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MORPHO-HISTOLOGICAL ANALYSIS OF SHOOT REGENERATION AND LARGE-SCALE PROPAGATION OF AN ENDANGERED SPECIES *Rhododendron mucronulatum* Turcz.

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An efficient system for the *in vitro* propagation of the endangered medicinal and winter-hardy ornamental plant, *Rhododendron mucronulatum* Turcz., through high frequency shoot induction from seedlings and *in vitro*-derived apical shoots was developed. While testing different zeatin (Z) concentrations in the seedling culture, both axillary shoot regeneration and adventitious shoot formation on hypocotyls were observed. The highest frequency of shoot regeneration from seedlings (80 %), including formation of adventitious buds on hypocotyls (25 %), was recorded in the presence of 1.0 μM Z. The highest adventitious shoot numbers per explant (31.12 ± 6.19) were formed under 2.5 μM Z. Histological examination confirmed that adventitious buds directly originated from parenchymal cells of the hypocotyl. The effect of different concentrations of 2-isopentenyladenine and Z alone as well as in combination with indole-3-acetic acid (IAA) on axillary shoot proliferation from apical shoot explants was studied. The highest regeneration frequency (100 %) and shoot multiplication (9.70 ± 0.63) with a maximum length (15.75 mm) were obtained by using a combination of 0.1 μM IAA with 1.0 μM Z. The most efficient root formation was achieved through 4-hour pulse treatment with 148.0 μM indole-3-butyric acid followed by *ex vitro* rooting in a mixture of peat and sand (1 : 1). This study contributes to conventional and genetic-engineering breeding programs for creating new frost-resistant cultivars and developing a strategy for *R. mucronulatum* germplasm conservation, as well as commercial large-scale propagation.

Keywords: *Rhododendron L., adventitious shoot differentiation, axillary shoot proliferation, zeatin, morpho-histological analysis.*

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INTRODUCTION

Rhododendron mucronulatum Turcz. (*Ericaceae*) known as the Korean Rosebay is a winter-hardy species native to the Russian Far East, Northern China, Mongolia, Japan and Korea. As a result of high anthropogenic pressure, *R. mucronulatum* is included in the List of Rare Plants of the Russian Far East (Kharkevich, Katchura, 1981) but, despite this, no conservation action has been taken.

Due to the content of wide range of phenolic compounds, essential oils, ascorbic acid and other biologically active substances this species is used as a medicinal plant, which has antitussive, fungicidal, antioxidant, antimicrobial and anti-inflammatory properties (Committee…, 2005; Baranova et al., 2012), and can treat complications of diabetes (Mok, Lee, 2013). In addition, *R. mucronulatum* is highly valued as an attractive ornamental plant. It is a dense upright deciduous shrub, which can reach up to 3 m in height. Its flowers bloom in clusters at the branch tips in early spring before the leaves expand. *R. mucronulatum* is regarded as the earliest blooming species among the other cultivated rhododendron species. The wide funnel-shaped flowers, 4.5–5.0 cm across, typically range from pink to

mauve or a rosy purple in color, with white being rarely observed. Some forms have petals overlapping each other, providing the effect of a double flower (Vrishch et al., 2010). Its elliptic, medium green leaves are, in accordance with their species name, mucronate (have sharp, pointed tips) up to 12 cm in length. Dark green foliage of shrub turns an attractive yellowish and reddish hue in fall. *R. mucronulatum* possesses such valuable characteristics as cold resistance, a highly ornamental quality, the polymorphism of its corolla color and its early blooming attracts the attention of many breeders.

In recent years, rhododendron breeders have aimed to create new ornamental cultivars for cold climate zones using interspecific hybridization through crosses between highly ornamental taxa with the cold-hardy wild species (Muras, Klein, 1998). However, the most promising approach is a genetic engineering technique allowing the transfer of desired properties through Agrobacterium transformation or microprojectile bombardment of rhododendron tissues (Mertens et al., 1996; Pavingerová et al., 1997). Unfortunately, both techniques encounter the problem of *in vitro* regeneration (Deroles et al., 2002). Therefore, *in vitro* propagation systems for each rhododendron genotype need to be developed. In spite of the existence of many publications on the clonal micropropagation of *Rhododendron* species and cultivars of a high commercial interest (Eeckhaut et al., 2010), there have been few attempts to apply this approach to the reproduction of species native to Asian Russia (McCown, Lloyd, 1982). Moreover, the developed techniques are not universal and the protocols used for the micropropagation of evergreen rhododendrons have not been efficient for wild deciduous and semi-evergreen species due to some genotypic differences (Eeckhaut et al., 2010; Zaytseva et al., 2016).

To introduce *R. mucronulatum* as a new ornamental plant and to create a germplasm resource base for conservation and breeding programs, it is necessary to develop the technology for producing a large amount of microclones. Since the seeds are of genetic heterogeneity, the use of them as starting material for establishing cultures and micropropagation is a preferable way to preserve the genetic diversity of the species (Benson et al., 2000), which is highly important for developing and strengthening a *R. mucronulatum* protection strategy. Besides, the use of seedlings as explants induces a broad range of morphogenic responses since the seedling tissues exhibit a high regenerative potential. Both the axillary shoot proliferation and *de novo* organogenesis associated with cell dedifferentiation seem to be quite possible in the seedling culture (Mulwa, Bhalla, 2006; Paul et al., 2011). The study of the localization of target cells, where initiation of *de novo* organogenesis or somatic embryogenesis occurs is important since the necessity of genetic transformation in woody plants has been increasingly growing in recent years. Therefore histological examinations of morphogenesis processes are needed. To our knowledge, such studies have not been conducted on *in vitro* regeneration from *R. mucronulatum* seedlings. The objectives of the present study were (a) to reveal the morphogenic responses of seedlings using histological analysis and (b) to develop efficient protocols for large-scale and rapid multiplication of *R. mucronulatum* from seedlings and *in vitro*-derived apical shoots.

MATERIALS AND METHODS

The effect of zeatin on shoot induction from seedlings. The seedlings obtained after *in vitro* germination in accordance with our early report (Zaytseva, Novikova, 2015) were exploited for explant preparation. For shoot regeneration, 3-wk old seedlings with removed roots were used as an initial explant (seedling explants). These seedling explants were placed on 0.6 % (*w*/*v*) agar-solidified Anderson's medium (AM) supplemented with different concentrations of zeatin (Z) (0, 1.0, 2.5, 5.0, 10.0 μM). After 8 wk of cultivation, the frequency and the type of morphogenic response and the number of axillary and adventitious shoots per explant were assessed. Fifteen explants were used per treatment in each of three replicate experiments. The pH of the medium was adjusted to 5.0 before autoclaving (121 °C; 1.05 kg · cm^{-2}), and plant growth regulators (PGRs) were added to the medium post-autoclaving. All cultures were incubated at (23 ± 2) °C under cool white fluorescent light (Philips, Pila, Poland) at an intensity of 40 μmol $m^{-2} \cdot s^{-1}$ with a 16-h photoperiod.

Morpho-histological analysis of adventitious shoot formation. Seedling explants cultured on AM supplemented with 1.0 μM Z were collected at 0 d, 2 d, 3 d, 5 d, 7 d, 10 d, 12 d, 14 d, 21 d, 5 wk and 8 wk from the start of the experiment and prepared for examination by light microscopy. Explants were fixed in glacial acetic acid (99.9 %), formalin (40 %), and ethyl alcohol (96 %) in the proportions 7 : 7 : 100 (v/v/v). The samples were dehydrated and embedded in Paraplast® (Sigma-Aldrich®) in accordance with Z. P. Pausheva (1988) and sectioned at 5 μm, using a microtome (HM-325 Microm, Walldorf, Germany). Sections were stained

with 0.05 % toluidine blue (Sigma-Aldrich®) for 3 min (Pausheva, 1988). Histological analysis was conducted using a microscope equipped with Axioplan 2 imaging, Axioskop-40, an AxioCam MRc5 camera and AxioVision 4.8 software (all from Carl Zeiss). The morphology of the regenerants was studied using Stereo Discovery V12 microscope and AxioCam HRc camera (all from Carl Zeiss, Gottingen, Germany).

The effect of plant growth regulators on shoot multiplication. For shoot multiplication, 3-nodal apical shoots were isolated from microshoots derived from seedling explants cultivated with 2.5 μM Z as shown above (item 2.2). To reveal the optimal conditions for bud break in apical shoots, the study was carried out in two steps. At the first stage, the explants were cultivated on AM, supplemented with either 2-isopentenyladenine (2-iP) or Z in the same concentrations (0, 0.5, 1.0, 2.5, 5.0 or 10.0 μM). Then, once the efficient cytokinin concentration was found (1.0 μM Z), the apical shoots were placed on AM, supplemented with 1.0 μM Z and indole-3-acetic acid (IAA) in various concentrations (0.1, 0.25, 0.5, 1.0, 2.0 μM). After 8 wk of culture the bud break frequency, number of shoot per explant (length ≥ 5 mm) and shoot length were assessed. Fifteen explants were used per treatment in each of the three replicate experiments.

Shoot rooting and acclimatization. The elongated regenerants (h ≥ 10 mm) were rooted in different conditions (*in vitro* or *ex vitro*). To stimulate root formation, two methods were tested: (a) direct 6-wk cultivation on AM, supplemented with 10.0 or 25.0 μM indole-3-butyric acid (IBA) and (b) 4-h presoaking with a solution of 148.0 μM IBA (pulse treatment). After IBA-pulse treatment the regenerants were placed either *in vitro* on AM0 or *ex vitro* in the mixture of peat (pH = 4.0–5.0) and sand (1 : 1, *v*/*v*). The rooting frequency, number and length of roots were assessed after 6 wk. Fifteen microshoots were used three times for each treatment. The plantlets were maintained under a cool white fluorescent light (27 μmol · m^{-2} · s^{-1}) with 16-hour photoperiod at (23 ± 2) °C. The acclimatization of *in vitro* rooted plantlets was carried out in a mixture of peat and sand (1 : 1, *v*/*v*) for 6 wk in high humidity. The plants acclimatized were transferred to greenhouse in 10-cm diameter pots. After 6 months of growing in the greenhouse, the *R. mucronulatum* young shrubs were transplanted in the open field conditions.

Statistical analysis. All data was analyzed by one-way ANOVA to assess treatment differences and interactions using STATISTICA 8 (StatSoft Inc., Tulsa, OK). The data is presented as means ± SE (standard error). Significance between means was tested by Duncan's test ($P \leq 0.05$).

RESULTS AND DISCUSSION

The effect of zeatin on the morphogenic reactions of seedlings. One of the determining factors affecting the success of *in vitro* plant propagation is the choice of plant growth regulators (PGRs). According to our data, the addition of 1.0 μM Z to the medium induced morphogenic reaction in 80 % of explants including direct formation of adventitious buds on hypocotyls of 25 % of them (Fig. 1, *a*, *b*).

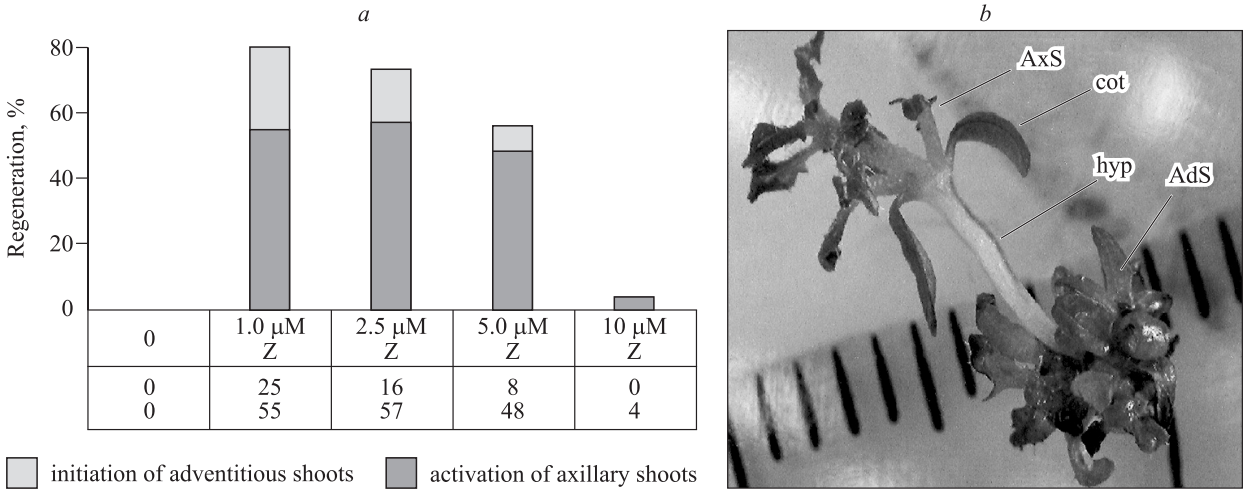

Fig. 1. Effect of Z on shoot regeneration from *R. mucronulatum* seedlings: *a* – frequency of axillary shoot proliferation and adventitious shoot regeneration after 8 weeks of cultivation; bars represent the standard error; *b* – morphology of regenerants after 6 wk of culture on AM supplemented with 2.5 μM Z. AdS – adventitious shoots; AxS – axillary shoots; hyp – hypocotyl; cot – cotyledons.

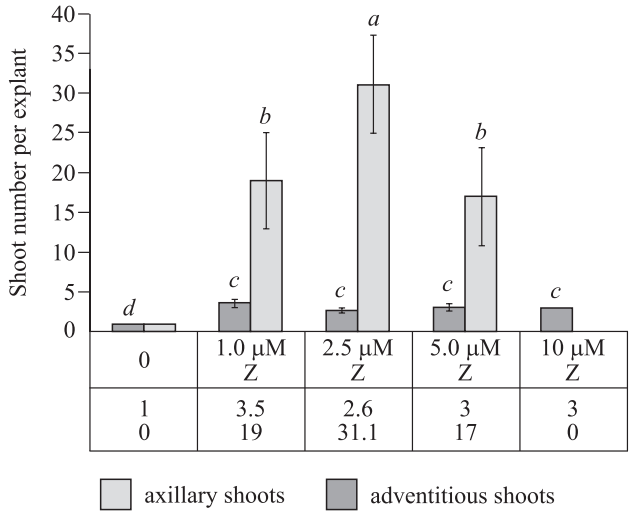

Fig. 2. Effect of Z concentration on the number of axillary and adventitious shoots in vitro seedling culture of *R. mucronulatum*. Means followed by the same letter are not significantly different ($P \leq 0.05$) according to Duncan's test. Bars represents the standard error.

Adventitious shoot numbers were the highest (31.12 ± 6.19 shoots per explant) at 2.5 μM Z (Fig. 2). Increasing Z concentrations up to 5.0 μM led to a decline in both the percentage of explants capable of morphogenic response and the frequency of adventitious bud formation (Fig. 1, *a* and Fig. 2). However, there was no significant effect of Z in 1.0–10.0 μM range on the number of axillary shoots (Fig. 2).

Thus, while testing different zeatin concentrations, we observed various morphogenic reactions in *R. mucronulatum* seedling culture. In low concentrations, this growth regulator simultaneously triggered both the axillary shoot regeneration, by reducing apical dominance and the direct adventitious shoot formation via the induction of cell dedifferentiation processes, and competence acquisition to further morphogenesis on hypocotyls of *R. mucronulatum* seedlings. Earlier reports demonstrated that the seedling apexes of *R. smirnowii* and *R. catawbiense* possessed the highest morphogenic response, whereas the hypocotyl tissues had an extremely low regenerative capacity (Kutas, 2009). To the contrary, our study found hypocotyls to be more morphogenic than the apexes of seedlings.

Adventitious shoot differentiation from seedlings. To identify the cells involved in *de novo* organogenesis from hypocotyl of *R. mucronulatum*, a histological analysis was conducted. Light microscopy of transverse sections of the hypocotyls at the time of explant isolation (0 d) demonstrated a single-layered epidermis, parenchyma of three or four cell layers and the vascular bundle (Fig. 3, *a*). Incubating explants for 12 d on AM with 1.0 μM Z caused the increase in diameter of the hypocotyl on

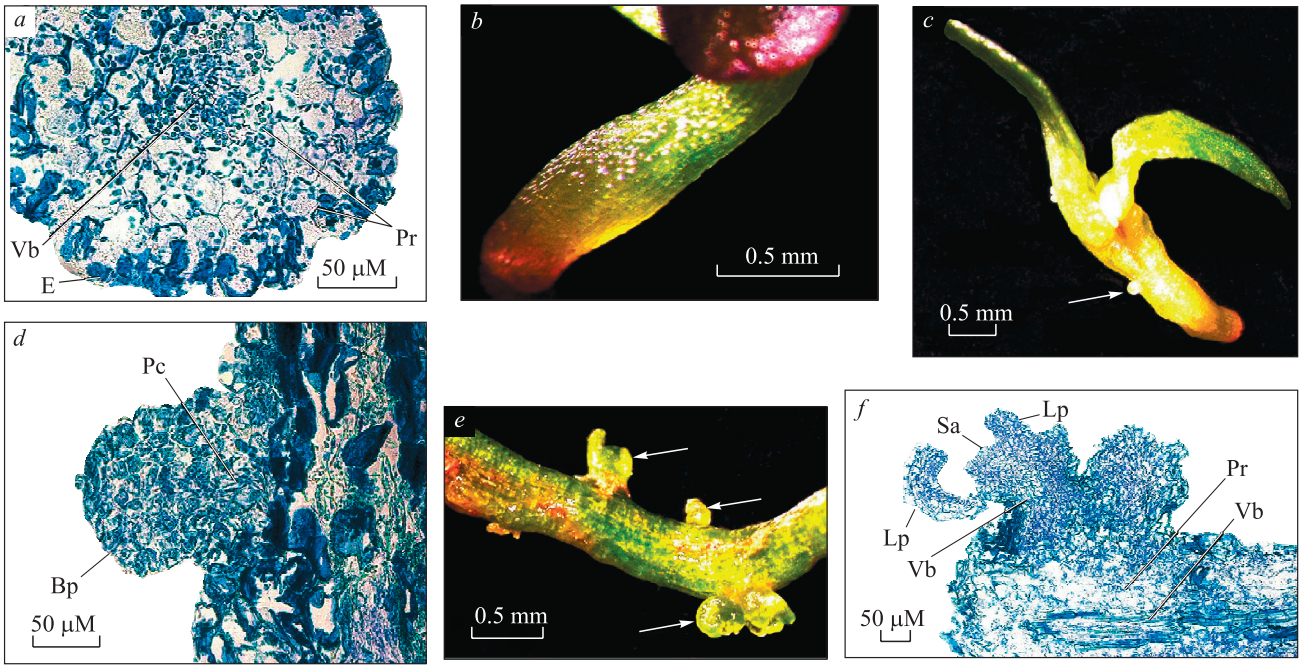

Fig. 3. Morpho-histological observations of adventitious bud formation from hypocotyl of *R. mucronulatum* seedling explants: *a* – cross section through hypocotyl at 0 d; *b* – the increased cut end of hypocotyl after 12 d of culture; *c* – development of adventitious bud primordia (arrow) at 21 d; *d* – longitudinal section through hypocotyl on early stage of bud primordia development from parenchymal cell at 21 d; *e* – formation of adventitious buds (arrow) at 5 wk; *f* – longitudinal section of adventitious buds with shoot apex, leaf primordia and vascular bundle at 5 wk of culture. Bp – bud primordium; E – epidermis; Lp – leaf primordium; Pr – parenchyma; Pc – procambium; Sa – shoot apex; Vb – vascular bundle.

the cut end (Fig. 3, *b*). Further cell divisions in the parenchyma and epidermis gave rise to a number of meristematic centers, which resulted in differentiation of adventitious bud primordia directly on the surface of the hypocotyl (Fig. 3, *c*, *d*). Completely developed adventitious buds with shoot apex, leaf primordia and vascular bundle were observed by 5 wk (Fig. 3, *e*, *f*). Adventitious bud formation occurred along the whole length of the hypocotyl surface, but more often near the cut end (Fig. 1, *b*).

Thus, a direct *de novo* organogenesis was found to occur in the culture of *R. mucronulatum* seedlings under Z. The initial stages of morphogenesis were localized in the outer layers of parenchyma. According our observations, hypocotyl tissues near the cut end were of the highest regenerative ability. This fact has already been demonstrated in *Eucommia ulmoides* (Chen et al., 2008). Regenerants of *R. mucronulatum* in the early stages of development also looked similar to somatic embryos (Fig. 3, *c*, *e*), however, morpho-histological examination revealed the lack of root apex and bipolar structure in subsequent stages. Histological observations for definition of initiation cells in *de novo* organogenesis from different part of seedlings in several species were carried out. The highest morphogenic potential was shown to be of epidermal, subepidermal and parenchymal tissues of immature cotyledon of *Macadamia tetraphylla* (Mulwa, Bhalla, 2006) and *Murraya koenigii* (Paul et al., 2011). The study on localization of target cells, which have the potential to regenerate is important for forest tree genetic transformation and clonal propagation (Ravi Kumar, 2000).

The effect of 2-iP, Z and IAA on axillary bud proliferation from apical shoots. The effect of different concentrations of Z, 2-iP and IAA on *in vitro* shoot regeneration from apical shoots of *R. mucronulatum* micropropagated plantlets was tested. The explants cultivated on AM0 within 8 wk demonstrated the monopodial growth only without axillary bud proliferation. Addition of 2-iP or Z in AM disrupted apical dominance of shoot tips and induced the proliferation of axillary shoots depending upon the type and concentration of cytokinin (Table 1). Generally, the regeneration frequency decreased with increasing concentrations of cytokinins. At the same time, Z was found to be more effective as the trigger of axillary shoots development than 2-iP used traditionally for rhododendron multiplication. Thus, the exposure of culture to 1.0 μM Z induced 100 % regeneration frequency, the highest shoot multiplication (7.09 shoots per explant) and shoot length (l = 10.80 mm) whereas addition of 1.0 μM 2-iP in medium led to axillary bud proliferation of 79 % explants and about 1.77 shoots per explant only. With increasing Z concentration these parameters declined.

Addition of 10.0 μM Z in medium completely depressed the shoot multiplication (Fig. 4, *a*). The effect of 2-iP on shoot multiplication and length was not significant in comparison with Z and did not depend on 2-iP level (Fig. 4, *a*, *b*). The results showed that the highest number of axillary shoots with length more than 10 mm was produced in the presence of 1.0 μM Z.

Application of auxin in the range of concentrations (0.1–2.0 μM IAA) in combination with opti-

Table 1. Effect of different concentrations of 2-iP, Z and IAA on axillary shoot regeneration from *R. mucronulatum* apical shoots. The meanings of Data presented ± SE. Meanings followed by the same letter are not significantly different ($P \leq 0.05$) according to Duncan's test

PGRs, μM			Proliferation frequency, %	Shoot number per explant	Shoot length, mm
2-iP	Z	IAA			
–	–	–	84.00	1.00 ± 0.00 e	9.01 ± 0.19 d
1.00	–	–	79.00	1.77 ± 0.33 e	5.00 ± 0.17 ef
2.50	–	–	55.00	1.43 ± 0.38 e	5.61 ± 0.20 ef
5.00	–	–	46.00	2.35 ± 0.41 e	4.65 ± 0.39 f
–	1.00	–	100.00	7.09 ± 0.28 b	10.80 ± 0.25 cd
–	2.50	–	93.00	6.00 ± 0.53 bcd	6.82 ± 0.27 e
–	5.00	–	51.00	5.10 ± 0.41 cd	4.43 ± 0.31 f
–	10.0	–	0.00	0.00 ± 0.00 e	0.00 ± 0.00
–	1.00	0.10	100.00	9.70 ± 0.63 a	15.75 ± 0.82 a
–	1.00	0.25	100.00	6.76 ± 0.77 bc	12.20 ± 0.46 bc
–	1.00	0.50	100.00	7.33 ± 0.84 b	12.96 ± 0.78 b
–	1.00	1.00	100.00	5.57 ± 0.43 bcd	11.30 ± 0.41 bcd
–	1.00	2.00	100.00	4.40 ± 0.31 d	9.63 ± 0.57 d

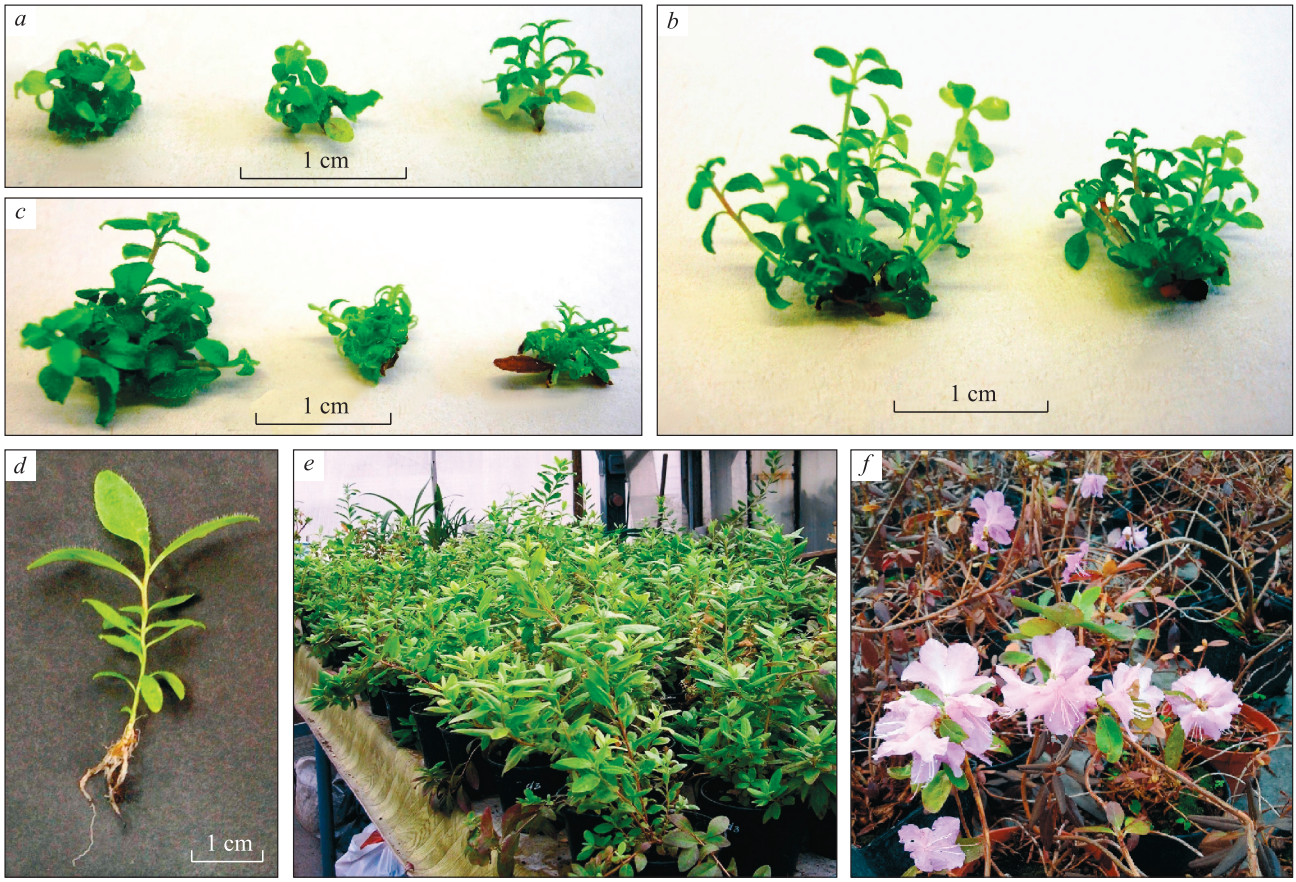

Fig. 4. Large-scale micropropagation of *R. mucronulatum* via *in vitro*-derived apical shoots: *a* – 8-wk shoots obtained under 1.0, 2.5, 5.0 μM 2-iP (from left to right); *b* – 8-wk shoots obtained under 1.0, 2.5, 5.0 μM Z (from left to right); *c* – 8-wk shoots obtained under 1.0 μM Z and 0.1 μM IAA; *d* – shoot rooted *ex vitro* in peat and sand after IBA-pulse treatment; *e* – plant grown in the greenhouse for 6 months; *f* – flowering plants after 1 year from rooting start.

mal Z concentration (1.0 μM) was effective both for regeneration frequency (100 %) and shoot multiplication (Table 1). The best results were achieved in combination 0.1 μM IAA with 1.0 μM Z, which promoted an average 9.7 shoots per explant with maximum length (15.75 mm) (Fig. 4, *c*).

Our data also revealed that shoot length was greater in all variants tested with IAA application in comparison with control and cytokinin variants. At the same time, the inverse dependence of IAA concentration with shoot number and their length was observed. Further increase of IAA concentration up to 2.0 μM resulted in decrease of proliferation activity and shoot elongation.

For rhododendron micropropagation, 2iP and IAA have been successfully used during shoot proliferation of several *Rhododendron* species and hybrids (Iapichino et al., 1991; Briggs et al., 1994; Hsia, Korban, 1997). Moreover, it was found that the high levels of these PGRs were most commonly exploited for maximal shoot proliferation of some evergreen *Rhododendrons* (Kamenicka et al., 1998). Almeida et al. (2005) reported that increasing Z concentration promoted shoot multiplication of *R. ponticum* and the shoot growth was higher in apical shoots and it was not stimulated by the presence of auxin. In the present study, on the contrary, the low levels of Z (1.0 μM) both alone and especially in the combination with IAA (0.1 μM) were demonstrated to be the most effective in terms of shoot multiplication and shoot length of *R. mucronulatum*. The application of 2-iP in range testing was found to not be favorable for the multiplication of *R. mucronulatum* in comparison with Z. At the same time, the efficiency of 2-iP for clonal micropropagation of evergreen cultivars was shown, although the higher concentrations of this cytokinin (more than 10.0 μM) were applied (Iapichino et al., 1991; Kita et al., 2005; Mao et al., 2011). Thus, the morphogenic response to exogenous PGRs in *Rhododendron* genus strongly depends on the genotype.

Rooting and acclimatization. To stimulate root formation, two types of treatments were used: (1) 6-wk cultivation on AM supplemented with IBA or (2) 4-h IBA-pulse treatment. The rooting percent was low in culturing *in vitro* on AM contain-

Table 2. Effects of rooting conditions and different IBA treatments on rooting frequency, root number and root and shoot length of *R. mucronulatum.* The Data presented are means ± SE. Means followed by the same letter are not significantly different ($P \leq 0.05$) according to Duncan's test

Rooting condition		Rooting, %	Root number	Root length, cm	Part of plants with secondary roots, %	Shoot length, cm
IBA-pulse treatment	*ex vitro* peat : sand	89.00	1.60 ± 0.09 a	6.41 ± 0.61 a	88.00	2.90 ± 0.22 a
	in vitro AM0	31.00	0.72 ± 0.23 b	2.00 ± 0.63 b	40.00	1.32 ± 0.11 c
in vitro AM 10.0 μM IBA		10.00	1.00 ± 0.00 b	0.20 ± 0.00 c	0.00	1.70 ± 0.10 b
in vitro AM 25.0 μM IBA		0.00	–	–	–	–

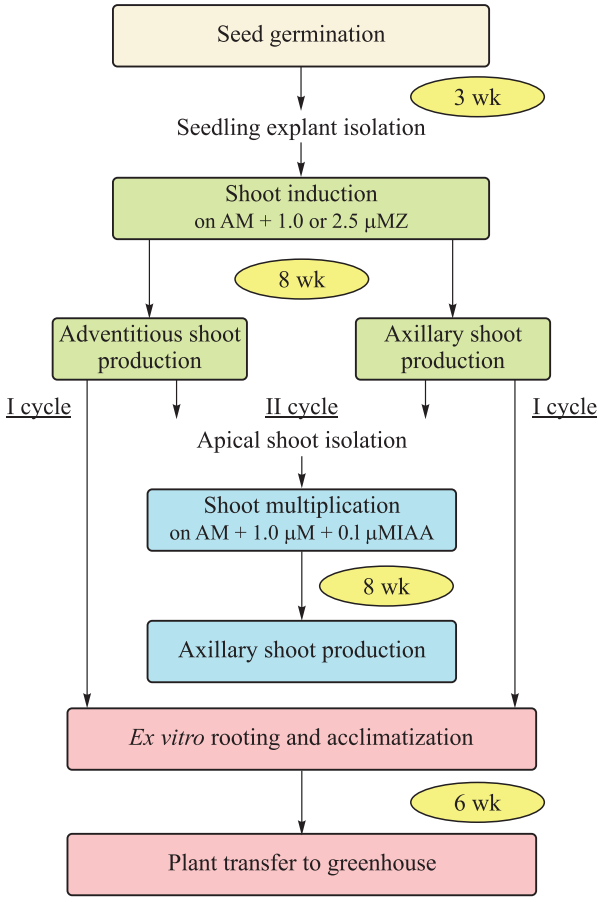

Fig. 5. Schematic representation of the plant micropropagation protocols from *in vitro* germinated *R. mucronulatum* seeds: I cycle – one multiplication cycle, one explant type (seedling); II cycle – two multiplication cycles, two explant types (seedling and apical shoots).

ing 10.0 or 25.0 μM IBA as well as on AM0 after IBA-pulse treatment (table 2). Moreover, plantlets rooted *in vitro* needed subsequent acclimatization. As compared with *in vitro* rooting technique, IBA-pulse treatment followed by *ex vitro* rooting in a mixture of peat and sand showed the most efficiency for *R. mucronulatum* rooting. The plants rooted in this way were of the well-development root and shoot system, which were successfully acclimatized (Fig. 4, *d*, *e*). The short pretreatments with the solutions of different exogenous auxins were used for rooting in various plant species: *Pistacia vera* (Benmahioul et al., 2012), olive (Leva, 2011), *Arnebia hispidissima* (Phulwaria, Shekhawat, 2013). In the present study, the effectiveness of IBA-pulse treatment in the combination with *ex vitro R. mucronulatum* rooting was shown to result in improving the quality of roots, and reducing the time of obtaining the acclimatized plants twofold and, therefore, decreasing the production cost.

To summarize, acclimatized plant production from *R. mucronulatum* seeds based on the developed *in vitro* multiplication system takes 17 or 25 wk (Fig. 5), depending on the shoot production cycle (I or II) and results in obtaining an average of 27 or 267 plants per seed, respectively. Moreover, it was observed that the ontogenesis rate of *in vitro* derived plants of *R. mucronulatum* increased twofold as compared with open field plants. After 1–2 months of *ex vitro* growing, the plants started to branch out and after 1–1.5 years most of them was in blossom (Fig. 4, *f*), which is an incontestable advantage for breeding.

CONCLUSIONS

A rapid regeneration system for the conservation and large-scale propagation of *R. mucronulatum* from seedlings and *in vitro* derived apical shoots was developed. The morphogenic potential of hypocotyl for shoot multiplication was demonstrated to be considerably higher than the seedling apex. The early stages of adventitious shoot organogenesis were localized in the upper cell layers of the hypocotyl parenchyma. This finding could be useful for genetic engineering through *Agrobacterium*-mediated or microprojectile bombardment trans-

formation due to expanding regeneration ability of the seedlings. Moreover, the efficient micropropagation protocol of *R. mucronulatum* using axillary shoot proliferation based on high activity of low levels of Z and IAA, and *ex vitro* rooting after IBA-pulse treatment was shown. The present study can strengthen conservation, breeding and the cultivar development on the base of *R. mucronulatum* as an important medicinal and ornamental plant for some cold environments.

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In vitro material from collection № USU_440534 «Collection of living plants indoors and outdoors» was used in the study.

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МОРФОГИСТОЛОГИЧЕСКИЙ АНАЛИЗ РЕГЕНЕРАЦИИ ПОБЕГОВ И МАССОВОЕ РАЗМНОЖЕНИЕ *Rhododendron mucronulatum* Turcz. КАК РЕДКОГО ВИДА

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Разработана эффективная система для размножения *in vitro Rhododendron mucronulatum* Turcz. – редкого лекарственного и морозостойкого декоративного растения – путем индукции побегообразования из проростков и микрочеренков с апикальными почками. При исследовании действия различных концентраций зеатина (Z) в культуре проростков наблюдали как пролиферацию пазушных меристем апикальных почек проростков, так и регенерацию адвентивных побегов на гипокотилях. Максимальная частота регенерации из проростков (80 %), включая формирование адвентивных почек на гипокотилях (25 %), отмечена в присутствии 1.0 μM Z. Максимальное число побегов на эксплант (31.12 ± 6.19) сформировалось под действием 2.5 μM Z. Гистологический анализ показал, что адвентивные почки развивались из паренхимных клеток гипокотилей. Исследовали влияние различных концентраций 2-изопентиладенина и Z как в комбинации с индолил-3-уксусной кислотой, так и без нее на пролиферацию пазушных меристем апикальных микрочеренков. Наибольшие показатели частоты регенерации (100 %) и числа побегов на эксплант (9.70 ± 0.63) с максимальной высотой (15.75 мм) получены при использовании комбинации 0.1 μM ИУК и 1.0 μM Z. Наиболее эффективным способом укоренения оказалась 4-часовая импульсная обработка побегов 148.0 μM индолил-3-масляной кислотой с последующим укоренением *ex vitro* в смеси торфа и песка (1 : 1). Проведенное исследование будет способствовать разработке стратегии сохранения *R. mucronulatum*, созданию традиционных и основанных на генной инженерии селекционных программ для получения новых морозоустойчивых сортов, а также использоваться для массового коммерческого размножения.

Ключевые слова: *Rhododendron L., дифференциация адвентивных побегов, пролиферация пазушных побегов, зеатин, морфогистологический анализ.*