Micropropagation of *Uncaria rhynchophylla* – a Medicinal Woody Plant©

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INTRODUCTION

Uncaria rhynchophylla (kagikazura or the cat's claw herb) is a plant species used in traditional Chinese medicine and also kampo (Japanese study and adaptation of traditional Chinese medicine), and is a woody plant found widely in Japan and China. It contains alkaloids rhynchophylline (Fig. 1), iso-rhynchophylline, hirstine, and others (Shi et al., 2003) which are good for treating high blood pressure and dementia. In addition (+)-Catechin and (-)-epicatechin are also found in the plant (Hou et al., 2005). It is in four of the 148 Kampo medicine formulae. Kampo herbal medicines are regulated as pharmaceutical preparations and their ingredients are exactly measured and standardized. Access to Kampo herbal medicines is guaranteed as part of Japan's national health plan for each of its citizens. For the purpose of micropropagation and development of a basis for useful substance production by breeding and cell culture, a tissue culture procedure was developed for this species.

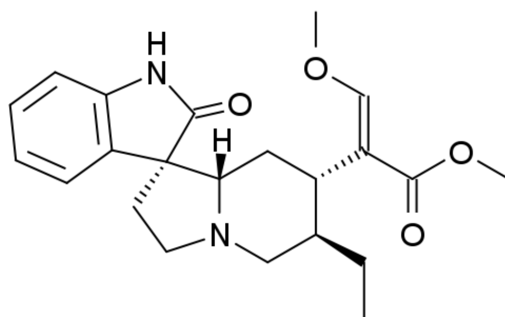

Fig. 1. Rhynchophylline.

MATERIALS AND METHODS

Branch stem segments containing hooks (formed from reduced branches) from wild *U. rhynchophylla* were collected from the forest in Kochi prefecture, Shikoku island, Japan (Fig. 2B). Surface sterilization of stem segments was done using 70% ethyl alcohol for 1 min, 5% hydrogen peroxide for 10 min, then washed well twice with sterile water for eliminating surface microorganism. For initial culture, MS (Murashige and Skoog, 1962), ½ DCR (Gupta and Durzan, 1985), SH (Schenk and Hildebrandt, 1972), and ½ LP (Quiolin and Lepoivre, 1977) media plus different hormonal combinations of 6-benzylaminopurine (BAP), kinetin, Zeatin, and NAA were compared. For subculture and rooting of the shoots, ½ LP and ½ MS medium containing 1 µM IBA were used. For habituation a small scale controlled environment plant culture system [Terrace® System (MKB Dream Co., Japan)] was used. Culture condition was maintained at the constant temperature of 25°C under 16 h photoperiod of 70 µM·m^{-2} s^{-1} provided by cold-cathode fluorescent lamp. Propagated plantlets were first cultured in the greenhouse then planted out to the field.

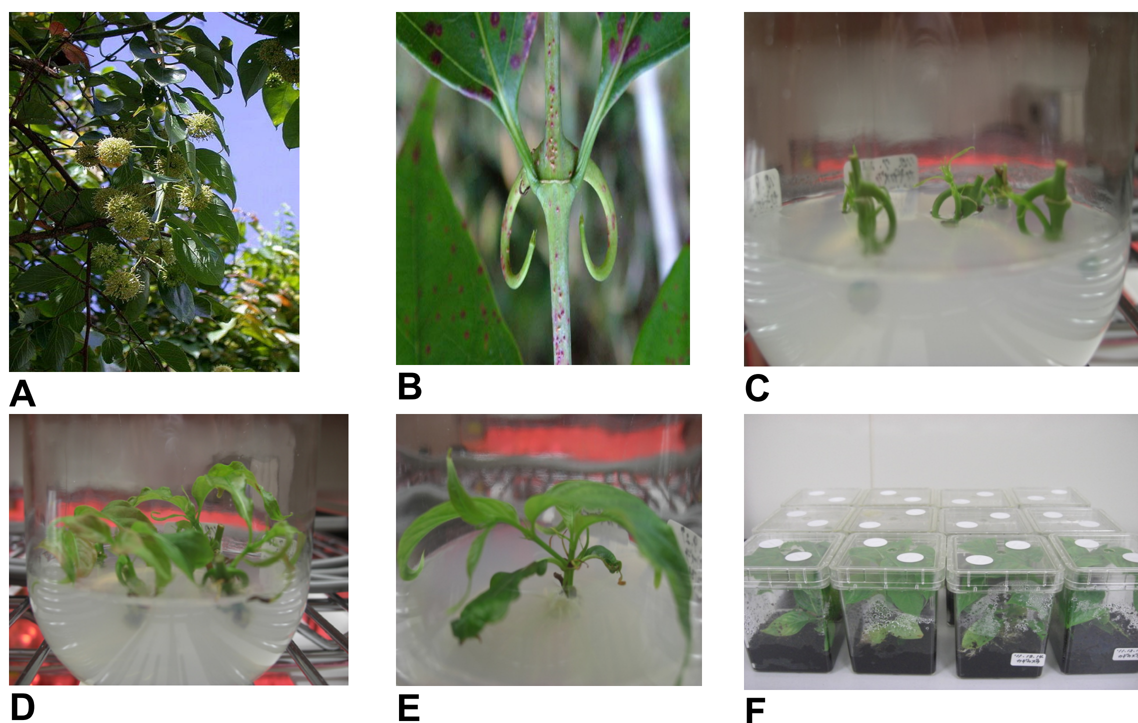

Fig. 2. Process of in vitro propagation of *Uncaria rhynchophylla*. (A) *Uncaria rhynchopylla*, (B) stem spine, (C) shoot induction from spine, (D) shoot elongation, (E) Rooting, (F) Regenerated in vitro plantlets in plant boxes.

RESULTS AND DISCUSSION

Shoots were induced from stem hooks of kagikazura in the ½ MS medium containing BAP or zeatin (Fig. 2C). A ½ MS medium containing 4 µM BAP was the best for shoot induction (Table 1). Callus induced around the stem segments were continuously subcultured in fresh ½ LP medium containing 0.5 µM BAP and 1 µM 2,4-D. These cell lines can be used for the possible secondary metabolite production and for chemical constituents breeding by somaclonal variation or molecular genetics technology. A higher concentration of BAP was better for induction of buds from subcultured stem segments (Table 2). Regenerated plants were obtained by rooting of these shoots on ½ MS medium containing IBA (Fig. 2E, Table 3). A low concentration of IBA was better for rooting (Table 3). Rooted plantlets were cultured in Giffy 7® with 60 ml of 0.1% Hyponex® medium in plant boxes (65×65×100 mm) (Fig. 2F). Each plant box contained one regenerated plantlet. Culture condition were at 25°C constant temperature under a 16-h photoperiod of 50 $\mu M \cdot m^{-2} \cdot s^{-1}$ by cold cathode fluorescent lamps. Then after 2 months, they were habituated in a small scale controlled environment plant culture system [Terrace® System (MKB Dream Co., Japan)] which provides 100% humidity and automatic watering for 1 month (Fig. 3), and then grown in a greenhouse for 6 months. Field planting was successful (Fig. 4). Selection of clones with higher chemical content is planned.

Improving the propagation rate and selection of tree clones with higher contents of rhynchophylline is planned in the future.

Table 1. Shoot induction from stem segments of kagikazura.

Shoot medium	Plant growth regulator (μM)	Shoot induction (%)	Number
½ MS	Zeatin (4)	73 (11/15)	1.2 ± 0.1
½ MS	BAP (4)	85 (11/13)	1.4 ± 0.1
½ MS	Kinetin (4)	10 (1/10)	1
½ MS	Zeatin (4), NAA (0.5)	27 (3/11)	1.3 ± 0.3
½ MS	BAP (4), NAA (0.5)	60 (6/10)	1.2 ± 0.2
½ MS	Kinetin (4), NAA (0.5)	0 (0/10)	0

N=4~16.
Abbreviation: BAP = 6-benzylaminopuring.

Fig. 3. Habituation.

Fig. 4. Field grown *Uncaria rhynchophylla*.

Table 2. Effects of 6-benzylaminopurine (BAP) concentration on bud differentiation from segments of kagikazura (½ LP medium).

BAP (μM)	Average no. of induced buds ± SD
0	0 ± 0
1	0.6 ± 0.25
10	1.6 ± 0.35
50	2.4 ± 0.25

N=10.

Table 3. Effects of indole-3-butyric acid (IBA) on rooting of kagikazura (½ Murashige and Skoog medium).

IBA (μM)	Rooting percentage ±SD
0.1	93 ± 5
0.5	80 ± 9
2.5	0 ± 0
12.5	0 ± 0

N=15.

ACKNOWLEDGMENTS

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