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Research article

Plant regeneration of Korean wild ginseng (*Panax ginseng* Meyer) mutant lines induced by γ-irradiation (^{60}Co) of adventitious roots

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ABSTRACT

An efficient *in vitro* protocol has been established for somatic embryogenesis and plantlet conversion of Korean wild ginseng (*Panax ginseng* Meyer). Wild-type and mutant adventitious roots derived from the ginseng produced calluses on Murashige and Skoog (MS) medium supplemented with 0.5 mg/L 2,4-dichlorophenoxyacetic acid and 0.3 mg/L kinetin; 53.3% of the explants formed callus. Embryogenic callus proliferation and somatic embryo induction occurred on MS medium containing 0.5 mg/L 2,4-dichlorophenoxyacetic acid. The induced somatic embryos further developed to maturity on MS medium with 5 mg/L gibberellic acid, and 85% of them germinated. The germinated embryos were developed to shoots and elongated on MS medium with 5 mg/L gibberellic acid. The shoots developed into plants with well-developed taproots on one-third strength Schenk and Hildebrandt basal medium supplemented with 0.25 mg/L 1-naphthaleneacetic acid. When the plants were transferred to soil, about 30% of the regenerated plants developed into normal plants.

1. Introduction

Panax ginseng Meyer is an important medicinal herb that is widely cultivated in Korea, China, and Japan. The root has been used as a drug for over 2000 years in oriental countries. Its use is rapidly expanding in Western countries as complementary and alternative medicine [1]. Ginsenosides are the major pharmacologically active components in *P. ginseng*. More than 30 types of ginsenosides have been identified from the genus [2,3].

Ginseng is a perennial plant that grows slowly and has a long production cycle (4–6 years). And > 3 years of juvenile period are required for producing seeds [4,5]. This has made the generation of superior genotypes by conventional breeding difficult. Therefore, attempts have been made to achieve a more rapid and increased production of the ginsenosides using other methods such as classical tissue culture [6], bioreactor culture [7], *Agrobacterium*-mediated hairy root production [8,9], using elicitors in cell cultures [10–12], and mutation breeding by γ-irradiation [13,14]. The last method has been used in many other plant species and has provided a large number of variants useful for plant breeding [15–17]. Mutagenesis by γ-irradiation has been shown to enhance ginsenoside production in *P. ginseng* [13,14]. Recently, we have also generated mutant cell lines by applying γ-irradiation on *P. ginseng* adventitious roots which were derived from Korean wild ginseng root [18]. Among the selected mutant cell lines, line 1 showed the highest total ginsenoside content of seven major ginsenosides

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Table 1
Effects of 2,4-Dichlorophenoxyacetic Acid (2,4-D) and Kinetin on the Frequency of Callus Formation from Ginseng Adventitious Roots[1)]

2,4-D (mg/L)	Kinetin (mg/L)	Number of root explants	Number of root explants forming callus
0.5	0	30	4.3 ± 1.0^{de}
	0.3	30	16.2 ± 1.8^{a}
	0.5	30	9.1 ± 1.7^{de}
1	0	30	7.2 ± 1.2^{bc}
	0.3	30	5.4 ± 1.1^{cd}
	0.5	30	2.3 ± 0.8^{ef}
2	0	30	0.0 ± 0^{f}
	0.3	30	0.0 ± 0^{f}
	0.5	30	0.0 ± 0^{f}

[1)] Data were collected after 6 wk of culture. The results represent the means ± standard error of the mean of values obtained from three experiments. Different corresponding letters within a column are significant different at $p < 0.05$ by Duncan's multiple range test

(Rg1, Re, Rb1, Rb2, Rc, Rf, and Rd). The total ginsenoside content of the mutant line was 2.3 times higher than in the wild-type line [18]. Using γ-irradiation, we have created a useful mutant line for breeding of the ginseng plant. However, there are no reports on *in vitro* plant regeneration with mutant lines of ginseng adventitious root.

Plant tissue culture system is considered a valuable tool in the plant improvement program. Somatic embryogenesis has been used as a preferred method for rapid *in vitro* propagation of many plant species [19–21]. *P. ginseng* is a difficult species to manipulate *in vitro*; however, its regeneration has generally been accomplished using somatic embryogenesis in callus derived from mature root tissues [22–24], callus derived from zygotic embryo [25,26], protoplast derived from callus [27], and cotyledons [4,28–30]. The development of efficient *in vitro* culture methods has facilitated the use of mutation technique for improvement of vegetative propagation of ginseng adventitious roots [13,14,18]. At present no information is available on the regeneration of a mutant adventitious root line that has been selected from γ-irradiated *P. ginseng* adventitious roots.

In this paper, we report on an efficient procedure for the regeneration of wild-type and mutant cell lines of *P. ginseng* adventitious roots through somatic embryogenesis.

2. Materials and methods

2.1. Callus induction and proliferation

Adventitious roots derived from Korean wild ginseng were provided by Sunchon National University, Sunchon, Korea. The adventitious roots were generated as described previously [7,31,32] and have been maintained in our laboratory for over 10 years. A mutant adventitious root line has been generated from the wild-type adventitious roots by γ-irradiation [18]. For embryogenic callus induction, wild-type and mutant adventitious roots were sectioned into 10 mm in length and were placed on Murashige and Skoog (MS) solid medium supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D), kinetin, and 3% sucrose. The media were solidified with 0.3% Gelite. Callus induction frequency was tested on MS solid medium supplemented with various concentrations of 2,4-D (0.5 mg/L, 1 mg/L, 1.5 mg/L, 2 mg/L) and kinetin (0 mg/L, 0.3 mg/L, 0.5 mg/L). All media were adjusted to pH 5.8 prior to autoclaving. Thirty pieces of adventitious roots were placed on each petri dish. Three replicates were prepared for each treatment. All cultures were incubated at 25°C in the dark. Callus formation was observed after 4 wk of culture. After 6 wk of culture, the frequency of callus induction was estimated. The induced callus was subcultured at 3-wk intervals on the same medium for induction of embryogenic callus and maintenance.

2.2. Induction of somatic embryos

Embryogenic callus induced from the segments of adventitious roots was used for induction of somatic embryos. A 10 g piece of embryogenic callus was incubated in a 15 L airlift bioreactor containing 5 L MS liquid medium with 0.5 mg/L 2,4-D and 3% sucrose for proliferation. After 3 wk, the proliferated embryogenic callus was used as explants for induction of somatic embryogenesis.

To examine the effect of 2,4-D on somatic embryo induction, proliferated callus was placed on a solid MS medium supplemented with different concentrations of 2,4-D (0 mg/L, 0.5 mg/L, 1 mg/L). Ten clumps of embryogenic callus (about 5 mm in diameter) were cultured on petri dishes containing 40 mL of medium and the experiment repeated three times. All cultures were incubated at

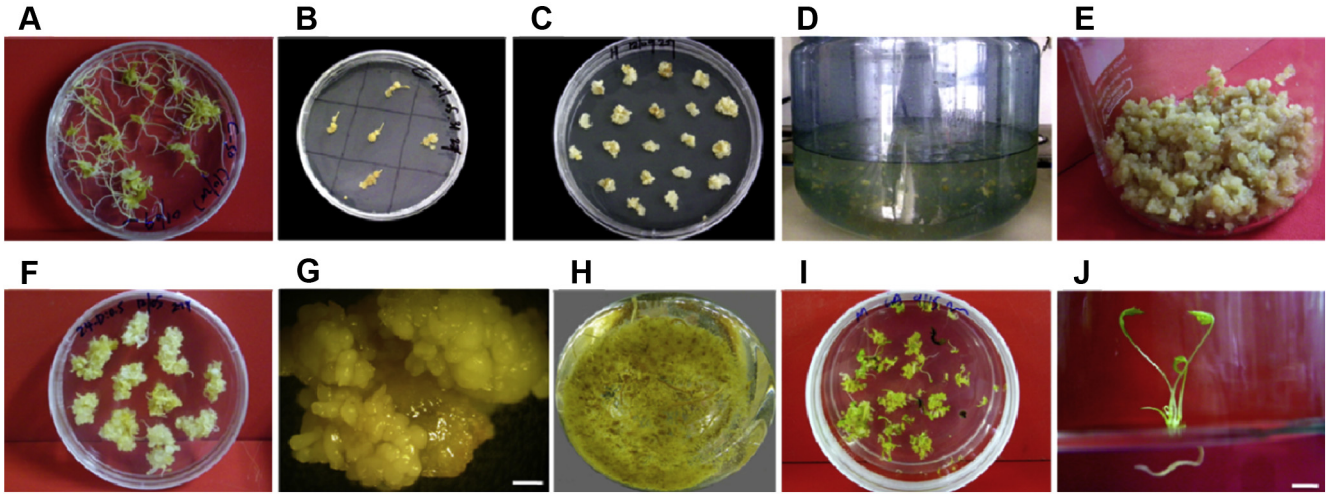

Fig. 1. Somatic embryogenesis and regeneration of plantlet from adventitious roots of *Panax ginseng*. (A) Adventitious roots derived from Korean wild ginseng root. (B) Callus induction from adventitious root explants. (C) Embryogenic callus derived from adventitious roots. (D) Proliferation of embryogenic callus in an airlift bioreactor. (E) Proliferated embryogenic cell clumps from bioreactor culture. (F) Somatic embryos formed on embryogenic callus. (G) Magnified image from (F; scale bar = 2 mm). (H) Proliferation of somatic embryos in conical flasks. (I) Maturation and germination of somatic embryos on MS medium supplemented with 5 mg/L GA_3. (J) Well-developed plantlet derived from somatic embryo (scale bar = 0.8 cm).