Banno, K., K. Yoshida, S. Hayashi, and **K. Tanabe.** 1989. *In vitro* propagation of Japanese pear cultivars. J. Jpn. Soc. Sci. 58:3742.

Nemeth, G. 1986. Induction of rooting. p.49-64. In: Y.P.S. Bajaj (ed.). Biotechnology in agriculture and forestry. Vol. 1. Springer-Verlag, Berlin.

Arrilaga, I., T. Marzo, and **J. Segura.** 1991. Micropropagation of juvenile and adult *Sorbus domestica* L. Plant Cell Tissue Organ Cult. 27:341-348.

Micropropagation of Venus Fly-Trap (*Dionaea muscipula* Ellis)

T. Yamamoto
Minami-Kyushu University, Takanabe, Miyazaki 884

T. Shiba
Shiba Orchid Nursery, Nyuta Shilitomi, Miyazaki 889-14

M. Horikawa
Tsubakiya Co., Minamikata, Hougou, Hiroshima 729-04

Experiments were carried out on a large-scale propagation of Venus fly-trap through leaf explant culture. When whole leaf explants excised from a donor plant grown in vitro were cultured on half-strength LS media with different concentrations of BA, adventitious shoots were mainly formed from the petiole of the explants, and few formed from the leaf blade of the explants. The medium supplemented with 2 mg liter^{-1} BA was the most effective for organogenesis. The shoots grew into plantlets which were transferred to the medium with 0.1 mg liter^{-1} NAA. The differentiation and subsequent growth of the rhizomes was better in the medium solidified with Gelrite than in that with agar. The adventitious shoots formed in a row on the rhizome in the medium. These shoots were excised from the rhizome and were transferred to the medium for further proliferation. By these procedures, a large number of regenerated plantlets were obtained, and the plants after acclimatization have grown well in pots.

INTRODUCTION

Venus fly-trap (*Dionaea muscipula* Ellis) is an interesting insectivorous plant which belongs to the family Droseracea. The plant is native to the eastern coast of the United States, and wild species have been reported to be threatened with extinction (Ayensu, 1981). The plant can be used indoors as a potted ornamental plant, and is sometimes used as teaching material for children. The propagation of the plant is usually from seed, however, it is not easy. There are several reports on micropropagation of Venus fly-trap using shoot tips (Hutchinson, 1984), leaves

(Parliman, 1982; Minocha, 1985; Kukulczanka, 1989), and rhizomes (Parliman,1982) as explants. These reports demonstrate that the use of cytokinin with auxin promotes in vitro proliferation of the plant through the formation of adventitious buds or lateral buds. The present paper describes the effect of benzyladenine (BA) on the formation of adventitious shoots from leaf petiole explants and the formation of adventitious shoots from the rhizome of the plant regenerated in vitro.

MATERIALS AND METHODS

Petiole segments (1.5 cm in length) were excised from a potted Venus fly-trap obtained from a market. After sterilization with 3% hydrogen peroxide, the segments were dipped in 1% citric acid solution for 5 min and placed on a half-strength Linsmaier Skoog (LS) medium which was solidified with 0.7% agar and supplemented with 1 mg liter^{-1} BA and 0.1 mg NAA liter^{-1}. The pH of the medium was adjusted to 5.8 before autoclaving. About 45 days after the beginning of culture, adventitious shoots were formed on the petiole explants. When the shoots attained about 1 cm in height, these were transferred to the medium for formation of the rhizome. The regenerated plantlets grew in the culture vessel into plants with many leaves through the induction of axillary buds. From the plants grown in vitro, leaf explants (about 1 cm in length) including petiole and leaf blade were excised, and cultured on a half-strength LS medium supplemented with BA of various concentrations. Plantlets formed from the petiole were separated from the explants and subcultured for the formation of rhizomes, on a medium which was supplemented with 0.1 mg l^{-1} NAA and solidified with 0.2% Gelrite or 0.7% agar. The regenerated plants with fully developed rhizomes were transferred to pots (9-cm diameter) containing a vermiculite or sphagnum medium for acclimatization.

RESULTS AND DISCUSSION

When whole leaf explants excised from a donor plant grown in vitro were cultured on a half-strength LS medium, adventitious shoots were mainly formed from the petiole of the explant after 40 to 50 days culture, while little formed from the leaf blade of the explant. Figure 1 shows the formation of adventitious shoots from the petiole. Since formation of callus could not be observed on the cultures, the shoots were considered to be directly formed from the surface tissue of the petiole. Afterwards the explants were covered with the proliferated shoots. As shown in Table 1, 2 mg litre^{-1} BA was the most effective for the formation of adventitious shoots. In this case, the mean value of the number of shoots obtained per explant was six after 4 months of culture, and then further increases in the number of shoots were observed. Adventitious shoots were not formed from some explants, because their tissue showed a brown colour during culture and withered. The promotive effect of cytokinin on the formation of adventitious shoots from the petiole has been reported with various plants, e.g. *Smilax*

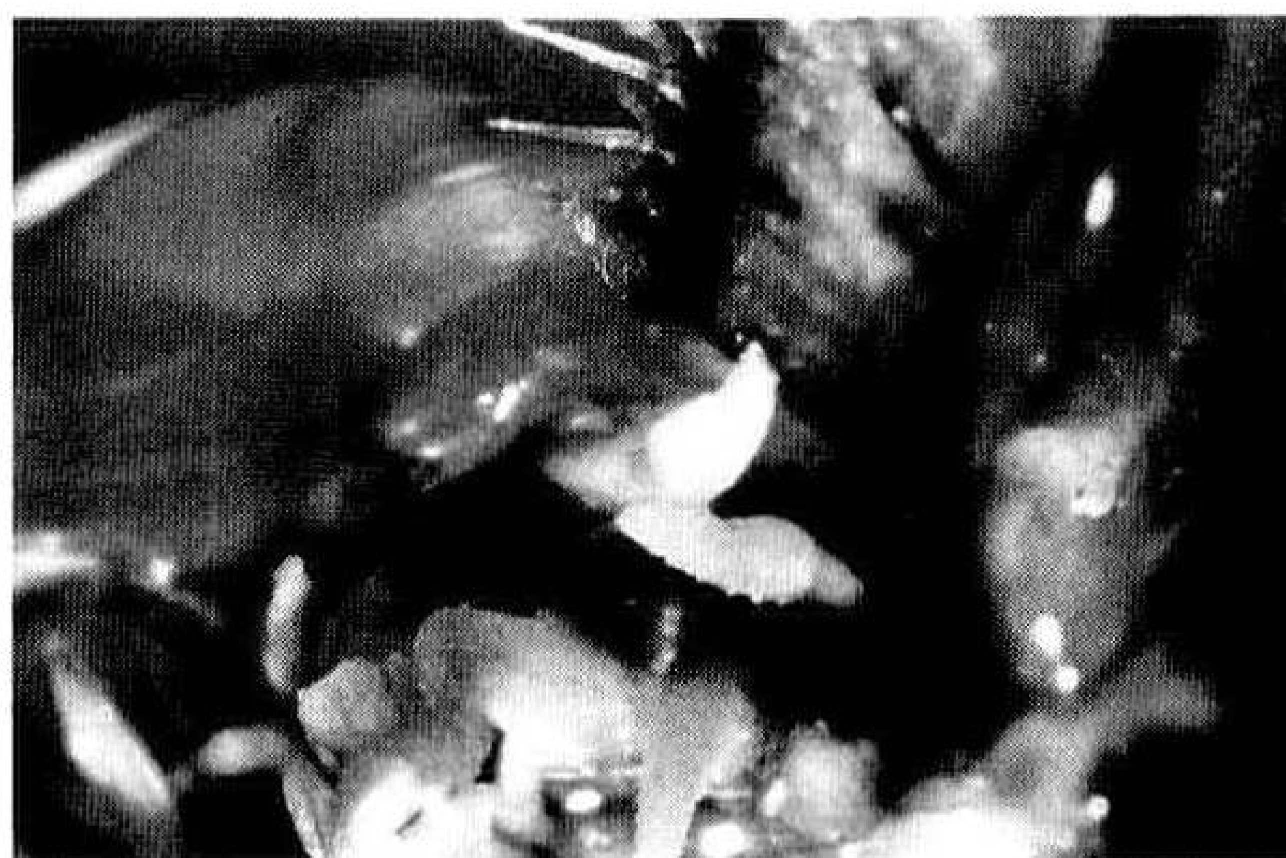

Figure 1. Formation of adventitious shoots from the petiole of leaf explant.

(Yamamoto, 1992) and *Vitis* (Cheng, 1989). The petiole can be considered to have a high regeneration potential because it has relatively young vascular tissue.

Table 1. Effect of BA on formation of adventitious shoots from leaf explant.

BA (mg liter^{-1})	Explants with shoots (%)	Plantlets per explant
0	29[X]	2.7
1	60	4.5
2	68	6.0
3	42	3.4
4	44	2.1
5	50	2.7

[X] Values were scored after 4 months of culture. Basal medium was ½ LS with 0.2% agar.

The adventitious shoots in the culture vessel grew into plantlets having three to four leaves. The plantlets were separated from the explants, and cultured on the medium for rhizome formation. Table 2 shows the effects of hormones on the rate of rhizome formation after 17 days of culture. Each value is expressed as a percentage of the plantlets with rhizome. In the medium without NAA, the rate of rhizome formation decreased with the increase in BA concentrations in the shoot formation medium. This indicates the preventative effect of BA on rhizome formation. However, the effect of BA was suppressed by the addition of 0.1 mg liter^{-1} NAA to the medium (Table 2).

Table 2. Effect of hormones on formation of rhizome from the shoots.

Shoot formation medium (BA mg liter^{-1})	Support	Rhizome formation media 0	0.1(NAA mg liter^{-1})
0	G[X]	73[Y]	73
1	G	45	64
2	G	27	64
2	A	18	64
3	G	36	73

[X] G= Gelrite, A = Agar
[Y] Values (%) were scored after 17 days of culture in the rhizome formation media.

The differentiation and subsequent growth of the rhizome was better in the medium solidified with Gelrite than in that solidified with agar. When the rhizome developed fully, adventitious shoots were formed in a row on the rhizome in the medium as shown in Figure 2. More than 10 shoots could be excised from one rhizome, and these were subcultured for further proliferation. We can consider that

Figure 2. Formation of adventitious shoots on rhizome in the medium with 0.1 mg liter^{-1} NAA and 0.2% Gelrite.

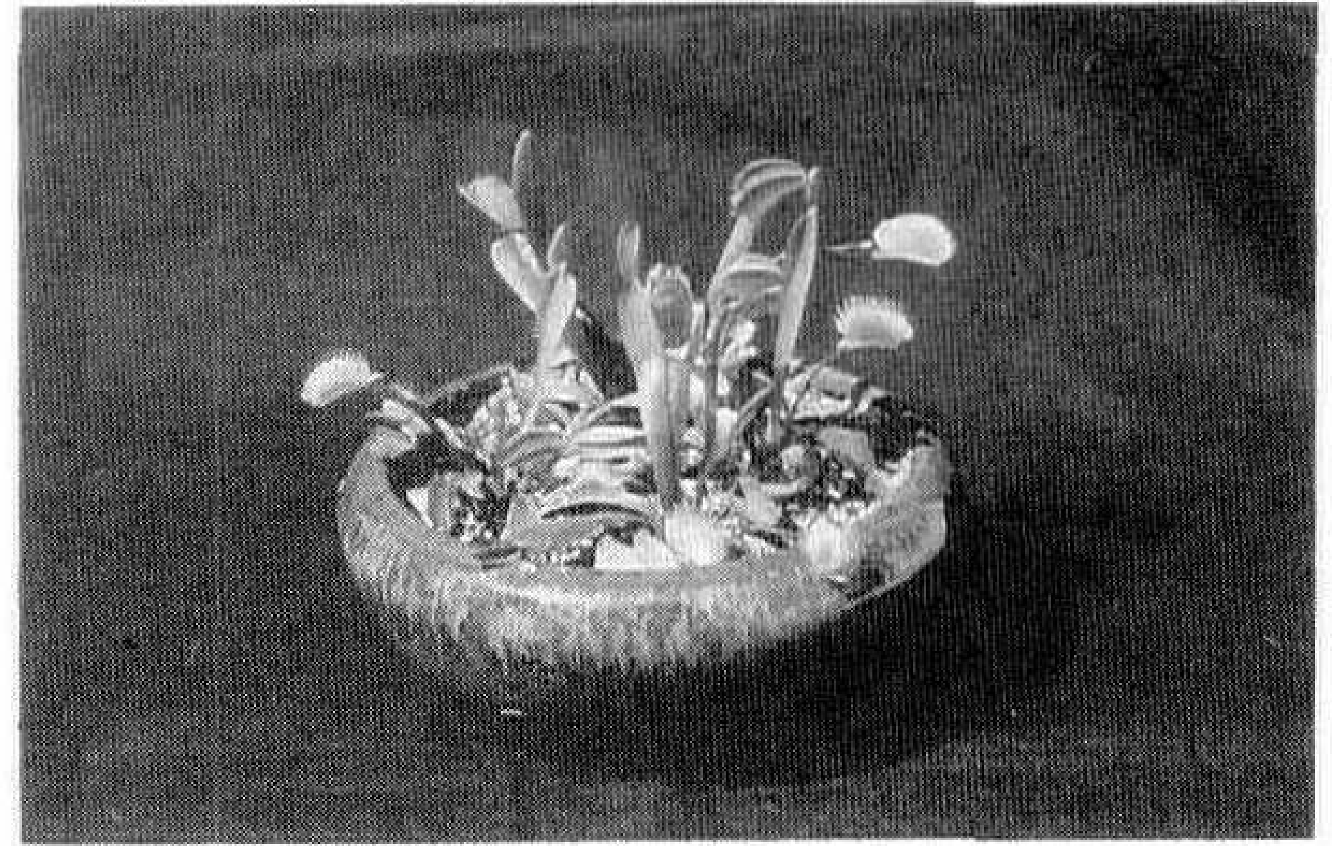

Figure 3. The growth of regenerated Venus fly trap after acclimatization.

there is a two-step process in the propagation of Venus fly-trap in the present culture system. At first, the plantlets can be obtained through the formation of adventitious shoots from the petiole of explants. Next, adventitious shoots are formed from a fully developed rhizome of the plantlet. The former is promoted by use of the half-strength LS medium supplemented with 2 mg liter^{-1} BA, and the latter by use of the medium solidified with Gelrite and supplemented with 0.1 mg liter^{-1} NAA. Either media, vermiculite, or peat moss, are suitable for acclimatization. Figure 3 shows an example of the growth of a regenerated plant. At Shiba orchid nursery, a large number of Venus fly-traps are being produced by this method of micropropagation.

LITERATURE CITED

Cheng, Z-M. and **B.l. Reisch.** 1989. Shoot regeneration from petioles and leaves of *Vitis ×laburuseana* 'Catawba'. Plant CelI Rep. 8:403-406.

Ayensu, E.S. 1981. Assessment of threatened plant species in the United States. pp. ???? In: H. Synge, ed., The biological aspect of rare plant conservation. John Wiley and Son Ltd.

Hutchinson, J.F. 1984. In vitro propagation of *Dionaea muscipula* Ellis (Venus fly-trap). Scientia Hort. 22:189-194.

Kukulczanka, K., B.Czastka, and **A. Arczewska.** 1989. Regeneration from leaves of *Dionaea muscipula* Ellis cultured in vitro. Acta Hort. 251:155-160.

Minocha, S. C. 1985. In vitro propagation of *Dionaea muscipula*. HortScience 20:216-217.

Parliman, B.J., P.T. Evans, and **E. A. Rupert.** 1982. Tissue culture of single rhizome explants of *Dionaea muscipula* Ellis ex L., the Venus fly-trap, for rapid asexual propagation. J. Amer. Soc. Hort. Sci. 107:305-310.

Parliman, B. J., P. T. Evans, and **A. R. Mazur.** 1982. Adventitious bud differentiation and development in leaf cuttings of *Dionaea muscipula* Ellis ex. L. (Venus Fly-Trap) cultured in vitro. J. Amer. Soc. Hort. Sci. 107:310-316.

Yamamoto, T. and **H. Oda.** 1992. Various methods for seedling production of *Smilax oldhami* Miq. by tissue culture. Acta Hort. 319:143-148.