Plant Tissue Cult. & Biotech. **26**(2): 143-149, 2016 (December) **PTC&B**

In vitro Conservation of *Campanula sclerophylla* Kolak - Endemic Endangered Species of Western Caucasus

T. M. Kolomiets, V. I. Malyarovskaya and L. S. Samarina

Laboratory of Plant Biotechnology, Physiology and Biochemistry, Russian Research Institute of Floriculture and Subtropical Crops, Jan Fabritsiusa str., 2/28, 354002 Sochi, Russia

Key words: In vitro conservation, *Campanula sclerophylla*, Endemic, Endangered species

Abstract

The study was conducted to develop micropropagation and slow-growth conservation protocol for endangered relict plant species *Campanula sclerophylla*. The best multiplication rate up to 7.2 shoots per explant was obtained on half strength MS supplemented with 3 mg/l BAP and 1 mg/l IAA. The effects of temperature/light conditions, sorbitol and ABA on shoot growth of *Campanula sclerophylla* were studied. Addition of 3 g/l sorbitol and 5 mg/l ABA inhibited shoot length and multiplication of *C. sclerophylla*. Explants were stored for 9 months without subculture at 7ºC and light intensity of 23 lux.

Introduction

Campanula sclerophylla Kolak is one of the north-Colchis narrow local endemics of western Caucasus. This is extremely endangered relict plant species of the Tertiary period with about 100 individual plants growing in Ahtsu canyon in the valley of the river Mzymta and Shangen canyon in the valley of the river Psou (Sochi, Russia). *Campanula sclerophylla* grows in crevices of limestone rocks of canyons foothills in the well lighted places (Timukhin 2006). Habitat for this species is highly fragmented with limited connectivity between populations.

According to the recent estimation in the last 50 years more than 300,000 plants species on our planet have become extinct (Blîndu and Holobiuc 2008). The western Caucasus is a natural habitat of many endemic relict species and many of them are on the verge of extinction due to industrial development of the region which led to the subsequent destruction of natural ecosystems.

*Author for correspondence: <q11111w2006@ya.ru>.

Field collections are not always reliable way of conservation but slow growth *in vitro* collections provide reliable preservation of many plant species with subculture period varied from several months to 2 - 3 years (Cruz-Cruz et al. 2013). Some attempts were made recently for *in vitro* propagation and conservation of endemic and endangered plant biodiversity of Western Caucasus (Sokolov et al. 2012, Suprun et al. 2014, Kolomiets et al. 2014a, b, Kolomiets et al. 2015a, b). However, protocol for *in vitro* conservation of *Campanula sclerophylla* has not yet been established and as such the present work was undertaken to study effects of culture media composition and temperature/light conditions on *in vitro* propagation and slow-growth conservation.

Materials and Methods

Apical buds of *Campanula sclerophylla* Kolak were collected as explants from natural habitat in winter-spring 2014 (Fig. 1). Surface sterilization was carried out by the previously described protocol (Sokolov et al. 2012), then meristems were isolated and placed on Van-Hoof (Van-Hoof 1971) culture media for *in vitro* growth initiation (Sokolov et al. 2013). Microshoots derived from meristems were transferred to half strength MS and Van-Hoof culture media with BAP, Kn, IAA, GA_3. Experimental culture media are presented in Table 1. All culture media were supplemented with 20 g/l sucrose and 7 g/l agar, pH was set to 5.8 prior to autoclaving at 121ºC for 25 min. Explants were cultured under the cool white fluorescent lamps with photoperiod 16/8 hrs and respective humidity (70%).

Table 1. Growth regulators added in basal media (mg/l).

Basal media	Variant	BAP	Kn	IAA	GA_3
Half strength MS	I	2.0	-	0.5	-
	II	3.0	-	1.0	-
	III	-	2.0	0.5	-
	IV	-	3.0	-	0.5
Van-Hoof	V	2.0	-	0.5	-
	VI	3.0	-	1.0	-
	VII	-	2.0	0.5	-
	VIII	-	3.0	-	0.5

Auxin and cytokinin free half strength MS basal media was used for slow-growth storage. The effects temperature/light conditions on shoot length and additional shoot number was studied after 2, 3, 5, 9 months of *in vitro* storage. There were standard conditions (temperature 23ºC and light intensity 1300 lux), medium conditions (temperature 23ºC and light intensity 560 lux) and low

conditions (temperature 7ºC and light intensity 23 lux). The effects of 3 g/l sorbitol, 5 mg/l ABA and combination of 3 g/l sorbirtol + 5 mg/l ABA added in culture media on shoot length and shoot number were studied after 2 and 3 months of conservation.

Each treatment included 15 replicates and experiments were repeated three times. Results were assessed by a standard analysis of variance for a completely randomized design using ANOVA test and MS Excel 2008 package.

Results and Discussion

Best multiplication (2.9 - 7.2 additional shoots/explant) was obtained on half strength MS (variants I - IV) compared to Van-Hoof media (2.2 - 3.1 shoots/explant - variants V - VIII) (Fig. 1A). Van-Hoof basal media was infrequently used for propagation of explants but in our previous study this medium was found to be effective in the stage of tissue culture initiation from apical buds and meristems of chrysanthemum etc. (Kolomiets and Malyarovskaya 2013). But the results obtained in this study have not confirmed efficacy of Van-Hoof media for *Campanula* propagation.

The best combination of growth regulators for multiplication of *C. sclerophylla* was 3 mg/l BAP and 1 mg/l IAA (variant II) provided the highest multiplication rate 7.2 additional shoots per explant 5 - 8 cm length (Fig. 1A, B). Decreasing of BAP and IAA concentration (Variant I) resulted decreasing multiplication rate up to 3 shoot per explant.

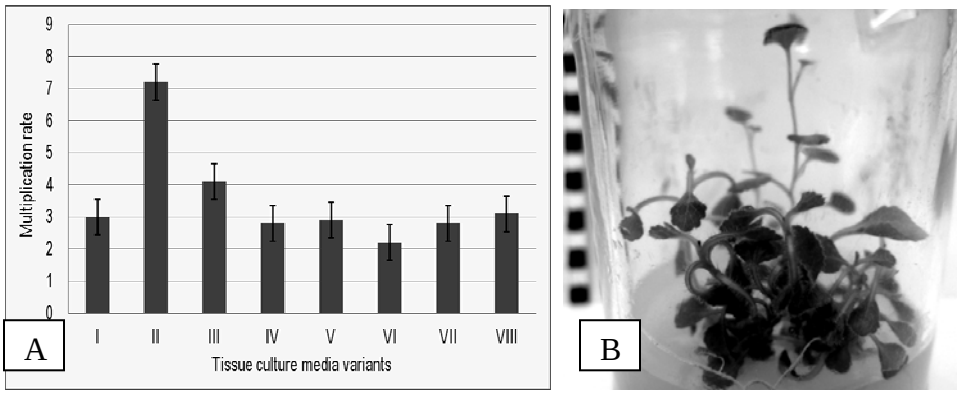

Fig. 1. Multiplication rate of *Campanula sclerophylla* depending on plant media composition.

Addition of 2 mg/l Kn and 0.5 mg/l IAA in basal medium resulted decreasing multiplication rate in 1.8 times comparing to the Variant II. Variant IV with 3 mg/l Kn and 0.5 mg/l GA_3 also resulted not high multiplication rate (2.7 shoots/explant). Our results correspond with others who obtained best multiplication using BAP 1 - 4

mg/l for *Campanula polymorpha* and *C. glomerata* on MS medium (Joung et al. 2002, Paunescu 2010).

It was revealed that at low temperature 7ºC and light intensity of 23 lux growth of shoots became slower: shoot length increased only 2 cm more and became 3.5 cm in total after 9 months of conservation (Fig. 2A). For the first 5 months of *in vitro* storage no additional shoot development was observed, and only after 9th month the average number of shoots increased from 1.5 to 2.5 (Fig. 2B). In these growth conditions shoots were strong with 1.5 mm width and leaves were intense green with average diameter 7 mm (data are not presented).

Maximum growth of explants was observed in standard conditions at the temperature 23ºC and light intensity of 1300 lux: shoot length increased more than two times and shoots length reached 6.5 cm after five months of *in vitro* conservation (Fig. 2A). Number of adventitious shoot increased from 2.8 - 4.5 on the 5th months of *in vitro* storage in standard conditions (Fig. 2B). Shoots thinning, yellowing of leaves and decreasing of plant vigor were observed after 5 months of conservation in standard conditions without subculture (data are not presented).

Explants cultured at the temperature 23ºC and light intensity of 560 lux showed medium growth parameters comparing with two other growth conditions. The shoot length of explants increased almost three times from the 3rd to 9th months of conservation and reached 6.5 cm. Three additional shoots were developed in these growth conditions after 9 months of *in vitro* storage.

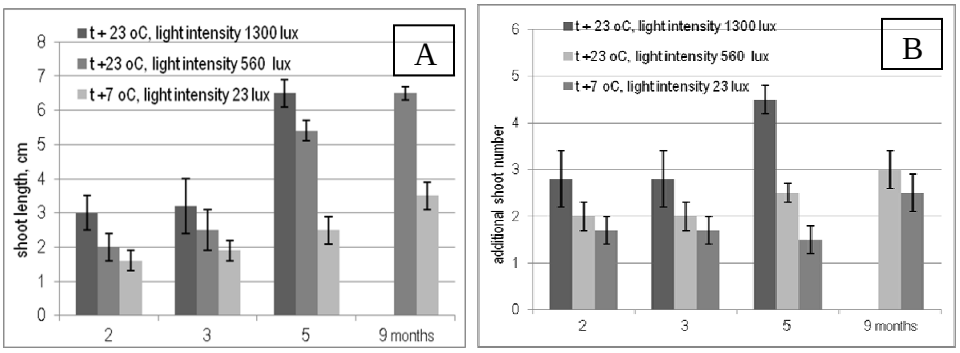

Fig. 2. Effect of temperature/light conditions on shoot length (A) and additional shoot number (B) of *Campanula sclerophylla*.

Addition of 3 g/l sorbitol alone in culture media has no inhibited effect on shoots growth: shoot length reached 3 cm after two months in culture and differs not comparing with control (Fig. 3A). The number of additional shoots per explant increased in standard temperature/light conditions and became 2.8

comparing with control 0.9 shoots/explant as an effect of 3 g/l sorbitol addition. The difference was not significant at the low temperature/light conditions.

Addition of sorbitol as an osmotic agent in media was noted in many reports as useful tool of increasing subculture period during slow-growth conservation (Huang et al. 2014). Usually authors use 1 - 3% sorbitol in culture media for growth retardation. In our work the possible reason of enhancing effect of sorbitol on multiplication was too low concentration 0.3% (3 g/l) and we suppose plant tissues could use it as an additional carbohydrate source.

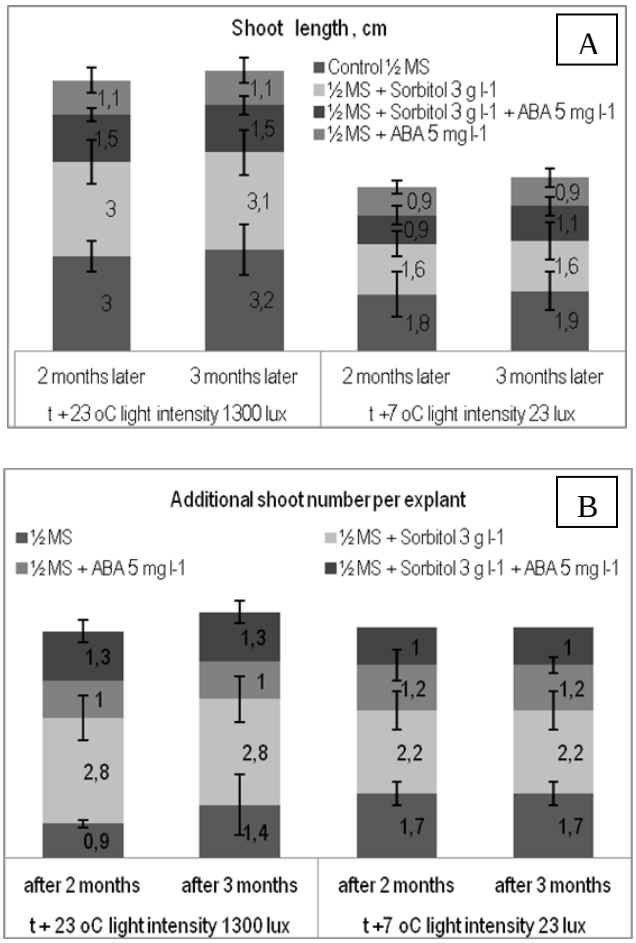

Fig. 3. Effect of ABA, sorbitol and temperature/light conditions on A - shoot length and B - additional shoot number of *Campanula sclerophylla.*

Addition of 5 mg/l ABA and both ABA + sorbitol decreased shoot length significantly. The shoot lengths were 1.1 and 1.5 cm, respectively compared to control (3.2 cm) after 3 months of *in vitro* conservation in standard conditions. At

the low temperature/light conditions the shoot length decreased up to 0.9 - 1.1 cm (Fig. 3A).

Addition of 5 mg/l ABA alone and in combination with 3 g/l sorbitol in culture media caused no significant effect on shoot number. The average number of new formed shoots per explant was 1 - 1.3 compared to control 0.9 - 1.7 (Fig. 3B). However, our experiments showed that 5 mg/l ABA alone caused 41.6% death of explants after 3 months of conservation (data are not presented).

These results correspond with others where ABA was added together with other retardants and has an effect in concentrations 2 - 5 mg/l but did not decrease the survival rate (Huang et al. 2014). But in contrast to our results addition of ABA 0.5 - 3 mg/l increased the survival rate of grape explants compared to control after 10 months of conservation (Pan et al. 2014). Possibly for *C. sclerophylla* 3 mg/l ABA is a too high concentration, and for best results it could be useful to reduce it in further experiments.

References

Blindu R and **Holobiuc I** (2008) Conservarea *ex situ* a speciilor de plante din lista roşie a plantelor superioare în România. Biotehnologii plante pentru secolul. **21**: 153 -168.

Cruz-Cruz CA, **González-Arnao MT** and **Engelmann F** (2013) Biotechnology and Conservation of plant biodiversity. Resources **2**: 73-95.

Huang HP, **Wang J**, **Huang LQ**, **Gao SL**, **Huang P** and **Wang DL** (2014) Germplasm preservation *in vitro* of *Polygonum multiflorum* Thunb. Pharmacogn Mag. **10**(38): 179-184.

Jounga YH, **Liaoa MS**, **Roha MS**, **Kamoa K** and **Songb JS** (2002) *In vitro* propagation of *Campanula glomerata*, 'Acaulis' from leaf blade explants. Scientia Horticulturae **92**(2): 137-146.

Kolomiets TM and **Malyarovskaya VI** (2013) Development of biotechnological tools for propagation of high quality planting material of Chrysanthemum cultivars. (In Russian) Subtropical and Ornamental Horticulture **48**: 115-122.

Kolomiets TM, **Sokolov RN** and **Malyarovskaya VI** (2014a) *In vitro* propagation of Eryngium maritimum L. (In Russian). Subtropical and Ornamental Horticulture **50**: 196-204.

Kolomiets TM, **Malyarovskaya VI**, **Gvasaliya MV**, **Samarina LS** and **Sokolov RN** (2014b) Micropropropagation of subtropical, ornamental and endangered species of Western Caucasus: original and developed protocols. (In Russian). Agricultural Biology **3**: 49-58.

Kolomiets TM, **Malyarovskaya VI** and **Bekhterev VN** (2015a) Effect of light spectrum on biometrical and physiological parameters of *Eryngium maritimum* L. propagated *in vitro*. (In Russian). Problems of agro-industrial development of the region. **4**: 22-28.

Kolomiets TM, **Malyarovskaya VI** and **Gubaz SL** (2015b) Management of *in vitro* genetic resources of subtropical fruit and ornamental crops and endemic species of Western Caucasus. (In Russian). Pomicult. and small fruits culture in Russia. **43**: 99-104.

Pan XJ, **Zhang WE** and **Li X** (2014) *In vitro* conservation of native Chinese wild grape (*Vitis heyneana* Roem. & Schult) by slow growth culture. Vitis **53**(4): 207-214.

Paunescu A (2010) *In vitro* propagation of *Campanula polymorpha* Witas. - An endemic plant of Carpathian Mountains. Analele Universităţii din Oradea - Fascicula Biologie. **17**(2): 235-238.

Silva TL and **Scherwinski-Pereira JE** (2011) *In vitro* conservation of *Piper aduncum* and *P. hispidinervum* under slow-growth conditions. Pesq. Agropec. Bras. **46**(4): 384-389.

Sokolov RN, **Samarina LS** and **Kolomiets TM** (2012) Preservation of rare endemic species of Black Sea Coast *Mzymtella sclerophylla in vitro*. (In Russian). Proceedings of the VI Russian conference of young scientists "Scientific support of agriculture", Krasnodar. pp. 50-51.

Sokolov RN, **Kolomiets TM** and **Malyarovskaya VI** (2013) Tissue culture initiation of rare plant species of Western Caucasus. (In Russian). Multidisciplinary scientific Journal of Kuban Agrarian University **10**(094) [articleID] 0941310013

Suprun II, **Kolomiets TM**, **MalyarovskayaVI**, **Sokolov RN**, **Samarina LS** and **Slepchenko NA** (2014) ISSR markers study for genotyping of rare species of Western Caucasus: *Lilium caucasicum*, *Galánthus woronowii*, *Pancratium maritimum*. (In Russian). Multidisciplinary Scientific Journal of Kuban Agrarian University. **103**(09).

Timukhin IN (2006) Inventory of major taxonomic groups and plant communities of the Sochi National Park. (In Russian). Book edited by BS Tuniev. Moskow. p. 150.

Van-Hoof P (1971) Methods de culture *in vitro* de meristemes pour l'obtention d' oeillets non virosses. Parasitica **2**: 27-35.