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研究簡報

日本當歸(*Angelica acutiloba* K.)莖培植體之植株再生

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摘 要

日本當歸莖段培植體，在 0.6 mg/L 2,4-D (2,4-dichlorophenoxy acetic acid)、0.05 g/Ll 抗壞血酸(ascorbic acid)和 1.05 g/L 檸檬酸(citric acid)的 MS(Murashige and Skoog)培養基中，2 週內可誘導出黃白色、緊密狀的癒合組織。癒合組織在原培養基中繼代培養，每月可增加 2 到 4 倍鮮重。將黑暗中生長的癒合組織移到光環境下，會生成綠色、結節狀的癒合組織並進一步自發性的分化形成多芽體。移植多芽體培植體到含 0.02 mg/L NAA(naphthyleneacetic acid)和 0.1 mg/L kinetin 的 MS 根誘導培養基時，可在 3 週內在莖的基部長出不定根而成爲完整的植株。這些在試管內再生成功的完整植株，可以移植到盆土中作溫室栽培。

【關鍵詞】莖培植體、癒傷組織、藥用植物、日本當歸、植株再生

In Vitro Plant Let Regeneration of the Medicinal *Angelica Acutiloba* K. Via Stem Explants

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Abstract

Callogensis, shoot proliferation and plant let regeneration have been achieved for *Angelica acutiloba* K. through stem explants. Various explants excised from leaf, stem and root were collected from established aseptic plants. The stem explants of *Angelica acutiloba* K. induced compact and yellow–white calli from the proximal ends on Murashige and Skoog (MS) salts supplemented with 0.6 mg/L 2, 4-D, 0.05g/L ascorbic acid and 1.05 g/L citric acid within two weeks in darkness. Calli were maintained by subculturing on the same medium for callus induction and proliferated 2–4 folds (fresh weight) in 1 month. Upon lighting condition, green compact calli were produced and turned into nodular appearance which subsequently differentiated into multiple shoots vigorously, typical of light-dependent development. Shoots with well-developed leaves were planted individually on a root-induction MS medium containing 0.02 mg/L naphthyleneacetic acid and 0.1 mg/L kinetin. Adventitious roots protruded from the basal parts of the stems within 3 weeks after transferring to the rooting medium. The *in vitro* regenerated plantlets

were successfully transferred to soil.

Keywords: stem explants, callus, medicinal plant, *Angelica acutiloba* K., plant regeneration

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INTRODUCTION

There are more than 90 species of *Angelica* in planta, meaning "healing power" from the Latin. *Angelica* plants are generally perennial of 30–100 cm, height with purplish stems and strongly aromatic. The roots of *Angelica* plants (Chinese name Duggi) is well-known crude drug used in Chinese traditional medicine as an analgesic, emmenagogue, and tonic. The Chinese pharmacopoeia recorded that Danggui is derived from root of *Angelica sinensis*. However, *Angelica acutiloba* K. which are mainly found in Japan, is commonly used as the substitutes of Danggui in the market of southeast Asia. In Japanese herbal medicines, the roots of *Angelica acutiloba* K. is a well-known component herb used in the treatment of gynaecological diseases and arthritis. Several natural chemicals have been identified from the root of *Angelica acutiloba* K. These included polysaccharides, ligustide and choline, etc. These compounds have been reported to have mitogenic, anti-cholinergic and analgesic activities (Kumazawa et al., 1982; Tsuchida et al., 1987), respectively. It is recorded that 70 formulae in China and 56 formulae in Japan contain Danggui (Huang and Song, 2001). Besides the common usage in Asia, Danggui is also used as a health food product for women's care in Europe and America. Therefore, the demand for Danggui is enormous throughout the world.

In recent years, various explants have been used in many laboratories, producing many regenerated *in vitro* plantlets throughout the world (Chen and Chang, 2006; Ernst, 1994; Morel, 1960; Teng et al., 1997). However, there is no information about stem-derived callus and its regeneration of *Angelica acutiloba* K.

Due to the high economic value of this plant, especially in Taiwan which imports large amounts annually, *in vitro* culture was undertaken to develop a more expeditious method for multiplication and also for special flavor or compound production. This report deals with callus inducing, proliferation and subsequent plant let regeneration through stem explants of *Angelica acutiloba* K. in basal Murashige and Skoog medium (Murashige and Skoog, 1962) with different growth regulators. These stem-derived calli differentiated into plantlets, which can be successfully grown in soil.

MATERIALS AND METHODS

Plants of *Angelica acutiloba* K. were kindly given by Taiwan Agricultural Research Institute and grown in a greeenhouse under natural conditions. The leaves, stems and roots were dissected from the healthy plants(ca. 30 cm height), washed in running water thoroughly, surface sterilized with 2.5％ sodium hypochlorite for 7 minutes. Then rinsed twice in sterile distilled water. These explants(leaf, stem and root)were cut into pieces(ca. 0.5 cm in length)and cultured on callus inducing Murashige and Skoog (MS) medium containing 0.6 mg/L 2, 4-D, 0.05g/L ascorbic acid and 1.05 g/L citric acid and 30 g/L sucrose. The medium pH was adjusted to 6.0 prior to addition of 2 g/L gelrite and sterilized by autoclaving at 121℃ for 15 min. These cultures were wrapped with aluminum foil to insure complete darkness and maintained in such medium incubated at 25 ± 1 ℃ in dark. Clusters of compact calli were placed on the shoot induction medium (SIM) containing the same ingredients as callusing medium except for light condition.

After 2 weeks on SIM medium, multiple shoots obtained from shoot induction medium (1.5–2.0 cm in length) were excised and placed on the root induction medium (RIM) consisting of Murashige and Skoog basal salts supplemented with 0.02 mg/L NAA and 0.1 mg/L kinetin. The cultures were maintained at 26 ± 2 ℃ on a 16 h photoperiod (30 μmol $m^{-2} \cdot s^{-1}$) in the culture room. Five weeks after the initiation of rooting, the deflasleed-plantlets were transplanted to the soilless pots, covered with a transparent lid and placed in a culture room. After 1 week, the transparent lids were removed and transferred to a greenhouse.

RESULTS AND DISCUSSION

Among leaves, stems and roots dissected from the healthy plants of *Angelica acutiloba* K., only stem segments placed on callus inducing medium (CIM) expanded in size and showed yellow coloration. After 12 days in culture of MS medium supplemented with 0.6 mg/L 2, 4-D, 0.05g/L ascorbic acid and 1.05 g/L citric acid, off-white to pale yellow callus (Fig. 1)were induced from stem explants of *Angelica acutiloba* K. These stem derived calli were maintained by subculturing on the same medium for callus induction and proliferated 2–4 folds in (fresh weight) within 1 month. However prolonged the cultural dutation (more than 1 month) of the callus under the same medium resulted in numerous etiolated shoot clusters formation even in darkness (Fig. 2). When these etiolated multiple shoots were transferred to a growth chamber with a 16 h night photoperiod, they turned green within 2 days. The stem explants derived calli also turned green and nodular appearance when they were transferred to light condition and clusters of shoots started to become visible around 19–21 days after plating (Fig. 3).

Fig. 1 Off-white to pale yellow callus induced from the stem explants of *Angelica acutiloba* K. in darkness.

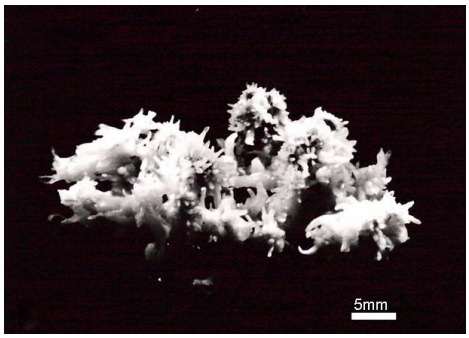

Fig. 2 Prolonged culture of the stem derived calli in the callusing medium resulted in etiolated shoot clusters.

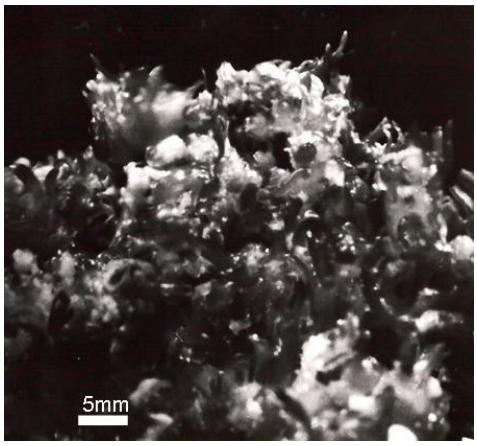

Fig. 3 Calli turned green and nodular appearance in growth chamber of 16 hr photoperiod condition.

With no root protruding, the multiple shoot clusters grew well in the same medium under illumination or in darkness (Fig. 4). However, transfer of these stem derived calli to either MS medium plus 0.02 mg/L NAA and 0.1 mg/L kinetin or growth regulator-free medium gave rise to root protruding(Fig. 5) and subsequently regenerated plantlets that were successfully transferred to soil (Fig. 6). The rootless microshoots in the callus inducing medium also formed roots in this growth regulator supplemented medium. The regenerated plants grew in a greenhouse are similar to the parent plants in morphology (Fig. 6).

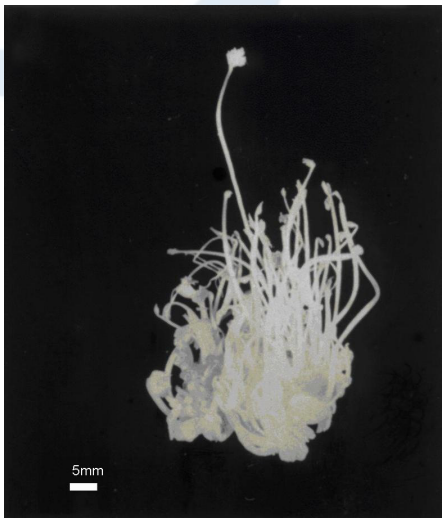

Fig. 4 *In vitro* etiolated shoot cluster, without root formation, cultured in darkness.

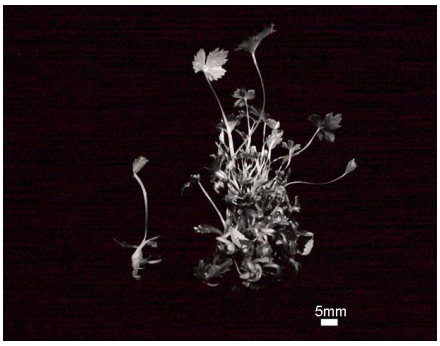

Fig. 5 Profuse shoot cluster and single shoot derived from multiple shoots formation under photoperiod condition. No root protruding.

Fig. 6 *In vitro* regenerated plantlets of *Angelica acutiloba* K., 1 (left) and 2 (right) months after transferring to rooting medium. Plantlets with healthy roots protruding from the stem bases, were successfully transferred to soil.

We demonstrated here that 2, 4-D in MS medium containing ascorbic acid and citric acid gave the results of callus induction on excised stem explants of *Angelica acutiloba* K. The developmental stage of the explant, plant growth regulators, explant type, growth medium and light regime were known to influence regeneration. The plant growth regulators used in this study for *in vitro* regeneration of *Angelica acutiloba* K. are similar to those used for most of the Umbelliferae species (Ammirato, 1983; Huang et al.,1996 and 1997). That is, growth initiation on a medium with an addition of auxin followed by subsequently plant regeneration when the induced calli are transferred to a free of growth regulators. Our results also clearly showed that a low concentration of NAA (0.02 mg/L) and kinetin (0.1 mg/L) in combination would stimulate rooting of the shoots (Fig. 6). Our results also followed the suggestion that embryo initiation probably occurs during the primary culture and that the presence of auxin in the medium prevented their maturation (Ammirato, 1983; Halperin, 1966; Halperin and Jensen, 1967).

Miura et al. (1988) have used floral buds of *Angelica acutiloba* K. as explants to induce somatic embryoids by cell suspension culture with different basal medium and hormone concentrations. In comparison, our method for *in vitro* plant regeneration of *Angelica acutiloba* K. is by steam explants and its induced callus culture. Besides, this callus could be subcultured and maintained on MS medium containing charcoal (1 g/L) without loss their totipotency for regeneration.

As mentioned earlier in this report, aerial parts of *Angelica acutiloba* K. did not protrude their roots in the callus inducing medium (Fig. 4, 5). Nevertheless, these aerial parts of the plants grew vigorously in either light regime or darkness in the glass vessel. The step-wise growth phenomena could be used in investigation of the role of aerial parts and light on the pharmacolongically active constituents in the roots of *Angelica acutiloba* K., namely ligustilide and choline.

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