Formation of plantlets from callus cultures of ginseng (*Panax ginseng*)[1]

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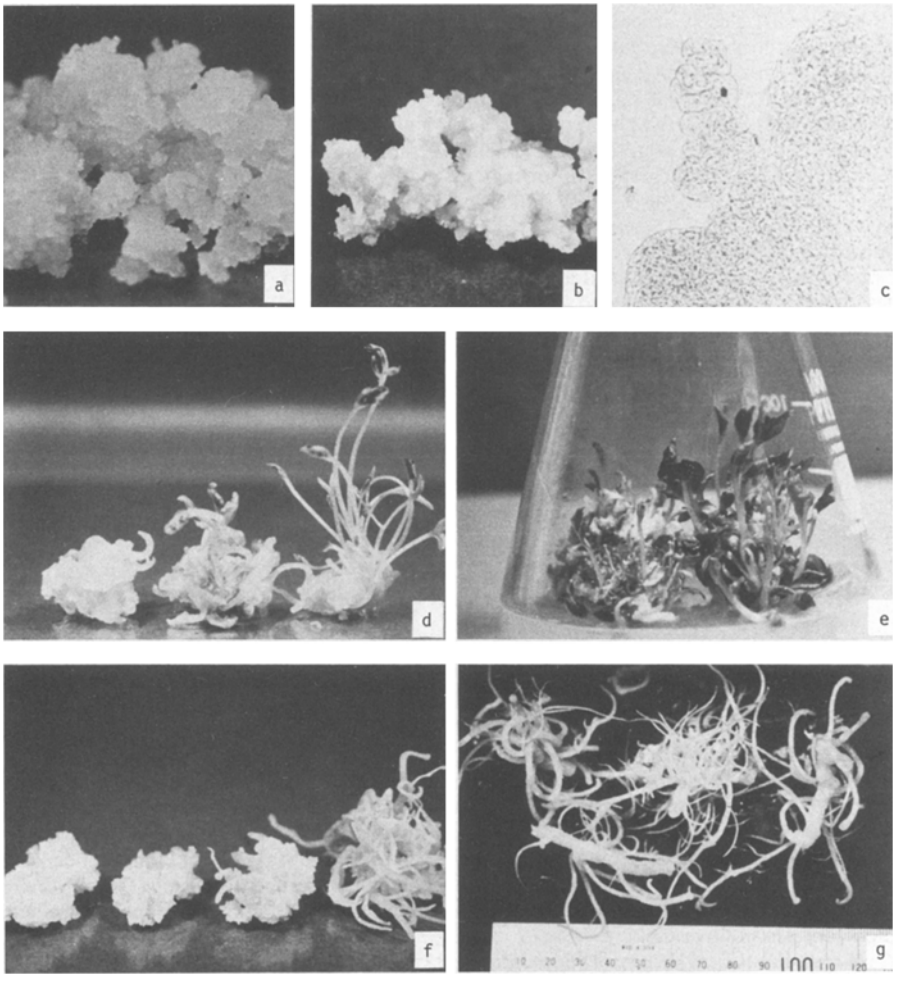
Summary. From callus cultures of *Panax ginseng* C. A. Mayer, shoots and roots were systematically formed at a high rate (in all flasks) under optimal culture conditions. The chemical constituents and the morphological structures of the differentiated plantlets closely resembled those of the native plants.
Key words. Panax ginseng; Araliaceae; plant tissue culture; differentiation; cloning plant; root formation; ginseng saponin production.

The ginseng (*Panax ginseng* C. A. Mayer, Araliaceae) is a herbaceous plant growing wild in Eastern Asia and cultivated in Northern China, Korea and Japan. Its root has been widely used as a tonic in the Orient since ancient times. However, the ginseng root is very expensive because of its long-term (5–7 years) and troublesome cultivation. Therefore, a number of investigators in the world have studied the production of the active ingredients, i.e. saponins, by tissue culture. In our earlier papers[2–4], it has already been shown that ginseng callus tissues derived from the petiole produce the same kind of saponins in a chemical and pharmacological sense as those of the original plants. Moreover, we have reported on the culture methods for effective saponin production[5] and on mass culture by jar fermentor using new strains selected from the callus or the original plant[6]. In the present study, a systematic method was investigated to obtain the various steps of differentiated tissues such as shoot, root and plantlet.

The ginseng callus, which was named 'Pg-3 DK callus' as shown in figure a, was induced on Murashige and Skoog's medium containing 2,4-D (2,4-dichlorophenoxyacetic acid) 1 ppm and kinetin 0.1 ppm from a 5-year-old ginseng root cultivated in Korea, in December 1978. The DK callus was maintained in a good state on the same medium at 25 °C in the dark and subcultured at 4-week intervals for 5 years. Immediately after transferring to the medium without 2,4-D, a rigid and a compact callus, which had a higher potential for organizing apices, as shown in figure b, appeared in all flasks from the original callus. In practice, the development of many nodules from the compact calli were observed at a high frequency (in all flasks) under a microscope (fig. c). Thereafter, the compact calli were transferred to media containing various kinds of plant growth regulators and subcultured for several generations.

Especially on transferring to the medium supplemented with only kinetin 1 ppm and then culturing at 20 °C under illumina-



Systematic formation of shoots, roots and plantlets from callus cultures of *Panax ginseng*. *a* Ginseng callus derived from native ginseng root on MS medium containing 2,4-D (2,4-dichlorophenoxyacetic acid) 1 ppm and kinetin 0.1 ppm (DK callus). *b* Meristemoids induced from the DK callus (a) on the medium without 2,4-D. *c* A cross section of the meristemoids (b), × 20. *d* Shoots formed from the meristemoids (b) on the medium containing kinetin 1 ppm, stepwise from left to right. *e* Cloning plantlets developed from shoots (d) on the K 1 medium under illumination. *f* Roots formed from meristemoids (b) on the medium containing IBA (indole-3-butyric acid) 1 ppm, stepwise from left to right. *g* Roots cultured in the liquid medium containing IBA 2 ppm and kinetin 0.1 ppm in the dark.

Comparison of the saponin contents between various ginseng cultured tissues and original plant

Callus or tissue	Growth ratio*	Dry wt (g) per 100 g fr. wt	Saponin content (mg) per 100 g fr. wt Rb group	Rg group**	Total	Rb group / Rg group	Total saponin per dry mass (wt %)
Static							
DK callus (Fig. 1a)	4.30	2.48	2.4	8.4	10.8	0.29	0.44
K 1 shoot (Fig. 1d, e)	5.81	2.96	15.1	22.3	37.4	0.67	1.26
IBA 1 root (Fig. 1f)	3.40	3.09	27.6	25.1	52.7	1.10	1.71
Suspension							
IBA 2 K 0.1 root (Fig. 1g)	6.22	6.31	41.6	38.8	80.4	1.07	1.27
Plant							
Aerial part (stem and leaf)		9.53	21.7	62.1	83.8	0.35	0.88
Root		23.91	59.0	37.3	96.3	1.58	0.40

* Growth ratio was determined by the increase of the fresh weight after 4-week culture. The values are the quotient of the fresh weight after 4 weeks culture and the fresh weight of the inoculum. ** The amount of Rb group was calculated as the total of ginsenosides Ra, Rb_1, Rb_2, Rc and Rd, having protopanaxadiol as the sapogenin, and Rg group was calculated as the total of ginsenosides Re, Rf, Rg_1, Rg_2 and Rh, having protopanaxatriol. Each value in the cultured tissues shows the average of duplicate estimations in 4 flasks of 3 different cultures. Each value for the original plant is the average of duplicate estimations in 3 different samples.

tion, 2500–4000 lx, 16 h/day with warm fluorescent light in a phytotron cabinet, the compact calli actively generated many green shoots. Figure d shows differentiation of shoots progressing stepwise from left to right. The shoot formation was actively promoted under the same culture conditions as those described above, and finally many clonal plantlets were formed in a flask (fig. e). On the other hand, when the compact calli were transferred to the medium supplemented with IBA (indole-3-butyric acid) 1 ppm (IBA 1 medium) and cultured for several generations on the same medium in the dark, many roots were formed as shown in figure f. Subsequently, the roots were separated and subcultured on the same medium, and finally cultured in the liquid medium (fig. g). It was observed under a microscope that the morphological structures of the isolated roots (named IBA 1 strain) closely resembled those of the original plant. Thereafter, the roots were cultured on media containing various auxins and cytokinins or their combinations as described in our earlier paper[5]. The most efficient saponin production by the ginseng cultured tissues was observed in the combination of IBA 2 ppm and kinetin 0.1 ppm (named IBA 2 K 0.1 medium). The roots cultured under the best conditions finally grew to 9–12 mm in a diameter in a flask on a rotary shaker or in a vessel in a jar fermentor.

Subsequently, the saponin contents in various calli and differentiated tissues were determined according to the TLC method described in our earlier papers[3–5]. The values are shown in the table in comparison to the saponin content of the native plant. As a result, it was demonstrated that the shoots (K 1) and the roots (IBA 1) produce larger amounts of saponins than the original callus (DK callus); 3.5 times as much in K 1 tissues and 4.9 times in IBA 1. The saponin content in suspension culture (IBA 2 K 0.1 medium) was comparable to those of the aerial part and the root of the plant on a fresh weight basis. On the other hand, the saponin content of the cultured roots on a dry weight basis was 1.71% in static culture and 1.27% in suspension, and those were 3–4 times higher than that in the plant root, 0.40%. Moreover, the ratio of the ginsenoside Rb group, which has protopanaxadiol as the sapogenin, to the Rg group, protopanaxatriol, was calculated for a quality evaluation of the ginseng saponin, and compared between the various cultured tissues and the native material. The ratios in DK callus and K 1 shoot resembled those in the aerial part of the native plant, while in the root cultures, i.e. IBA 1 root and IBA 2 K 0.1 suspension, they resembled that in the plant root.

Two other groups[7,8] have already reported that shoots and roots are differentiated from ginseng callus cultures. However, all the tissues developed spontaneously from the primary callus immediately after the callus induction. This experiment sheds light for the first time on the systematic methods suitable for obtaining plantlets, or shoots and roots, which have the desired quality, from the ginseng callus subcultured for several generations, over 5 years, after the induction. Now, we are trying to grow plants on soil from the differentiated plantlets.

1 Part 44 in the series 'Studies on Plant Tissue Culture'. For Part 43 see Yoshikawa, T., and Furuya, T., Planta med. *(1985)* 110.
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Cattle grazing behavior regulates tick populations

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Summary. Analysis of field population data showed that host-seeking larvae of the tick *Boophilus microplus* were less successful in attaching to their hosts when larval densities were high. Experimental results showed that cattle hosts detected and avoided high densities of larvae in pasture. The finding reveals a previously unknown population-regulating mechanism for ticks, which are important pests of livestock.
Key words. Parasitism; behavior; population; tick; cattle; host; *Boophilus;* density dependence.