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# Callus Induction and Plant Regeneration of American Ginseng


Andrew S. Wang[1]

*Sungene Technologies Cop, 2050 Concourse Drive, San Jose, CA 95131*



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***Abstract.*** **Friable embryogenic callus of American ginseng *(Panax quinquefolium* L.) was induced from root pith on Murashige and Skoog medium supplemented with 2 mg 2,4-D and 1 mg KIN/liter. Optimal callus growth occurred on medium containing 1.5 mg dicamba/liter. Plants were regenerated on MS medium supplemented with various concentrations of plant growth regulators (PGRs); the best PGR combination was 0.5 mg IBA and 0.1 mg NAA/liter. Chemical names used: (2,4 -dichlorophenoxy) acetic acid (2,4-D); 3,6-dichloro-o-anisenic acid (dicamba); 6-benzylaminopurine (BA); gibberellic acid (GA); indole-3-butyric acid (IBA); kinetin (KIN); and naphthaleneacetic acid (NAA).**


The root of ginseng *(Panax* spp.), cultivated in northern China, Korea. the United States, Canada, and the Soviet Union, has been extensively used as a cure-all drug or tonic in the Orient (Choi, 1988). American ginseng, grown in North America, is *Panox quinquefolium* L. (Carlson, 1986; Proctor and Bailey, 1987). In 1987 and 1988, the United States exported 578 and 448 t, respectively, of American ginseng root (valued at nearly $100 million) to other countries, mainly in Asia (US Bureau of Census). The majority of exported ginseng is produced in Wisconsin (Wis. Dept. of Agr.). Wisconsin harvested 592 and 472 t of the ginseng root in 1987 and 1988, respectively.

Improvement of ginseng root production through conventional breeding is slow and difficult, since the plant is a perennial and requires 3 to 4 years to flower and 4 to 5 years to harvest. Tissue-culture technology might be able to accelerate the rate of improvement. For Korean ginseng *(Panax ginseng* C.A. Meyer), callus cultures have been successfully established (Butenko et al., 1968), plantlets have been regenerated from root-derived embryogenic callus (Chang and Hsing, 1980a; Choi, 1988; Furuya et al., 1986), and in vitro flowering of somatic embryos from root-derived callus has been reported (Chang and Hsing, 1980b). Callus cultures of Korean ginseng have been transformed with *Agrobacterium rhizogenes* to produce many hairy roots that synthesize the same saponins and ginsenoides as did untransformed roots (Yoshikawa and Furuya, 1987).

Although callus cultures have been established using leaves, roots, and stems of American ginseng (Butenko et al., 1968; Jhang et al., 1974), plant regeneration from these cultures has not been reported. This paper reports studies on embryogenic callus induction, callus growth, and plant regeneration of American ginseng.

A 4-year-old fresh field-grown ginseng mot was washed in 70% alcohol for 40 see, then blotted and dried with paper towels. The cleaned ginseng root was then sterilized in 2.5% sodium hypochlorite for 20 min under vacuum with occasional agitation, rinsed with sterile, distilled water three times, and sliced into 2-mm sections. Pith tissue of each section was removed with a cork borer 1 cm in

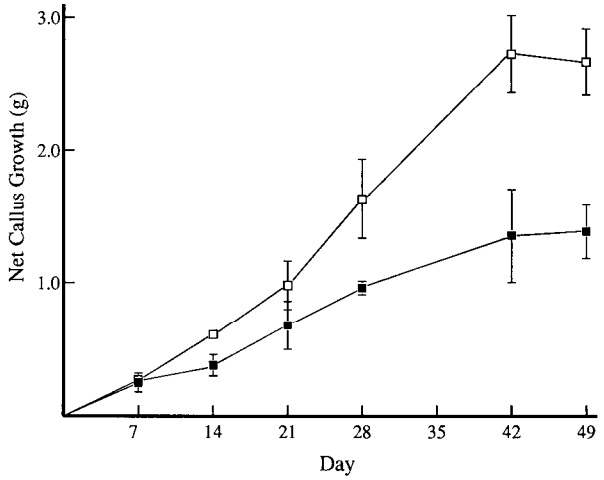


Fig. 1. Net callus growth of American ginseng cultured in MS medium containing 2 mg dicamba ( ❑ ) or 2 mg 2,4-D ( ■ )/liter. Each point represents the average callus growth from at least four plates. Each plate initially contained 50 ml of the maintenance medium and 1 g of callus. The vertical bars represent the SE of the mean.


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[1]Present address: Northrup King Co. Research Center, Highway 19, Stanton, MN 55081.

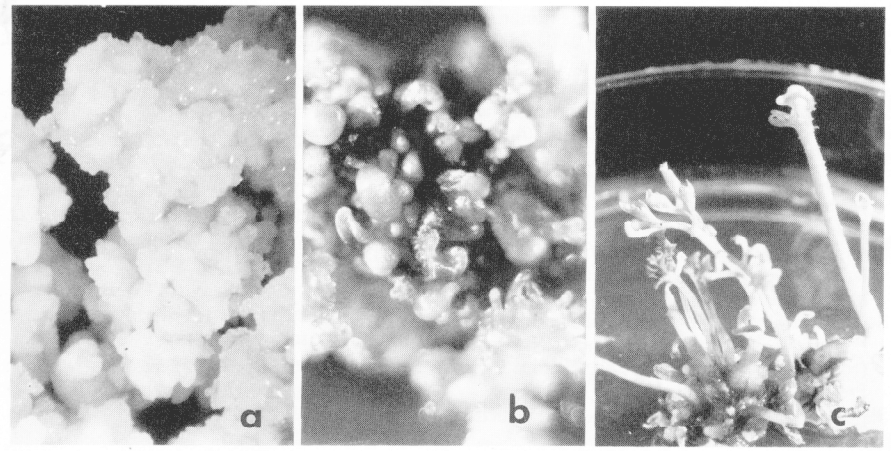


Fig. 2. Friable embryogenic callus **(a),** mature somatic embryos **(b),** and regeneratcd plantlets **(c)** of American ginseng.

Table 1. Effects of the plant growth regulators (PGR) BA, GA, IBA, KIN, and NAA on plating efficiency of American ginseng cultures.[z]

| PGR concn (mg·liter$^{-1}$) | Plating efficiency (%)[y] | | | | |
|---|---|---|---|---|---|
| | BA | GA | IBA | KIN | NAA |
| 0.10 | 0 | 17 | 72 | 5 | 56 |
| 0.50 | 5 | 56 | 56 | 22 | 22 |
| 1.00 | 33 | 89 | 33 | 22 | 0 |
| 2.00 | 22 | 89 | 27 | 17 | 5 |

[z]0.5 g of embryogenic callus was evenly spread on a 60 × 20-mm petri dish that. contained 20 ml of MS medium supplemented with various PGRs and maintained at 27C with a 16-hr photoperiod.
[y]All percentages based on 18 plates.

diameter, transferred onto a callus induction medium, and incubated in the dark at 27C.

The callus induction medium consisted of Murashige and Skoog (MS) salts (Murashige and Skoog, 1962), thiamine HCl (10 mg·liter$^{-1}$), casein hydrolysate (100 mg·liter$^{-1}$), surcrose (30 g·liter$^{-1}$), Difco-Bacto agar (8 g·liter$^{-1}$), 2,4-D (2 mg·liter$^{-1}$), and KIN (1 mg·liter$^{-1}$). Dark-greenish, compact calli were observed on the root-pith sections 4 weeks after initiation.

Four weeks_ later, the calli, were transferred to a maintenance medium similar to the initiation medium except that KIN was omitted and gelrite (Kelco) (2 g·liter$^{-1}$) was used instead of agar. Friable yellowish callus formed on the edge of the compact callus 2 to 3 weeks after the transfer. This friable callus grew very slowly. To enhance callus growth, dicamba (2 mg·liter$^{-1}$) was substituted for 2,4-D in the maintenance medium. Not only did the callus grow faster on the medium containing dicamba than on the 2,4-D medium (Fig. 1), but the callus became embryogenic (Fig. 2a). The callus has been maintained in the dark for more than 3 years and subculture at 3-week intervals.

The friable embryogenic callus grows well in a liquid maintenance medium supplemented with dicamba. American ginseng seems to develop more readily on a medium supplemented with dicamba, in contrast to Korean ginseng, which grows better on a medium containing 2,4-D (unpublished data).

For plant regeneration, embryogenic callus was transferred onto an embryo-maturation medium that was composed of MS salts, B5 vitamins (Gamborg et al., 1968), NAA (0.4 mg·liter$^{-1}$), 2,4-D (1 mg·liter$^{-1}$), sucrose (30 g·liter$^{-1}$), and agar (7 g·liter$^{-1}$) and maintained at 27C with a 16-hr photoperiod under cool-white fluorescent light (60 µmol·s$^{-1}$·m$^{-2}$). The somatic embryos matured in about 3 weeks (Fig. 2b). Mature somatic embryos were then transferred to MS medium supplemented with various concentrations of BA, GA, IBA, KIN, or NAA and also grown at 27C with a photoperiod of 16 hr.

The effect of these plant growth regulators on germination of somatic embryos was studied (Table 1). Overall, GA gave the highest plating efficiency (89%) and BA and KIN the lowest ( ≈20%). Also, plantlets derived from callus grown on medium containing BA, GA, or KIN were vitreous and abnormal, and roots were not formed. These plantlets were difficult to maintain.

Plantlets obtained from callus grown on medium containing IBA or NAA were normal, although their growth was relatively slow. Therefore, the regeneration medium was modified by using a combination of IBA and NAA in ratios of 0.1:0.1, 0.1:0.5, 0.5:0.1, and 0.5:0.5 mg·liter$^{-1}$. Plantlets regenerated on medium supplemented with 0.5 mg IBA and 0.1 mg NAA/liter were the healthiest (Fig. 2c), with a regeneration frequency of 30%.

This study reports the initiation of friable embryogenic callus from American ginseng root and plant regeneration from its somatic embryos. The method should be useful for genetic improvement of ginseng.

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