IN VITRO MULTIPLICATION OF *JOVIBARBA SOBOLIFERA*

SMARANDA VANTU[1]

Keywords: Jovibarba sobolifera, micropropagation, preservation

Abstract: *Jovibarba sobolifera* and its subspecies (subsp. *hirtum*, subsp. *allionii*, subsp. *arenaria*) live in the eastern and southern Alps, the Carpathians and the western Balkans south to northern Albania. In Romania was noticed the presence of two species: *Jovibarba heuffelii* and *Jovibarba sobolifera.* The aim of the paper is the preservation of *Jovibarba sobolifera*, a rare species from the Red List of Romanian Flora, based on unconventional strategies through clonal micropropagation. The composition of the phenolic compounds recommend also this species as a potential source of pharmaceutically active compounds and natural biopesticides. The cytokinin growth regulators (benzylaminopurine and kinetine) are added to shoot culture media to promote axillary shoot growth. Single shoots or shoot clusters have been cultivated to a different medium for rooting "*in vitro"* before being transferred to the external environment.The regenerated plants have shown a positive reaction, indicating a high level of adaptability.

INTRODUCTION

Jovibarba is a small genus of three species of succulents in the family Crassulaceae (Byalt, 1997, 1998) endemic to Europe (Gudžinskas, 1996, Puchalski and Gawryś, 2007, Witkowski et al., 2003). Only three species are accepted as distinct by the *Flora Europaea*: *Jovibarba globifera* (syn. *J. sobolifera*; *Sempervivum globiferum*) (Ham Roeland, 1994, Ham Roeland, 1995, Gallo, 1996), *Jovibarba heuffelii* (syn. *J. velenovskyi*; *Sempervivum heuffelii*), *Jovibarba hirta* (syn. *Sempervivum hirtum*)**.**

Jovibarba globifera is an evergreen perennial growing to 0.1m by 0.2 m. It is in leaf all year, in flower in July. It requires dry or moist soil. *Jovibarba sobolifera* belongs to the succulent plants, that store water in their enlarged fleshy leaves with a developed water storage parenchyma.

This allows them to survive in arid or cooler environments. This species has long widespread roots and the leaves (sub) sessile are aggregated into rosettes. In addition to sexual propagation by seeds, the species *Jovibarba sobolifera* also propagates by dividing rosettes.

Jovibarba sobolifera is on the red list of endangered species from Romania, originating from its natural habitat: Apuseni Mountains (Dihoru and Dihoru,1993-1994; Boscaiu et al.**.,**1994; Oltean et al..,1994; Ciocarlan, 2000).

The increase of interest in possibilities of the use of naturally occurring pharmaceutically substances from plants suggests that these plants can be used as a potential source of alkaloids (Stevens et al., 1992), tannins (Stevens et al., 1995) and phenolic compounds (Gnedkov**,** 1970, Szewczyk and Krzaczek, 2004). Plant phenolics, e.g. phenolic acids and flavonoids, are currently considered as one of the most promising groups of potential anticarcinogens (Nakamura et al., 2003, Tapiero et al.., 2002) showed that phenolics possess a wide spectrum of biochemical activities such as antioxidant , anticarcinogenic.

Tissue culture offers alternative method of plant propagation which is independent of the vegetative season. High multiplication rate and good health status of micropropagated plants are the additional features of that method.

MATERIAL AND METHODS

The initial plant material (rosette explants) was surface disinfected with 70% ethanol for 1 min followed by 10% solution of calcium hypochlorite for 10 min. After rinsing with sterile distilled water the explants were cultured in Erlenmayer flasks, each with 25 ml of MS medium (Murashige and Skoog, 1962) solidified with 0.7% (w/v) agar. Media MS of full strength of mineral salts, with salts reduced by 50% and by 25% were tested at induction stage.

To establish favourable conditions for shoot multiplication K (kinetine) at concentration of 0.1 mg /l, 1.0 mg/l, 2.0 mg/l and BAP (benzylaminopurine) at concentrations of 0.1 mg/l, 1.0 mg /l and 2.0 mg/l were applied. The experiments on micropropagation were done on MS medium in 18 variants. Each variant differs through mineral salts concentration and type of cytokinines (Table 1).

Microcuttings (15-20 mm long) were rooted in MS medium of full and reduced to 50% strength salts. Media for rooting were enriched with 1.0 mg/l, 2.0 mg/l, or 5.0 mg/l IAA (indole-3-aceticacid).

Culture conditions were a 16 h photoperiod and 23±1oC. The observations and measurement were recorded after four weeks of culturing. The experiment was conducted in five replications, each consisted of 5 flasks. The experiment was repeated twice.

Table 1-The variants of MS medium

Caulogenesis induction			Roots induction	
Mineral salts	**Benzylaminopurine mg/l**	**Kinetine**	**Mineral salts**	**Indole-3-acetic acid mg/l**
100%	0,1	0,1	**100%**	1,0
	1,0	1,0		2,0
	2,0	2,0		5,0
50%	0,1	0,1	**50%**	1,0
	1,0	1,0		
	2,0	2,0		2,0
25%	0,1	0,1		
	1,0	1,0		5,0
	2,0	2,0		

RESULTS AND DISCUSSIONS

The sterilization procedure was satisfactory, resulted in 90% of disinfected explants (Photo 1, 2).

A high level of adventitious shoot regeneration with a frequency of nearly 100% was obtained, when fragments of rosette were used as explants.

The newly-formed shoots number varied with the concentration of mineral salts and concentration of growth regulators. The satisfactory growth of new shoots were observed on medium containing 100% MS salts with BAP at concentration 2.0 mg /1(Photo 3).

Comparison of multiple shoots development on different culture media revealed that benzylaminopurine was the most effective cytokinins in induction process.

The cytokinin growth regulators added to shoot culture media to promote axillary shoot growth, usually inhibit root formation.

Single shoots (Photo 4, 5, 6) must therefore be moved to a different medium for rooting *in vitro* before (Photo 7) being transferred as plantlets to the external environment. Individual shoots were excised from shoot clusters and cultured for rooting on MS media of full and reduced to 50% strength salts, each of them with IAA (indole-3-aceticacid) at different concentrations: 1.0 mg/l, 2,0 mg/l, or 5.0 mg/l.

The first roots were observed on 12th day after exposing shoots to high concentration of auxins and the highest percentage of rooted shoots was noted on MS medium. The rate of root induction within following days depended on the concentration of mineral salts and on concentration of auxins.

Rooted shoots (Photo 7) were obtained on MS medium supplemented with 5 mg/l IAA achieving 100% rooting and roots of good quality. The medium with low nutrient concentration was found to be more effective in root induction than medium rich in mineral salts.

Acclimatizing plants originated from *in vitro* conditions is particularly difficult since their specific character. The plant prefers light (sandy) soils and requires well-drained soil. Crassulaceae generally occupy arid and semi-arid, usually rocky habitats.

The regenerated plants were transferred in soil and grown in a controlled environment chamber with 16 hours photoperiod at 24°C (Photo 8).

Using substrate supplemented with perlite and peat (1:1), improved both plant growth and percentage of established plants**.** Applying a mixture of perlite and peat at acclimatization of plantlets increased their quality by improving the root system.

CONCLUSIONS

The high multiplication rate of shoots was noted on MS media with 100% mineral salts.
High level of BAP at concentrations 2.0 mg/1 can lead to an increased production of adventitiously regenerated shoots.
The MS medium with salts reduced by 50% gave the best roots multiplication rate of *Jovibarba sobolifera***.**
MS medium supplemented with 5 mg/l IAA was the most effective for roots inducing.
During the acclimatisation process was achieved a positive effect of using a mixture of perlite and peat (1:1).

REFERENCES

Boscaiu, N., Coldea, G., Horeanu , C. (1994) : *Lista rosie a plantelor vasculare disparute, periclitate, vulnerabile si rare din Flora Romaniei, Ocrotirea Naturii si Mediului Inconjurator*, **38**(1), 45-56
Byalt,V.V. (1997) : Zametki o tolstyankovykh (Crassulaceae) Vostochnoi Evropy. (Notes on the family Crassulaceae of Eastern Europe.), in *Bot. Zhurn*. **82**(2): 79-82
Byalt,V.V.(1998): Zametki po sistematike semeistva tolstyankovykh (Crassulaceae). (De familia Crassulaceae notae systematicae.) in *Novosti Sistematiki Vysshikh Rastenij*, Moscou, **31**: 120-122
Ciocarlan, V. (2000)- Flora ilustrata a Romaniei, Pterydofita et Spermatophyta, Ed Ceres, Bucuresti
Dihoru, G., Dihoru, A. (1993-1994) : Plante rare, periclitate si endemice in Flora Romaniei – Lista rosie, Acta. Bot. Hort. Bucurestiensis, 173-197
Gallo, L. (1996): Notes on *Jovibarba globifera* subsp. allionii, in: *The Sempervivum Society Newsletter* **42**: 2
Gnedkov, P.A.(1970): Phytochemical study of some succulent plants of the Crassulaceae family, in *Farmatsevt. Zh*., Kiev, **25(6**) : 28-31
Gudžinskas, Z. (1996): Notes on the genera *Sempervivum* and *Jovibarba* in Lithuania, in *Botanica Lithuanica*, (Bulletin del'Institut de Botanique de l'Université de Vilnius) **2**(1): 27-35
Gumenyuk, L.A., Borysenko, A.N.(1979): Sempervivum ruthenicum, a new source of biologically active compounds in *Farmatsevt. Zh*., Kiev, **(3**): 72-73
Ham Roeland C.H.J van (1994): *Phylogenetic implications of chloroplast DNA variation in the Crassulaceae*, Ph. D. Thesis, Université d'Utrecht (NL) : 153 pp.
Ham Roeland C.H.J van (1995): Phylogenetic relationships in the Crassulaceae inferred from chloroplast DNA variation: 16-29, in't Hart Henk & Eggli Urs – *Evolution and Systematics of the Crassulaceae*, Backhuis Publishers, Leiden : 192 pp.
Murashige-Skoog, A. (1962).: A revised medium for rapid growth and bioassay with tobacco tissue culture; Physiol. Plant., **15**, 473-49
Nakamura, Y., Watanabe, S., Miyake, N., Kohno, H., Osawa, T., (2003) Dihydrochalcones: evaluation as novel radical scavenging antioxidants. J. Agric. Food. Chem. **51**, 3309-3312.
Oltean, M., Negrean, G., Popescu, A., Roman, N., Dihoru, G.,Sanda, V. Mihăilescu, S. (1994): Red List of vascular plants from Romania. – In: **Oltean, M.** (coord.), Studii, sinteze, documentaţii de ecologie. Romanian Acad., Inst. of Biology, **1**: 1-52
Puchalski, J., Gawryś, W. (2007): Collections of protected law and endangered plants and of species protectednby Bern Convention in Polish Botanical Gardens, *Biuletyn Ogrodów Botanicznych, **16:*** 47–184
Stevens, Jan Frederik, 't Hart Henk, Hendriks, Henk, Malingré, Théo M. (1992): Alkaloids of some european and macaronesian sedoideae and sempervivoideae, in *Phytochemistry* **31**: 3917-3924
Stevens Jan Frederik, 't Hart Henk, Roeland C.H.J.(1995): Distribution of Alkaloids and Tanins in the Crassulaceae, in *Biochemical Sistematics and Ecology* **23(2**): 157-165
Szewczyk, K., Krzaczek, T. (2004): Sterols in leaves and roots of *Jovibarba sobolifera* Sims Opiz Hen and Chickens Houseleek, Herba Polonica **50(1**): 28-32
Szewczyk K., Krzaczek T., (2004): Phenolic acids in leaves and roots from *Jovibarba sobolifera* (SIMS.) Opiz. Herba polonica **50**, 37-41
Tapiero, H., Tew, K.D., Ba, G.N., Mathe, G., (2002): Polyphenols: do they play a role in the prevention of human pathologies? *Biomed. Pharmacother*. **56**, 200-207.
Witkowski, Z. J., Krol, W., Solarz, W. (2003): Carpathian List of Endengered Species, Institute of Nature Conservation Polish Academy of Sciences, Europress, Krakow Poland

1.”Al. I. Cuza” University, Faculty of Biology, 700505 IASI, ROMANIA
e-mail: s-vantu@yahoo.com

Photo 1- Explants sources

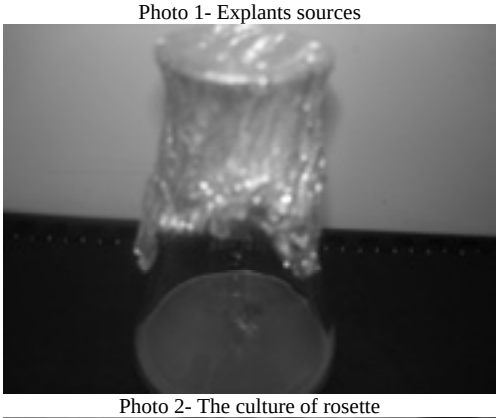

Photo 2- The culture of rosette

Photo 3- Multiple shoots development

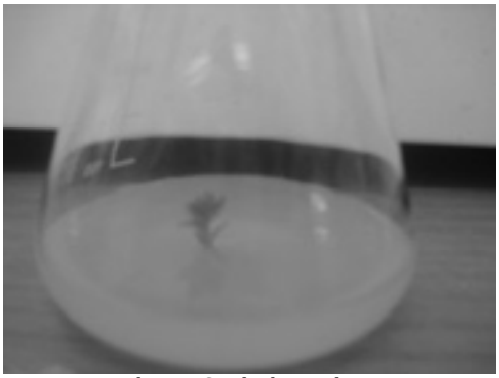

Photo 4- Single shoot culture

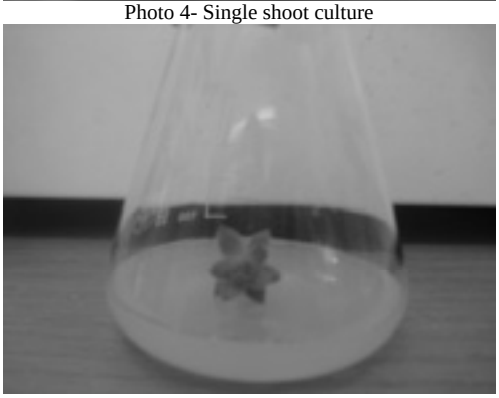

Photo 5- Stage of rosette development

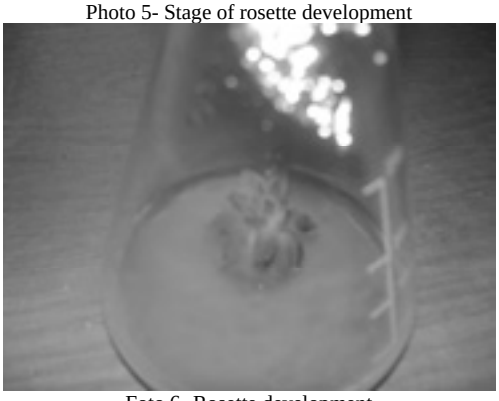

Foto 6- Rosette development

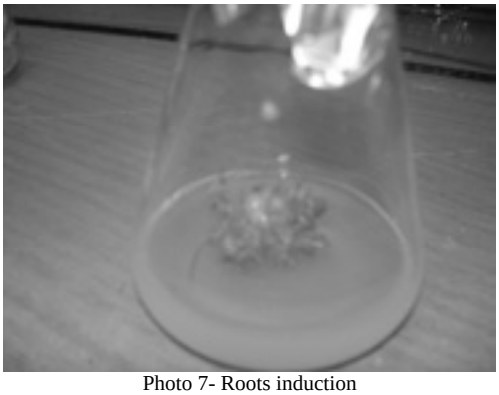

Photo 7- Roots induction

Photo 8- Plant acclimatisation