RESEARCH NOTE
Bot. Bull. Academia Sinica **13**: 118-120 (1972)

INDUCTION OF CALLUS FROM STEM SECTION OF *CRYPTOMERIA JAPONICA*[1][2]

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Dedifferentation and redifferentiation of woody plants on chemically defined medium have been generally difficult. Only cultures of callus from *Pinus* (Bornes and Naylor, 1958; Harvey, 1967; Brown, 1968; Bonga and Fowler, 1970) and plantlet from Aspen callus (Winton, 1968) have been obtained. During the past year, we were working to induce callus from the shoot of *Cryptomeria japonica*. Successes in dedifferntiation and redifferentiation will make asexual propagation and breeding of this woody plant possible.

The defined synthetic agar media developed by Murashige and Skoog (MS) (1962) and White (1943) with the addition of coconut milk (15%) and 2, 4-Dichlorophenoxyacetic acid were used for callus initiation. The concentrations of 2.4-D tested in this experiment were 0, 1, 2, 4, 6, 8 and 16 ppm. Medium was solidified with 1% agar and autoclaved for 5 minutes at 1.3 kg/cm^2, pH value was adjusted to 5.5.

Stem sections of 1-2 cm long taken from a well growing shoot of two years old seedling were used for callus induction. After leaves were shorten, sections were first rinsed with detergent in the ultrasonic cleaner for 10 minutes, then under running tap water for 15 minutes, followed by sterilization in 3% sodium hypochlorite solution for 3 minutes. They were washed in sterile water and placed in tubes each containing 8-10 ml. solid medium and covered with aluminium foil. The tubes were incubated at room temperature (25°C) under dark condition. When callus began to proliferate from the stem pieces, they were fixed in formalin-alcohol-acetic acid, dehdrated in normal butyl alcohol series, infiltrated with paraffin. Sections were cut into 8μ thickness and stained with Ehrlich's acid hematoxylin. They were then mounted in canada balsam for microscopic observation.

(1) Research supported by Taiwan Forestry Bureau.
(2) Paper No. 126 of the Scientific Journal Series, Institute of Botany, Academia Sinica.
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It was found that MS medium induced callus better than the White's medium. This may be accounted for by the difference in nitrogen source in these two media, ie., MS medium contains both NH_4^+ (20.6 m equivalents/1) and NO_3^- ions (39.4 m equivalents/1) while White's medium contains only NO_3^- ions (3.2 m equivalents/1).

High level of 2, 4-D (6 ppm) in MS medium appears to promote callus initiation better than other concentrations. The rates of callus initiation, size, color, and smoothness are all different at various 2, 4-D contents. On MS medium containing 6 ppm of 2, 4-D, callus induction was quicker. Induced callus has smoother surface and lighter yellow color.

Microscopic observation showed that the callus was originated in the cortex layer of the stem (Fig. 2 and 4). These cells then broke through the epidermis and grew on the surface of the stem (Fig. 6).

Further study of redifferentiation into embryoide from the callus is still under investigation.

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柳杉 (*Cryptomeria japonica*) 癒合組織的誘導

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利用柳杉的幼嫩枝條在含有 2,4-D (6 ppm) 及椰子胚乳 (15%) 的 Murashige and Skoog 氏培養基下培養，在黑暗狀態下經三星期的培養可使枝條誘導出癒合組織。經製成石臘切片在顯微鏡下觀察發現癒合組織的形成始原於莖部皮層之薄壁細胞羣。

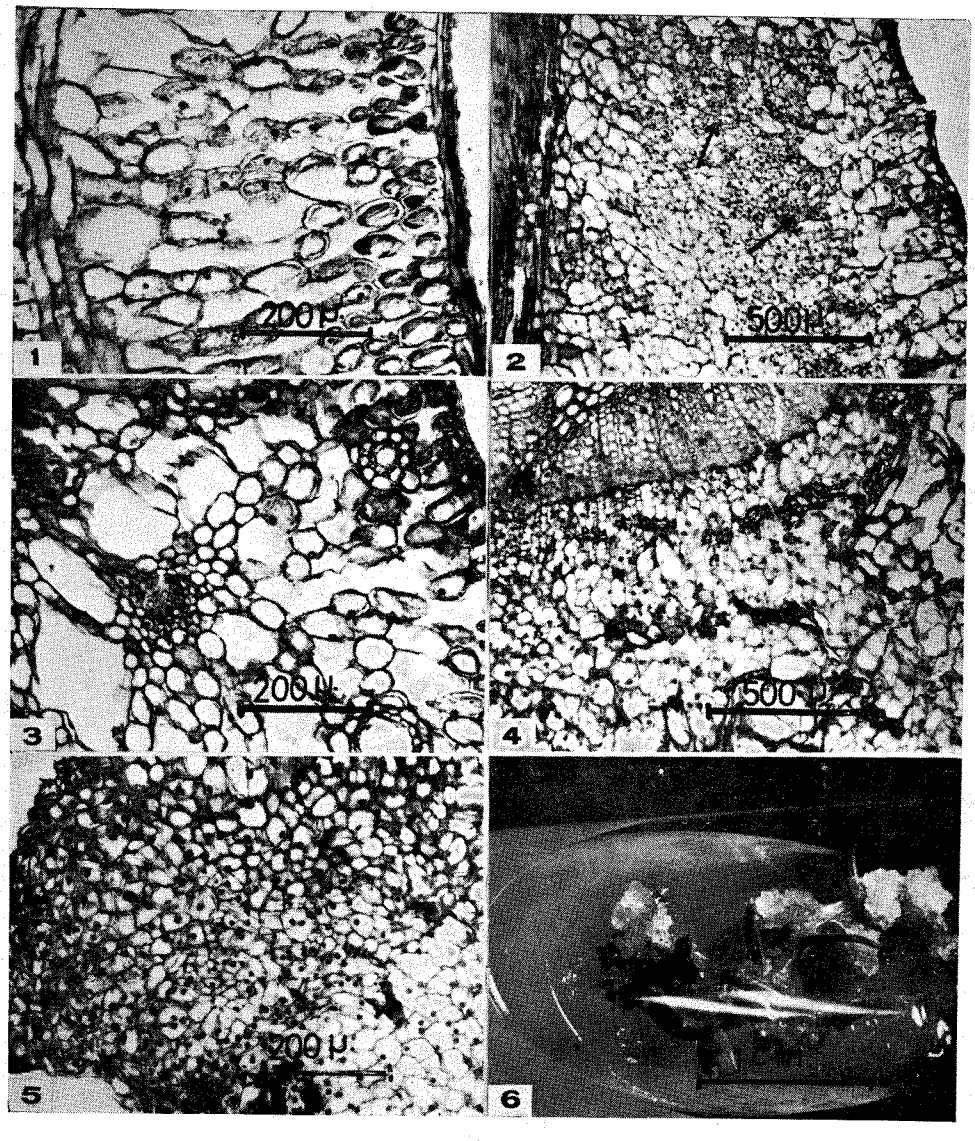

Fig. 1-6

1. Longitudinal section of untreated stem of ***Cryptomeria japonica.***
2. Longitudinal section of ***Cryptomeria japonica*** stem after 3 weeks culture on MS medium containing 2,4-D (6 ppm). Heavily stained cells (arrow) were found in the parenchymatous cells of cortex, they are the origin of callus.
3. Cross section of untreated stem of ***Cryptomeria japonica.***
4. Cross section of treated stem of ***Cryptomeria japonica*** showing heavily stained cells in cortex.
5. The section of callus tissue.
6. Well growing callus of ***Cryptomeria japonica*** in test tube.