

The optimal germination of somatic embryos induced from mature cotyledon explants of *Panax ginseng* C. A. Meyer by gibberellic acid treatments

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### Objectives

In the present report, we describe somatic embryos derived from mature zygotic embryos of *Panax ginseng*. In addition, the effect of GA<sub>3</sub> on the developmental embryo stage and the optimal GA<sub>3</sub> concentration were investigated to obtain the more efficient plant regeneration.

### Materials and Methods

- Plant materials : Stratified seeds of *P. ginseng* C. A. Meyer
- Culture conditions : All media were adjusted to pH 5.8 and autoclaved at 121°C for 15 min. The culture room was maintained at 23 ± 2°C, under a 16-h photoperiod (24 μmol m<sup>-2</sup> s<sup>-1</sup> from white-fluorescent tubes)
- Direct somatic embryogenesis : Cotyledons excised aseptically from zygotic embryos were placed abaxial side down on MS basal medium supplemented with 5% sucrose and 1% agar.
- Germination of somatic embryos
  - Media : 30 ml of MS medium supplemented with gibberellic acid (GA<sub>3</sub>), 3% sucrose, 0.8% agar.
  - GA<sub>3</sub> concentration : 0, 5, 10, or 20 mg l<sup>-1</sup> GA<sub>3</sub>, with 40 embryos.

### Results

Somatic embryos on growth regulator-free medium can be directly produced from cotyledon explants of *Panax ginseng* C. A. Meyer. When the embryo developmental stage was torpedo and cotyledon, germination rate of embryos on MS medium supplemented with gibberellic acid (GA<sub>3</sub>) was very high. However, conversion percentage to plantlets at the cotyledon stage was higher than that at the torpedo. This result shows that the embryo of the cotyledon stage was the most suitable for germinating by GA<sub>3</sub>. Germination rates of embryos cultured on the medium including any concentrations of GA<sub>3</sub> was all high. When the well-developed embryos were

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cultured continuously on medium with high concentration of GA<sub>3</sub> from 10 to 20 mg l<sup>-1</sup>, conversion percentage to plantlets was lower than that of low concentration from 3 to 5 mg l<sup>-1</sup>. Shoot with abnormal shape grew at high concentration. These results indicated that treatment of GA<sub>3</sub> high concentration negatively affects embryo germination for obtaining a good plantlet. The optimal treatment of GA<sub>3</sub> provide the basis for efficiently obtaining healthy plantlets derived from ginseng somatic embryos and may contribute developing an efficient acclimatization system.

Table 1. Effect of GA<sub>3</sub> and developmental stage of somatic embryo on germination and conversion of germinated embryos.

Embryo developmental stage	Germination rate of embryos (%) <sup>a</sup>	Conversion to plantlets in 5 weeks (%)
Globular	0c	0d
Heart	32.6±5.0b	12.8±2.0c
Torpedo	92.5±8.0a	67.4±7.0b
Cotyledon	95.5±10.1a	83.2±8.5a

<sup>a</sup> Particular embryos were cultured on MS medium supplemented with 5 mg l<sup>-1</sup> GA<sub>3</sub> for 5 weeks. Means followed by the same letters are not significantly

Table 2. Influence of different concentration of GA<sub>3</sub> on germination and conversion of germinated embryos.

Concentration (mg l <sup>-1</sup> ) of GA <sub>3</sub>	Germination rate of embryos (%) <sup>a</sup>	Conversion to plantlets in 5 weeks (%)
0	0b	0c
3	91.1±5.3a	85.6±3.8a
5	95.4±7.0a	87.2±7.9a
10	89.3±6.6a	61.2±4.2b
20	87.7±11.3a	55.7±5.1b

<sup>a</sup> Mature somatic embryos were cultured on MS medium supplemented with GA<sub>3</sub> for 5 weeks. different at P=0.05 of Duncan's Multiple Range Test.

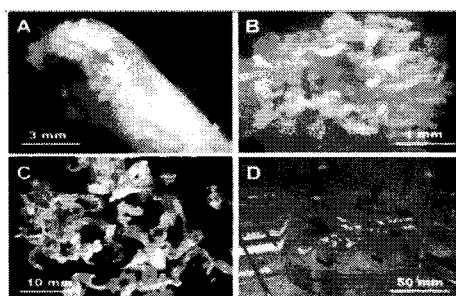


Figure 1. Direct somatic embryogenesis from mature zygotic embryos. (A) Direct somatic embryos from a zygotic embryo cultured on growth regulator-free MS medium. (B) Embryo germination after 2 weeks of 5 mg l<sup>-1</sup> GA<sub>3</sub> treatment. (C) Rooted plants from elongated shoots. (D) Plants with a taproot before the acclimation

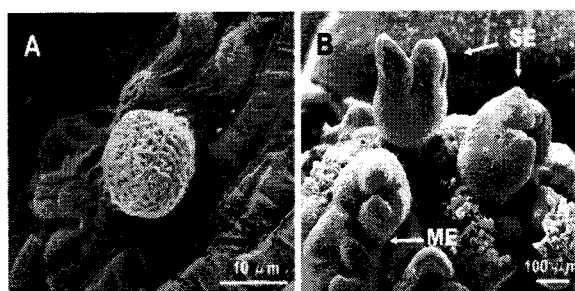


Figure 2. Scanning electron microscope photographs of somatic embryos. (A) a single cell shown to globular stage of embryo. (B) Mature somatic embryos cultured on growth regulator-free MS medium after 5 weeks. SE-single embryo, ME-multiple embryo fused with other embryo.