Propagation In Vitro of *Nothapodytes amamianus* an Endangered Medicinal Tree©

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INTRODUCTION

Wadatsuminoki (*Nothapodytes amamianus* Nagam. & Mak. Kato, Icacinaceae) is an endangered species only naturally found as a new species in 2004 in the southern part of Amamioshima Island located in the south of Japan (Nagamasu and Kato, 2004). It produces a useful alkaloid, camptothecin (Fig. 1), which is a raw material of cancer drug irinotecan. Its related species, *N. foetida*, is currently cultivated for drug raw material production. For application of wadatsuminoki in the commercial usage and species conservation, propagation from limited number of trees is crucial. There are several reports about conservation of endangered species using in vitro culture (Sugii and Lamoureux, 2000; Ishii et al., 2004; and Ishii et al., 2005). So, screening of in vitro culture conditions of this species was carried out for the first time. There is a report about tissue culture of a closely related species *N. foetida* (Sundravelan et al., 2003), however its production of useful chemicals was low. Although several papers have been reported on in vitro culture of camptothecin (Fig. 1) producing plants such as *Camptotheca acuminate* (Jain and Nessler, 1996) or *Ophiorrhiza pumila* (Sudo et al., 2001), this is the first report for in vitro culture of *N. amamianus*.

MATERIALS AND METHODS

Branches from 2-year-old seedlings of *Nothapodytes amamianus* which seeds were collected from the natural mother tree. Surface sterilization of shoot and stem segments was done using 70% ethyl alcohol for 1 min, 0.1% mercury chloride for 10 min, and 5% hydrogen peroxide for 10 min, then washed well twice with sterile water for eliminating surface microorganisms. 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 (different in the combination of hormones such as 10 μM BAP or zeatin, and 0.027 μM NAA) were compared. For subculture and rooting of the shoots, ½ LP, CD, and ½ MS media containing 1 μM IBA were used. For habituation, nursery trays were used. Culture condition was maintained at the constant temperature of 25 °C under 16 h photoperiod of 70 $\mu M \cdot m^{-2} \cdot s^{-1}$ by fluorescent lamp. Propagated plantlets were first cultured in the greenhouse then planted out to the field.

RESULTS AND DISCUSSION

In the initial culture, shoots were rooted in the ½ DCR medium containing 3 $g \cdot L^{-1}$ activated charcoal after 2 months (Fig. 2). Shoots were induced from three subcultured root segments out of 15 in the ½ MS medium containing 2 μM BAP (Fig. 3) and further root initiation occurred. Those plantlets grew well in the ½ LP medium containing 5 $g \cdot L^{-1}$ activated charcoal (Fig. 4). Axillary buds were also induced from

the dissected segments (2 cm length) of in vitro cultured shoots of *N. amamianus* in the ½ LP medium containing 10 μM BAP. Regenerated shoots were rooted in the ½ MS medium containing l μM IBA.

Rooted plantlets were further grown in the ½ LP medium containing 5 $g \cdot L^{-1}$ activated charcoal then habituated successfully in the nursery trays at 100% humidity in the covered tray (Fig. 5). Propagated plantlets were grown in the greenhouse for 3 months (Fig. 6) then planted out in the field. From one shoot stem explants, about 50 plantlets were obtained by in vitro culture of *N. amamianus* after 6 months. Improving the propagation rate and selection of trees with higher contents of camptothecin is necessary in the future.

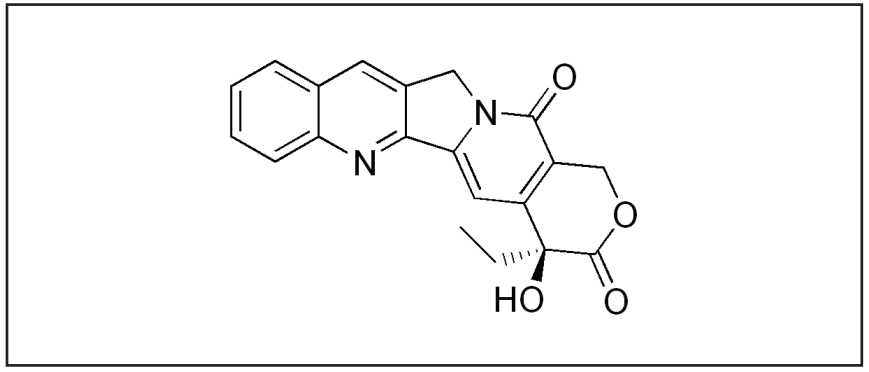

Figure 1. Camptothecin structure.

Figure 2. Rooting of shoot and growth of *Nothapodytes amamianus*.

Figure 3. Shoot induction from root segments of *Nothapodytes amamianus*.

Figure 4. Regenerated plantlet of *Nothapodytes amamianus*.

Figure 5. Habituation.

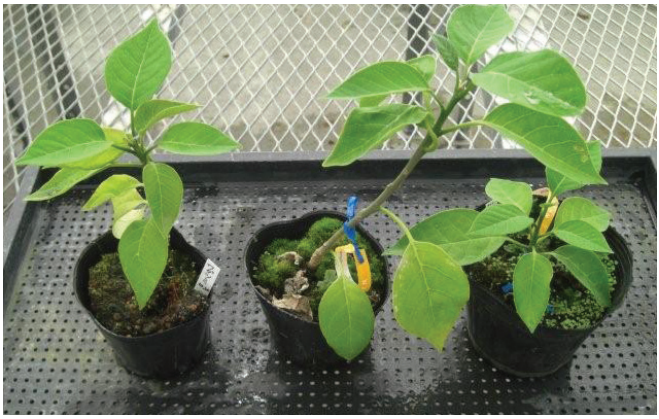

Figure 6. Habituated plantlets of *Nothapodytes amamianus*.

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