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RESPONSE OF DROSERA KAIETEURENSIS AND DROSERA INTERMEDIA PLANTLETS AND LEAF EXPLANTS TO COMBINATIONS OF CYTOKININS AND AUXINS

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Abstract

This research focused on in vitro propagation of Drosera kaieteurensis and Drosera intermedia using several growth regulators. Half-strength Murashighe-Skoog medium (1/2MS) was employed for all treatments and controls. D. kaieteurensis plantlets were placed in medium supplemented with 0.5 mg/l kinetin (KIN) or 0.05 mg/l KIN+0.05 mg/l α-naphthaleneacetic acid (NAA). Leaves were transferred to 1/2MS containing 2 mg/l activated charcoal (AC) and either 10 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) or 10 mg/l 6-benzyladenine (BA) and grown in darkness (D) or under an 18/6h light/dark regime (L). D. intermedia plantlets and leaves were cultured in zeatin (Z) (0.1, 0.5 mg/l) or KIN (0.05, 1 mg/l) alone or in combination with NAA (0.05, 0.1, 1 mg/l). After 8 weeks, D. kaieteurensis plantlets developed best in the absence of growth regulators. When leaves were employed 10 mg/l BA+AC+L ensured maximum plant size while shoot formation was optimal in 10 mg/l 2,4-D+AC+D and root growth in the control medium. For D. intermedia plantlets, the lack of growth regulators resulted in larger plants and 0.1 mg/l Z stimulated significant root and shoot multiplication. Shoot and root production in both species was better when using plantlets.

Key words*: auxins, cytokinins, Drosera kaieteurensis, Drosera intermedia, multiplication.*

INTRODUCTION

Drosera kaieteurensis and *Drosera intermedia* are part of the genus *Drosera* (Droseraceae), a group of carnivorous plants that includes more than 200 species (McPherson, 2010) distributed in Australia, Africa, South America, North America, Asia and Europe (Rivadavia et al., 2003; Coritico and Fleischman, 2016). Their leaves have been converted into active sticky traps that can also digest prey by means of enzyme secretion (Banasiuk et al., 2012).

D. kaieteurensis was first recognized as a new tropical species by Brummer-Dinger in 1955 but its multiplication in vitro has not been investigated. *D. intermedia* has become popular in horticulture and homeopathic medicine. *Drosera* plants have been found to synthetize naphthoquinones and flavonoids with antibacterial properties and apoptotic activity against cancer cell lines (Banasiuk et al., 2012; Braunberger et al., 2015; Devi et al., 2016). In this context overharvesting and loss of habitat are serious threats to their conservation (Grevenstuk et al., 2010). *D. intermedia* is considered critically endangered (Laslo et al., 2011; Sprainaitytė, 2015) and has been encountered in the Cluj and Alba counties of Romania (Boşcaiu et al., 1994; Laslo et al., 2013). The aim of this research was to assess the effects of several growth regulators on in vitro propagation of *D. kaieteurensis* and *D. intermedia*.

MATERIALS AND METHODS

The experiments used plant material from established *in vitro* cultures of *D. kaieteurensis* and *D. intermedia* maintained on half-strength MS medium (Murashige and Skoog, 1962) without plant growth regulators. These cultures originated from seeds kindly provided by Grădina botanică Alexandru Borza, Cluj-Napoca. All reagents were acquired from Duchefa Biochemie, The Netherlands.

The employed medium, MS with half strength macroelements, half strength microelements and full strength vitamins (1/2MS) was

enriched with 30 g/l sucrose and 2 mg/l activated charcoal (AC) for the appropriate treatments. All media were solidified with 5 g/l phytagel and pH was adjusted to 5.5-5.6 before autoclaving at 121°C for 20 min. After sterilization 1/2MS was supplemented with plant growth regulators (PGR) according to species and experimental design. Control media without any PGR were prepared for all treatments.

In the first experiment *D. kaieteurensis* plantlets (10-12 mm in height) were placed in medium supplemented with 0.5 mg/l kinetin (KIN) or 0.05 mg/l KIN + 0.05 mg/l α-naphthaleneacetic acid (NAA). At the same time, leaves were transferred to 1/2MS containing AC and either 10 mg/l 2,4-dichlorophenoxyacetic acid (2,4 D) or 10 mg/l 6-benzyladenine (BA). These were placed in darkness (D) or under an 18/6 h light/dark regime (L). In the second experiment *D. intermedia* plantlets (10-15 mm in height) or leaves were cultured in zeatin (Z) (0.1, 0.5 mg/l) or KIN (0.05, 1 mg/l) alone or in combination with NAA (0.05, 0.1, 1 mg/l).

After 8 weeks plant height, rosette diameter, number of roots, root length, number of shoots, and number of buds were recorded for both species and types of explant. These measurements were used to calculate number of roots and shoots per explant. Seven 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 test 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 the first experiment when *D. kaieteurensis* plantlets were used to start the culture plant height and rosette diameter were similar for the control and medium supplemented with 0.05 mg/l KIN + 0.05 mg /l NAA (Table 1).

Both were significantly higher than the values measured for 0.5 mg/l KIN. The number of shoots for each explant and root development outlined by root length and number of roots/explant followed the same trend, the control being significantly better than media with PGR.

Table 1. Effect of PGR on multiplication starting from *D. kaieteurensis* plantlets

Treatment	Plant height	Rosette diameter	Roots/ explant	Root length	Shoots/ explant	Buds/ explant
Control	2.77± 0.10a	2.41± 0.14a	9.64± 3.13a	1.13± 0.19a	12.31± 2.08a	0.39± 0.16a
0.05 KIN + 0.05 NAA	2.64± 0.16a	2.63± 0.21a	4.81± 1.97ac	0.70± 0.35ac	6.21± 0.92b	0.96± 0.47a
0.5 KIN	1.19± 0.12b	1.75± 0.09b	0.00± 0.00bc	0.00± 0.0bc	0.32± 0.14c	4.92± 1.21b

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

When *D. kaieteurensis* leaves were used as explants (Table 2) plant development expressed as plant height and rosette diameter had the highest values in the presence of 10 mg/l BA combined with AC and subjected to an 18-h photoperiod.

Table 2. Effect of PGR on multiplication starting from *D. kaieteurensis* leaves

Treatment	Plant height	Rosette diameter	Roots/ explant	Root length	Shoots/ explant	Buds/ explant
Control L	1.91± 0.08a	2.30± 0.10ab	9.68± 0.44a	3.15± 0.10a	4.00± 0.27ac	0.00± 0.00ac
Control D	2.60± 0.09a	0.00± 0.00a	2.33± 0.37b	1.41± 0.07b	3.56± 0.02ac	0.38± 0.09ac
2,4D 10 + AC + L	1.59± 0.05a	1.93± 0.11ab	9.93± 0.87a	1.12± 0.04b	3.89± 0.27ac	0.24± 0.14ac
2,4D 10 + AC + D	2.17± 0.05a	0.00± 0.00a	1.01± 0.51b	0.27± 0.12c	4.75± 0.53a	0.60± 0.61b
10 BA + AC + L	4.38± 2.10a	4.66± 2.13b	8.77± 1.75a	1.15± 0.23b	3.43± 0.29bc	0.04± 0.06c
10 BA + AC + D	2.45± 0.09a	0.00± 0.00a	2.00± 0.67b	0.37± 0.10c	4.23± 0.08ac	0.29± 0.17ac

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

On the other hand, the largest number of shoots/explant was induced by supplementation with 10 mg/l 2,4 D in darkness. The high 2,4 D concentration did not produce callus but instead resulted in etiolated and chlorotic shoots (Figure 1.C) that needed to be acclimated to normal lighting conditions. Root growth was significantly better when leaves were exposed to light and placed in 1/2MS + 10 mg/l 2,4 D or control medium.

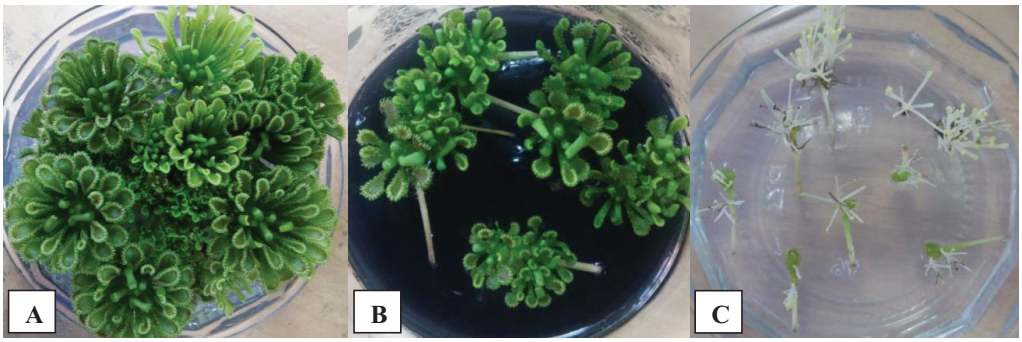

Figure 1. Multiplication of *D. kaieteurensis*: A. using plantlets placed in 1/2MS without PGR; B. using leaves placed in 1/2MS + 10 mg/ml BA + AC in an 18-h photoperiod; C. using leaves placed in 1/2MS without PGR and darkness.

The experiment on *D. kaieteurensis* showed that medium without PGR could support the development of a high number of roots/explant and a significant number of shoots/explant that evolved further into large plants (Figure 1.A). Low concentrations of PGR, namely 0.05 mg/l KIN + 0.05 mg/l NAA were also effective particularly with respect to plant size. The use of leaves as explants gave rise to bigger plants in the presence of 10 mg/l BA and light while root development was better in the absence of PGR and shoot production was favoured by 10 mg/l 2,4 D in darkness.

The second experiment employed *D. intermedia* plantlets (Table 3) and leaves (Table 4).

Table 3. Effect of PGR on multiplication starting from *D. intermedia* plantlets

Treatment	Plant height	Rosette diameter	Roots/ explant	Root length	Shoots/ explant	Buds/ explant
Control	2.47± 0.25a	2.63± 0.35a	6.91± 1.65ad	0.52± 0.10abc	7.07± 1.20a	0.00± 0.00a
0.1 Z	1.53± 0.09b	1.62± 0.07bc	13.57± 1.98b	0.47± 0.02abc	11.98± 1.23b	0.00± 0.00a
0.5 Z	1.61± 0.07bc	1.67± 0.15bcd	1.53± 1.75c	0.89± 0.37b	4.09± 0.90ad	0.00± 0.00a
0.05 KIN+ 0.05 NAA	2.74± 0.29a	2.02± 0.10ac	9.08± 3.67ab	0.51± 0.04abc	6.81± 0.64a	0.00± 0.00a
1 KIN+ 1 NAA	0.95± 0.21b	0.93± 0.19b	1.78± 2.52cd	0.06± 0.03c	2.00± 0.51cde	0.00± 0.00a
0.1 Z+ 0.05 NAA	2.35± 0.12ac	2.20± 0.14ac	5.83± 1.59ac	0.33± 0.02abc	5.19± 0.72ae	0.00± 0.00a
0.1 Z+ 0.1 NAA	1.56± 0.01b	1.35± 0.02bd	4.33± 1.07ac	0.19± 0.02ac	5.11± 0.28ae	0.00± 0.00a

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

Plants resulting from the first type of explant had the most substantial number of roots/explant in the presence of 0.1 mg/l Z (Figure 2.A) followed by the combination 0.05 mg/l KIN + 0.05 mg /l NAA. Zeatin was also responsible for significantly longer roots and the highest number of shoots/explant.

If leaves were used to start *D. intermedia* in vitro cultures the best results for most recorded parameters were observed in medium without PGR (Table 4). Buds were more numerous in 0.5 mg/l Z (Figure 2.C.) than in any other medium.

Table 4. Effect of PGR on multiplication starting from *D. intermedia* leaves

Treatment	Plant height	Rosette diameter	Roots/ explant	Root length	Shoots/ explant	Buds/ explant
Control	1.77± 0.28a	1.76± 0.23a	1.88± 0.33a	0.46± 0.05a	1.56± 0.17a	0.00± 0.00a
0.1 Z	1.07± 0.08b	1.35± 0.04b	0.71± 0.14bc	0.23± 0.02b	1.32± 0.22ab	0.77± 0.09b
0.5 Z	0.71± 0.07bd	0.96± 0.05bd	1.38± 0.28ac	0.44± 0.05a	1.01± 0.12b	1.73± 0.30c
0.05 KIN+ 0.05 NAA	0.00± 0.00cd	0.00± 0.00ce	0.00± 0.00b	0.00± 0.00cd	0.00± 0.00cd	0.00± 0.00a
1 KIN+ 1 NAA	0.00± 0.00cd	0.00± 0.00ce	0.00± 0.00b	0.00± 0.00cd	0.00± 0.00cd	0.00± 0.00a
0.1 Z+ 0.05 NAA	0.37± 0.20bd	0.35± 0.19de	0.79± 0.42bc	0.07± 0.04d	0.07± 0.04d	0.36± 0.23ab
0.1 Z+ 0.1 NAA	0.55± 0.29bd	0.50± 0.27de	0.19± 0.10b	0.08± 0.04bd	0.03± 0.02d	0.00± 0.00a

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

Results of the experiment on *D. intermedia* were similar to those of Rejthar et al. (2014) who also found that low concentrations (0.1 and 0.5 mg/l) of Z provided the best results for shoot proliferation. In our case, the same PGR also provided the best root development.

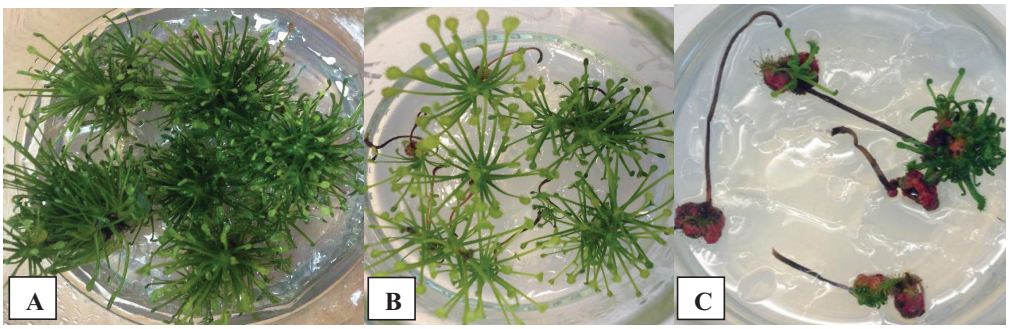

Figure 2. Multiplication of *D.intermedia*: a. starting from plantlets placed in 1/2MS + 0,1 mg/l Z; B. starting from leaves placed in 1/2MS without PGR; C. starting from leaves placed in 1/2MS + 0,5 mg/l Z

Laslo et al. (2013) recommended a moderate (2 mg/l) dose of Z and a small (0,1 mg/l) dose of indole-3-butyric acid (IBA) for the multiplication of *D. intermedia*. In the current experiment, addition of NAA to medium with Z resulted in larger plants but decreased shoot numbers and did not improve root development. It is also worth pointing out that a five-fold increase in Z concentration had a significant negative effect on root development and shoot number. This is in contrast to the results of Jayaram et al. (2007) who found that 0.5 mg/l Z yielded the maximum number of shoots in *Drosera indica*.

If our work is compared to that of Grevenstuk et al. (2010) who used 0.1 mg/l KIN it becomes apparent that low concentrations of KIN and NAA used in combination decreased shoot numbers but favoured plant size.

Results for these two species are similar to our research on *D. rotundifolia* and *D. capensis* (Miclea and Zăhan, 2017) which found that a low concentration of KIN (0.5 mg/ml) or no PGR were most suitable for shoot and root production in both species.

High concentrations of cytokinins and NAA have been effective for the induction of multiple shoots and roots in *Drosera burmannii* starting from shoot tips, but 6-benzylamino-purine (BAP) + NAA and KIN + NAA were more effective than Z + NAA (Yanthan et al., 2017).

CONCLUSIONS

This research found that medium without PGR was the best support for development of *D. kaieteurensis* when starting from plantlets. If leaves were cultured under an 18-h photoperiod 1/2MS + 10 mg/l BA gave rise to the highest plants and the control medium to the longest roots. Complete darkness and 10 mg/l 2,4-D resulted in the production of etiolated and chlorotic shoots instead of callus. In *D. intermedia* the lowest Z concentration provided the best results for shoot and root proliferation. Addition of NAA to Z and the use of KIN + NAA combinations suppressed shoot production but improved plant size. For both species our recommendation would be to use plantlets as explants.

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