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Mass Propagation of Sundew, *Drosera rotundifolia* L. through Shoot Culture

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Abstract

In order to establish *in vitro* propagation method of sundew, *Drosera rotundifolia* L., the effects of MS medium concentration, cytokinin type and concentration, pH, and auxin type and concentration on shoot proliferation and root formation were investigated using shoots at 3 month after seed germination. The highest shoot production was obtained with the half strength of MS (½ MS) medium than with any other strength of MS medium tested. Addition of kinetin or BA in ½ MS medium was strongly suppressed shoot proliferation. The suppression of shoot proliferation was more effective in BA-supplemented ½ MS medium than kinetin-supplemented. The optimum pH of the media for shoot proliferation was pH 5.7-6.7. Shoots were subcultured in ½ MS medium supplemented with 0.5mg/L 2,4-D for rooting every 8 weeks. All subcultured shoots produced extensive root systems after 5 to 6 week culture. Plantlets after root development were planted in plastic pots filled with moss. The survival rate of plantlets was almost 100%. On subculturing every 8 weeks, hundreds of the plants were propagated from a single plant within a year.

Introduction

The variety of biological species has been embossed as an index of national resources. But, the wild plant habitat has been destroyed and various species of wild plants come to an extermination crisis, because of industrial development and population increase. Molt (1989) also argued that the wild plant species have to be preserved positively because about 9 thousand wild plant species will come to an extermination crisis by 2,000 year. IUCN(The International Union for Conservation of Nature and National Resources) reported in 1992 that more than 20 thousand species among 300 thousand plant species will come to an extermination crisis by 2,000 year.

The sundew plant, a perennial carnivorous plants, belongs to *Drosera peltata* var. nipponica OHWI family. It is protected by the Ministry of Environment Plant 75 in Korea (Park, 1994). It grows in humid areas such as marsh and in low fertility soil with a low pH which ranges from 3 to 5 (James and Patricia, 1993). It solves their nutritional needs by capturing prey with the sticky mucilaginous secretions at the end of the leaf tentacles. The plants are completely covered with droplets of a sticky liquid.

Extracts of sundew plant's leaves were used to treat external disorders including warts, corns and sunburn, or internal disorders including tuberculosis, asthma, whooping cough, arteriosclerosis, eye and ear inflammations, liver pain, syphilis and so on (James and Patricia, 1993).

The plant tissue culture technic plays an important role on the germplasm preservation and micropropagation of plants that are on the brink of an extermination crisis or rarity. Several types of carnivorous plants have been successfully propagated in vitro, including the Venus fly trap, *Dionaea muscipula* Ellis (Beebe, 1980; Parliman et al., 1982; Hutchinson, 1984), Australian Pitcher Plant, *Cephalotus follicularis* (Adams et al., 1979a) and others (Adams et al., 1979b; Carroll, 1982; Mohan Ram et al., 1972).

In order to establish an efficient *in vitro* method for propagation on of sundew, *Drosera rotundifolia* L. the effects of MS medium concentration, cytokinin

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type and concentration, pH, and auxin type and concentration on shoot proliferation and root formation were investigated.

Materials and Methods

Seed sterilization and germination

The sundew seeds were collected in Wando island and stored at 4℃ prior to use. The seeds were surface sterilized in a solution of 20% (v/v) commercial bleach and 0.01% (v/v) Tween-20 for 20 min, and then washed with sterilized distilled water. The seeds were placed on ½ MS medium (Murashige and Skoog, 1962) containing 3% sucrose, 0.2% gelrite and pH 5.7. The seeds were germinated at 25℃ under 16 h/8 h photoperiod with a light intensity of 30 $\mu Em^{-2}s^{-1}$.

Maintenance of proliferating shoot culture

Cultures were established from shoot 3 month after seed germination and maintained in a proliferating state by subculturing every 8 weeks. The basal medium was ½ MS medium containing 3% sucrose, 0.2% gelrite and pH 5.7 without hormone.

Factors studied

The following factors were investigated as individual experiments. In each case, 20 replications per treatment were used. The shoot proliferation and root formation were observed after 8 week culture.

(1) MS medium concentration - the effect of MS, ½ MS, ¼ MS and ⅛ MS medium was evaluated.
(2) Cytokinin type and concentration - the effect of BA or kinetin, each at 0.5, 1, 2 and 5mg/L, was evaluated using ½ MS medium.
(3) pH - ½ MS medium was adjusted to a pH of 3.7, 4.7, 5.7 and 6.7 .
(4) Auxin type and concentration - the effect of 2,4-D or NAA, each at 0.5, 1, 2 and 5mg/L, were evaluated using ½ MS medium.

Results and Discussion

Effects of MS medium concentration

The sundew seeds germinated within 2-3 weeks on ½ MS medium (Figure 1a, 1b). The germination rate of seeds was 100% when the seeds were treated at 4℃ for more than 30 days. But the seeds without cold treatment at 4℃ for at least 4 weeks did not germinate at all.

Because the carnivorous plants grow normally in nutrient-poor areas, the effects of the strength of MS medium on shoot proliferation were tested with ½ MS, ¼ MS and ⅛ MS medium in addition to standard MS medium. The shoot proliferation was greatly influenced by MS medium strength as shown in Table 1. The number of shoot was the highest on ½ MS medium (Figure 1c) and the lowest on ⅛ MS medium. This data was similar with the previous result of Jang et al. (1997) that the shoot proliferation from leaf segment of sundew was the best on ⅓ MS medium and poor when diluted to ⅙ MS and on standard MS medium. The shoot length was the longest on standard and ½ strength MS medium (3.0-3.5cm long), and the shortest on ⅛ MS medium (1.7cm long).

Table 1. Effect of MS medium concentration on shoot proliferation from shoot of sundew, *Drosera rotundifolia* L., after 8 weeks culture.

MS medium concentration	No. of shoot(±SE)	Shoot length(cm±SE)
MS	30.7 ± 4.3	3.0 ± 0.6
½ MS	55.0 ± 6.2	3.5 ± 0.9
¼ MS	21.2 ± 3.5	2.1 ± 0.4
⅛ MS	5.6 ± 1.6	1.7 ± 0.2

Effects of cytokinin type and concentration

Cytokinins generally inhibit root development and promote shoot growth (Pennazio, 1975). Cytokinins has been applied to induce and proliferate the shoot in micropropagation of plants. We examined the effects of cytokinins on shoot proliferation of sundew. As shown in Table 2, Addition of cytokinins (kinetin or BA) in ½ MS medium at the concentration of 0.5-5.0 mg/L strongly supressed the shoot proliferation. The suppression of shoot proliferation was more in BA-supplemented than in kinetin-supplemented. Interestingly, all cultures in BA-supplemented medium produced shoots with poorly developed leaves, and also cultures in cytokinin-containing media produced short, thick and black roots.

Effects of pH

The carnivorous plants grow in a low pH soil which ranges from 3 to 5 (James and Particia 1993). Parliman et al. (1982) initially grew their cultures of Dionaea muscipula on the medium of pH 5.8, but later successfully used media of pH 4.9. Since pH affect nutrient uptake and shoot proliferation, the effects of pH levels on shoot proliferation of sundew were examined (Table 3). The optimum pH for shoot proliferation was from pH 5.7 to 6.7 and was severely inhibited in more acidic media. And the optimum pH for roots development was from pH 5.7 to 6.7.

Effects of auxin type and concentration

The effects of auxins on root formation were shown in Table 4. The root formation rate maintained 100% level on ½MS medium supplemented with 0.5-2mg/L 2,4-D or 0.5-1mg/L NAA. These data were different from the result of Hutchinson (1984) that the addition of NAA in propagation of *Dionaea muscipula* Ellis in vitro was completely inhibitory to root initiation. The disparity may mainly come from the different plant source. The root number and root length were best on ½MS medium supplemented with 0.5mg/L 2,4-D and 0.5mg/L NAA.

After one subculture, the young shoots were growed enough to separate into individual. Separated shoots produced extensive root systems after 6 to 8 weeks, in contrast to the weak-rooted plants in the wild (Juniper et al., 1989). Plantlets without roots had a quite long leg phase before they start to grow, compared to plantlets which already had roots at the moment of planting out. Roots showed a normal appearance, which were black, unbranched and entirely covered with root hairs. Interestingly, they originated in the axils of the leaves. About 90% of the plantlet produced flowers on ½MS medium without hormones. But the plantlet cultures on ½MS medium with hormones did not produce flowers.

Potency of in vitro mass propagation through shoot culture

Plantlets were removed from culture and planted to plastic pots filled with pure moss. Pots were covered with plastic boxes to ensure high humidity.

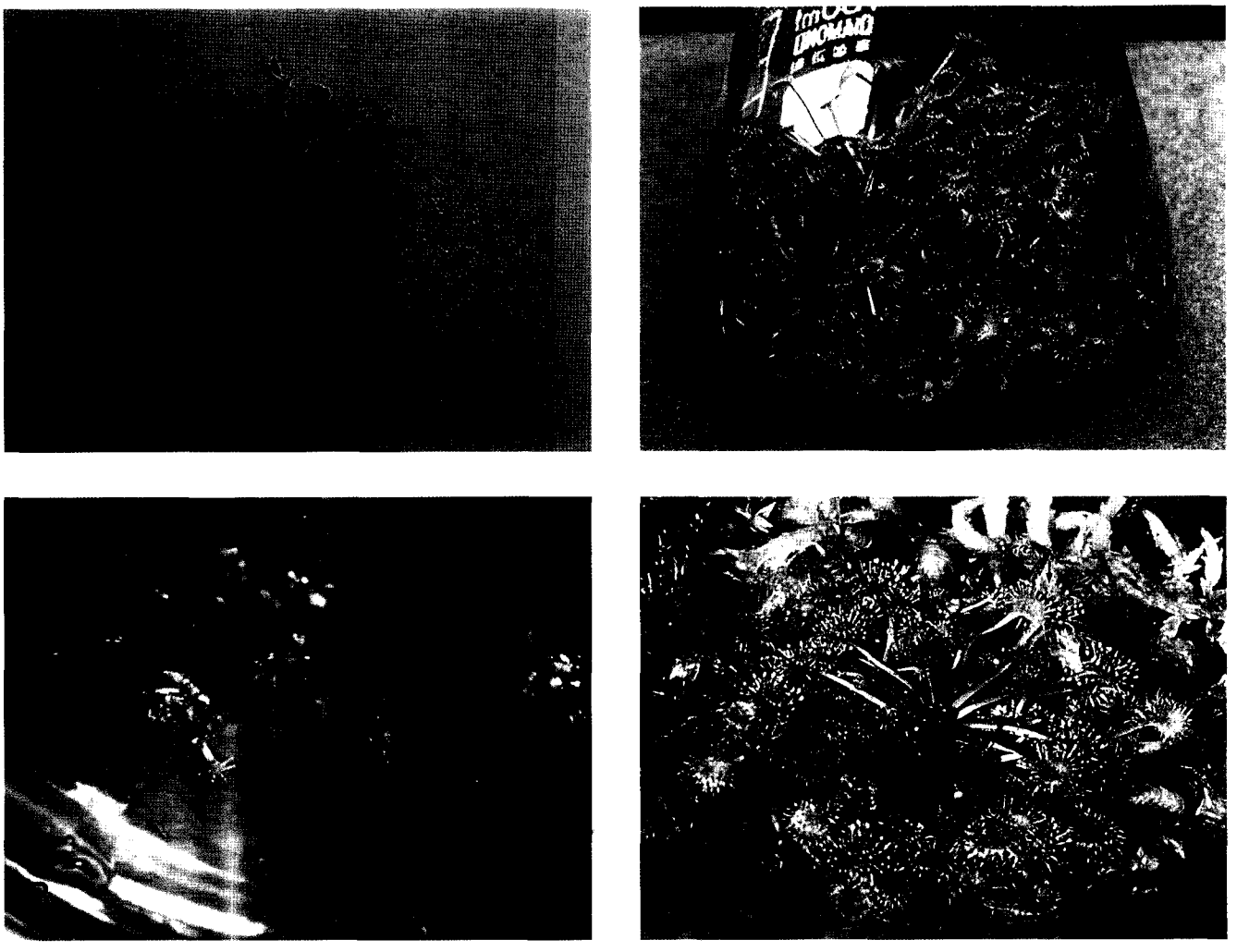

Figure 1. Micropropagation through shoot cultures of sundew, *Drosera rotundifolia* L. (A) Ripe spikes and seeds of sundew. (B) Seedlings germinating from seeds after 2-3 weeks culture on ½MS medium. (C) Multiple shoots formation from shoot after 8 weeks culture on ½MS medium. (D) Well-established plant 3 weeks after transplanting to pot. Arrows indicate the production of sticky liquid.

Table 2. Effect of cytokinins on shoot proliferation from shoot of sundew, *Drosera rotundifolia* L., after 8 weeks culture on ½ MS medium.

Cytokinins (mg/L)		No. of shoot(±SE)	shoot length(cm±SE)
Control		55.0 =/-6.1	3.5=/-0.9
Kinetin	0.5	16.8 ± 2.1	2.2 ± 0.3
	1	24.8 ± 3.6	3.7 ± 0.5
	2	10.0 ± 1.9	2.0 ± 0.3
	5	5.2 ± 0.9	1.6 ± 0.2
BA	0.5	3.5 ± 0.7	1.7 ± 0.3
	1	9.9 ± 1.3	1.9 ± 0.2
	2	14.1 ± 3.0	2.5 ± 0.5
	5	14.3 ± 2.8	2.9 ± 0.3

Table 3. Effect of pH on shoot proliferation from shoot of sundew, *Drosera rotundifolia* L., after 8 weeks culture on ½ MS medium.

pH	No. of shoot(±SE)	shoot length(cm±SE)
	9.2 ± 3.0	1.7 ± 0.3
4.7	18.7 ± 3.6	2.0 ± 0.4
5.7	55.0 ± 6.2	3.5 ± 0.9
6.7	48.0 ± 5.4	3.2 ± 0.8

Table 4. Effect of auxins on root formation from shoot of sundew, *Drosera rotundifolia* L., after 6 weeks culture on ½ MS medium.

Auxins (mg/L)		Root formation rate(%)	No. of root(±SE)	Root length (cm±SE)
2,4-D	0.5	100	5.5 ± 1.2	2.1 ± 0.3
	1	100	3.0 ± 0.7	1.2 ± 0.3
	2	100	2.1 ± 0.5	0.7 ± 0.2
	5	75	1.3 ± 0.4	0.5 ± 0.1
NAA	0.5	100	4.6 ± 1.3	1.8 ± 0.4
	1	100	2.6 ± 0.4	1.0 ± 0.4
	2	85	2.0 ± 0.2	0.6 ± 0.2
	5	60	1.2 ± 0.3	0.5 ± 0.2

Growth conditions were the same as they were cultured. The survival rate was 100% and the young plants grew well (Figure 1d). After 2-3 weeks, the leaf glands already started to produce sticky liquid (Figure 1d). The *in vitro* root system continued to grow, indicating their normal function. This is in contrast with many other plants which usually form a new root system after transfer to soil, due to the non-functional roots. In 2-3 months after transfer to soil, newly formed leaves and secretory glands were functional and able to catch insects. They reacted normally, which means that glands bend towards the insects and the leaf top folds down to enclose the insects completely.

The present experiment proved the possibility of a mass propagation of sundew, *Drosera rotundifolia* L., *in vitro* where one shoot is subcultured every 8 weeks, hundreds or even thousands of the valuable plants can be propagated in a year.

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