In vitro Multiplication of the Pitcher Plant *Sarracenia Purpurea*

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Abstract

The aim of the current research was to find the best plant growth regulators for the multiplication of *Sarracenia purpurea*. Murashige and Skoog medium (MS) was prepared with macronutrients and micronutrients at 1/3 strength, full strength vitamins, supplemented with 30 g/l sucrose and 5 g/l phytagel and autoclaved. After cooling 0.5 mg/l α -naphthaleneacetic acid (NAA), 5 mg/l 6-benzyladenine (BA) or 0.5 mg/l NAA + 3 mg/l BA were added. Young *S. purpurea* plants were selected and transferred to media with or without plant growth regulators and cultured for 12 weeks. At the end of this time frame number of roots, root length (cm) and number of shoots were evaluated and differences were analysed by the analysis of variance and interpreted using the Tuckey test. The largest number of roots grew in medium supplemented with 0.5 mg/l NAA but the the absence of plant growth regulators increased their length. The best conditions for shoot multiplication were provided by supplementing 1/3MS with 5 mg/l BA.

Keywords: *in vitro* propagation, *Sarraccenia purpurea*

INTRODUCTION

Sarracenia purpurea L. (Sarraceniaceae), is a perennial carnivorous plant widely distributed in North America (Harris *et al.*, 2012). It grows in bog environments where the acidic soil is poor in nutrients, particularly nitrogen so it adapts by trapping insects in highly modified and fused leaves (pitchers) and consuming them (Rogers *et al.*, 2010). *S. purpurea* has come to the attention of hobby horticulturists (Northcutt and Davies, 2012) and although many *Sarracenia* species are endangered, mainly by loss of habitat (Uhnak, 2003), poachers still harvest wild plants illegally to sell to collectors (Stiefel, 2000). This species is well known for its uses in traditional medicine (Guerrero-Analco *et al.*, 2014), it can inhibit poxvirus replication at the level of early viral transcription (Arndt *et al.*, 2012), afford protection

against diabetic neuropathy (Eid and Haddad, 2014) but also cause apoptosis in renal cells (Li *et al.*, 2018). In this context micropropagation becomes an alternative source to produce large numbers of plants to be sold or used for the extraction of secondary metabolites. The aim of our research was to find the most fitting plant growth regulators for the multiplication of *S. purpurea*.

MATERIALS AND METHODS

Reagents were acquired from Duchefa Biochemie, The Netherlands. Murashige and Skoog medium (1962, MS) was prepared with macronutrients and micronutrients at 1/3 strength (1/3MS), full strength vitamins, supplemented with 30 g/l sucrose, solidified with 5 g/l phytagel and pH was adjusted to 5.4-

Tab. 1. Effects of growth regulators on plant multiplication in *S. purpurea*

Treatment	No. roots/explant	Root length (cm)	No. shoots/explant
Control	4.56±0.95a	4.12±0.82a	1.14±0.19a
0.5 mg\l NAA	6.79±0.45c	2.60±0.38a	1.87±0.19a
5 mg\l BA	0.48±0.17bd	0.45±0.13bc	3.75±0.36b
0.5 mg\l NAA + 3 mg\l BA	0.30±0.20d	0.18±0.13c	2.12±0.43a

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

Fig. 1. *S. purpurea* shoots with poorly developed roots (a) and with well developed roots (b) 12 weeks after being placed in culture medium.

5.5 before autoclaving at 121°C for 20 min. After cooling 0.5 mg\l α -naphthaleneacetic acid (NAA), 5 mg\l 6-benzyladenine (BA) or 0.5 mg\l NAA + 3 mg\l BA were added to the medium and it was dispersed in culture vessels. Young *S. purpurea* plants from established *in vitro* cultures were selected and transferred to media with or without plant growth regulators and kept for 12 weeks in the growth 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 $\mu\text{mol}/\text{m}^2/\text{s}$. At the end of this time the number of roots, root length (cm) and number of shoots were assessed and differences were analysed by the analysis of variance and interpreted using the Tuckey test with $p < 0.05$ being considered statistically significant. These were performed using GraphPad InStat version 3.05 (GraphPad Software, San Diego California, USA).

RESULTS AND DISCUSSION

The average number of roots calculated for each explant showed that root production was significantly better in the presence of 0.5 mg\l NAA than for any of the other treatments (Tab.

1.). Higher concentrations of growth regulators greatly reduced the number of roots generated by each explant while their complete absence had a less marked but still significant effect. Root length was smaller when root number was highest (0.5 mg\l NAA) but it increased in medium with no growth regulators.

The number of shoots for each explant reached the maximal value when 5 mg\l BA was added to the culture medium and decreased if both an auxin (NAA) and a cytokinin were present (BA). This result is in line with the known effect of auxins and cytokynins as root and shoot inductors, respectively.

With regard to shoot multiplication the results of our experiment are similar with those of Northcutt and Davies (2012) who generated the largest number of shoots using 3 mg/l BA.

When root growth is analysed it becomes apparent that although the number of roots was highest in medium supplemented with 0.5 mg\l NAA which runs parallel to the study of Northcutt and Davies (2012), these roots were longer in the control, which is an interesting and surprising development.

CONCLUSION

In *S. purpurea* we observed that adding a low concentration of NAA (0.5 mg\l) to the 1/3MS culture medium resulted in the growth of roots while the absence of plant growth regulators increased root length. The best conditions for shoot multiplication were provided by the addition of 5 mg\l BA to 1/3MS.

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