РАЗДЕЛ II

БИОТЕХНОЛОГИИ

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IN VITRO REGENERATION OF *RHODIOLA ROSEA* FROM ROOT EXPLANTS

T.A. Ak-Lama[1], A.A. Erst[1,2]

[1] *Central Siberian Botanical Garden SB RAS, Novosibirsk*
[2] *Ningbo Osaki Biotech Co., Ltd., China*

✉ annaerst@yandex.ru

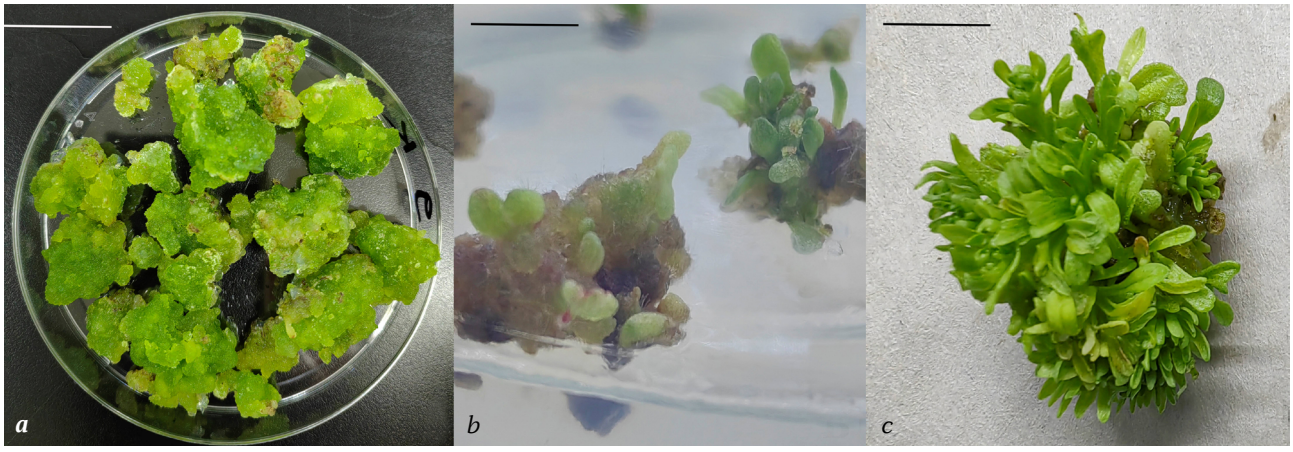
Abstract

The regeneration protocol developed in this study provides a basis for germplasm conservation and for further investigation of medicinally active constituents of the rare medicinal plant *Rhodiola rosea*.

Rhodiola rosea L. is a widely used medicinal plant in traditional medicine across northern countries, Eastern Europe, and Asia. It is prized for its beneficial effects, including stimulation of the nervous system, enhancement of physical and mental performance, alleviation of fatigue, and reduction of depression. Over 140 chemical compounds have been identified in the roots and rhizomes of *R. rosea*, with rosavins and salidroside being the primary active constituents [1].

Although this species is listed in the Red Book of the Russian Federation, the high commercial value and strong demand on both Russian and global markets have led to continued illegal harvesting from wild populations. In vitro propagation techniques offer an effective approach for the mass reproduction and conservation of the genetic resources of many endangered plant species [2].

In vitro regeneration has been successfully achieved for numerous medicinal and aromatic plants, including several species within the genus *Rhodiola* [3]. Specifically, shoot regeneration from leaf explants of *Rhodiola* species has been well documented [4–7]. However, to date, there are no reports describing shoot regeneration of *R. rosea* from root



Shoot regeneration and callus formation in *R. rosea* in vitro culture: *a* — non-morphogenic callus from leaf explant on MS medium supplemented with 5 μM TDZ and 5 μM NAA (Bar: 20 mm); *b* — adventitious shoots from root explant on MS medium supplemented with 5 μM TDZ and 5 μM NAA (Bar: 5 mm); *c* — adventitious shoots on MS medium supplemented with 0.5 μM TDZ (Bar: 10 mm)

explants. Root explants present several advantages over other explant types, including higher regeneration capacity and greater susceptibility to *Agrobacterium*-mediated transformation.

The objective of this study is to establish an efficient in vitro propagation protocol for *R. rosea* utilizing root explants.

In this study, sterile *R. rosea* sprouts were used and divided into two types of explants: root and cotyledon leaf. Our results demonstrated that Murashige and Skoog (MS) medium supplemented with 5 μM thidiazuron (TDZ) and 5 μM α-naphthylacetic acid (NAA) induced shoot regeneration in 86 % of sterile root explants. The multiplication coefficient reached 5.27 shoots per explant (see figure, *b*). In contrast, cotyledon leaf explants resulted only in callus formation without shoot regeneration (see figure, *a*). Subsequent two-stage cultivation of the regenerated microshoots — first on MS medium containing 5 μM TDZ for 2 weeks, followed by transfer to medium with reduced cytokinin concentration (0.5 μM) — promoted prolific shoot proliferation, yielding clusters of shortened microshoots averaging 47.13 shoots per explant (see figure, *c*).

In summary, an efficient regeneration system for *R. rosea* from root explants was successfully developed. Adventitious shoots formed on MS medium supplemented with 5 μM TDZ and 5 μM NAA. The rapid regeneration protocol established in this study provides a reliable source of explants for large-scale micropropagation, conservation of genetic resources, and further investigation of the medicinal active compounds of this valuable and rare species.

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