Note

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Somatic Embryogenesis and Plantlet Regeneration on Several Species of Licorice

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Abstract

Leaf explants of four species of licorice (*Glycyrrhiza glabra* L., *G. uralensis* F., *G. echinata* L., and *G. squamulosa* F.) were cultured on Murashige and Skoog (MS) basal medium. *G. glabra* L. and *G. uralensis* F. formed adventitious roots from calli on the medium containing NAA ($1mgl^{-1}$). Both calli induced from *G. echinata* L. and *G. squamulosa* F. formed somatic embryos on the medium containing NAA ($1mgl^{-1}$) and BAP ($1mgl^{-1}$), or 2,4-D ($0.5mgl^{-1}$) alone. Plantlets regenerated from the somatic embryos were transplanted to soil.

There have been many attempts to use cell culture or hairy root culture of licorice to produce its useful metabolite (Dorisse *et al.*, 1988; Hayashi *et al.*, 1988; Ko *et al.*1989). However, somatic embryogenesis and plantlet regeneration of licorice requires the preparation of homogeneous plantlets for the production of glycyrrhizin by hydroponics (Kakutani *et al.*1997) and modification of their metabolite pathways using transgenic techniques (Saito *et al.*1990). We have recently succeeded in plantlet regeneration through somatic embryos from leaf explants of two of four species of licorice.

The four species of *Glycyrrhiza glabra* L., *G. uralensis* F., *G. echinata* L., and *G. squamulosa* F. maintained in the Takeda's Kyoto Herbal Garden were used as the source of material. The nodal segments of those plants were cut, sterilized in 70% ethanol for 30s and 5% sodium hypochlorite for 15min. The surface-sterilized segments were rinsed several times in sterile water and then cultured on 1/3 Murashige & Skoog (MS) medium (Murashige *et al.* 1962) supplemented with 1% sucrose and 0.2% gelrite. The culture was maintained at 25 ℃ under 16h photo period of 4,000-lux provided by fluorescent tubes. For the induction of callus, leaf explants($5 \times 5mm^2$) were cut from shoots (10-cm) grown after one month of culture and incubated on solid MS medium containing plant growth regulators. The combinations of plant growth regulators was a crossed series of 6-Benzylaminopurin (BAP) and 1-Naphthaleneacetic acid (NAA) , and a crossed series of 2,4-dichlorophenoxyacetic acid (2,4-D) and BAP. Twenty-five combinations of the plant growth regulators were prepared in five concentrations (0 mgl^{-1}, 0.1 mgl^{-1}, 0.5 mgl^{-1}, 1 mgl^{-1} and 5 mgl^{-1}).

All explants formed calli on each medium with plant growth regulator after 10 to 20 days of culture. Each callus grew according to increase of the plant growth regulator concentration. However, no difference was observed in callus growth with the addition of NAA or 2.4-D. Calli from *G. glabra* and *G. uralensis* were friable and white or yellow, while calli from *G. echinata* and *G. squamulosa* were compact and brown. *G. glabra* and *G. uralensis* particularly formed adventitious roots from calli on the medium containing NAA ($1mgl^{-1}$ or $5mgl^{-1}$) after 30 days of culture, but these adventitious roots were thin and of insufficient extension **(Fig. 1)**.

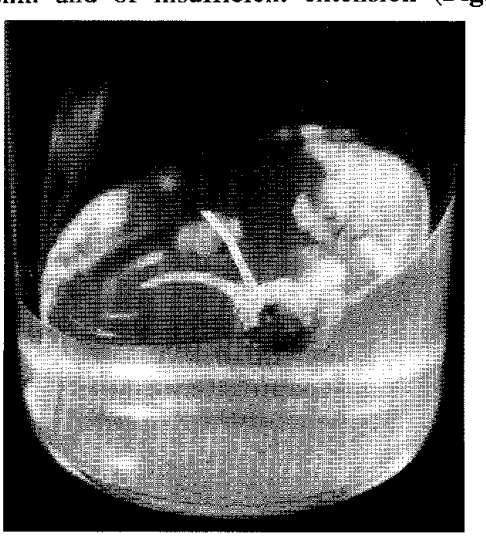

Fig. 1 Adventitious roots from the callus tissue of *G. glabra* L. on the medium containing $1mgl^{-1}$

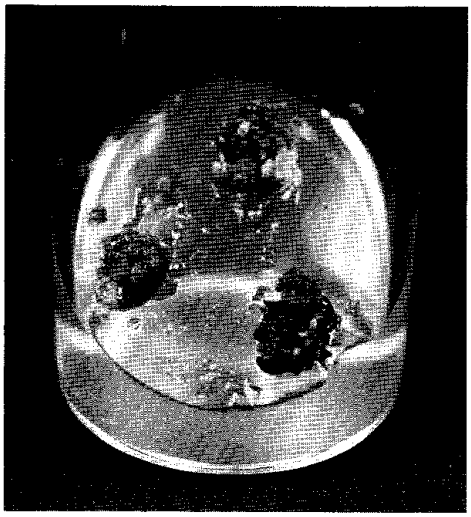

Fig. 2 Somatic embryos developing from callus of *G. echinata* L..

Fig. 4 Shoot formation from somatic embryos of *G. echinata* L..

Fig. 3 Somatic embryos developing from callus of *G. squamulosa* F. on the medium containing 0.5mgl^{-1} 2,4-D.

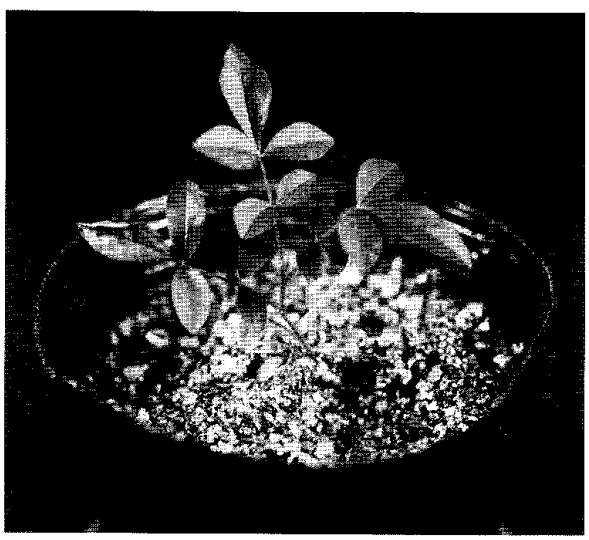

Fig. 5 Acclimatized regeneration plantlets via somatic embryos of *G. echinata* L. (30days after cultivation).

Both calli induced from *G. echinata* and *G. squamulosa* formed somatic embryos after 30 days of culture. Although somatic embryos of *G. echinata* were induced on the medium containing NAA (1mgl^{-1}) and BAP (1mgl^{-1}), those of *G. squamulosa* were obtained on the medium containing 2,4-D (0.5mgl^{-1}) with BAP (1mgl^{-1}) or 2,4-D (0.5mgl^{-1}) alone (**Fig. 2** and **3**). Both somatic embryos developed into the shoots (ca. 20 shoots / callus) after 60 days of culture (**Fig. 4**). These shoots transferred onto the MS medium without plant growth regulators, elongated and took root after 7 days of culture. After rinsing with sterile water, these regenerated plantlets were transferred to potting soil with vermiculite and then acclimatized (**Fig. 5**).

In this experiment, *G. echinata* and *G. squamulosa* producing no glycyrrhizin regenerated the plantlets from the somatic embryos, but *G. glabra* and *G. uralensis* producing glycyrrhizin failed to regenerate the plantlets. To reveal the relationship between glycyrrhizin productivity and plantlet regeneration, further research is required.

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