Notes Plant Tissue Culture Letters, **12**(1), 91-93(1995)

Callus Formation and Plant Regeneration from Petiole-Derived Calli of *Angelica sinensis*

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Plant regeneration from callus has been studied in many plants[1-4]. Callus induction has been reported from various explants, such as leaf[1], shoot[2] or embryo[3]. Callus from the root, leaf, petiole, cotyledon and hypercotyl of *Angelica sinensis* (Oliv). Diels, and regenerated plantlets by somatic embryogenesis and adventitious bud from calli were reported by Zhang and Cheng[4]. Since it was reported that 2, 4-D induced chromosome deviation[5], in this paper we tried to induce and culture callus of *A. sinensis* with NAA instead of 2, 4-D.

Leaf petioles of *A. sinensis* (0.5-1.0 cm in length) were cultured on Murashige and Skoog (MS) medium[6] containing 2% sucrose and 0.2% gellan gum (as gelling agent) for callus induction and plant regeneration. The pH was adjusted to 5.90 ± 0.05 in the experiment. The cultures were maintained at 25°C in 16 hr illuminatin per day at about 1500 lux light intensity.

For callus induction leaf petiole segments were surface-sterilized with 75% ethanol for 2-3 min. and 2% sodium hypochlorite solution for 20 min. and followed by three rinses with sterilized distilled water. Petiole segments about one cm in length were cultured on MS basal medium containing 80 μM naphthaleneacetic acid (NAA).

The calli of *A. sinensis* were induced on the cut-end of leaf petioles on the solid MS medium containing 65 μM NAA in the dark after one month (**Fig. 1**). The calli were yellow-white and soft. When the calli were transferred to the medium containing 80 μM NAA cultured under 1500 lux illumination of 16 hr photoperiod, green-white calli (embryoids) were obtained (**Fig. 2**). Calli were precultured with the medium containing 80 μM NAA and 10 μM kinetin, root proliferation from the calli were observed within 6-8 weeks of culture (**Fig. 3**). Green spots and adventitious roots appeared when calli were subcultured on the medium containing 80 μM NAA and 20 μM kinetin with illumination (**Fig. 4**). The green spots differentiated into shoots after further incubation. Root initiation was observed after transferring the shoots to the solid MS medium containing 0.4 μM NAA and 0.5 μM kinetin (**Fig. 5**). Regenerated plants were transplanted in pots with sandy-clay with 10% (v/v) vermiculite and cultivated in a glass chamber in greenhouse for 2-3 weeks (**Fig. 6**). Cultivated whole plants thus obtained and acclimated at 25°C in the greenhouse showed the same morphology and flavor as its mother plants although chromosome and other genetic characteristic were not studied.

The combination of NAA and kinetin as used in the media in our experiments was enough for inducing the calli from leaf petioles and initiating bud primordia and adventitious roots from the calli. Ishimaru[7] also reported that NAA and IAA slightly stimulated the formation of the callus on the petiole segments of *Rheum palamatum* L.. Zhang and Chang[4] reported that callus induction frequency was as high as 87% on the medium with the combination of 2, 4-D (3.1 μM), NAA (26.8 μM) and 6-BA (2.2 μM). In our works we used NAA as auxin instead of 2, 4-D for inducing

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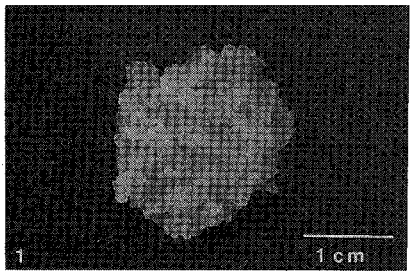

Fig. 1 Calli induced from petioles of *Angelica sinensis* cultured on MS medium containing 65 μM NAA in the dark.

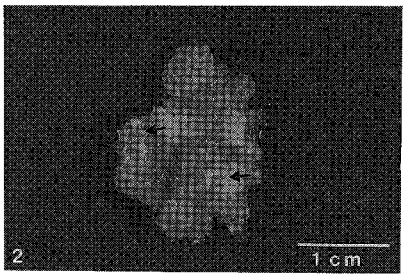

Fig. 2 Partionally green calli cultured on MS medium containing 80 μM NAA in 16 h/day light (1, 500 lux). Note embryoid (arrow).

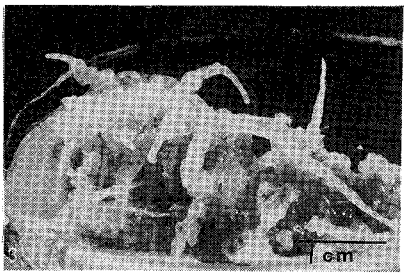

Fig. 3 Root proliferation from the calli cultured with 80 μM NAA and 10 μM kinetin.

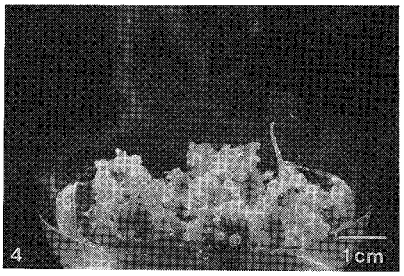

Fig. 4 Green spots and adventitious roots appeared on the calli cultured with 80 μM NAA and 20 μM kinetin under the illumination.

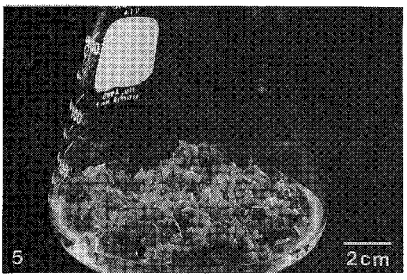

Fig. 5 Adventitious shoots and roots formed on the calli cultured on MS medium containing 0.4 μM NAA and 0.5 μM kinetin.

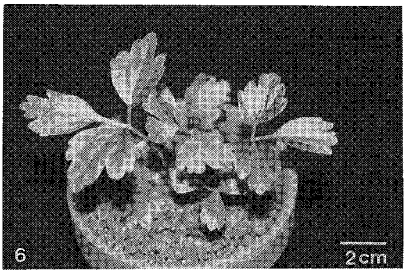

Fig. 6 Regenerated plants in pot cultivated in greenhouse.

callus. Callus induction frequency was about 80-90%. Further, calli were successfully subcultured on media only supplemented with 80 μM NAA. A one-cm-diameter callus in the NAA medium could grow to five-cm-diameter callus in one month. Embryogenic calli (embryoids) were also induced on MS medium supplemented with NAA and kinetin. Each embryoid continuously cultured in the subculture-medium further developed into a small plantlet. Without using 2, 4-D as phytohormone the morphologically abnormal plantlets from the embryoids from five-subcultured calli did not appeared in the culture medium. In our laboratory the media supplemented with NAA and kinetin were also used for inducing calli of *Levisticum officinale*[8] and *Coriandrum satibrum*[9], the plants of Umbeliferae. The media with NAA and kinetin, but not 2, 4-D, were used for inducing

callus and maintaining cell lines.

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