PLANT REGENERATION THROUGH SOMATIC EMBRYOGENESIS IN ROOT-DERIVED CALLUS OF GINSENG

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Summary

Callus culture was initiated from explants of mature root tissues of ginseng (*Panax ginseng* C. A. Meyer) on MS medium enriched with 2, 4-D. The aging callus produced numerious embryoids in the same medium. Reculture of these embryoids in the media (1/2 MS or B5) supplemented with benzyladenine and gibberellic acid resulted in profuse plantlet regeneration.

Introduction

Ginseng callus has been successfully grown from various tissues, including leaves (Butenko et al., 1968; Jhang et al., 1974), anthophores (Butenko et al., 1968), stem (Butenko et al., 1968; Jhang, et al., 1974) and roots (Butenko et al., 1968; Jhang et al., 1974; Chang and Hsing, 1978a). However, the development of organized structures from callus has been limited to roots (Butenko, et al., 1968; Jhang et al., 1974; Chang and Hsing, 1978a), shoots (Jhang et al., 1974; Chang and Hsing, 1978c) and embryoids (Butenko et al., 1968; Chang and Hsing, 1978b). The suggestion of somatic embryogenesis in ginseng tissue cultures was first made by Butenko and her colleagues (1968). Tissues of leaf, petioles, anthophores and roots of ginseng were noted to exhibit an high capacity of callus formation and a tendency to spontaneous somatic embryogenesis during the first 12–18 months. With further subculture the capacity of cultured tissue for spontaneous differentiation decreased and finally ceased. However, they failed to make these embryolike structures develop further. Pursuing this work on culture of ginseng root tissues, we have obtained embryoids from root-derived callus in a defined condition (Chang and Hsing, 1978b). This communication deals with the subsequent attainment of plantlets.

Materials and Methods

Roots of ginseng (*Panax ginseng* C. A. Meyer) were surface sterilized in 2.5% sodium hypochlorite solution for 15 min. with frequent agitation and rinsed successively 5 times in autoclaved distilled water. For callus induction, explants of pith tissues of mature roots were excised aseptically and cultured on the surfaces of agar-gelled media. The basal medium (MS) consisted of Murashige and Skoog salts with (in mg/l) myo-inositol, 100; nicotinic acid, 0.5; pyridoxine HCl, 0.5; thiamine HCl, 0.1; glycine, 2.0; casin hydrolysate, 1,000; sucrose 30,000; Difco-Bacto agar, 10,000. Plant growth regulators were added

as optional adjuvents. This basal medium was also employed for subculture of callus and embryoid induction. All cultures were maintained in growth chambers at 26 ± 1°C in the dark. To achieve plant regeneration from embryoids a B5 formulation of Gambory et al (1968) and a medium containing one half of the Murashige and Skoog salts (1/2MS) were used. The cultures were maintained either at 26 ± 1°C in the dark or 26 ± 2°C under 12:12 light-dark regime. The lighting was from New Asian (Taipei, Taiwan, ROC) Day Light Fluoresent Lamps emitting 160 μEm^{-2}.

Results and Discussion

No development was apparent during the first 3 weeks. Shortly thereafter the root explants in the basal medium supplemented with 1 mg/l 2,4-dichlorophenoxyacetic acid (2, 4-D) showed a callus development of a golden color. The growth of the ginseng callus in this medium was slow particularly when compared with the growth of tobacco callus then currently being maintained in our laboratory. After 10 weeks the callus had enlarged to 1 cm in diameter. At 6–8 week intervals thereafter, the callus was subdivided and cultured in fresh medium to increase its quantity. The subcultured callus grew more vigorously, was friable, and exhibited a pale-yellow coloration. Prolonged culture without transferring to fresh medium resulted in pale-brown colored callus. This callus survived as long as one year. Examination of the 8-month old callus tissue growing in MS plus 1 mg/l 2, 4-D revealed the differentiation of numerous globular and heart-shape stage embryoids (Fig. 1). They were snow-white, soft and easily separable from the callus. Most of the embryoids were bipolar with two prominent cotyledons and resembled morphologically zygotic embryos of the same species. Abnormal cotyledonary embryoids were commonly observed. A giant poly-cotyledonary embryoid can be seen in Fig. 2.

None of the embryoids proceeded to form a normal plantlet when still maintained in the old medium or transferred to a fresh medium with the same nutrient components. A portion of the embryoids did not pass through the normal stages of development but swelled considerably, callused and ultimately became transform into a mass of callus. This embryoid-derived callus, capable of continuous growth, was subcultured and produced a fresh crop of embryoids, thus providing an unlimited source of embryoids.

When the embryoids were taken from the embryoid-generating medium and subcultured with a small amount of the callus tissue in either 1/2MS or B5 medium containing I mg/l benzyladenine and 1 mg/l gibberellic acid, they germinated (Fig. 3), developing etiolated shoots (Fig. 4) in the dark and normal green shoots under lighting (Fig. 5). In many cases more than one plant developed from one embryoid. These may have been derived from adventitious shoot-bud formation or socalled "secondary embryoids".

Development of ginseng seedlings from seeds in the field usually requires one and a half years due to the immature zygotic embryo. Our success in plantlet regeneration through somatic embryogenesis in callus that originated in root explants signifies simply a potential of clonal propagation of ginseng on an accelerated scale through tissue culture. The mature root tissues of phenotypically desirable ginseng plants could be used as the source of embryods to propagate elite genotypes, thus avoiding the uncertainty encountered when working with phenotypically undefined embryonic tissues.

Enhanced production of embryoids from root callus in a shorter period (less than 8 months) is in progress. Further work in also being conducted with other tissues of plantlets derived from embryoids, including leaves, petioles and cotyledons. Observations on the further development of embryoid-derived plantlets are continuing.

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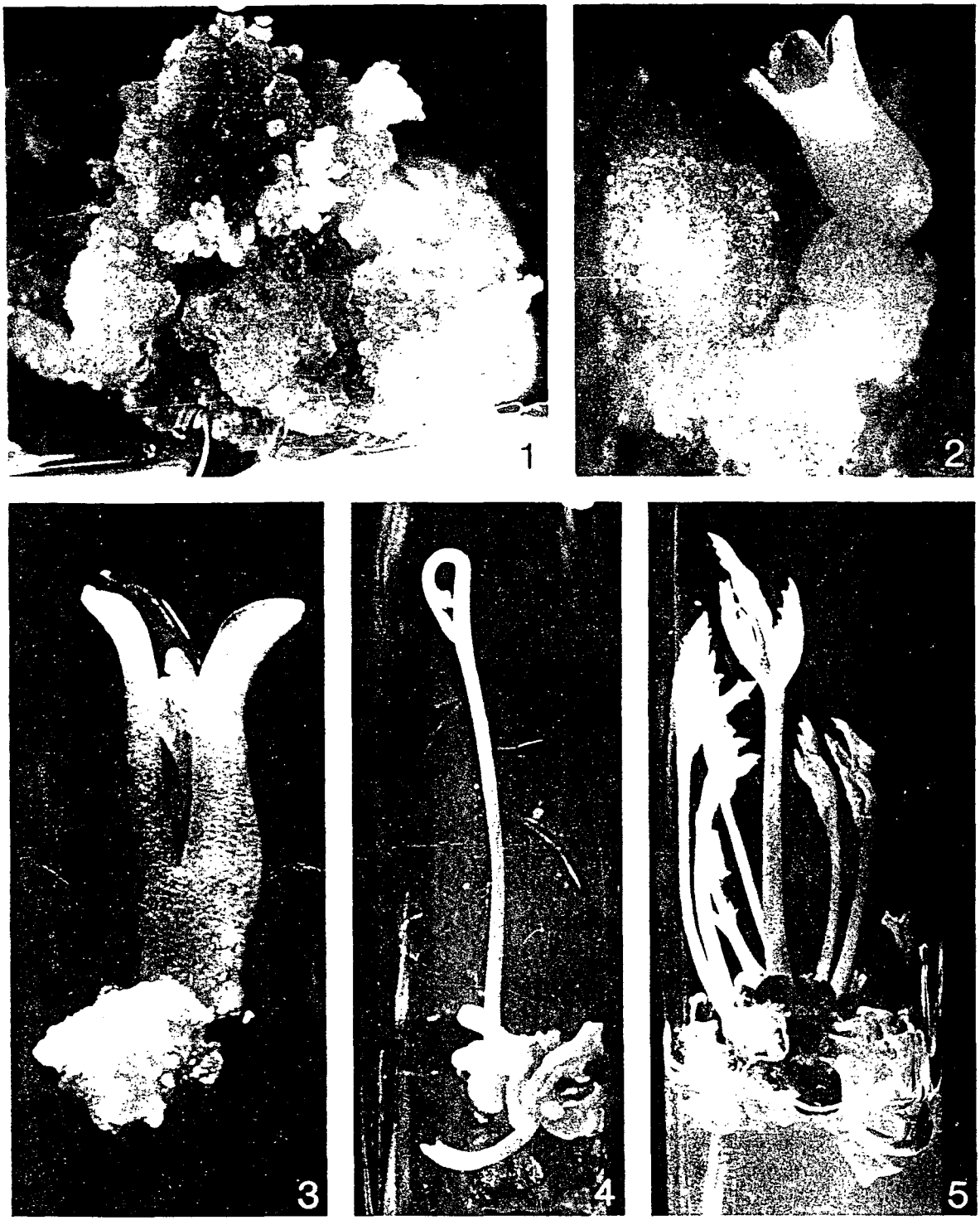

Fig. 1. Cluster of embryoids on the surface of an aging callus
Fig. 2. An aberrant poly-cotyledonary giant embryoid
Fig. 3. Germinated embryoid in 1/2 MS medium enriched with benzyladenine and gibberellic acid 1 mg/l each
Fig. 4. An etiolated plantlet derived from an embryoid maintained in 1/2 MS plus 1 mg/l benzyladenine and 1 mg/l gibberellic acid in the dark
Fig. 5. A green plantlet derived from an embryoid maintained in B5 medium plus 1 mg/l benzyladenine and 1 mg/l gibberellic acid under lighting
Scale bar: (1): 8 mm; (2): 2.5 mm; (3):1 mm; (4–5): 6 mm

Chairman: Now the time is open to discussion

Reuckert: Did you already analyze and find out about the content of ginsenoside? And if yes, what ginsenoside did you find?

Chang: We haven't done any chemical analysis on the ginseng component yet. Because I am not very good at chemistry. However, some of my colleagues are interested in my suspended culture and they would analysis for me.

Questioner (from Korea): Is there any change difference between the age of ginseng for your embryogenesis.

Chang: Do you mean the genetic variation?

Questioner (from Korea): I mean the different age of ginseng, four year or six year? Did you use four year old ginseng? I think this was commercial when published about six years old.

Questioner (from Korea): In the average what percent of green plant did you get from your this culture?

Chang: You mean how many percentage are about the regeneration? I would say about 90%, very easy. We just isolated the small pieces of the callus embryo for the medium.

Questioner: Do you mean that over 50 percent of regeneration?

Chang: I will say more than that. I will say 90 percent.

Questioner (from Korea): So it seems to put way to using this for publication purpose or something like that. It's a very fine paper particularly with regard to the flowergensis but the embryogenesis you observed that you had to wait about six months with the respect to the compact white tissue to form in order to get the embryoids. Is that true with all cases or are you able to get at young callus tissue as well as this? I don't know it's rare event or common event where you get the compact cell formation that they lead to embryoids.

Chang: What we are talking about, what I was talking about is the first time we have to spend six month to get at that embryo. After we get the embryo we have to spend six month to get at the embryo. After we get the embryo we put the embryo to a medium we don't want to become the plant to induce the callus. So the callus we call the embryonic callus. After we get the embryonic callus, it is very easy to get lots of embryo from them. We don't need to spend six months. We can get very easy in one month. That's why we get a lot of the embryo known.

Questioner (from Korea): Can you comment on the embryogenesis in the suspension culture with regard to ginseng?

Chang: Not yet. We are still working on that.

Questioner (from Korea): In our study we have many contamination of callus. How do you think about it of your study. And the second, which condition did you use to induce of embryogenesis?

Chang: Excuse me, the first question is how do we avoid the contanimation. Is that correct? You say you have lots of contamination. Is that correct?

Questioner (from Korea): Yes. In our study we had many contamination of the callus. How do you think about it?

Chang: I see. We were just using very ordinary procedure for plant tissue culture. We were using the water to clean our tissue. We didn't get any trouble about the contamination.

Questioner (from Korea): What method do you use? I would say it's not examined. We used the water with alcohol to clean the surface. Surface sterilized and then put in the media. Of course, we get a small percentage of contamination.

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