POSSIBILITIES FOR THE PROPAGATION AND RE-POPULATION OF PEAT BOGS WITH CARNIVOROUS SPECIES

Doina Clapa[1)], A. Fira[1)], I. Pacurar[2)], Anca Sotropa[2)]
[1)]Fruit Research Station Cluj, 5 Horticultorilor Street, 400457 Cluj-Napoca, Cluj- Napoca, Romania; www.scdpcluj.ro, doinaclapa@yahoo.com
[2)]University of Agricultural Sciences and Veterinary Medicine, 3-5 Manastur Street, 400372, Cluj-Napoca, Romania

Abstract. *Our paper presents the possibilities regarding the in vitro propagation of species Drosera rotundifolia si Pinguicula vulgaris, two of the few carnivorous species in Romania. These two species can be found in humid, swampy areas, especially in peat bogs, the perimeters in which they live are natural reservations, protected areas or Natura 2000 sites, being protected by law. The in vitro propagation of these species was done with the aim of obtaining a large number of plants in a short time, starting from leaf fragments, for the conservation of the bodiversity of these sites.*

Keywords: *Drosera rotundifolia, Pinguicula vulgaris, in vitro,* acclimation

INTRODUCTION

The European Union embarked on the path of stopping the decline of biodiversity and it takes part in a global convention for the significant reduction of the loss in biodiversity. Natura 2000 is essential for attaining this goal, as it is the network of environmental protection areas at the level of the European Union, created in order to ensure the survival of the most valuable species and habitats in Europe. It is not limited to natural reservations, but it is based on a broader principle of conservation and sustainable use, according to which people and nature can coexist in harmony.

The variety of habitats and species in this network has increased due to the succesive broadening of the European Union. In 1995, the joining of Sweden and Finland lead to the creation of the Boreal Region. In 2004, Hungary, the Slovak Republic and the Czech Republic added the Pannonian Region, Romania and Bulgaria contributed with the Stepic and Pontic Regions, further increasing the rich diversity of fauna and flora of the network. On the territory of the European Union 9 biogeographical regions were identified. Romania is the country on the territory of which there are the most biogeographical regions, which are five (Alpine, Continental, Pannonian, Stepic and Pontic).

Drosera rotundifolia and ***Pinguicula vulgaris*** are two of the few carnivorous plants that can be found in our country. They are especially in peat bogs, they are protected species and the areals in which they live are Natura 2000 sites. ***Drosera rotundifolia*** (sundew) is a genus of insect-foraging plants, it is part of family *Drosaceae*, Genus *Drosera* comprises about 200 species such as: *Drosera amazonica, Drosera grantsaui, Drosera viridis, Drosera affinis, Drosera alba, Drosera aliciae, Drosera anglica, Drosera arenicola, Drosera bequaertii, Drosera biflora, Drosera brevifolia, Drosera burkeana, Drosera capensis, Drosera capillaries, Drosera cayennensis, Drosera cendeensis, Drosera collinsiae, Drosera colombiana, Drosera communis, Drosera cuneifolia, Drosera dielsiana, Drosera*

elongate, Drosera ericgreenii, Drosera esmeraldae, Drosera felix, Drosera filiformis, Drosera glabripes, Drosera graomogolensis, Drosera hamiltonii, Drosera hilaris, Drosera hirtella, Drosera hirticalyx, Drosera humbertii, Drosera insolita, Drosera intermedia, Drosera kaieteurensis, Drosera katangensis, Drosera linearis, Drosera madagascariensis, Drosera Montana, Drosera natalensis, Drosera neocaledonica, Drosera nidiformis, Drosera oblanceolata, Drosera panamensis, Drosera peruensis, Drosera pilosa, Drosera ramentacea, Drosera roraimae, Drosera rotundifolia, Drosera solaris, Drosera slackii, Drosera spatulata, Drosera stenopetala, Drosera trinervia, Drosera uniflora, Drosera villosa, Drosera yutajensis

The roots of *Drosera* are rather weakly developed. The leaves are situated as a basal rosette. Their petiole is long, and the lamina is covered with hairs. At the end of spring and the beginning of summer the pinkish-white or violet, pentamere flowers appear, grouped into a scorpioid inflorescence. An insect that seats on a leaf gets stuck into the secretion of the clubby hairs. By struggling to free itself, it gets stuck to many other hairs. The hairs secrete an abundant, sticky liquid that suffocates the insect. Then the insect is digested by certain proteolytic enzymes in a few days, only the chitinous exoskeleton being left the insect.

Pinguicula vulgaris (fam. *Lentibulariaceae*, ord. *Lamiales*) is a rare carnivorous species, found in humid, swampy areas, especially in peat bogs. It has thick, fleshy leaves with glandular hairs that produce a sticky, mucilaginous secretion, which has the role of attracting and digesting small insects on the spot. The species has decorative role by its rosettes with thick, fleshy leaves and flowers with different colours. In our country it can be found in the Bucegi Mountains. In this context, the objectives of this paper are the protection of these species, their propagation and the re-populaton of peat bog sites with these two species.

Drosera rotundifolia can be propagated by seeds, division of shoots or leaf cuttings in spring. In vitro propagation has also proved to be an extremely effective method for this species (Bekesiova *et al*., 1999; Cachiţă-Cosma *et al*., 2008; Ichiishi *et al*., 1999; Jayaram *et al*., 2009; Sauerwein *et al*., 1994; Turcuş, 2009). The propagation of *Pinguicula vulgaris* can be done by conventional methods, this is done efficiently by leaf cuttings, which regenerate plantlets in the basal part, as well as by in vitro culture.

MATERIAL AND METHOD

After testing several culture media for the in vitro propagation of the 2 varnivorous plant species (Clapa et al., 2008, Clapa et al., 2009) it was found that the most advantageous nutritive media were Murashige & Skoog (MS) with 0.1 mg/l benzyladenine (BAP) gelled with 6 g/l Plant-Agar for *Pinguicula* and Murashige & Skoog (MS) with 5 mg/l Kinetin gelled with 6 g/l Plant-Agar or hormone-free Murashige & Skoog medium for *Drosera* (Table 1). As explants, for multiplying *Drosera rotundifolia* rosettes were used, whereas for *Pinguicula vulgaris* rosettes or leaf fragments were used. For both species, the plantlets obtained on the multiplication media could be acclimated in hydroculture, but for this stage better results were obtained by using plantlets from hormone-free media for *Pinguicula* and from MS with macroelements reduced to half concentration for *Drosera* (Table 2).

Table1.

The variants of media used for the in vitro multiplication of *Pinguicula vulgaris* and *Drosera rotundifolia*

Components	*Pinguicula vulgaris*	*Drosera rotundifolia*
Salts	MS*	MS*
Myo-inositol	100 mg/l	100 mg/l
Vitamin B1	1 mg/l	1 mg/l
Vitamin B6	0.5 mg/l	0.5 mg/l
Nicotinic acid	0.5 mg/l	0.5 mg/l
Sugar	30 g/l	30 g/l
Plant Agar	6 g/l	6 g/l
BAP	0.1 mg/l	-
Kinetin	-	5 mg/l
pH adjusted to 5.8		

*Murashige & Skoog 1962

Table 2

The variants of media used for the regeneration of plants for acclimation in *Pinguicula vulgaris* and *Drosera rotundifolia*

Components	*Pinguicula vulgaris*	*Drosera rotundifolia*
Macroelements	MS*	MS* 1/2
Microeements	MS*	MS*
Myo-inositol	100 mg/l	100 mg/l
Vitamin B1	1 mg/l	1 mg/l
Vitamin B6	0.5 mg/l	0.5 mg/l
Nicotinic acid	0.5 mg/l	0.5 mg/l
Sugar	30 g/l	30 g/l
Plant Agar	6 g/l	6 g/l
pH adjusted to 5.8		

* Murashige & Skoog 1962

In order to obtain *Pinguicula vulgaris* plants for acclimation, on the hormone-free media, Plant Agar was used for gelling at 6 g/l and 3 g/l as well as Vege-Gel at 6 g/l (Table 4). 720 ml jars were used, containing 100 ml of media/vessel, as well as Magenta GA7 vessels containing 70 ml of media/vessel.

The plantlets regenerated on the media presented herein were transferred ex-vitro into plastic trays with tap water with neutra pH and cultured for 1 month for acclimation. The acclimated plants were then transferred into pots containing acid peat in order to grow them for transfer into the protected areas.

RESULTS AND DISCUSSION

For *Pinguicula vulgaris*, rosettes and leaf fragments were used as explants. The rosettes regenerated plantlets especially from the wounded surfaces and the leaf fragments regenerated plantlets from their basal part. After 3 months of in vitro culture the plantlets regenerated from leaves got rooted on the same medium in the same vessels, without transfer to fresh media.. Such, in every Magenta vessel, hundreds of rooted plantlets resulted by direct organogenesis. The plantlets were long and thin. From the rosettes, plantlets regenerated that grew unevenly, some of them vigorous, others long and thin. The multiplication rate was higher in the leaf

explants as compared to the ones grown from rosettes (Fig. 1). The highest multiplication rate (97.44) was obtained on the medium MS+0.1 mg/l BAP by using leaves as explants (Table 3).

Fig. 1. *Pinguicula vulgaris*: In vitro multiplication and *ex vitro* acclimation

Table 3.

The multiplication of species *Pinguicula vulgaris* on media gelled with agar, using different explant types

Variant	Nutritive medium	Gelling agent	Type and no. of inoculi/vessel	No. of plantlets resulted/explant	Multiplication rate
1	MS+0.1 mg/l BAP	6 g/l Plant-Agar	1 shoot (rosette)	54	54
2	MS+0.1 mg/l BAP	6 g/l Plant-Agar	9 shoots (rosettes)	237	26.33
3	MS+0.1 mg/l BAP	6 g/l Plant-Agar	9 leaves	877	97.44

On hormone-free MS vigorous plantlets resulted, very suitable for acclimation, but with lower multiplication rates (Table 4). The multiplication rate/rosette/jar was of 326 for the MS medium gelled with Vege-Gel and 212 when using 3 g/l Plant Agar.

Table 4

The influence of the gelling agent upon multiplication rate in *Pinguicula vulgaris*

Variant	Nutritive medium	No. of plantlets resulted/vessel	Multiplication rate/rosette
1	Hormone-free MS + 3 g/l Plant agar	212	212
2	Hormone-free MS + 6 g/l Vege-Gel	326	326

When using large basal leaves the average multiplication rate was of 34, whereas by using small apical leaves average multiplication rate was of 65. 5 explants were inoculated into each vessel (Table 5).

Table 5.

The influence of explant type upon multiplication rate in *Pinguicula vulgaris*

Variant	Inoculum type	Magenta GA7 vessel	No. of plantlets resulted/vessel	Multiplication rate
1a	5 large basal leaves	1	25	34
		2	43	
2a	5 small apical leaves	1	89	65
		2	41	

In *Drosera rotundifolia* the highest multiplication rate, 52.2 was obtained when using MS + 5 mg/l kinetin (Table 6). The plantlets regenerated on the hormone-free MS medium with macroelements reduced to ½ proved to be more suitable for acclimation (Fig. 2).

Table 6

Multiplication rate in *Drosera rotundifolia*

Variant	Nutritive media	No. of inoculi	No. of rosettes resulted/vessel	Multiplication rate (no. of rosettes resulted/inoculum)
1	Hormone-free MS	5	169	33.8
2	Hormone-free MS, no agar	5	114	22.8
3	MS+ 2 mg/l kinetin	5	168	33.6
4	MS+ 5 mg/l kinetin	5	256	51.2
5	MS+ 10 mg/l kinetin	5	154	30.8

Fig. 2. *Drosera rotundifolia*: in vitro multiplication and *ex vitro* acclimation

The *Pinguicula* plantlets transferred *ex vitro* in the trays containing water directly from the regeneration media survived in a proportion of 80 % during acclimation, whereas the ones cultured on hormone-free MS in a proportion of 100

%. The Drosera plantlets obtained on the MS medium with macroelements reduced to half got acclimated in hydroculture in a proportion of 100 %. After being planted into peat, in both species around 25 % of the plantlets did not survive due to unfavorable conditions.

CONCLUSIONS

Pinguicula vulgaris and *Drosera rotundifolia* are two species of protected insectivorous plants that can be easily saved by using the method of *in vitro* propagation.

The most favorable nutritive media recommended for the *in vitro* propagation of these species are Murashige & Skoog (MS) with 0.1 mg/l benzyladenine (BAP) gelled with 6 g/l Plant agar for *Pinguicula vulgaris* and Murashige & Skoog (MS) with 5 mg/l kinetin and gelled with 6 g/l Plant Agar or hormone-free Murashige & Skoog medium for *Drosera rotundifolia*.

The optimal explant type is the rosette for *Drosera rotundifolia* and leaves or leaf fragments for *Pinguicula vulgaris*.The plantlets obtained *in vitro* can be successfully acclimated *ex vitro* in hydroculture and then cultured in the greenhouse in acid peat or peat + perlite mixture. It is recommended that they should be kept in a shady place.

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