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순화된 인삼 조직배양묘의 현장 재배시 적응성 및 진세노사이드 조성 안정성

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Adaptability and Ginsenoside Compositional Stability of Acclimatized *Panax ginseng* Plantlets Derived from Somatic Embryogenesis under Field Conditions

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ABSTRACT

Background: *Panax ginseng* C. A. Meyer is a major medicinal crop in Korea; however, its low seed propagation efficiency limits the distribution of new cultivars. Somatic embryogenesis has been developed as an alternative propagation method. Nevertheless, the adaptability and compositional stability of acclimatized *in vitro*-grown roots (IGRs) under field conditions remain unclear.
Methods and Results: IGRs of the 'Cheonryang' cultivar were produced by somatic embryogenesis, acclimatized in a greenhouse, and categorized into three weight groups (< 0.8 g, 0.8 – 1.4 g, > 1.4 g). They were transplanted into an experimental field, with seed-derived seedlings serving as controls. The emergence rate increased with IGR weight, reaching 88.0% in the > 1.4 g group, which was comparable to the control (86.6%). Multi-stem formation occurred frequently in IGRs, reaching 46.2% in the > 1.4 g group. Flowering was observed in IGRs ≥ 0.8 g but not in controls. Although aerial traits were smaller in IGRs, growth improved with increasing initial root weight. IGRs > 1.4 g were similar in weight and length to the controls but exhibited larger diameters and more rhizomes. Ginsenoside composition and total content did not differ significantly between groups.
Conclusions: These findings confirm the field adaptability and compositional equivalence of acclimatized IGRs, supporting their practical use in large-scale propagation.

Key Words: *Panax ginseng*, Somatic Embryogenesis, *In Vitro*-Grown Roots, Field Acclimatization, Ginsenoside

INTRODUCTION

The family Araliaceae comprises approximately 55 genera and 1,500 species, most of which are utilized as medicinal crops (Wen *et al.*, 2001). Among them, *Panax ginseng* C. A. Meyer is a representative medicinal crop in Korea, and its root has been used as traditional medicine in East Asia for thousands of years owing to its beneficial effects on human health (Potenza *et al.*, 2022). *P. ginseng* grows naturally only in eastern Asia, particularly between 33° and 48° N latitude (Woo *et al.*, 2004). This perennial herb loses its aerial parts in winter, while the root with rhizome survives and produces new shoots the following spring. Flowering is more strongly influenced by the vegetative growth period than by photoperiod or temperature, requiring more than three years after sowing for the roots to reach a size suitable for flowering (Kwon *et*

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al., 1998). Furthermore, the seed production cycle is long and the yield is low (Lee *et al.*, 2023a), limiting the distribution of new cultivars to farmers.

Plant tissue culture techniques offer a promising solution for crops with low propagation efficiency (Koufan *et al.*, 2022). Somatic embryogenesis is an effective method that enables the rapid propagation of genetically uniform plants (Guan *et al.*, 2016), and direct somatic embryogenesis carries a lower risk of genetic variation than organogenesis (Gaj, 2001). In *P. ginseng*, direct somatic embryogenesis can produce about 40 plantlets from a single seed in one year (Lee *et al.*, 2023a), and acclimatization in artificial soil under greenhouse conditions can achieve survival rates close to 90% (Lee *et al.*, 2021).

However, the emergence, flowering, growth, and ginsenoside content of acclimatized *in vitro*-grown roots (IGRs) after transplantation under field conditions have rarely been reported. To address this gap, the present study evaluated the adaptability of IGRs produced via direct somatic embryogenesis under field conditions. Specifically, we compared the emergence rates and flowering of IGRs of different weights with seed-derived controls, examined phenotypic traits of aerial and underground parts, and assessed the equivalence of ginsenoside content between acclimatized IGRs and controls.

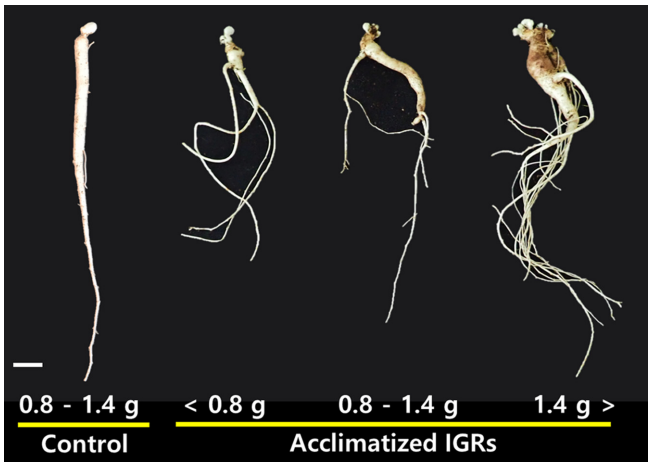

Fig. 2. Morphological observation of seed-derived control and acclimatized IGRs classified by weight before transplantation into the field. The control represents one-year-old ginseng roots (0.8 – 1.4 g) derived from seed; IGRs, *in vitro*-grown roots derived from plant tissue culture, classified into three weight categories (< 0.8 g, 0.8 – 1.4 g, and > 1.4 g). Scale bar; 1 ㎝.

MATERIAL AND METHOD

1. Plant materials

The production process of the plant materials used in this study is shown in Fig. 1. IGRs were obtained via somatic embryogenesis of the cultivar 'Cheonryang', following the method described by Lee *et al.* (2023b). IGRs obtained from plant tissue culture were acclimatized for five months in a glass greenhouse maintained at approximately 25°C, following the procedure of Lee *et al.* (2023c). After harvest, the acclimatized IGRs were subjected to cold treatment at approximately 2°C for one month in cold storage. The acclimatized IGRs were then classified into three weight categories (< 0.8 g, 0.8 –1.4 g, and > 1.4 g) and used as transplanting materials in field conditions (Fig. 2). The seed-derived control consisted of one-year-old 'Cheonryang' seedlings harvested in March 2023 from the experimental field of the National Institute of Horticultural and Herbal Science (Eumseong, Chungcheongbuk-do, Korea), and only seedlings weighing 0.8

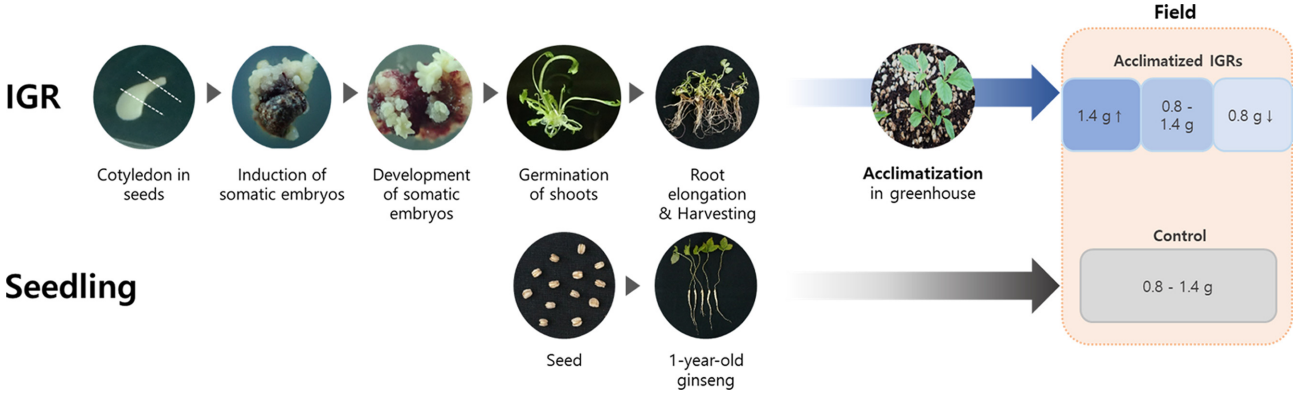

Fig. 1. Schematic overview of plant material production and transplantation. The processes of IGR production, seedling preparation, and field transplantation are illustrated. IGR indicates *in vitro*-grown roots derived from plant tissue culture.

– 1.4 g were used in the experiment.

2. Field transplantation and cultivation method

The acclimatized IGRs and the control were immersed in 25 ㎎/ℓ gibberellic acid 3 (GA_3) (Duchefa Biochemie, Haarlem, Netherlands) solution for 1 hour, following the method of Lee *et al.* (2021), and transplanted into the experimental field of the National Institute of Horticultural and Herbal Science (Eumseong, Chungcheongbuk-do, Korea) in April 2023. The spacing between plants was 10 ㎝ × 10 ㎝, with 36 plants per treatment, and the experiment was replicated three times. The cultivation practices, including the installation of shading facilities, followed the methods described by Kim *et al.* (2023).

3. Growth characteristics of aerial parts

The emergence rate of each treatment was evaluated 30 days after transplantation and calculated as the proportion of emerged plants among the total number of transplanted individuals. For each emerged plant, the number of stems was recorded. Plants with a single stem were classified as single-stem type, whereas those with two or more stems were classified as multi-stem type (Fig. 3). The proportions of single- and multi-stemmed plants were subsequently calculated for each treatment. Fifty days after transplantation, the number of flowering plants was recorded for each treatment. Ninety days after transplantation, aerial growth characteristics were assessed using 35 plants per treatment. The measured traits included stem length, stem diameter, leaf length, and leaf width.

4. Growth characteristics of underground parts

One hundred fifty days after transplantation, when the aerial parts had senesced, the roots were harvested. Growth characteristics of the roots, including root length, diameter, and fresh weight, were measured using 20 plants per treatment. Root length was measured from the rhizome to the tip of the longest root, and root diameter was measured at the thickest part of the root. Root fresh weight was determined immediately after harvest, with the aerial parts removed. The number of rhizomes was examined by photographing the samples under a binocular microscope (S8AP0, Leica, Wetzlar, Germany).

5. Ginsenoside analysis

Harvested roots derived from the control and each acclimatized IGRs treatments classified by weight before transplantation were freeze-dried. The dried samples were ground into powder using a mortar and pestle to prepare samples for ginsenoside content analysis. Ten ginsenosides, including the protopanaxadiol (PPD) type (Rb1, Rb2, Rb3, Rc, and Rd) and the protopanaxatriol (PPT) type (Re, Rf, Rg1, Rg2, and Rh1), were analyzed. Ginsenoside contents were determined according to the method described by Kwon *et al.* (2023). For sample preparation, each powdered root sample was extracted by immersing it in 70% methanol and homogenizing it with ultrasonication for 30 min. The supernatant obtained after centrifugation was filtered through a 0.45 ㎛ membrane filter (Agilent, Santa Clara, CA, USA) for purification. The purified samples were injected into a UPLC system (Nexera X2, Shimadzu, Kyoto, Japan) equipped with a Halo RP-amide column (4.6 × 150 ㎜, 2.7 ㎛; Thermo Fisher Scientific, Wilmington, DE, USA). Ginsenoside contents were analyzed in triplicate at 50°C. The flow rate was set at 0.5–0.8 ㎖/min, and UV detection was performed at 203 ㎚.

6. Statistical analysis

All statistical analyses were performed using R software (version 4.3.3; R Foundation for Statistical Computing, Vienna,

Fig. 3. Characteristics of single-stem and multi-stem types in *P. ginseng*. (A) Single-stem type; a single stem emerging from a single root. (B) Multi-stem type; two or more stems emerging from a single root. Red arrows indicate the stems. Scale bar; 1 ㎝.

Austria). For comparisons between two treatments, homogeneity of variance was first tested using Levene's method, followed by a two-sample t-test to evaluate significance ($p < 0.05$). For comparisons of multivariate data, significance was first assessed using analysis of variance (ANOVA), and when significant differences were detected, Duncan's multiple range test (DMRT) was applied for post-hoc analysis ($p < 0.05$).

In addition, since root weight was found to influence ginsenoside contents of IGRs, analysis of covariance (ANCOVA) was performed using root weight as a covariate. This approach allowed us to test for differences between control and IGR plants while statistically adjusting for the effect of initial root weight. Least-squares means (LSmeans) and 95% confidence intervals were calculated to evaluate adjusted group differences.

RESULTS

1. Emergence and flowering characteristics of aerial parts

Photographs illustrating the emergence characteristics of acclimatized IGRs of different weights compared with the control are presented in Fig. 4. The highest emergence rate (88.0%) was observed in acclimatized IGRs weighing > 1.4 g, which was not significantly different from that of the control (86.6%) (Table 1). The emergence rate of acclimatized IGRs

Table 1. Shoot emergence and development according to transplanted root type.

Treatment		Number of transplanted plants	Number of emerged plants	Emergence rate (%)	Proportion of stem type (%)		Number of flowering plants
					Single	Multi	
IGR	< 0.8 g	360	119	33.1±8.1[c]	93.1	16.9	0
	0.8 – 1.4 g	144	94	61.8±6.6[b]	73.6	26.4	1
	> 1.4 g	108	104	88.0±3.2[a]	53.8	46.2	7
Control		216	187	86.6±2.8[a]	96.8	3.2	0
p-value				< 0.001			

Significant differences were determined using one-way ANOVA. Different letters indicate significant differences according to Duncan's Multiple Range Test (DMRT, $p < 0.05$).

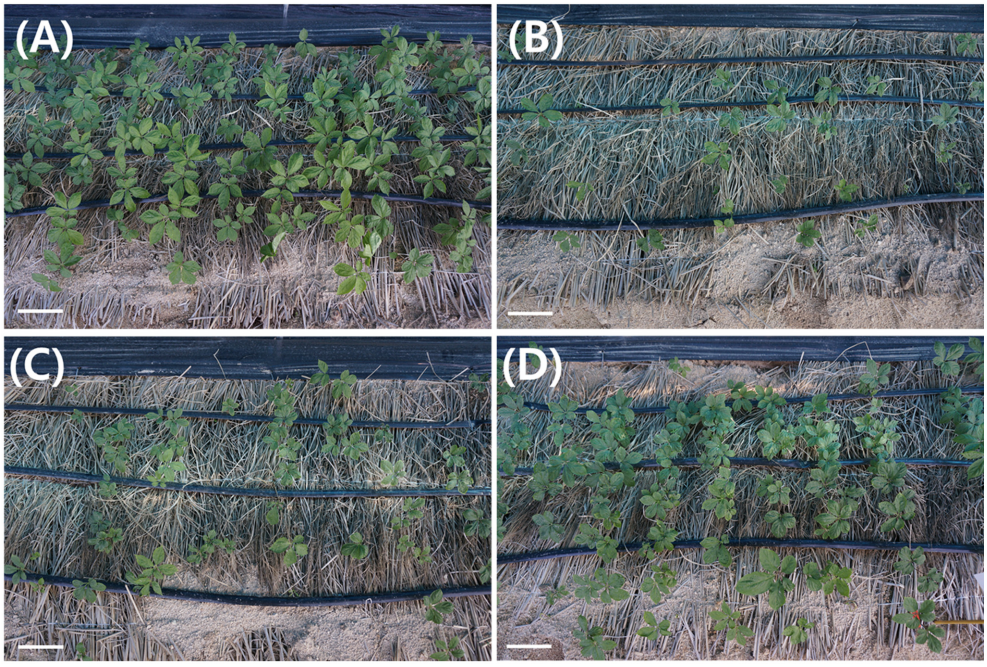

Fig. 4. Shoot emergence and growth characteristics of 60 days after transplantation. (A) Control (seed-derived seedlings). 1.4 g. (B) IGRs weighing < 0.8 g. (C) IGRs weighing 0.8 – 1.4 g. (D) IGRs weighing > 1.4 g. IGRs, *in vitro*-grown roots derived from plant tissue culture. Scale bar; 10 ㎝.

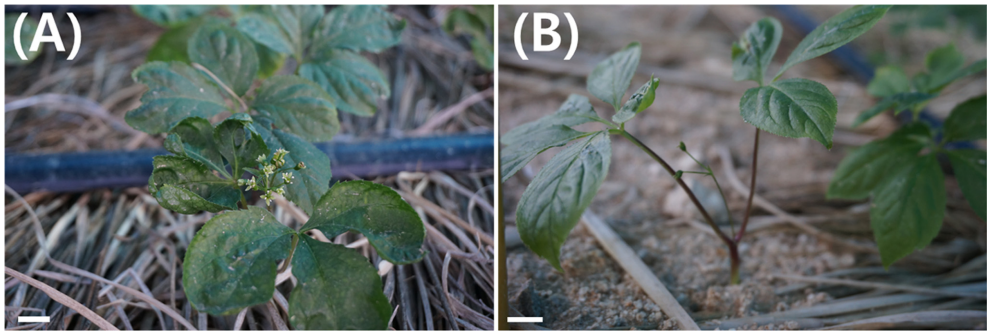

Fig. 5. Flowering characteristics of *P. ginseng* after field transplantation. (A) Acclimatized IGRs weighing > 1.4 g. (B) Control (seed-derived seedlings). IGRs, *in vitro*-grown roots derived from plant tissue culture. Scale bar; 1 ㎝.

weighing 0.8 – 1.4 g was 61.8%, while that of IGRs weighing < 0.8 g was the lowest at 33.1%. Among the emerged plants, stem number was further assessed: 96.8% of the control plants produced single-stem type, whereas acclimatized IGRs showed a markedly higher proportion of multi-stem type. The proportion of multi-stem individuals increased with IGR weight, reaching 46.2% in the > 1.4 g group.

Fifty days after transplantation, flowering was evaluated. No flowering was observed in the control or in acclimatized IGRs weighing < 0.8 g. In contrast, flowering occurred in one plant of the 0.8 – 1.4 g group and in seven plants of the > 1.4 g group (Fig. 5).

2. Growth characteristics of aerial parts

The results of evaluating the main traits of aerial parts, including stem length, stem diameter, leaf length, and leaf width, 90 days after transplantation are summarized in Table 2. Overall, the control plants exhibited significantly superior aerial growth compared with all acclimatized IGR groups. Stem length was significantly longer in the control than in all IGR groups, although among the IGR groups, stem length increased proportionally with root weight. Stem diameter was greatest in the IGRs weighing > 1.4 g group, followed by the control, the 0.8 – 1.4 g group, and the < 0.8 g group. Leaf length and width were also significantly greater in the control, but both

Table 2. Shoot growth characteristics according to transplanted root type.

Treatment		Stem length (㎝)	Stem diameter (㎜)	Leaf length (㎝)	Leaf width (㎝)
IGR	< 0.8 g	1.6±0.4^{d}	1.76±0.27^{c}	4.0±0.7^{d}	2.3±0.4^{d}
	0.8 – 1.4 g	2.3±0.6^{c}	2.03±0.32^{b}	4.7±0.9^{c}	2.7±0.5^{b}
	> 1.4 g	3.5±1.3^{b}	2.32±0.48^{a}	5.6±0.8^{b}	3.3±0.7^{c}
Control		4.8±0.9^{a}	2.07±0.30^{b}	7.4±0.7^{a}	3.7±0.6^{a}
p-value		< 0.001	< 0.001	< 0.001	< 0.001

Shoot growth was investigated 90 days after transplantation using 35 plants per treatment. Significant differences were determined using one-way ANOVA. Different letters indicate significant differences according to Duncan's Multiple Range Test (DMRT, $p < 0.05$).

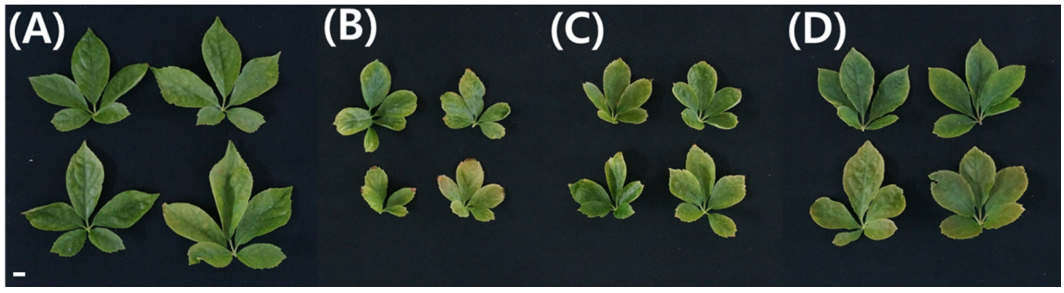

Fig. 6. Representative leaf characteristics according to transplanted root type. (A) Control (seed-derived seedlings). (B) Acclimatized IGRs weighing < 0.8 g. (C) Acclimatized IGRs weighing 0.8 – 1.4 g. (D) Acclimatized IGRs weighing > 1.4 g. IGRs, *in vitro*-grown roots derived from plant tissue culture. Scale bar; 1 ㎝.

traits tended to increase with root weight among IGRs. Despite these size differences, no distinct differences in leaf morphology were observed between the acclimatized IGR-derived plants and the control (Fig. 6).

3. Growth characteristics of underground parts

After senescence of the aerial parts, the underground parts were harvested and their growth characteristics were examined. Greater initial weight of the acclimatized IGRs led to higher root fresh weight at harvest. In particular, acclimatized IGRs weighing > 1.4 g showed root fresh weight and length comparable to those of the control, but exhibited a 27.6% greater root diameter. The number of rhizomes was higher in acclimatized IGRs than in the control and tended to increase proportionally with IGR weight (Table 3). Morphologically, across all weight categories, acclimatized IGRs showed more active lateral root development than the control, resulting in atypical root morphology (Fig. 7).

4. Ginsenoside analysis

The results of ginsenoside analysis between acclimatized IGRs and seed-derived controls after 150 days of field growth are summarized in Table 4. Initially, one-way ANOVA among IGR weight groups (< 0.8 g, 0.8 – 1.4 g, > 1.4 g) revealed significant differences in several ginsenosides (data not shown) (e.g., Rb1, Rb2, Rc, Rd, Rf, Rg1, Rg2, and Re), indicating that root weight may influence compositional variation. To account for this effect, ANCOVA was subsequently performed using root weight as a covariate. The ANCOVA results demonstrated that, after adjusting for root weight, no significant differences were observed in the levels of individual ginsenosides, total ginsenoside content, or the PPD/PPT ratio between acclimatized IGRs and seed-derived controls. This indicates that the apparent variation among IGR subgroups was primarily attributable to weight differences rather than intrinsic differences in metabolic composition.

Table 3. Root growth characteristics according to transplanted root type.

Treatment		Root weight (g)	Root length (㎝)	Root diameter (㎜)	Number of Rhizomes
IGR	< 0.8 g	1.0±0.4^{c}	8.3±3.4^{b}	7.43±2.01^{c}	2.1±0.6^{b}
	0.8 – 1.4 g	1.6±0.6^{b}	8.5±4.0^{b}	10.01±3.07^{b}	3.0±1.3^{a}
	> 1.4 g	3.7±1.0^{a}	13.8±3.2^{a}	15.03±3.97^{a}	3.5±1.2^{a}
Control		3.9±1.4^{a}	11.9±2.8^{a}	10.88±2.46^{b}	1.2±0.4c
p-value		< 0.001	< 0.001	< 0.001	< 0.001

Root traits were analyzed 150 days after transplantation using 20 samples per treatment. Each root type was examined with 20 samples. Significant differences were determined using one-way ANOVA. Different letters indicate significant differences according to Duncan's Multiple Range Test (DMRT, $p < 0.05$).

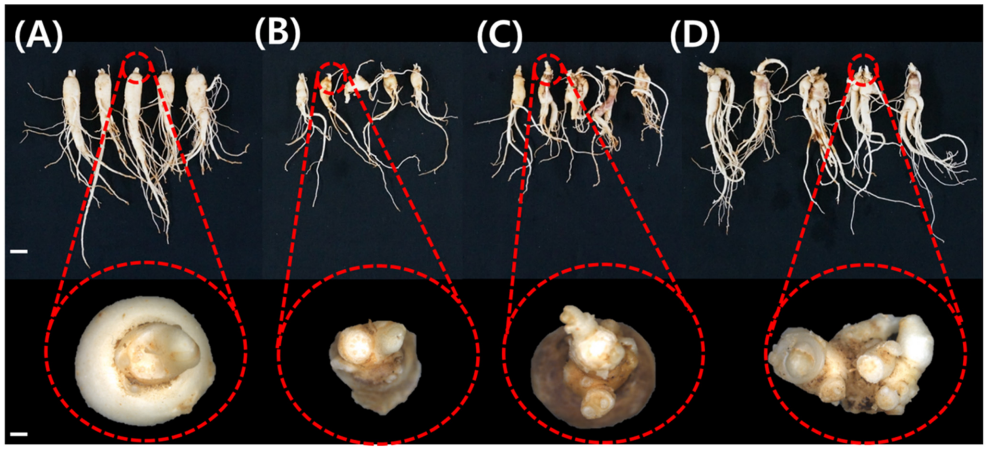

Fig. 7. Representative root characteristics according to transplanted root type. (A) Control (seed-derived seedlings). (B) IGRs weighing < 0.8 g. (C) IGRs weighing 0.8 – 1.4 g. (D) IGRs weighing > 1.4 g. IGRs *in vitro*-grown roots derived from ginseng tissue culture. Upper Scale bar; 1 ㎝, lower scale bar; 1 ㎜.

Table 4. Ginsenoside content by transplanted root type, adjusted for root weight by ANCOVA.

	PPD (㎎/g)					PPT (㎎/g)					PPD/PPT	Total (㎎/g)
Type	Rb1	Rb2	Rb3	Rc	Rd	Re	Rf	Rg1	Rg2	Rh1		
Control	0.27±0.02	0.11±0.01	0.02±0.00	0.12±0.01	0.10±0.01	0.33±0.03	0.17±0.02	0.16±0.02	0.07±0.01	0.01±0.00	0.80±0.04	1.31±0.05
IGR	0.29±0.03	0.10±0.02	0.02±0.00	0.13±0.02	0.09±0.02	0.35±0.03	0.18±0.02	0.17±0.02	0.07±0.01	0.01±0.00	0.79±0.05	1.34±0.06
Type(T) *p*-value	0.42	0.56	0.71	0.38	0.44	0.40	0.36	0.41	0.39	0.55	0.51	0.47
Weight(W) *p*-value	0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.002	< 0.001	< 0.001	0.004	0.12	0.09	< 0.001
T x W *p*-value	0.78	0.62	0.65	0.80	0.76	0.70	0.82	0.79	0.83	0.87	0.85	0.74

Values are adjusted to the overall mean root weight (≈1.1 g) by ANCOVA. IGR; *in vitro* grown roots derived from plant tissue culture.

DISCUSSION

For the practical application of micropropagation of new ginseng cultivars through plant tissue culture, high adaptability of tissue-cultured plantlets is a prerequisite. In the study of Choi *et al.* (1998), the survival rate of regenerated ginseng plantlets acclimatized to soil was only 36%. Kim *et al.* (2013a) reported that the survival rate in the field condition was limited to 46.7 – 53.3% when regenerated plantlets had a root length greater than 4 ㎝ or a weight above 0.4 g. Kim *et al.* (2016) proposed IGRs as a novel source for soil transplantation, and their high survival rate was later demonstrated by Lee *et al.* (2021). However, to date, no studies have documented the emergence and growth of acclimatized IGRs after transplantation under field conditions.

In the present study, acclimatized IGRs were classified according to root fresh weight and subsequently transplanted into the field, where the emergence rate was found to increase in proportion to initial root weight (Fig. 4, Table 1). In particular, the acclimated IGRs with a root weight greater than 1.4 g exhibited a very high emergence rate of 88.0%. This finding is in agreement with the report of Lee *et al.* (2023c), who demonstrated that the emergence rate increased with IGR weight prior to acclimatization in the greenhouse. Our findings provide the first empirical evidence that transplantation of acclimatized IGRs with greater root weight (> 1.4 g) is particularly effective in enhancing survival rates under field conditions.

In ginseng plants germinated from seeds, it is generally typical for young plants of 1 – 2 years of age to produce only a single stem (Lee *et al.*, 2021). In seed-derived ginseng plants, it is generally observed that individuals two years of age or younger produce only a single stem (Lee *et al.*, 2021). Consistent with this, in the present study the control group derived from one-year-old seedlings was predominantly exhibited the single-stem type (Table 1). In contrast, acclimatized IGRs showed a markedly higher proportion of multi-stemmed individuals, and this proportion increased progressively with root weight. Similarly, several studies have reported that ginseng plantlets derived from IGRs exhibit a high frequency of multi-stem type emergence (Kim *et al.*, 2016; Lee *et al.*, 2021). Given that the cultivar ‘Cheonryang’ used in this study is typically characterized by a single stem (Kim *et al.*, 2013b), the multi-stem type observed in the acclimatized IGRs may be attributed to alterations in endogenous plant hormone composition under *in vitro* conditions, which likely promoted the concurrent development of multiple dormant buds. Kwon *et al.* (2023) suggested that cytokinin serves as a key regulatory factor in bud initiation and the increase of stem number in *P. ginseng*. Further research is needed on quantitative profiling of endogenous cytokinins and other plant hormones in IGRs, along with the expression of associated genes.

Consistent with previous reports indicating that flowering in *P. ginseng* generally occurs only in plants older than three years (Kim *et al.*, 2016), no flowering was observed in the two-year-old control plants in the present study (Fig. 5). Interestingly, some acclimatized IGRs with an initial root weight exceeding 0.8 g exhibited flowering. Flowering has previously been reported in regenerated ginseng plants older than three years (Kim *et al.*, 2013b); however, our study is the first to demonstrate flowering in acclimatized IGRs during the same year of transplantation. Lee *et al.* (1990) reported the induction of flowering *in vitro* through ginseng tissue culture, attributing the response primarily to the action of 6-

benzyladenine (BA) and GA_3. In the present study, although flowers that bloomed from acclimatized IGRs in the year of transplantation did not produce seeds, they successfully yielded normal seeds in the subsequent year (data not shown). For successful seed production following flowering, sufficient nutrient reserves stored in the root are essential. Thus, it is plausible that acclimatized IGRs lacked sufficient storage capacity to support seed development in the transplantation year. If IGRs with a root weight of more than 2 g can be produced in the future, it is expected that seeds could be obtained immediately after field transplantation, and subsequent studies should be conducted to verify this possibility.

In the present study, the aerial parts of plants derived from acclimatized IGRs were consistently smaller than those of the control plants (Table 2). Consistent with these findings, a previous study reported that IGR-derived plants showed reduced stem length, stem diameter, leaf length, and leaf width compared with two-year-old seedlings (Lee *et al.*, 2021). This difference is attributable to the higher proportion of multi-stemmed plants among tissue-cultured individuals. Because multi stems arise from a single root, they grow competitively and consequently exhibit smaller size than single-stemmed plants (Lee, 1996).

The present study revealed that, among the acclimatized IGR treatments, higher root weight was consistently associated with enhanced growth traits (Table 2). Lee *et al.* (2023c) likewise reported that plant growth tended to improve in positive correlation with the weight of IGRs. Although size differences were observed, no distinct differences in leaf morphology were found between the control plants and those derived from acclimatized IGRs (Fig. 6). Ginseng plantlets produced through somatic embryogenesis have been reported to be genetically stable in previous studies (Lee *et al.*, 2021; Lee *et al.*, 2023b,c).

After the aerial parts had senesced and the underground parts were harvested, the roots of acclimatized IGRs weighing less than 1.4 g were found to have significantly lower weight and length than those of the control. In contrast, acclimatized IGRs weighing more than 1.4 g displayed root weight and length comparable to the control, while exhibiting a significantly greater root diameter (Table 3). Consistent with our findings, Lee *et al.* (2021) reported that the roots of acclimatized IGRs cultivated under greenhouse conditions were significantly thicker than those of conventionally grown ginseng. Moreover, the number of rhizomes was higher in acclimatized IGR treatments than in the control, and plants with greater root weight tended to develop more rhizomes. An increased rhizome number in *P. ginseng* plants derived from tissue culture has also been reported in previous studies, consistent with our observations (Kim *et al.*, 2016). This distinctive root morphology of acclimatized IGRs likely reflects the initial morphological differences between the IGRs used as transplanting material and the seed-derived control plants (Fig. 2). While the controls developed a long taproot with a single rhizome, the IGRs generated through somatic embryogenesis were characterized by a short, thick taproot with multiple rhizomes. A similar trend was observed even after one year of cultivation in the experimental field (Fig. 7), and this morphological tendency remained evident when the tissue-cultured plantlets were grown for up to three years, indicating that the trait is stably maintained over multiple cultivation years (Kim *et al.*, 2013a). It should also be emphasized that these morphological differences were evident even before transplantation, suggesting that they are intrinsic features of somatic embryogenesis-derived plantlets. Previous studies have consistently reported enhanced lateral root development and shortened primary root structure in IGRs compared with seed-derived seedlings (Lee *et al.*, 2021; Lee *et al.*, 2023a). Such traits may result not only from hormonal alterations during *in vitro* culture but also from differences in culture environments, including high sucrose concentrations, limited gas exchange, light conditions, and relative humidity, all of which are known to affect root development and acclimatization capacity (Kim *et al.*, 2023; Kim *et al*., 2025).

Ginsenosides are the primary bioactive constituents of *P. ginseng*. Approximately 300 ginsenosides have been identified in *Panax* species (Yang *et al.*, 2014), and these compounds are generally classified into two major groups: PPD and PPT. The predominant ginsenosides, such as Rb1, Rb2, and Rg1, exhibit mechanisms of action comparable to those of steroid hormones (Lee *et al.*, 2015). Various biological activities of ginsenosides have been continuously reported (Chung *et al.*, 2016; Shi *et al.*, 2019). To achieve large-scale micropropagation of new ginseng cultivars through plant tissue culture, it is therefore critical to confirm the stability of ginsenoside content in plants derived from tissue-cultured plantlets. In this study, acclimatized IGRs and seed-derived control plants that had been transplanted and cultivated for one year in the experimental field were compared for the contents of 10 ginsenosides, including Rb1 and Rb2 (Table 4). One-way ANOVA across

IGR weight groups initially revealed significant differences in several ginsenosides, suggesting that compositional variation might be associated with root size at transplantation. However, ANCOVA with root weight as a covariate demonstrated that these differences disappeared after statistical adjustment. No significant differences remained in the levels of individual ginsenosides, the total ginsenoside content, or the PPD/PPT ratio between IGRs and seed-derived controls. This indicates that the apparent variation among IGR subgroups was largely attributable to weight effects rather than inherent metabolic instability of tissue culture-derived plantlets. These findings are consistent with previous reports indicating that the ginsenoside content of acclimatized IGRs does not differ significantly from that of conventionally grown ginseng roots (Lee *et al.*, 2023b). Together, this provides further evidence that large-scale micropropagation of ginseng via tissue culture does not compromise the stability of its major bioactive constituents.

Through this study, it was demonstrated that acclimatized ginseng plantlets derived from plant tissue culture can stably emerge and flower under field conditions, while showing no compositional differences compared with seed-derived plants. Large-scale micropropagation of new ginseng cultivars via plant tissue culture is therefore expected to facilitate the rapid distribution of elite and stress-tolerant cultivars to farmers, thereby contributing to improved cultivation stability in *P. ginseng* production.

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