Original Research Article

In vitro Induced Mitotic Polyploidy in *Drosera capensis* L.

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Abstract

The objective of this study was to induce mitotic polyploidization in *Drosera capensis*. Tetraploid plants of *D. capensis* were induced successfully by treating leaf segments *in vitro* with oryzalin solution with four different concentrations (20, 40, 60 or 80 µM) for 12, 24 or 48 hours. Three tetraploid (2n = 4x = 80) plants were obtained in three treatments (20 µM for 48 h, 60 µM for 24 h and 80 µM for 12 h). Tetraploidy was confirmed by flow cytometry. The survival rate of these plants was not significantly influenced by oryzalin concentration or exposure time.

Keywords: *Drosera capensis L.*; *Droseraceae*; flow cytometry; oryzalin; polyploidization.

INTRODUCTION

Droseraceae is a family of herbaceous carnivorous plants which comprises three genera: *Aldrovanda* (waterwheel plant), *Dionaea* (Venus flytrap) and *Drosera* (sundew). *Drosera* genus includes about 160 species globally distributed with two centers of diversity – Australia and South Africa (Barthlott et al., 2007).

Species are valued for their ornamental as well as medicinal properties. From an ornamental point of view, the plants are appreciated for their evergreen foliage converged into trap mechanisms with sticky tentacles enticing preys. 1,4-naphtoquinones, especially plumbagin and ramentaceone, included in sundew tissues have significant antimicrobial, antituberculotic, and antifungal activity (Ziaratnia et al., 2009).

The aim of plant breeding is to obtain better quality, diversity, adaptation and performance of important crops from both agronomical and horticultural point of view. Breeding of new varieties can be achieved by mitotic polyploidization *in vitro*.

Polyploid plants differ from their diploid progenitors in morphological, ecological, physiological or cytological characteristics (Dewitte et al., 2009).

The objective of this work was to obtain tetraploid plants for horticultural purposes with possible different morphological characteristics.

MATERIAL AND METHODS

Plant material and culture establishment

Mature seeds of *Drosera capensis* L. were treated with 0.1% solution of gibberellic acid (GA_3) (Sigma) (24 hours soaking at room temperature) to break dormancy and induce more uniform germination. The chromosome number of *D. capensis* is 2n = 2x = 40 (Bennett and Cheek, 1990; Rivadavia, 2005).

The surface of seeds was sterilized in 70.0% ethanol solution for 40 s and then in 1.0% sodium hypochlorite (NaClO) solution with a drop of Tween 20 for 15 minutes. Subsequently, seeds were washed three times in sterilized distilled water. Seeds were placed in 100 ml Erlenmeyer's flasks containing half-strength MS medium (Murashige and Skoog, 1962) at pH 5.7. Seeds were cultivated in cultivation box under 16:8 h photoperiod with light intensity 40.5 µmol·m^{-2}·s^{-1} and temperatures of 25 °C and 20 °C, respectively. The plants were multiplied by leaf explants to achieve high number of viable plantlets required for polyploidy induction.

Induction of polyploidy

The modified method according to Fong (2008) was used. Leaf segments of *Drosera capensis* were tested in subsequent oryzalin concentrations: 20, 40, 60 and 80 µM during 3 exposure times: 12, 24 and 48 hours. Leaf explants on ½ MS medium were completely immersed in oryzalin solution for the entire exposure time. After the exposure time, leaf segments were removed, washed three times in sterilized distilled water, dried on filter paper and cultured in ½ MS medium without growth regulators. Ploidy level was checked after three months. Each treatment comprised 20 explants; in total 240 plants were tested.

Flow cytometry analysis

Small pieces of leaf tissue were chopped in a Petri dishes containing 500 µl of Otto I buffer. The crude suspension of isolated nuclei was filtered through a 50-µm nylon mesh. One ml of Otto II buffer, supplemented with 50 µg/ml RNase and 50 µg/ml propidium iodide, was added. Samples were analysed using Partec PAS flow cytometer (Partec GmbH, Münster, Germany) equipped with a high-pressure mercury arc lamp. The gain of the instrument was adjusted so that the peak representing control plant G_1 nuclei appeared on channel 100.

RESULTS AND DISCUSSION

An antimitotic agent, oryzalin, was used for polyploidy induction in *Drosera capensis*. Tetraploidy (chromosome number 2n = 4x = 80) was achieved in three treatments: 20 µM after 48 h, 60 µM after 24 h and 80 µM after 12 h (Table 1) with total efficiency of 1.25%. Tetraploids were detected by flow cytometry (Figures 1 and 2).

Results show that for lower oryzalin concentrations longer times of exposure are needed to achieve polyoploids and vice versa. In terms of survival rate of plantlets, there was no significant influence of oryzalin concentration observed, similarly to Väinölä (2000) (Table 1).

Polyploidization using oryzalin concentrations above 40 µM was achieved in ornamental plants e.g. in *Nepenthes gracilis* (Fong, 2008), *Hedychium muluense* (Sakhanokho et al. (2009), *Epidendrum* (Miguel and Leonhardt, 2011) and *Berberis thunbergii* (Lehrer et al., 2008).

In conclusion, the modified method according to Fong (2008) produced tetraploid plants of *Drosera capensis*. The efficiency of polyploidization was rather low in this study, but further increase of oryzalin concentration or exposure time could increase the rate of polyploidization. Tetraploids will be multiplied for further morphological and physiological observations.

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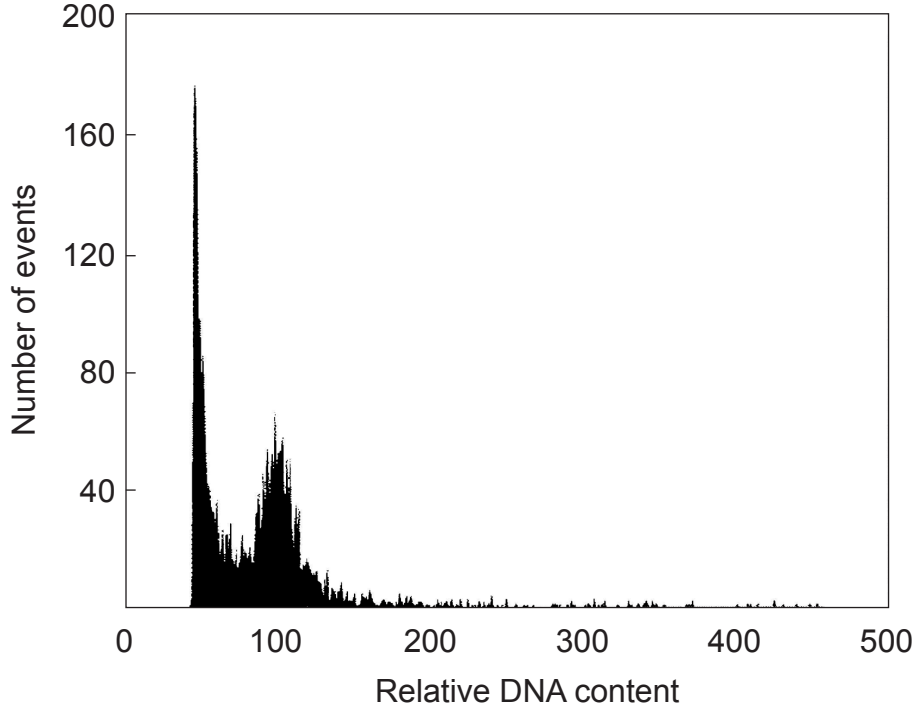

Figure 1. Histogram of relative DNA content with a peak corresponding to G0/G1 nuclei of the control plant.

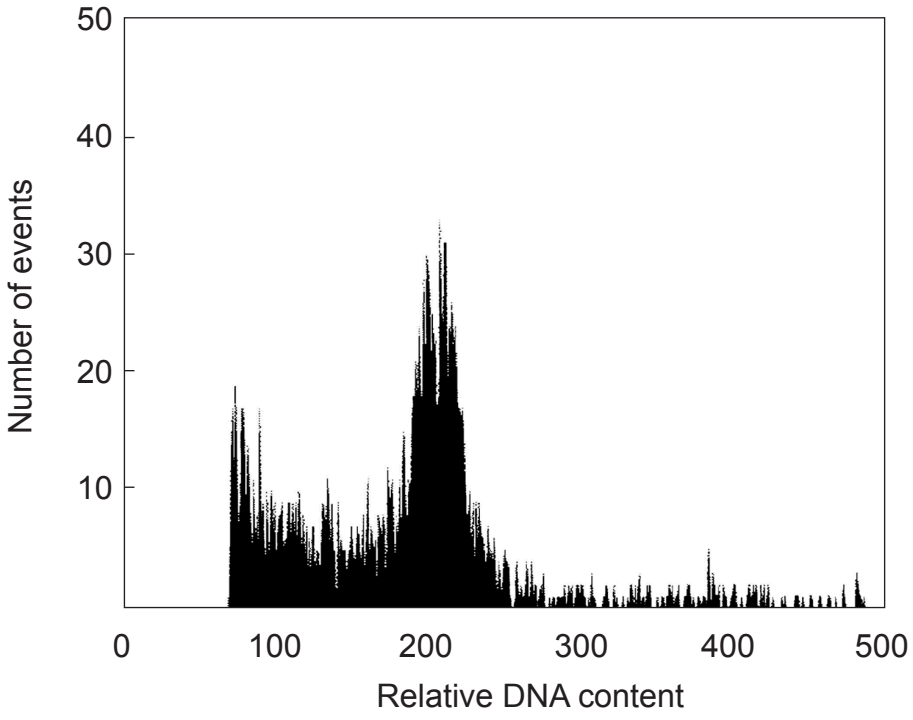

Figure 2. Histogram of relative DNA content with a peak corresponding to G0/G1 nuclei of the tetraploid.

Table 1. Effect of *in vitro* oryzalin treatment on the survival rate and number of polyploids in *Drosera capensis*

Oryzalin concentration (µM)	No. of explants (Leaf segments)	Treatment duration (h)	Survival rate (%)	No. of tetraploid plants detected by flow cytometry	Polyploidization efficiency (%)
0	20	12	90.0	0	0.0
	20	24	90.0	0	0.0
	20	48	90.0	0	0.0
20	20	12	20.0	0	0.0
	20	24	15.8	0	0.0
	20	48	10.0	1	5.0
40	20	12	10.0	0	0.0
	20	24	22.2	0	0.0
	20	48	29.4	0	0.0
60	20	12	16.7	0	0.0
	20	24	15.0	1	5.0
	20	48	10.0	0	0.0
80	20	12	18.8	1	5.0
	20	24	42.1	0	0.0
	20	48	18.8	0	0.0

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