. from Various Habitats

A. A. Erst[a], * and V. V. Yakubov[b], **

[a]*Central Siberian Botanical Garden, Siberian Branch, Russian Academy of Sciences Novosibirsk, 630090 Russia*
[b]*Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far Eastern Branch, Russian Academy of Sciences, Vladivostok, 690022 Russia*
**e-mail: annaerst@yandex.ru*
***e-mail: yakubov@biosoil.ru*

Received November 22, 2018; revised December 19, 2018; accepted December 24, 2018

Abstract—The morphogenetic potential of in vitro culture of the valuable medicinal plant *Rhodiola rosea* from six natural habitats was evaluated as a basis for developing effective methods for the reproduction and conservation of the rare species. The influence of different quality seeds from different habitats on germination in vitro, the dependence of the regenerative capacity of *R. rosea* shoots on the concentration and combination of 6-benzylaminopurine (BAP) and α-naphthylacetic acid (NAA), and the effect of shoot precultivation on media with growth regulators on the development parameters of regenerants on a hormone-free medium 1/2 MS were studied. The dependence of in vitro germination of *R. rosea* seeds on the habitat of samples and shelf life is shown. It was noted that the introduction of growth regulators into the nutrient medium led to an increase in the multiplication factor by 1.9–2.8 times and a decrease in the height of the shoots by 2.4–3.3 times. Variant no. 2 from Sakhalin oblast was characterized by the highest average shoot height and breeding rate. For variant No. 4 from Kamchatka krai, various morphogenic reactions (sprouting, callus formation, and flowering of plants) have been noted in an in vitro culture. For all studied variants, 100% rooting on a 1/2 MS medium is typical. For variants nos. 1 and 5, the positive effect of precultivation of explants on media containing 1 mg/L BAP alone or in combination with 1 mg/L NAA is shown to obtain optimal indicators of rhizogenesis and the development of regenerants. Significant differences in the parameters of growth and development of explants in in vitro culture depending on the composition of the nutrient medium and habitat of *R. rosea* are shown.

Keywords: Rhodiola rosea, in vitro morphogenesis, biodiversity conservation
DOI: 10.1134/S1995425519040036

INTRODUCTION

Rhodiola rosea L. (golden root) is an herbaceous perennial plant of the family Crassulaceae, widely distributed in the arctic and mountainous regions of Europe, Asia, and North America, as well as on the sea coasts of the Arctic Ocean and the Far Eastern seas. The species *R. rosea* is included in the Red Book of the Russian Federation (*Krasnaya kniga*, 2008); it has the status of a rare and vulnerable species in many European countries, the United States, and Canada (according to the IUCN classification).

About 140 components were found in the underground part of *R. rosea*, the most valuable of which are salidroside and rosavin (Ahmed et al., 2015). Due to the increase in market demand, the high rate of removal of plants from the natural habitat, reduction of the habitat of *Rhodiola rosea* in different countries, and programs for the in situ and ex situ preservation of the species are extremely important.

When developing approaches for the ex situ reproduction of rare and endangered species of plants from seeds, it is necessary to have knowledge of the features of their biology and ecology. It is important to take into account characteristics such as different quality and rhythms of seasonal seed germination (Tkachenko, 2009). According to literary data, it is known that the seeds of golden root are characterized by variability in terms of morphological features and heterogeneity in terms of quality indicators, including germination and germination energy (Kim, 1999).

During the in vitro reproduction of the golden root, it has been shown that callusogenesis, organogenesis, and regeneration are influenced by various factors, including the composition of the nutrient medium and the type of explant (Furmanowa et al., 1995; Ishmuratova, 1998; Yin et al., 2004; Hai-jun et al., 2006; Tasheva, Kosturkova, 2010; Erst et al., 2018). It is known that the golden root requires nutrient media optimization, depending on the habitats of the plants

Table 1. Ecological and geographical characteristics of the collection sites of the studied samples of *Rhodiola rosea*

Natural area/altitudinal belt	Variant no.	Collection site	Coordinates	Altitude a.s.l., m
Coniferous and deciduous forests	1	Sakhalin Region, Kunashir Island, Yuzhno-Kurilsk, on the rocks by the sea	N 44°01.704′, E 145°51.476′	30–50
	2	Sakhalin Region, Sakhalin Island, Korsakovsky District, the west coast of the Tonino-Aniva Peninsula at Tri Brata Cape, cliffs and rocky slopes along the seashore and in the glen by the river	N 46°12.365′, E 143°25.401′	15–50
	3	Sakhalin Region, west of Sakhalin Island, Uglegorsk District, Tatar Strait coast, Cape Lamanon, on the damp rocks of the northern exposure above the deep shallow	N 48°47.400′, E 141°51.325′	30–50
Forest tundra	4	Kamchatka Krai, Penzhinsky district, Parapolsky dol, valley of the Ichigininvayam River, lichen wasteland on sandy–pebble sediments	N 61°24.592′, E 165°02.116′	66
Middle taiga/mountain forest belt	5	Kamchatka Territory, Ust-Kamchatka District, Klyuchevskaya group of volcanoes, Ploskaya volcano, "Kopyto" tract, bank of a dried-up stream and slopes along the edge of the forest of *Betula ermanii*	N 55°57.278′, E 160°14.061′	929
Subalpine belt	6	Altai Republic. Southern slopes of the Iolog ridge. Karakol lakes	N 51°29.0′, E 86°23.0′	1800–2000

from which the explants were isolated. Ishmuratova (1998) showed that liquid nutrient mediums are preferable for *R. rosea* plants from wet habitats and solid agar nutrient mediums, containing from 0.45 to 0.6% agar, are preferable for plants from dry and moderately moistened habitats. Based on the analysis of literature data, Tasheva and Kostyurkov (2010) note that the optimal concentrations of cytokinin 6-benzylaminopurine (BAP) for plants in the Altai population of *R. rosea* were 10–15 times higher than for the species from Tibet. However, a comparative analysis of the regenerative in vitro capacity of *R. rosea* from different habitats of the Asian part of Russia, depending on the concentration and combination of growth regulators, is not given in the literature.

The aim of the study is to identify the morphogenetic potential of *R. rosea* in six habitats in in vitro culture as the basis for the development of effective methods for the reproduction and preservation of the species.

MATERIALS AND METHODS

The seeds of *Rhodiola rosea* selected in 2015 and 2016 from six different places of growth (variants #1–6) are used for the study (Table 1).

The in vitro cultivation of the golden root was carried out in the Laboratory of Biotechnology of the Central Siberian Botanical Garden (CSBG) at the Siberian Branch, Russian Academy of Sciences (SB RAS), in Novosibirsk. Sterile work was performed under laminar boxing conditions (Lamsystems, Russia). The surface sterilization of *R. rosea* seeds was carried out according to the following scheme: the seeds were immersed in 70% alcohol for 30s, then in a 20% Domestos solution (sodium hypochlorite) for 20 min (orbital shaker, 100 rpm), followed by washing three times in sterile distilled water. For in vitro seed germination, 0.6% aqueous solution of agar (Spain) was used. Seeds were introduced into a culture in 2017.

The apical buds of seedlings were used as explants for in vitro reproduction. Murashige and Skoog (MS) was the main culture medium for cultivation (Murashige and Skoog, 1962). At the stage of shoot multiplication, the nutrient medium was supplemented with BAP at a concentration of 0.5 and 1 mg/L in combination with NAA (α-naphthylacetic acid) 0.5 and 1 mg/L or without auxin. Half the composition of the MS medium (1/2MS) was used for rooting. The influence of growth regulators used at the stage of micropropagation (precultivation) on the parameters of growth and development of regenerants during one passage was also evaluated on this medium. The source of carbohydrates was sucrose (30 g/L) and the pH of the medium before autoclaving was adjusted to 5.8. The duration of the passage was 30–35 days.

The explants were cultivated in the following conditions: photoperiod: 16/8h of light/dark; illumination: 2–3 klx: and temperature: 24 ± 1°C. Seeds were germinated on a photoperiod of 16/8 h of light/dark.

To study the different quality of seeds, the following indicators were taken into account: seed length (mm), seed width (mm), elongation coefficient, and seed area (mm^2). For studying the in vitro morpho-

Table 2. Characteristics of *Rhodiola rosea* seeds from different habitats

Variant no.	Collection year	% of germination		Seed area, mm^2	Seed length, mm	Seed width, mm	Elongation factor
		3 days	6 days				
1	2015	15	15	0.49 ± 0.14	1.32 ± 0.17	0.55 ± 0.09	0.43 ± 0.05
2	2015	7	7	0.64 ± 0.09	1.67 ± 0.13	0.58 ± 0.06	0.35 ± 0.04
3	2015	10	12	0.60 ± 0.11	1.74 ± 0.19	0.53 ± 0.07	0.32 ± 0.05
4	2016	80	87	0.53 ± 0.08	1.48 ± 0.13	0.52 ± 0.06	0.37 ± 0.05
5	2016	81	89	0.52 ± 0.14	1.58 ± 0.30	0.50 ± 0.10	0.34 ± 0.07
6	2016	32	52	0.85 ± 0.16	2.01 ± 0.23	0.68 ± 0.06	0.35 ± 0.05

genesis, the following factors were taken into account: the multiplication factor—number of developed shoots in one explant (pcs./exp.), shoot length (mm), rooting frequency (%), and average length of roots (pcs./exp.).

When calculating germination, seeds that germinated cotyledons were considered germinated. Germination energy (%) was calculated on the 3rd and germination rate (%) on the 6th day of cultivation. The interval for calculations was chosen experimentally, since there is no GOST for seed germination indicators of *R. rosea*.

Statistical processing of the results and analysis of the data were performed using Microsoft Excel 7.0 and STATISTICA 6.0 (LSD test, ANOVA). All experiments were performed in two replications with 20–30 explants in each replicate. Data are presented as mean values and confidence intervals ($p \le 0.05$).

The processing of images of seeds was carried out using the SIAMS Photolab system for obtaining and processing images at the Central Clinical Center at the CSBG SB RAS).

RESULTS

In Vitro Seed Germination

As a result of the study, the variability of *R. rosea* seed size associated with the geographical distribution of populations was revealed: the seeds of Far Eastern samples were significantly smaller than seeds from the Republic of Altai. They differed in length most significantly: 1.2–1.5 times, the width of the seeds was more stable: the differences were up to 1.2 times. The maximum seed size was typical for variant 6 of golden root from the Republic of Altai (0.85 ± 0.16 mm^2) and the smallest was for variant 1 from Kunashir Island (0.49 ± 0.14 mm^2) (Table 2). At the same time, a decrease in the absolute sizes of seeds is largely associated with a reduction in their length (elongation factor 0.43 ± 0.05).

During in vitro seed germination, it was found that the reduction in germination depends on the shelf life. After 2 years of storage, germination did not exceed 15%, whereas after one year of storage for Kamchatka plants, germination was 87–89 and 52% for samples from the Republic of Altai. Under in vitro conditions, the mass germination of *R. rosea* seeds was observed on the 3rd day of cultivation.

In Vitro Morphogenesis

We have shown that 100% of the explants were able to thrive on all tested media. The height of the shoots and the multiplication factor depended on the nutrient medium used and the habitat of golden root (Table 3, Figs. 1, 2). The introduction of growth regulators into the nutrient medium led to an increase in the multiplication factor by 1.9–2.8 times and a reduction in the height of the shoots by 2.4–3.3 times for all studied variants. Variant 2 was characterized by the highest average shoot height and breeding rate, and variants 4 and 5 by the lowest ($p < 0.05$).

The largest number of shoots of *R. rosea* developed on a medium containing 1 mg/L BAP and 0.5 mg/L NAA in variant 2 (8 pcs./exp.), and the smallest were in variant 1 on the same nutrient medium (1.3 pcs./exp.). In general, an increase in the multiplication factor should be noted when introducing growth regulators into the nutrient medium when compared to the control, but no general patterns were observed for the variants (Fig. 1).

Variant 4 growing on the control MS medium was characterized by the highest shoots (45.5 mm). The

Table 3. Growth and development parameters of *Rhodiola rosea* in vitro culture, depending on the place of growth of the parent plants (average values for all nutrient media)

Variant no.	Number of shoots per explant	Shoot height, mm	Callusogenesis, +/–
1	2.8ab	9.7b	–
2	3.1a	18.1a	–
3	2.4ab	10.1b	–
4	2.1b	17.2a	+
5	2.2b	13.0ab	–
6	2.8ab	12.2b	–

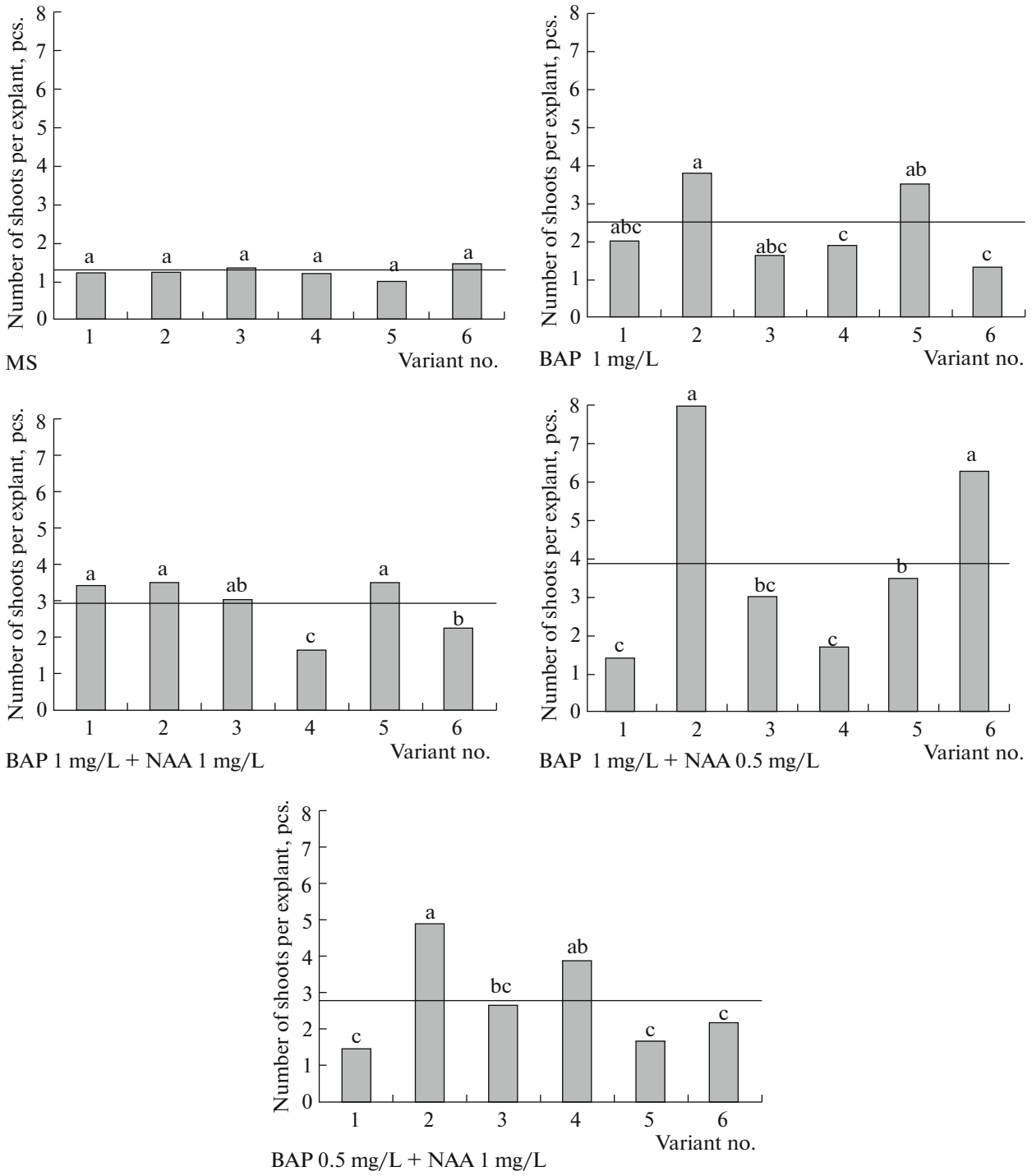

Fig. 1. Change in breeding ratio of *Rhodiola rosea* from different habitats depending the nutrient medium for cultivation. Note: the line on the graphs is the average for all habitats. Columns (values) denoted by the same letters are not significantly different at $p \leq 0.05$ (LSD-test, ANOVA).

introduction of cytokine BAP, alone or together with auxin NAA, led to a significant decrease in the height of shoots for all variants ($p < 0.05$) (Fig. 2). For variant 4, in vitro culturing revealed the formation of a nonmorphogenic callus on the medium supplemented with 1 mg/L BAP and 0.5 mg/L NAA. The further cultivation of callus on nutrient media containing BAP and NAA did not lead to the de novo regeneration of shoots. In addition, it was among the representatives of this variant that we observed the flowering of plants in an in vitro culture at the third passage on all the test nutrient media (Fig. 3).

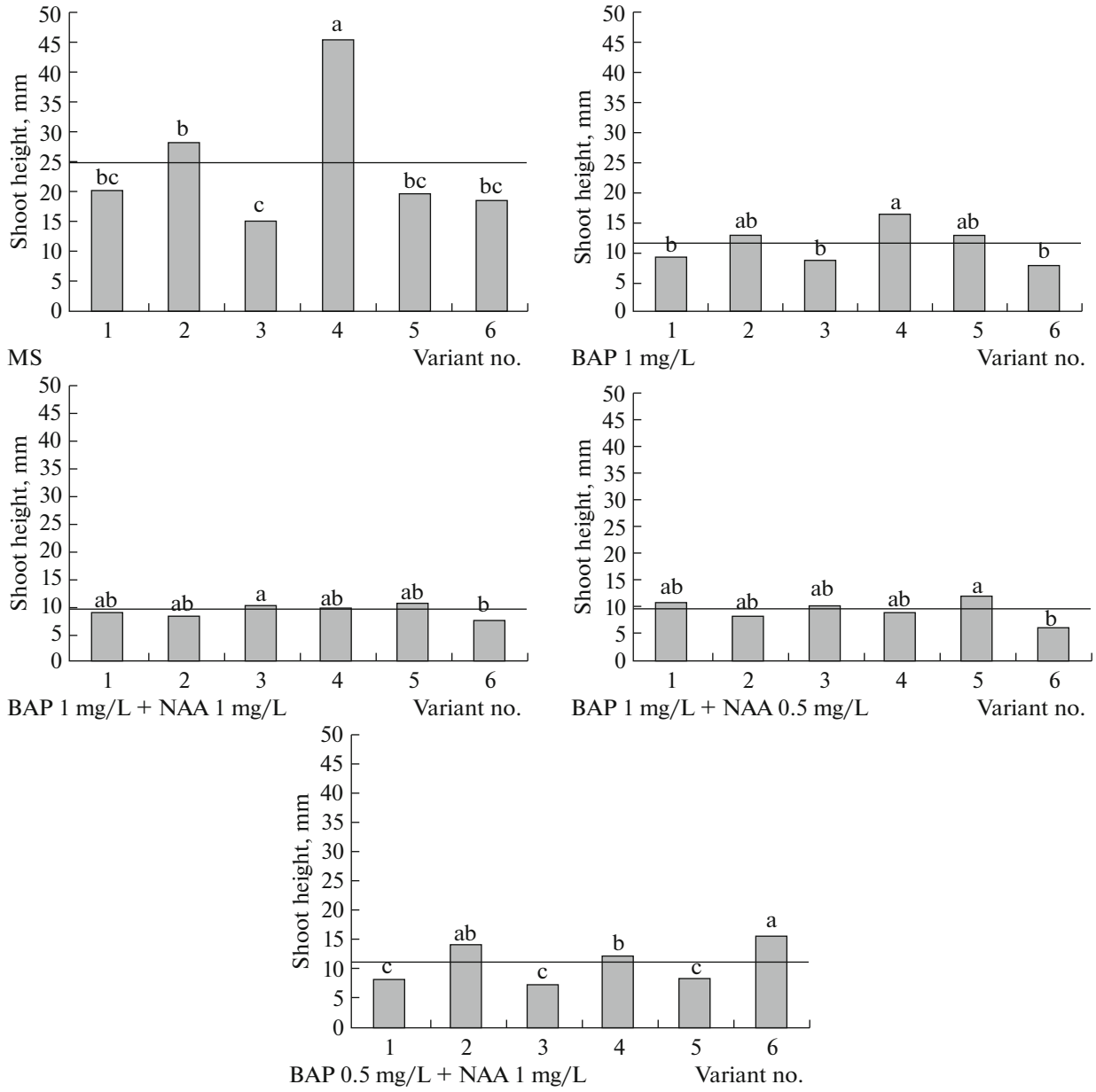

Fig. 2. Change in the shoot height of *Rhodiola rosea* from different habitats depending on the nutrient medium for cultivation. Note: the line on the graphs is the average for all habitats. Columns (values) denoted by the same letters are not significantly different at $p \le 0.05$ (LSD-test, ANOVA).

In Vitro Rooting

For all studied variants of *R. rosea*, 100% rooting on 1/2 MS medium is typical. The features of the influence of growth regulators used at the stage of micropropagation on the parameters of plant growth and development of the golden root from different populations at the in vitro rooting stage have been revealed (Tables 4, 5, 6). Therefore, for variant 3, cultivation on a medium supplemented with 1 mg/L BAP and 0.5 mg/L NAA with subsequent rooting of shoots in ½ MS led to a 1.6-time increase in the height of the shoots when compared to the control (Table 4). For variant 2, the opposite tendency, an increase in the height of shoots, was observed after cultivation on the control MS medium (Table 4). For variants 1 and 5, the development of several shoots on the rooting medium after cultivation on media with growth regulators is typical (Table 5). The average root length was longer when different combinations of growth regulators were used before the rooting stage. For variants 1 and 5, the maximum length of roots is typical for plants that were previously cultured on medium supplemented with 1 mg/L BAP and, for variants 2 and 4, with 1 mg/L BAP and 1 mg/l NAA (Table 6, Fig. 4).

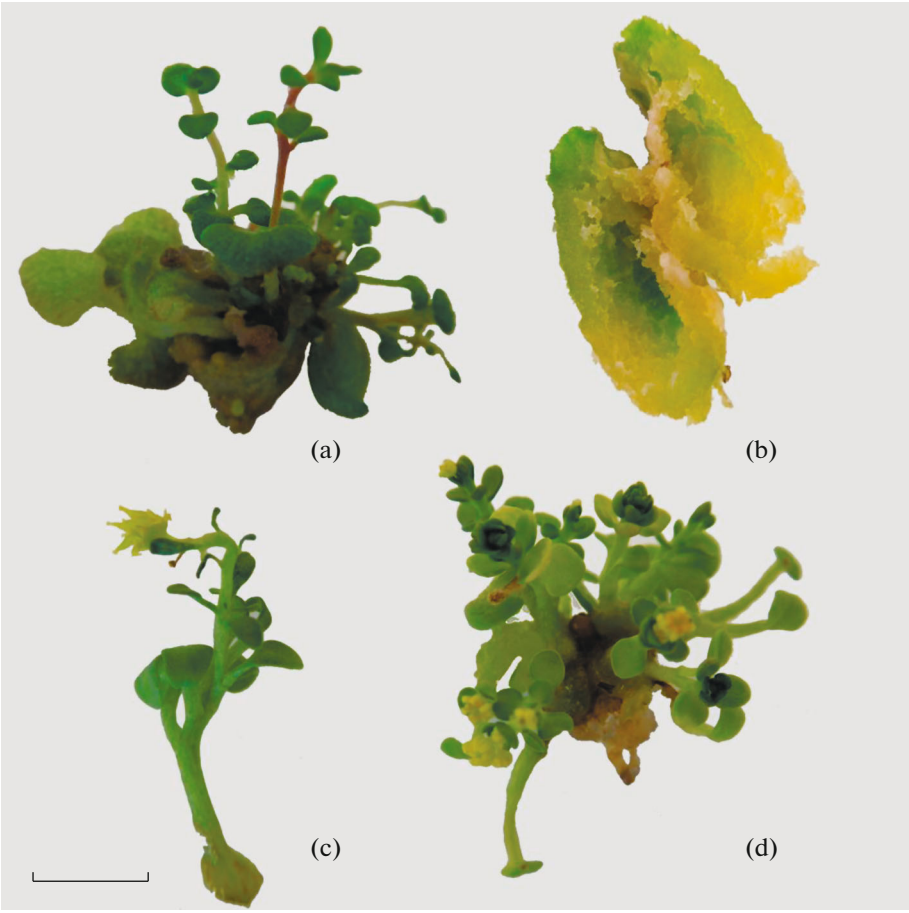

Fig. 3. Variant 4 of *Rhodiola rosea* under in vitro culturing in MS medium, supplemented with 1 mg/L BAP and 0.5 mg/L NAA: (a) shoot formation, (b) nemorphogenic callus (in section), (c) formation of pistillate flowers, (d) formation of staminate flowers.

DISCUSSION

In Vitro Seed Germination

The differences in germination parameters and germination energy, depending on the origin of the population and cultivation conditions, were previously shown for seeds of golden root. Ishmuratova and Satsyperova (1998) noted that the soil seed germination is rather low (11–23%) for *Rhodiola rosea* from Gorny Altai. For freshly harvested seeds of golden root from Bulgaria (Rila National Park), in vitro germination was 96% (Tasheva and Kosturkova, 2010). We have identified differences in the character of in vitro seed germination of golden root from different habitats. The larger seeds of variant 6 from the Altai Republic were characterized by a lower percentage of germination. The maximum percentage of germination for the studied variants was observed on the sixth day of in vitro cultivation.

Table 4. The effect of precultivation of *Rhodiola rosea* explants on different nutrient media on the height of the shoots (mm) in an in vitro culture after 30 days of cultivation on 1/2 MS

Nutrient medium for pre-cultivation	Variant no.					
	1	2	3	4	5	6
MS	26.8ab	35.4a	28.2b	25.1a	30.5a	25.4b
BAP 1 mg/L	23.0b	22.7b	32.4ab	27.3a	32.5a	26.5ab
BAP 1 mg/L + NAA 1 mg/L	35.3a	26.9b	25.3b	26.8a	26.6a	32.3a
BAP 1 mg/L + NAA 0.5 mg/L	29.4ab	23.1b	44.3a	30.2a	29.4a	24.5ab
BAP 0.5 mg/L + NAA 1 mg/L	23.7ab	23.7b	26.9b	25.4a	30.1a	26.9ab

Fig. 4. Rooting of variant 2 of *Rhodiola rosea* under in vitro culturing in 1/2 MS medium after precultivation on the hormone-free MS medium (a) and MS medium containing 1 mg/L BAP and 1 mg/L NAA (b).

In Vitro Morphogenesis

Factors such as plant genotype and combination and concentration of growth regulators in the nutrient medium were important for the in vitro regeneration processes of the representatives of the genus Rhodiola.

Table 5. Effect of precultivation of *Rhodiola rosea* explants on different nutrient media on the shoot formation (pcs.) in an in vitro culture after 30 days of cultivation on 1/2 MS

Nutrient medium for precultivation	Variant no.					
	1	2	3	4	5	6
MS	1.0b	1.4a	1.0a	1.0a	1.0b	1.0a
BAP 1 mg/L	2.7a	1.1a	1.0a	1.0a	1.9a	1.0a
BAP 1 mg/L + NAA 1 mg/L	2.7a	1.0a	1.0a	1.3a	1.3ab	1.0a
BAP 1 mg/L + NAA 0.5 mg/L	1.3b	1.3a	1.0a	1.1a	1.3ab	1.0a
BAP 0.5 mg/L + NAA 1 mg/L	1.3b	1.3a	1.1a	1.0a	1.1b	1.0a

Therefore, for *R. crenulata* and *R. yunnanensis*, the combination of 2.5 mg/L BAP and 0.1 mg/L NAA was optimal, which stimulated shoot formation by 71 and 84%. Nutrient medium containing higher concentrations of auxin NAA (0.5 mg/L) with the same concentration of BAP was used for the reproduction of *R. fastigata* and *R. sachalinesis*, and the regeneration was 80%. BAP, IAA, NAA, indole-3-butyric acid (IMC), and 2,4-dichlorophenoxy acetic acid (2,4-D) are the most commonly used growth regulators in the cultivation of golden root. The effect of zeatin, 2-isopentyladenine (2-IPA), kinetin and thidiazuron (TDZ) is also studied (Tasheva, Kosturkova, 2012). The introduction of benzyladenine or BAP in combination with NAA turned out to be effective to reproduce the Tibetan population of golden root, while the content of cytokinins should exceed the concentration of auxins (Yin et al., 2004). The positive result of sharing cytokinins and auxins is also shown for other plant species (Yan et al., 2009; Muraseva et al., 2015). Our

Table 6. Effect of precultivation of *Rhodiola rosea* explants on different nutrient media on the development of the root system (average root length, mm) in an in vitro culture after 30 days of cultivation on 1/2 MS

Nutrient medium for precultivation	Variant no.					
	1	2	3	4	5	6
MS	13.4bc	10.3b	13.0a	8.6b	15.2b	10.5a
BAP 1 mg/L	21.5a	7.9b	10.2a	10.3ab	37.9a	12.1a
BAP 1 mg/L + NAA 1 mg/L	15.5abc	16.0a	11.8a	15.7a	30.2a	13.0a
BAP 1 mg/L + NAA 0.5 mg/L	19.3ab	12.2b	12.4a	11.2ab	21.5ab	10.9a
BAP 0.5 mg/L + NAA 1 mg/L	11.5c	6.5b	11.9a	12.5ab	24.3ab	12.4a

studies also confirm the efficacy of using cytokinin BAP alone or in conjunction with NAA for the in vitro reproduction of the *R. rosea* samples studied. The morphogenic response of the explants to the introduction of growth regulators into the nutrient medium consisted of a significant increase in the multiplication factor and a decrease in the length of the shoots. The maximum multiplication factor is marked on MS medium containing 1 mg/L BAP and 0.5 mg/L NAA for variant 2 (8 pcs./exp.). For variant 4 (Kamchatka krai, Penzhinsky district), various morphogenic reactions (shoot formation, callusogenesis, in vitro flowering) were noted. In vitro flowering is considered a complex process regulated by such factors as plant growth regulators, a source of carbohydrates, pH of culturing medium, illumination, etc. (Heylen and Vendrig 1988; Zhang, 2007). Our study showed that the primary influence on this process is the origin of the golden root population, but not the in vitro cultivation conditions.

In Vitro Rooting

The success of the in vitro technologies depends largely on the stage of rooting of microshoots. The regeneration of roots in different species and varieties varies and depends both on the ability of plants to perceive the rooting factors and the rooting methods used. For *R. fastigata* and R. sachalinesis, effective rooting was observed on the medium containing IBA (87 and 73%, respectively). The effectiveness of this auxin at a concentration of 2 mg/L was shown for *R. rosea* (Bae et al., 2012). It is also known that the use of growth regulators before the rooting stage can significantly reduce plant rhizogenesis rates and, vice versa, rhizogenesis at the stage of shoot multiplication reduces the reproduction rate (Al-Khateeb, 2008). For example, for the successful in vitro rooting of *Vaccinium uliginosum* shoots, it is necessary to precultivate it on a hormone-free medium (Erst et al., 2018). In addition, it has been shown that the type and concentration of auxins used at the propagation stages preceding rooting influence the rooting ability of *Eucalyptus grandis* microshoots (Nakhooda et al., 2011). As a result of our study, it has been shown that microshoots of the studied *R. rosea* variants are rooted at 100% on a hormone-free 1/2 MS. Moreover, for variants 1 and 5, the highest rates of growth and development of regenerants (number of shoots and root length) were noted after the preliminary cultivation of explants on media containing 1 mg/L BAP or 1 mg/L BAP and 1 mg/L NAA.

CONCLUSIONS

Thus, we have shown the dependence of in vitro seed germination of *Rhodiola rosea* on the habitat of the samples and shelf life. No general patterns of morphogenic response among the studied samples of golden root, depending on the growing conditions of the mother plants and the applied growth regulators, have been identified. The introduction of growth regulators into the nutrient medium led to an increase in the multiplication factor 1.9–2.8 times and a reduction in the height of the shoots by 2.4–3.3 times. Variant 2 from Sakhalin oblast (Sakhalin Island) was characterized by the highest average shoot height and breeding rate. For variant 4 (Kamchatka krai, Penzhinsky district), the formation of a nonmorphogenic callus on the medium supplemented with 1 mg/L BAP and 0.5 mg/L NAA is noted. In addition, the flowering of plants was observed in an in vitro culture among the representatives of this variant. All studied variants were characterized by 100% rooting on a 1/2 MS medium. For variants 1 (Sakhalin oblast, Kunashir Island) and 5 (Kamchatka krai, Ust-Kamchatka district), the positive effect of precultivation of explants on media containing 1 mg/L BAP or 1 mg/L BAP and 1 mg/L NAA for optimal rhizogenesis and the development of regenerants was shown. Thus, the results of our study confirm the intraspecific variability of golden root and the need to optimize nutrient media for the cultivation of specific populations of the species. Developed biotechnologies can be the basis for programs to restore the natural populations of this endangered medicinal species.

ACKNOWLEDGEMENTS

In preparing the publication, materials from the bioresource scientific collection of the CSBG SB RAS "Collections of Living Plants in Open and Closed Ground," USU no. USU 440534, were used. Part of the work was performed on equipment from CCU "Microscopic Analysis of Biological Objects" at CSBG SB RAS.

FUNDING

This work was performed as part of the state assignment of the Central Siberian Botanical Garden, Siberian Branch, Russian Academy of Sciences, under the project "Assessing the Morphogenetic Potential of Plant Populations in North Asia by Experimental Methods" no. AAAA-A17-117012610051-5.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors declare that they have no conflict of interest.

Statement of the welfare of animals. This article does not contain any studies involving animals or human participants performed by any of the authors.

REFERENCES

Ahmed, F., Filion, V., Saleem, A., and Arnason, J.T., Phytochemistry of *Rhodiola rosea*, in *Rhodiola rosea*, Cuerrier, A. and Ampong-Nyarko, K., Eds., Boca Raton, FL: CRC Press, 2014, pp. 65–87.

Al-Khateeb, A.A., The problems facing the use of tissue culture technique in date palm (*Phoenix dactylifera* L.), *Sci. J. King Faisal Univ.*, 2008, vol. 9, pp. 85–104.

Bae, K.H., Ko, M.S., Kim, N.Y., Song, J.M., and Song, G.P., In vitro propagation and multiple shoot induction of *Rhodiola rosea* L. by axillary bud culture, *J. Plant Biotechnol.*, 2012, vol. 39, pp. 114–120.

Erst, A.A., Gorbunov, A.B., and Erst, A.S., Effect of concentration, method of auxin application and cultivation conditions on in vitro rooting of bog blueberry (*Vaccinium uliginosum* L.), *J. Berry Res.*, 2018, vol. 8, no. 1, pp. 41–53.

Furmanowa, M., Oledzka, H., Michalska, M., Sokolnicka, I., and Radomska, D., *Rhodiola rosea* L. (roseroot): In vitro regeneration and the biological activity of roots, in *Medicinal and Aromatic Plants VIII*, Biotechnology in Agriculture and Forestry Series vol. 33, Bajaj, Y.P.S., Ed., New York: Springer-Verlag, 1995, pp. 412–426.

Heylen, C. and Vendrig, J.C., The influence of different cytokinins and auxins on flower neoformation in thin cell layers of *Nicotiana tabacum* L., *Plant Cell Physiol.*, 1988, vol. 29, pp. 665–671.

Ishmuratova, M.M., Clonal micropropagation of *Rhodiola rosea* L. and *R. iremelica* Boriss. in vitro, *Rastit. Resur.*, 1998, vol. 34, no. 1, pp. 12–23.

Ishmuratova, M.M. and Satsyperova, I.F., Initial stages of ontogenesis and some biological features of development of *Rhodiola rosea* L. and *R. iremeloca* Boriss. introduced in Bashkiria, *Rastit. Resur.*, 1998, vol. 34, no. 1, pp. 3–11.

Kim, E.F., *Rhodiola rosea* (golden root) fam. Crassulaceae and biological principles of culturing, *Extended Abstract of Doctoral (Biol.) Dissertation*, Novosibirsk, 1999.

Krasnaya kniga Rossiiskoi Federatsii (rasteniya i griby) (The Red Data Book of Russian Federation: Plants and Fungi), Moscow: KMK, 2008.

Liu, H.-J., Guo, D., Yan, Q., Liu, Y.J., and Liu, C.Z., Tissue culture of four *Rhodiola species, Acta Bot. Boreali-Occident. Sin.*, 2006, no. 10, pp. 207–210.

Muraseva, D.S., Novikova, T.I., and Erst, A.A., In vitro propagation and conservation of rare species *Fritillaria meleagris* L. from floral explants, *Contemp. Probl. Ecol.*, 2015, vol. 8, no. 6, pp. 754–763.

Murashige, T. and Skoog, F., A revised medium for rapid growth and bioassays with tobacco tissue cultures, *Physiol. Plant*, 1962, vol. 15, no. 13, pp. 473–497.

Nakhooda, M., Watt, M. P., and Mycock, D., Auxin stability and accumulation during in vitro shoot morphogenesis influences subsequent root induction and development in *Eucalyptus grandis, Plant Growth Regul.*, 2011, vol. 65, pp. 263–271.

Tasheva, K. and Kosturkova, G., Bulgarian golden root in vitro cultures for micropropagation and reintroduction, *Central Eur. J. Biol.*, 2010, vol. 5, no. 6, pp. 853–863.

Tasheva, K. and Kosturkova, G., The role of biotechnology for conservation and biologically active substances production of *Rhodiola rosea*: endangered medicinal species, *Sci. World J.*, 2012, vol. 2012, art. ID 274942.

Tkachenko, K.G., Heterodiasporia and seasonal fluctuations in germination rhythms, *Nauchn. Ved. Belgorod. Gos. Univ.*, 2009, no. 11 (66), pp. 44–50.

Yan, M.M., Xu, C., Kim, C.H., Um, Y.C., Bah, A.A., and Guo, D.P., Effects of explant type, culture media and growth regulators on callus induction and plant regeneration of Chinese jiaotou (*Allium chinense*), *Sci. Hortic.*, 2009, vol. 123, no. 1, pp. 124–128.

Yin, W.B., Li, W., Du, G.S., and Huang, Q.N. Studies on tissue culture of Tibetan *Rhodiola rosea, Acta Bot. Boreali-Occident. Sin.*, 2004, vol. 24, pp. 1506–1510.

Zhang, T., In vitro flowering of *Perilla frutescens, In Vitro Cell. Dev. Biol.: Plant*, 2007, vol. 43, pp. 91–94.

Translated by M. Shulskaya