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Molecular Characterisation of Some Carnivorous Plants using RAPD Markers

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Abstract. In the present study we used 23 RAPD decamers to characterise 12 carnivorous plants species included in four different genera with the main goal to analyze the intra and inter relationships at DNA molecular level. RAPD analysis is a valuable tool for molecular characterisation of the carnivorous plants. Primers of OPB, OPA, OPE, AB, OPAL series generated the many bands (12-19) in the range of 200-1500 bp, with high level of polymorphism (77.7- 100%). The UPGMA dendrogram built using TreeView software, based on the genetic relationships between carnivorous plants, confirmed the high level of variability existing among *Drosera* species although between some analyzed species was not observed evident differences on the leaf morphology, prey capture system and plant height. RAPD assays showed that *Sarracenia purpurea* and *Nepenthes northiana* were clustered more closely than *Dionea muscipula.*

Keywords: carnivorous plants, DNA, RAPD, genetic variability

INTRODUCTION

Carnivorous plants, also called insectivorous plants, have attracted the attention on worldwide botanists, because of very curious trapping mechanisms and high level of specialized morphology (Juniper *et al*., 1989; Rivadavia *et al*., 2003). These plants are found in wild flora or cultivated worldwide because of their ornamental, medicinal and phylogenetic values.

The most studied carnivorous plants are included in *Droseraceae* family (sundews plants), comprising four genera (*Aldrovanda, Dionaea, Drosera, and Drosophyllum*) and about 115 species (nearly all of which belong to the genus *Drosera*). These species spread mainly in Africa, South America and Australia, also with some species grown in Northern Hemisphere (Hoshi *et al*., 2010). Sundews are perennial (or rarely annual) herbaceous plants, with height between 1.0 cm and 1.0 m, depending on the species. Climbing species form scrambling stems which can reach much longer lengths, up to 3.0 m. These plants catch and digest the prey using glandular tentacles with sticky secretions disposed on leaves surface. All species of sundew are able to move their tentacles in response to contact with digestible prey (insects and other tiny animals). According to Darwin (1875), glandular formations present in *Drosera* leaves secrete proteolytic enzymes similar to those in animals stomach. It also demonstrates that the substances solubilized and decomposed by the action of enzymes are absorbed by plant foliage. *Dionaea* genera is represented by a single species, *D. muscipula*, the well-known, quick-acting flytrap (Venus's-flytrap).

Sarraceniaceae (trumpet pitcher plants) is a family with three genera: *Darlingtonia, Sarracenia* and *Heliamphora,* indigenous from South-East region of United States of America (east seaboard, Texas, Great Lakes area) and southeastern Canada. Only *Sarracenia purpurea* species can be found in cold-temperate region. The plant's leaves have evolved into a funnel

in order to trap insects and digest the prey with proteases and other enzymes (Ellison *et al*., 2012) .

Nepenthes genus include insectivorous plants (popularly named tropical pitchers or monkey cups) grouped into *Nepenthaceae* family. The genus comprises aproximately 140 species, and numerous natural and many cultivated hybrids, mostly with climbing stems, spread from South-east of Asia, South-east of Africa, South of Australia, New Caledonia and archipelago Indonesian. Borneo and Sumatra are the areas with a great diversity of the tropical pitchers plants with many endemic species (Bauer *et al*., 2012).

Studies related to phylogeny of the carnivorous plants began with Charles Darwin in 1875 and the presumption that these plants had several independent origins among flowering plants, must be confirmed. During the phylogenetic evolution, carnivorous plants have adapted to grow in environments where the soil is poor in nutrients, especially nitrogen, such as acidic bogs and waterlogged soils. The trappings for insects have evolved as a means of providing a supplemental source of nutrients, especially nitrogen (Bhau *et al*., 2009).

At the present, there are a few reports (Ellison and Gotelli, 2009; Hoshi *et al*. 2010; Kawiak and Lojkowska, 2004) regarding molecular analysis of carnivorous plants considering that nuclear DNA markers does not provide conclusive data for a precise characterisation of these plants because of some biological characteristics such as the degree of ploidy, genetic structure, origins and evolution mode.

In the present study we used 23 RAPD decamers to characterise 12 insectivorous plants species included in four different genera (*Drosera*, *Dionaea*, *Sarracenia* and *Nepenthes*) with the main goal to analyze the intra and inter relationships at DNA molecular level.

MATERIALS AND METHODS

The biological material was represented by 12 carnivorous plants species (*Dionaea muscipula, Dosera aliciae, D. binata, D. burkeana, D. capensis* var. alba, *D. capensis* var. rubra, *D. capillaris, D. cuneifolia, D. intermedia, D. rotundifolia, Nepenthes northiana* and *Sarracenia purpurea* ssp. *purpurea*). The biological material was represented by 12 carnivorous plants species (*Dionaea muscipula*, *Dosera aliciae*, *D. binata*, *D. burkeana*, *D. capensis* var. alba, *D. capensis* var. rubra, *D. capillaris*, *D. cuneifolia*, *D. intermedia*, *D. rotundifolia*, *Nepenthes northiana* and *Sarracenia purpurea* ssp. *purpurea*). Young leaves from collection of "Alexandru Borza" Botanical Garden Cluj-Napoca were used for DNA isolation. DNA extraction from leaves was carried out according to Lodhi *et al*. (1994) protocol, modified by Pop *et al*. (2003). The concentration and purity of DNA were cuantified using a Nanodrop ND-1000 Spectrophotometer (Thermo Scientific).

To amplify the DNA samples, a number of 23 Operon Technologies (OPA, OPB, OPAB, OPC, OPD, OPE, OPF, OPG, OPO, OPX, OPAL, AB) primers (Microsynth AG) were used. PCR amplification (Williams, 1990 modified by Pop *et al*., 2003) was performed in 25 µl reaction volume containing 50 ng of template, 200 µM dNTP, 0.2 µM primer, 2.5mM $MgCl_2$, 2.5 mM 10 x buffer, 1 U Taq DNA Polymerase (Promega) and 2% PVP in a 96 Well Gradient Palm-Cycler CG1-96 (Corbett Research) programmed 1 cycle of 95^0C for 3 minutes for initial denaturation, followed by 45 cycles of 1 minute at 93^0C, primer annealing step at 34^0C for 1 minute and primer extension at 72^0C for 1 minute. After a final extension cycle (10min. at 72^0C) the samples were stored at 4^0C. For precision of results, all amplifications were repeated three times.

PCR amplicons and 100bp DNA Step Ladder sample (Promega) were separated on 1.4% agarose gel in 1 x TAE at 0.29 Volts/cm^2 for 90 minutes. Gels were visualized using

UV light Biospectrum AC Imaging System (UVP BioImaging Systems) after staining with 0.5 µg/µl ethidium bromide, for 25 minutes.

Gel images were analyzed using TL120 software (Nonlinear Dynamics). Amplified bands were scored present (1) or absent (0) and data entered into a binary matrix. The genetic distance between samples was calculated using Nei and Li/Dice coefficient of similarity (Nei and Li, 1979). Cluster analysis was conducted with an UPGMA algorithm using FreeTree software (Hampl *et al*., 2001) and a dendrogram was constructed, using the TreeView software (Page, 1996). Its consistency was assessed using bootstrap method in 1000000 repetitions. A synthetic outgroup was used for dendrogram rooting.

RESULTS AND DISCUSSIONS

The mean concentration for isolated DNA from leaves ranged from 176.78 ng/ mL to 1831.4 ng/mL, with purity between 1.6-1.9. In Fig. 1 is presented the DNA quantification graph generated with Nanodrop software at *Dionaea muscipula*.

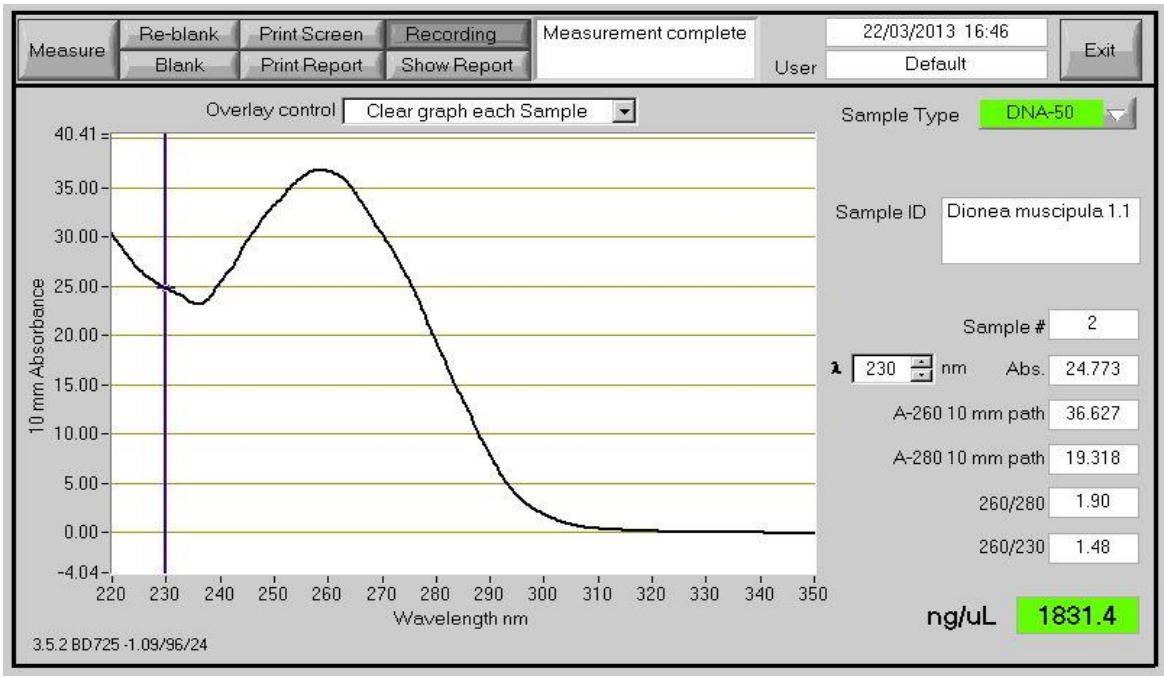

Fig. 1. Concentration and purity of isolated DNA from leaves at *Dionea muscipula* species

Lowest values of DNA quantity and purity were observed at *Drosera* sp., may be due to high level of polysaccharides present in the leaves (data not show).

In this study the RAPD technique was employed in order to assess the genetic relationships between some species of carnivorous plants. PCR amplicons generated with RAPD primers had the length between 200-1500 bp. The patterns obtained for primer OPB 18 are shown in Fig. 2. All the used primers generated scorable bands, with high level of polymorphism (77.7- 100%). As it can be seen in Tab. 1 a number of 245 polymorphic bands were generated with the used primers. The highest number of polymorphic bands were obtained using the OPB 17 primer (19 bands) and the lowest with the OPB 10 (six bands).

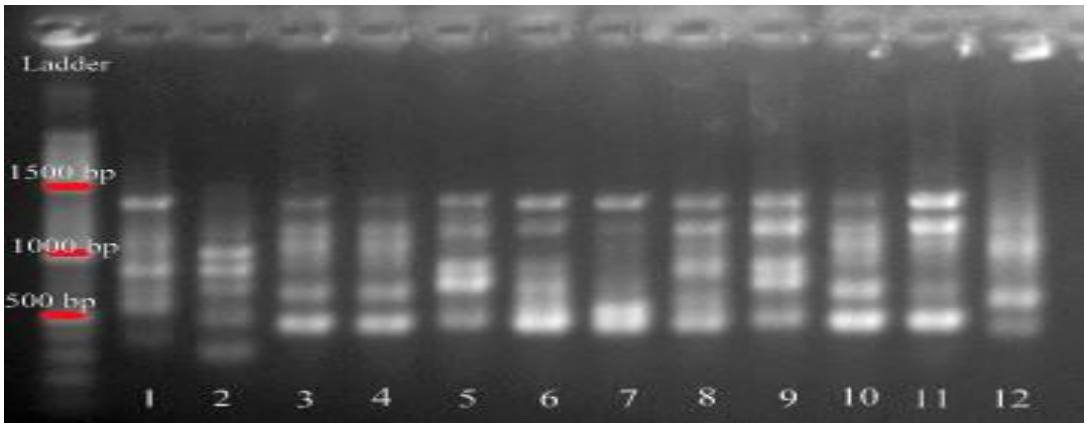

Fig. 2. RAPD profile generated with OPAB 18 primer. Ladder 100 bp; 1-*Dionaea muscipula*; 2- *Nepenthes northiana*; 3- *Drosera cuneifolia;* 4- *Dosera aliciae*; 5- *D. capensis* var. alba; 6- *D. capensis* var. rubra; 7- *D. burkeana*; 8- *D. binata*; 9- *D. intermedia*; 10- *D. rotundifolia*; 11- *D. capillaris*; 12- *Sarracenia purpurea* ssp. *purpurea*.

Tab. 1

Number of the generated bands with the used RAPD primers

Primer name	Number total of bands	Number of polymorphic bands	Number of monomorphic bands	% of polymorphism
OPA 01	12	12	0	100.0
OPA 02	9	8	1	88.88
OPA 03	11	11	0	100.0
OPA 04	13	13	0	100.0
OPA 09	10	10	0	100.0
OPA 17	9	7	2	77.77
OPB 08	14	14	0	100.0
OPB 10	6	6	0	100.0
OPB 11	9	8	1	88.88
OPB17	19	19	0	100.0
OPB 18	11	11	0	100.0
OPAB 11	8	7	1	87.50
OPAB 18	11	11	0	100.0
OPC 02	10	8	2	80.00
OPD20	10	7	3	70.00
OPE 11	14	12	2	85.75
OPE 14	14	14	0	100.0
OPF 02	10	9	1	90.00
OPG 07	13	12	1	92.30
OPO 14	10	9	1	90.00
OPX 03	14	12	2	85.71
OPAL 20	12	12	0	100.0
AB 11	13	13	0	100.0
Total	262	245	17	-

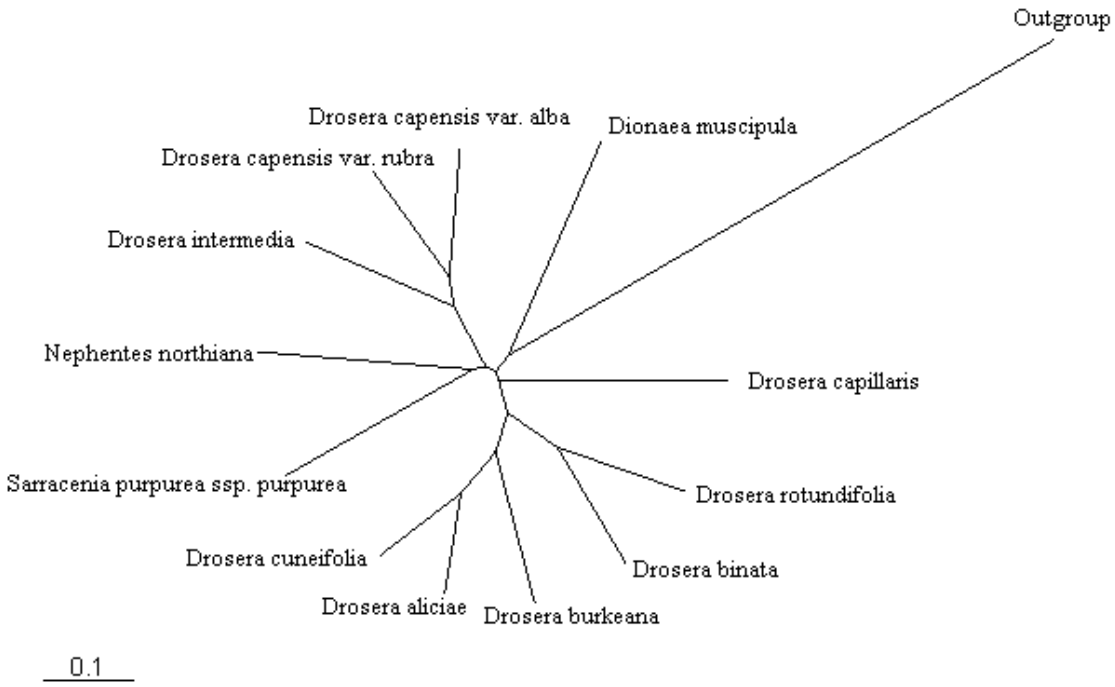

Fig. 3. UPGMA dendrogram generated using TreeView software, based on the genetic relationships between carnivorous plants, calculated using Nei Li/Dice coefficient with FreeTree software.

According to the dendrogram results produced by cluster analysis, the analysed carnivorous species are separated in two main groups (Fig. 3). The first group is represented by only *Dionea muscipula*. The second group includes two subgroups: one of them consist on different *Drosera* species (*D. capillaries, D. rotundifolia*, *D.binata, D. burkeana, D. aliciae* and *D. cuneifolia*) and the other one with a mixt structure with species (*Drosera capensis*, *Drosera intermedia*, *Nephentes northiana* and *Sarracenia purpurea*) from different genera (*Nephentes, Sarracenia* and *Drosera*).

The results obtained in this study shows that the species with similar phenotypic characters (plant form, catching system and trap form) belong to the same subgroup. Data registered in dendrogram indicates that the species *Dioneaea muscipula* is genetically the furthest comparing with the others analysed species, results confirmed by other authors (Albert *et al*., 1992; Ellison *et al*., 2009).

CONCLUSION

In this research, primers of OPB, OPA, OPE, AB, OPAL series shows the many bands (12-19) in the range of 200-1500 bp in three analysed genera, with high level of polymorphism (77.7- 100%).

The results obtained in this paper confirm the high level of variability existing among *Drosera* species although between some analyzed species was not observed evident differences on the leaf morphology, prey capture system and plant height.

RAPD assays showed that *Sarracenia purpurea* and *Nepenthes northiana* were clustered more closely than *Dionea muscipula*. In case of the analysed carnivorous plants, the potential of RAPD fingerprinting for species differentiation was successfully demonstrated.

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