Propagation of *Drosera rotundifolia* and *Drosera capensis* in an *in vitro* Culture System

Ileana MICLEA^{1*}, Marius ZĂHAN¹

¹Department of Animal Reproduction, University of Agricultural Sciences and Veterinary Medicine, 3-5, Mănăştur Street, 400372, Cluj-Napoca, Romania.

*Corresponding author, e-mail: ileana.miclea@usamvcluj.ro

Bulletin UASVM Animal Science and Biotechnologies 74(2)/ 2017

Print ISSN 1843-5262; Electronic ISSN 1843-536X

DOI:10.15835/buasvmcn-asb: 0018

ABSTRACT

Drosera rotundifolia and *Drosera capensis* (Droseraceae) are carnivorous plants grown as ornamentals and sources for homeopathic medicine. The aim of this study was to optimize nutrient and growth regulator concentrations for the *in vitro* propagation of these species. Half strength MS medium (1/2MS) was supplemented with kinetin (0.5, 2, 5 mg/l) or 6-benzyladenine (3, 5 mg/l) and plantlets were transferred to 1/2MS with or without cytokinins. After 8 weeks rosette diameter, plant height, number of roots, root length were recorded and plants were cultured in full strength MS, 1/2MS or 1/2MS with 0.5 mg/l α -naphthaleneacetic acid for the same period of time. Afterwards, plant characteristics (number of roots, root length, number of shoots, number of flower stalks) were assessed. For *D. rotundifolia*, shoot development and rosette diameter increased significantly in the medium with 0.5 mg/l kinetin and 3 mg/l 6-benzyladenine, while root development decreased. Plant growth regulator free medium was more suitable for root development than medium with α -naphthaleneacetic acid and thus supported the formation of significantly more flower stalks. For *D. capensis*, kinetin was detrimental for shoot development, the optimum medium for both shoot and root formation being MS without plant growth regulators.

Keywords: auxins, cytokinins, *Drosera rotundifolia*, *Drosera capensis*, *in vitro*

INTRODUCTION

The genus *Drosera* (sundews) is a large group of carnivorous plants comprising more than 200 species (McPherson, 2010). Numerous *Drosera* species are distributed worldwide across tropical to tundra regions, except for deserts and Antarctica. The largest number of species is concentrated in Australia and New Zealand but others can be found only in America, South Africa or Europe (Rivadavia *et al.*, 2003). The leaves of *Drosera* plants have developed sticky traps that attract, trap and digest prey using the resulting nutrients to supplement what they can obtain from poor growing substrates (Rice, 2011).

The unique biology and carnivorous habit of *Drosera* spp. have gained the interest of horticulturists and made sundews economically important ornamentals (Barthlott *et al.*, 2004). Besides their ornamental traits *Drosera* species

are also valued for their medicinal properties (Kawiak *et al.*, 2011) and are widely used in folk medicine. Extracts from these plants are included in herbal preparations used in the therapy of respiratory tract infections (Egan and van der Kooy, 2013). Therapeutic properties are thought to result from the presence of bioactive compounds which can be used in the production of pharmaceutical drugs (Banasiuk *et al.*, 2012). The main secondary metabolites in *Drosera* plants are 1,4 naphthoquinones, among which the most abundant are plumbagin and 7-methyljuglone (ramentaceone). Besides these sundews synthesize several minor, but unique, naphthoquinones as well as a broad spectrum of flavonoids such as myricetin, quercetin (Devi *et al.*, 2016; Braunberger *et al.*, 2015; Krolicka *et al.*, 2008).

Drosera rotundifolia has a prostrate growth

habit with leaves in contact with the soil (Anthony, 1992) and is one of the most broadly distributed species, occurring throughout much of the Holarctic (Wolf *et al.*, 2006). It is among the few species of carnivorous plants that can be found in Romania with a habitat made of acidic peat bogs in the mountains (Clapa *et al.*, 2009). It has been prescribed as a diuretic, a laxative, and treatment for a variety of kidney, stomach, and liver problems (Wolf *et al.*, 2006).

Drosera capensis is a small, erect perennial sundew native to the Cape region of South Africa (Pavlovič *et al.*, 2014) and its plumbagin production has been reviewed by Egan and van der Kooy (2013). Extract from *D. capensis* leaves could be effective in fighting antibiotic-resistant *Staphylococcus aureus* strains (Krolicka *et al.*, 2008).

The unfortunate side effect of the interest generated by sundews is overharvesting from natural sources. Together with a loss of their natural habitats this has led to their protection and inclusion in the European Red List of Threatened Plants (Kawiak *et al.*, 2003). Another result has been the surge in research regarding the micropropagation and the use of *in vitro*-grown plants as alternative sources of biomass (Hook, 2001).

This experiment aimed to find the most suitable nutrient concentration and to study the effect of several plant growth regulators (kinetin, 6-benzyladenine, α -naphthaleneacetic acid) for *in vitro* shoot development and rooting of *D. rotundifolia* and *D. capensis*.

MATERIALS AND METHODS

The experiments used plant material from established *in vitro* cultures of *D. rotundifolia* and *D. capensis* maintained on half-strength MS medium (Murashige and Skoog, 1962) without plant growth regulators. These cultures originated from seeds provided by the Cluj-Napoca Botanical Garden. Unless otherwise stated all reagents were acquired from Sigma-Aldrich, Germany.

The employed media were: full strength MS (fullMS) and MS with half-strength macronutrients, half-strength micronutrients and full vitamins (1/2MS). Both types of media were enriched with 30 g/l sucrose, solidified with 5 g/l phytagel and pH was adjusted to 5.4-5.5 before autoclaving at 121°C for 20 min. After sterilization half-strength

MS was supplemented with kinetin (0.5 mg/l, 2 mg/l, 5 mg/l) or 6-benzyladenine (BA) (3 mg/l, 5 mg/l), with cytokinin concentrations being chosen based on other published studies. *D. rotundifolia* and *D. capensis* plantlets (10-15 mm in length, 7-10 leaves) were selected from an established *in vitro* culture and transferred to 1/2 MS without plant growth regulators (1/2MS0) or to 1/2MS containing one of the two cytokinins. Plants were kept for 8 weeks in the growing chamber at 20-25°C under a 18/6 h light/dark regime provided by cool white fluorescent lamps with a light intensity of 40 $\mu\text{mol}/\text{m}^2/\text{s}$. Afterwards, rosette diameter, plant height, number of shoots, number of roots, root length, were measured and number of roots and shoots per explant was calculated. *D. rotundifolia* grows in horizontal rosettes which can be accurately defined by rosette diameter while in our experiment *D. capensis* formed stems which were better assessed by measuring plant height. Plants were placed in fullMS without plant growth regulators (fullMS0), 1/2MS0 or 1/2MS supplemented with 0.5 mg/l α -naphthaleneacetic acid (α -NAA) for another 8 weeks. At the end of this time the number of roots, and root length, number of shoots and flower stalks were assessed. These measurements were used to calculate number of roots, shoots and flower stalks per explant. Five plantlets per vessel were inoculated for each treatment and treatments were repeated 5 times. Data were analysed by the analysis of variance and differences were estimated using the Tuckey and t tests at $p < 0.05$. These were performed using GraphPad InStat version 3.05 for Windows 95 (GraphPad Software, San Diego California, USA).

RESULTS AND DISCUSSIONS

In *D. rotundifolia* kinetin and BA promoted significantly larger rosette diameter than medium without plant growth regulators, the best being 0.5 mg/l kinetin (Tab. 1.). The same treatment resulted in the largest number of shoots/explant while 3 mg/l BA was second and no shoots were generated in 1/2MS0. The largest number of roots/explant was counted for 0.5 mg/l kinetin but their average length was smaller than that of roots grown in medium lacking plant growth regulators. None of the other treatments resulted in growth and explants died before the end of the 8 weeks.

This suggests that plants develop better in

Tab. 1. Effect of cytokinins on growth and developmental characteristics of *Drosera rotundifolia* and *Drosera capensis*

Treatment	<i>Drosera rotundifolia</i>	Rosette diameter (cm)	Number of roots/explant	Root length (cm)	Number of shoots/explant
1/2MS0		1.73±0.071a	2.01±0.06a	2.40±0.08a	0.00±0.00
0.5 mg/l kinetin		3.61±0.101b	3.83±2.07a	1.30±0.63a	3.43±0.07a
2 mg/l kinetin		0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
5 mg/l kinetin		0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
3 mg/l kinetin		3.21±0.043c	0.00±0.00	0.00±0.00	2.45±0.06b
5 mg/l kinetin		0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Treatment	<i>Drosera capensis</i>	Plant height (mm)	Number of roots/explant	Root length (mm)	Number of shoots/explant
1/2MS0		2.81±0.41a	2.22±0.40a	2.92±0.17a	6.67±0.84a
0.5 mg/l kinetin		0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
2 mg/l kinetin		0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
5 mg/l kinetin		0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
3 mg/l BA		0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
5 mg/l BA		0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Note: Values expressed are mean ± standard error of the mean (SEM). Different letters between means within the same column denote significant differences ($p < 0.05$).

medium with reduced cytokinin concentration and that kinetin is better than BA. Our results are in contrast with those of Clapa *et al.* (2010) who found that 5 mg/l kinetin ensured a high proliferation rate in *D. rotundifolia* but are similar to those of Coelho (2009) who reported 0.1 mg/l kinetin in 1/4MS or 1/2MS as the best media for *D. intermedia* development. In the same species 0.1 mg/l zeatin provided the best results for shoot proliferation while kinetin and BA suppressed it (Rejthar *et al.*, 2014).

In our experiment *D. capensis* plants developed in MS0 but not in MS supplemented with growth

regulators. After 8 weeks of culture the plants were small as suggested by rosette diameter for *D. rotundifolia* and plant height for *D. capensis*. High concentrations of plant growth regulators: 2 mg/l, 5 mg/l kinetin and 3 mg/l, 5 mg/l BA did not promote plant development as shown by the growth characteristics. These plants were placed in 3 types of media for an additional 8 weeks to see if development could be improved by different nutrient concentrations or an auxin, namely α -NAA. For *D. rotundifolia* plants more roots grew in 1/2MS0 than in any other medium (Tab. 2.).

They were significantly longer in the

Tab. 2. Effect of nutrient concentration and α -NAA on growth and developmental characteristics of *Drosera rotundifolia* and *Drosera capensis*

Treatment	<i>Drosera rotundifolia</i>	Number of roots/explant	Root length (cm)	Number of shoots/explant	Number of flower stalks/explant
fullMS0		12.79±2.44a	3.05±0.26a	28.33±6.08a	8.31±1.35a
1/2MS0		14.77±1.52a	2.52±0.18a	22.12±5.78ab	4.88±1.28ab
0.5 mg/l α -NAA		11.67±3.29a	1.30±0.34b	4.97±1.72b	2.67±0.83b
Treatment	<i>Drosera capensis</i>	Number of roots/explant	Root length (mm)	Number of shoots/explant	Number of flower stalks/explant
fullMS0		8.23±3.23a	1.33±0.14ab	9.20±3.51a	0.10±0.10a
1/2MS0		5.17±1.23a	1.35±0.12a	6.06±1.87a	0.00±0.00
0.5 mg/l α -NAA		4.28±1.24a	0.74±0.21b	6.39±2.24a	0.00±0.00

Note: Values expressed are mean ± standard error of the mean (SEM). Different letters between means within the same column denote significant differences ($p < 0.05$).

absence of plant growth regulators regardless of nutrient concentration. Media without plant growth regulators also supported the formation of significantly more shoots and flower stalks per explant. The presence of numerous flower stalks suggests that the plants can undergo a full life cycle in this type of medium even in the absence of GA₃ which has been used to stimulate flowering in *D. capillaris* (Vásquez-Collantes *et al.*, 2014). In *D. capensis* the best medium was fullMS0 which resulted in the development of the largest number of roots and shoots per explant. For the same species nutrient concentration had very little influence on root length but its decrease resulted in reduced numbers of roots and shoots per explant.

Poorer root development in α -NAA supplemented MS is surprising given that this auxin is usually used for root induction. It is unexpected particularly given that various α -NAA concentrations have been previously used for rooting experiments in *Drosera* species: *D. cuneifolia* (Kawiak *et al.*, 2003), *D. indica* (Jayaram and Prasad, 2007) and *D. spatulata* (Perica, 1996). On the other hand Anthony (1992) reported that subcultured *D. rotundifolia* and *D. capensis* plantlets produced extensive root systems on 1/2MS without plant growth regulators but behaved poorly in media containing 0.02 mg/l BA and 0.01 mg/l NAA. This suggests that *D. rotundifolia* and *D. capensis* do not need α -NAA supplementation for successful rooting or that they need less than 0.5 mg/l. Our results contrast with those of Yanthan *et al.* (2017) who found that one-fourth strength MS supplemented with 4 mg/l BA and 4 mg/l or fortified with 4 mg/l kinetin and 4 mg/l NAA supported the growth of large numbers of shoots and roots.

The two species used in our study grew more vigorously and rooted better in media lacking plant growth regulators. This is comparable to the work of Anthony (1992) who reported that using 0.02 mg/l BA and 0.01 mg/l NAA in 1/2 MS or 1/4 MS resulted in large numbers of *D. rotundifolia* and *D. capensis* plantlets. Similar results were obtained by Jayaram and Prasad (2007) for *D. indica* with low concentrations of cytokinins (zeatin and kinetin) being the best while high concentrations resulted in retardation of shoot growth.

CONCLUSION

In the current experiment the most suitable medium for *D. rotundifolia* shoot production was half-strength MS supplemented with a low kinetin concentration (0.5 mg/l) while rooting was best in medium free of kinetin or α -NAA. In *D. capensis* half-strength medium without plant growth regulators was the best for shoot development and rooting was highest in full strength MS. Because *D. rotundifolia* and *D. capensis* are prized for their ornamental and medicinal properties this optimized protocol can be used for large scale propagation for commercial and conservation purposes.

REFERENCES

1. Anthony JL (1992). *In vitro* propagation of *Drosera* spp. HortScience 27(7):850.
2. Banasiuk R, Kawiak A, Krolicka A (2012). *In vitro* cultures of carnivorous plants from the *Drosera* and *Dionaea* genus for the production of biologically active secondary metabolites. J Biotechnol Comput Biol Bionanotechnol 93(2):87-96.
3. Barthlott W, Porembski S, Seine R, Theisen I (2004). The curious world of carnivorous plants: A comprehensive guide to their biology and cultivation. Portland: Timber Press.
4. Braunberger C, Zehl M, Conrad J, Wawrosch C, Strohhach J, Beifuss U, Krenn L (2015). Flavonoids as chemotaxonomic markers in the genus *Drosera*. Phytochemistry 118:74-82.
5. Clapa D, Fira A, Pacurar I (2009). *In vitro* multiplication, conservation and ex vitro acclimation of *Drosera rotundifolia*. Bulletin UASVM Horticulture 66(1):34-39.
6. Clapa D, Fira A, Pacurar I, Sotropa A (2010). Possibilities for the propagation and re-population of peat bogs with carnivorous species. Agricultura-Știință și practică 3-4 (75-76):21-26.
7. Coelho N (2009). *In vitro* propagation of insectivorous plants for phytochemical purposes. Master Thesis, Universidade do Algarve.
8. Devi SP, Kumaria S, Rao SR, Tandon P (2016). Carnivorous plants as a source of potent bioactive compound: naphthoquinones. Tropical Plant Biol 9(4):267-279.
9. Egan PA, van der Kooy F (2013). Phytochemistry of the carnivorous sundew genus *Drosera* (Droseraceae) – future perspectives and ethnopharmacological relevance. Chem Biodivers 10: 1774-1790.
10. Jayaram K, Prasad MNV (2007). Rapid *in vitro* multiplication of *Drosera indica* L.: a vulnerable, medicinally important insectivorous plant. Plant Biotechnol Rep 1:79-84.
11. Hook IL (2001). Naphthoquinone content of *in vitro* cultured plants and suspension of *Dionaea muscipula* and *Drosera* species. Plant Cell Tiss Organ Cult 67:281-285.
12. Jayaram K, Prasad MNV (2007). Rapid *in vitro* multiplication of *Drosera indica* L.: a vulnerable, medicinally important insectivorous plant. Plant Biotechnol Rep 1:79-84.

13. Kawiak A, Królicka A, Lojkowska E (2011). *In vitro* cultures of *Drosera aliciae* as a source of a cytotoxic naphthoquinone: ramentaceone. *Biotechnol Lett* 33(11):2309-2316.
14. Kawiak A, Królicka A, Lojkowska E (2003). Direct regeneration of *Drosera* from leaf explants and shoot tips. *Plant Cell Tissue and Organ Cult* 75:175-178.
15. Krolicka A, Szpitter A, Gilgenast E, Romanik G, Kamiński M, Lojkowska E (2008). Stimulation of antibacterial naphthoquinones and flavonoids accumulation in carnivorous plants grown *in vitro* by addition of elicitors. *Enzyme Microbial Technol* 42:216-221.
16. McPherson S (2010). Carnivorous plants and their habitats. vol 2, Redfern Natural History, Poole.
17. Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plant* 15:474-497.
18. Pavlovič A, Krausko M, Libiaková M, Adamec L (2014). Feeding on prey increases photosynthetic efficiency in the carnivorous sundew *Drosera capensis*. *Ann Bot* 113(1):69-78.
19. Perica MC (1996). *In vitro* growth and regeneration of *Drosera spatulata* Labill. on various media. *HortScience*: 31(6):1033-1034.
20. Rejthar J, Viehmannova I, Cepkova PH, Fernández E, Milella L (2014). *In vitro* propagation of *Drosera intermedia* as influenced by cytokinins, pH, sucrose, and nutrient concentration. *Emir J Food Agric* 26(6):558-564.
21. Rice BA (2011). Reversing the roles of predator and prey. In J. Seckbach & Z. Dubinsk (Eds.), *Cellular origin, life in extreme habitats and astrobiology. All flesh is grass: Plant-animal interrelationships* vol 16, (pp. 491-518) Berlin: Springer.
22. Rivadavia F, Kondo K, Kato M, Hasebe M (2003). Phylogeny of the sundews, *Drosera* (*Droseraceae*) based on chloroplast rbcL and nuclear 18S ribosomal DNA sequences. *Am J Bot* 90:123-130.
23. Vasquez-Collantes SG, Rojas-Idrogo C, Delgado-Paredes GE (2014). *In vitro* flowering and plantlets elongation in "sundew" *Drosera capillaris*. *International Journal of Plant, Animal and Environmental Sciences* 4(3): 508-517.
24. Wolf E, Gage E, Cooper DJ (2006). *Drosera rotundifolia* L. (roundleaf sundew): a technical conservation assessment. USDA Forest Service, Rocky Mountain Region. Available: <http://www.fs.fed.us/r2/projects/scp/assessments/droserarotundifolia.pdf> Accessed 22.08.2017
25. Yanthan JS, Kehie M, Kumaria S, Tandon P (2017). *In vitro* regeneration of *Drosera burmannii* Vahl.: a carnivorous plant of north-east India. *3 Biotech* 7(2):124.