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Plant Regeneration Through Somatic Embryogenesis in Root-derived Callus of Ginseng (*Panax ginseng* C. A. Meyer)

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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 ageing callus produced numerous embryoids in this medium. Reculture of these embryoids in media (1/2 MS or B5) supplemented with benzyladenine and gibberellic acid resulted in profuse plantlet regeneration.

Key words: Somatic embryogenesis – Root callus – Plantlet regeneration – Ginseng – *Panax ginseng*

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 for 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 a high capacity for callus formation and a tendency towards 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 embryo-like structures develop further. Pursuing this work on the 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 achievement 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 (1962) salts with (in mg/l): myo-inositol, 100; nicotinic acid, 0.5; pyridoxine HCl, 0.5; thiamine HCl, 0.1; glycine, 2.0; casein hydrolysate, 1,000; sucrose 30,000; Difco-Bacto agar, 10,000. Plant growth regulators were added as optional adjuvants. 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 Gamborg et al. (1968) and a medium containing one half of the Murashige and Skoog salts (½MS) were used. The cultures were maintained either at 26 ± 1 C in the dark or 26 ± 2 C under 16:8 light-dark regime. The lighting was from New Asian (Taipei, Taiwan, ROC) day light fluorescent lamps emitting 160 μEm^{-2}.

Results

No development was apparent during the first 3 weeks. Shortly afterwards the root explants in the basal medium supplemented with 1 mg/l 2,4-dichlorophenoxyacetic acid (2, 4-D) showed callus development with a golden colour. 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 weeks intervals thereafter, the callus was subdivided and cultured in fresh medium to increase its quantity. The subcultured callus grew more vigorously, was friable and pale-yellow in colour. Prolonged culture

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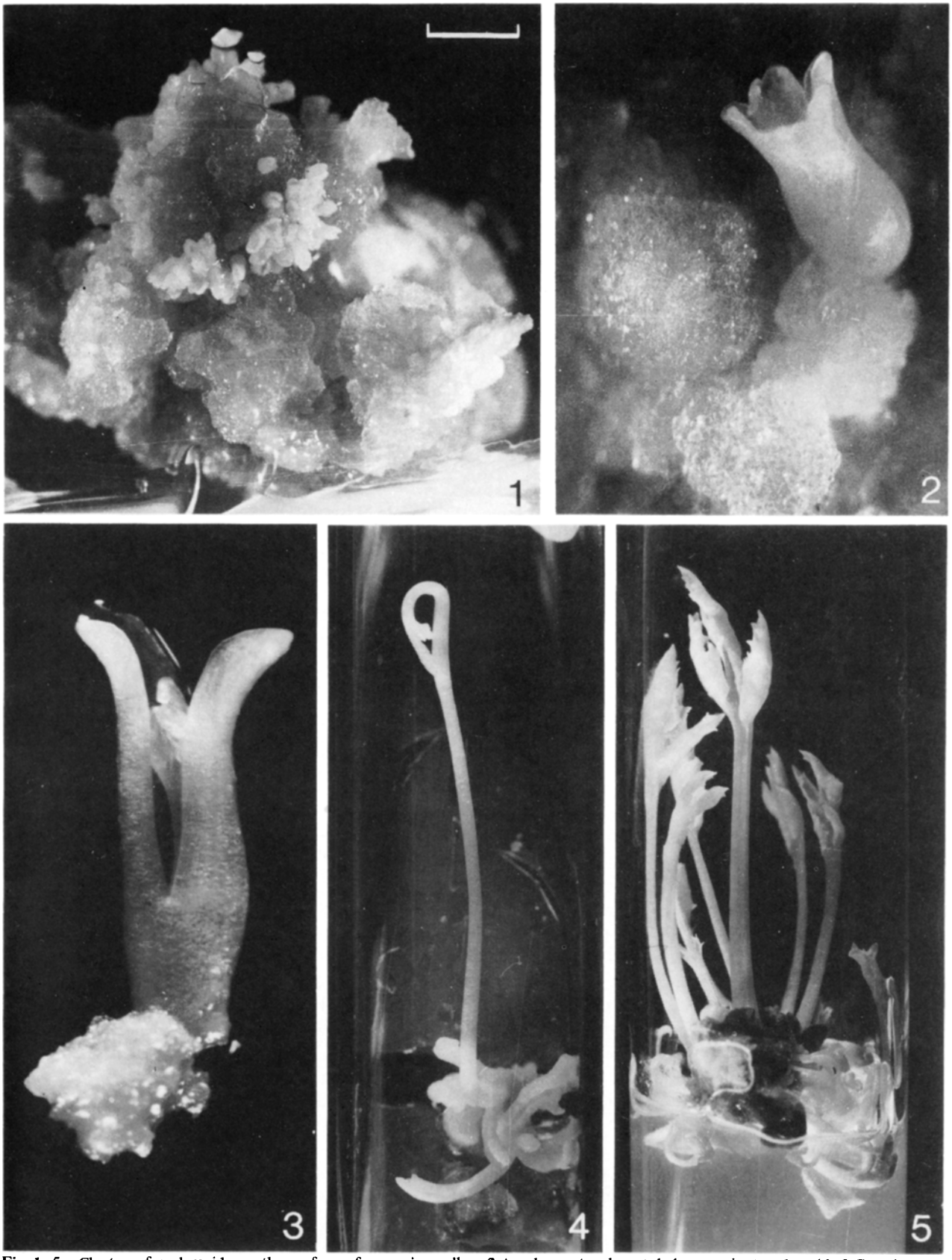

Fig. 1-5. Cluster of embryoids on the surface of an ageing callus; **2** An aberrant poly-cotyledonary giant embryoid; **3** Germinated embryoid in $\frac{1}{2}$MS medium enriched with benzyladenine and gibberellic acid 1 mg/l each; **4** An etiolated plantlet derived from an embryoid maintained in $\frac{1}{2}$MS plus 1 mg/l benzyladenine and 1 mg/l gibberellic acid in the dark; **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: Fig. 1. = 8 mm; Fig. 2. = 2.5 mm; Fig. 3. = 1 mm; Fig. 4-5. = 6 mm

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 separated from the callus. Most of the embryoids were bipolar with two prominent cotyledons and morphologically resembled zygotic embryos of the same species. Abnormal cotyledonary embryoids were commonly observed. A giant poly-cotyledonary embryoid can be seen in Figure 2.

None of the embryoids proceeded to form a normal plantlet when maintained in the old medium or transferred to a fresh medium with the same nutrient components. Some of the embryoids did not pass through the normal stages of development but swelled considerably, callused and ultimately were transformed 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 $\frac{1}{2}$MS or B5 medium containing 1 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 so-called 'secondary embryoids'.

Development of ginseng seedlings from seeds in the field usually requires one and a half years because of the immature zygotic embryo. Our success in plantlet regeneration through somatic embryogenesis in callus that originated from root explants signifies simply the potential for 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 embryoids 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 is 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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