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Establishment of high frequency plant regeneration system from leaf explants of *Pinellia koreana* via bulblets formation

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Abstract *Pinellia koreana* K-H Tae & J-H Kim is a recently discovered Korea endemic medicinal plant species whose natural habitat is rapidly destroyed by industrial development. Described in this paper are culture conditions for high frequency plant regeneration via bulblet formation from leaf explant cultures of *P. koreana*. Leaf explants formed white nodular structures and off-white calluses at a frequency of 91.2% when cultured on MS medium supplemented with 2 mg/L BA and 0.5 mg/L NAA. However, the frequency of white nodular structures and off-white calluses formation was slightly decreased with an increasing concentration of NAA up to 4 mg/L, where the frequency reached 31.7%. Most petiole explants did not form white nodular structures and off-white calluses except the combination treatment of 2 mg/L BA and 2 mg/L NAA. Upon transfer onto MS basal medium, over 90% of nodular structures gave rise to numerous bulblets and developed into plantlets. Plantlets regenerated from bulblets were transplanted to potting soil and grown to maturity at a survival rate of over 95% in a growth chamber. Therefore, the *in vitro* plant regeneration system of *P. koreana* obtained in this study will be useful for mass propagation and long-term preservation of genetic resources of *P. koreana*.

Introduction

Pinellia koreana K-H Tae & J-H Kim is a new plant species discovered at Chrisan in Korea (Tae and Kim, 2005). This plant differs from other plants belonging to the same genus *Pinellia* by having 3-foliolate leaves with bilobed lateral leaflets at maturity. The genus *Pinellia* is herbaceous medicinal plants belonging to the Araceae. The chemical composition of *Pinellia* is not well established as there are only a few reports on the occurrence of small amounts of alkaloids ephedrine in the tuber. Also a glycoprotein fraction was reported to have notable antiemetic effects (Kurata et al. 1998). And root contains toxins (presence of calcium oxylate), that are destroyed by drying or cooking. *Pinellia* is mostly used for indigestion, nausea, vomiting, gastritis, and ulcer (Lee and Cho 1987; Bown 1995).

The genus of *Pinellia* consists of a few species in eastern Asia (Kitamura et al. 1980). *Pinellia* is propagated by tubers and bulbils. However, the yield and quality of tubers are gradually reduced over cultivation because of viral diseases. Therefore, introduction of a mass propagation system using *in vitro* culture technology such as development of virus-free strains and genetic manipulation of tubers is necessary to enhance the productivity of tubers. Despite its medicinal importance, few scientific studies of *P. koreana* have been reported. Only several *in vitro* culture studies have reported on the related species *P. ternata* including protoplast culture and plant regeneration (He et al. 1996), formation of micro-tubers and plantlet regeneration by thidiazuron (Yoo and Lim 1997), and clonal propagation by bulbils (Chen et al. 1989; Tsay et al. 1989).

Also natural habitats of *P. koreana* decreased gradually because of industrialization. Tissue culture techniques may provide an alternative mean for its mass multiplication and *ex-situ* conservation of genetic resources. This study describes culture conditions for high frequency plant regeneration via bulblet formation from leaf explants cultures of *P. koreana.*

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Materials and methods

Plant material

Mature plants of *P. Koreana* K-H Tae & J-H Kim were collected from Chrisan and cultivated in Kyoungnam province. Leaf and petiole explants were surface-sterilized, in 70% (v/v) ethanol for 1 min followed by incubation in 0.4% (v/v) sodium hypochlorite solution for 20 min with occasional agitation. They were then rinsed four times with sterile distilled water. Petioles (approximately 2-3 mm in length) and leaves (approximately 5×5 mm^2 in area) were dissected with a forceps and scalpel. Petiole and leaf explants were placed onto culture induction medium in plastic Petri dishes (87×15 mm).

Bulblet and callus formation

To examine the effect of NAA and BA molar ratio on callus and bulblet formation, several combination treatments of NAA and BA were conducted. Leaf and petiole explants were placed onto MS (Murashge and Skoog 1962) medium containing 2 mg/L NAA and several concentrations of BA (0, 0.5, 1, 2 and 4 mg/L). Each treatment consisted of 10 explants per dish with three replicates. Also, leaf explants were placed onto MS medium containing one of several concentrations of NAA (0, 0.1, 0.5, 1, 2, or 4 mg/L) in combination with 2 mg/L BA. The culture medium used throughout the experiments was consisted of MS inorganic salts, 100 mg/L myo-inositol, 0.4 mg/L thiamine·HCl, 3% (w/v) sucrose, 0.4% (w/v) Gelrite. The pH of all media was adjusted to 5.8 before autoclaving. Twenty-five ml of medium were dispensed into plastic Petri dishes (87×15 mm). Unless mentioned otherwise, all cultures were incubated at 25℃ in the dark. After eight weeks of culture, data for the mean percentage of explants producing white nodular structures was calculated for each treatment.

Plant regeneration

To regenerate whole plants, white nodular structures (1 to 2 mm in size) were collected, and ten nodular structures were plated onto MS basal medium in each Petri dish. After four weeks of culture in the light (30 μmol $m^{-2}s^{-1}$ from cool-white fluorescent lamps with a 16-h photoperiod), the plant regeneration frequency was determined by counting the number of regenerated plantlets in five Petri dishes. Plantlets developed from bulblets were transplanted to potting soil, and maintained in a growth chamber (25℃ day/22℃ night, 70 μmol $m^{-2}s^{-1}$ from cool-white fluorescent lamps with a 16-h photoperiod).

Results and discussion

An efficient in vitro culture system was established for high frequency plant regeneration via bulblet formation from leaf explant cultures of a new *Pinellia* species, *P. koreana* (Fig. 1). After two weeks of culture, off-white calluses began to form on the cut edge of leaf explants (Fig. 1B). After four weeks of culture, white globular structure and off-white calluses began to form on the cut edge of leaf explants. After additional four weeks of culture, off-white calluses proliferated and white, small globular structures were developed (Fig. 1C). When transferred to MS basal medium, these white globular structures developed into bulblets after four weeks of culture, indicating that the globular structures were an early stage of bulblets (Fig. 1D). These off-white calluses were subcultured on the same medium and globular structures were formed on the surface of callus after four weeks of culture. Subsequently, most bulblets developed into plantlets with normal roots without any treatments at a conversion rate of over 90% (Fig. 1E). A few of bulblets were expanded and differentiated into calluses. Plantlets

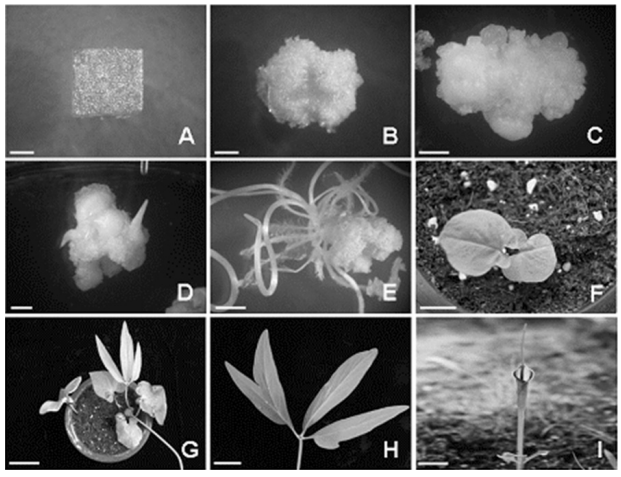

Figure 1. Plant regeneration from leaf explants of *P. koreana* via bulblets formation. A: Leaf explants; B: Off-white callus formation; C: White nodular structure formation; D: Bulblets formation from leaf-derived callus; E: Leaf and root development from bulblets; F: Regenerated plantlets transferred to soil; G: Morphological variation in leaf shape of regenerated plantlets; H: Morphological variation of plants after 2nd year cultivation; I: Flowering of mature plants. Scale bars represents 1 mm (A, B, C, D and E), 2 cm (F, H) and 5 cm (G, I), respectively

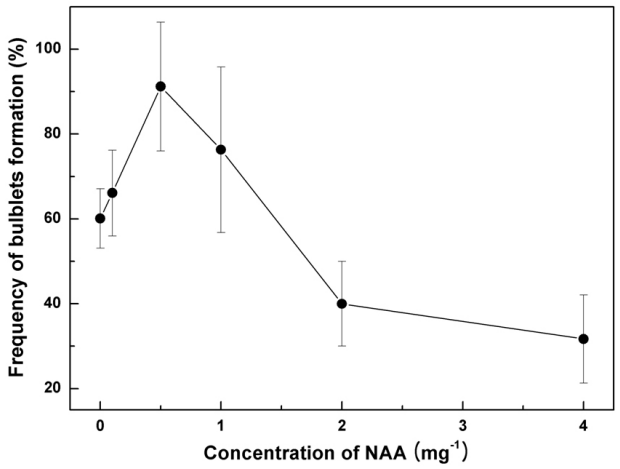

Figure 2. Effect of BA (2 mg/L) and concentrations of NAA on bulblets formation from leaf explants of *P. koreana*. Each treatment consisted of 20 explants with three replicates. Vertical bars represent standard deviations

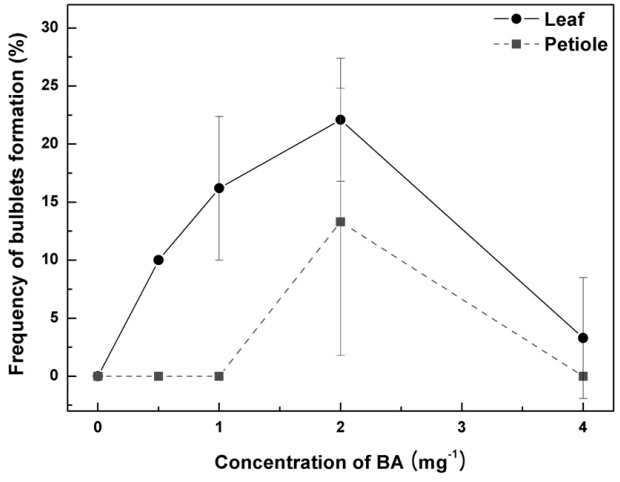

Figure 3. Effect of NAA (2 mg/L) and concentrations of BA on bulblets formation and callus formation from leaf (-●-) and petiole (-■-) explants of *P. koreana*. Each treatment consisted of 20 explants with three replicates. Vertical bars represent standard deviations

were successfully transplanted to potting soil and grown to normal plants at a survival rate of over 95% (Fig. 1F). Plantlets were successfully transplanted to potting soil and grown to maturity at a survival rate of over 95% (Fig. 1F). Regenerated plants showed numerous morphological variations in leaf shape at maturity (Fig. 1G). However we could not observed the typical characteristics in leaf shape and flowering of *P. koreana* after first year cultivation. After second year cultivation, a few regenerated plants showed 3-foliolate leaves with bilobed lateral leaflets (Fig. 1H). And they have grown to mature in the green house (Fig. 1I). Most studies of *in vitro* propagation of *Pinellia* species were achieved by micro-tuber formation (Chen et al. 1989; Tsay et al. 1989; Yoo and Lim 1997) and somatic embryogenesis (Kim et al., 2005). However, plant regeneration of *P. koreana* via bulblet formation was established in this study.

Leaf explants formed white nodular structures and off-white calluses at a frequency of 91.2% when cultured on MS medium supplemented with 2 mg/L BA and 0.5 mg/L NAA (Fig. 2). However, the frequency of white nodular structures and off-white calluses formation was slightly decreased with an increasing concentration of NAA up to 4 mg/L, where the frequency reached 31.7% (Fig. 2). In contrast, in the treatments of higher molar ratio of NAA over BA, the frequency of white nodular structures and off-white calluses formation from leaf explants was decreased and the highest frequency was 22.1% in MS medium supplemented with 2 mg/L NAA and 2 mg/L BA (Fig. 3). However, the frequency of white nodular structures and off-white calluses formation was sharply decreased with an increasing concentration of BA up to 4 mg/L, where the frequency reached 3.0% (Fig. 3). Most petiole explants did not form white nodular structures and off-white calluses except the combination treatment of 2 mg/L BA and 2 mg/L NAA. Considering these results, leaf was more optimal explants than petiole for bulblets formation of *P. koreana*. These results were similar to the report that leaf was more optimal for somatic embryogenesis of *P. tripartita* (Kim et al. 2005). However, in clonal propagation of *P. ternata*, there was no significant difference between leaf and petiole as original explants for bulbils formation (Tsay et al. 1989).

In this study, we established an *in vitro* plant regeneration system of *P. koreana* for the first time. Plant cell and molecular techniques would provide means for improving yield and quality of useful plant species. Therefore, the *in vitro* plant regeneration system of *P. koreana* obtained in this study will be useful for genetic manipulation, mass propagation, secondary metabolites production also for long-term preservation of genetic resources of the species.

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적 요

국내 신종 지리반하(*Pinellia koreana*)의 잎 절편배양으로부터 기내 소자구 형성을 통한 고효율 식물체 재생체계를 확립하였다. 잎 절편을 2 mg/L BA + 0.5 mg/L NAA 가 첨가된 MS 배지에 배양 시 소자구 및 백색 캘러스 형성빈도는 91.2%로 가장 높았다. NAA 처리 농도가 증가될수록 소자구 및 백색 캘러스 형성빈도는 감소하여 2 mg/L BA + 4 mg/L NAA 처리구에서는 그 빈도가 31.7%이었다. 한편 엽병 조직의 경우 2 mg/L BA + 2 mg/L NAA 처리구를 제외하고는 모든 처리구에서 소자구 형성이 이루어지지 않았다. 형성된 소자구는 MS 기본배지로 옮겨 배양한 결과 90% 이상이 식물체로 전환되었으며 이중 95% 이상이 토양순화에 성공하여 정상적인 식물체로 발달하였다. 본 연구를 통해 확립된 지리반하 식물체 재생체계는 신종 지리반하의 대량증식 및 기내 유전자원 보존 수단으로 활용될 수 있을 것으로 예상된다.

References

Bown D (1995) Encyclopaedia of Herbs and their Uses. Dorling Kindersley, London.

Chen CC, Gau TG, Tsay HS (1989) Studies on the callus formation and plant regeneration of *Pinellia ternata*. J Agric Res China 38:30-41

He Y, Zhu C, He M, hao S (1996) Protoplast culture and plant regeneration of *Pinellia ternata*. Plant Cell Reports 16:92-96

Kim SW, In DS, Tae KH, Liu JR (2005) High frequency plant regeneration from leaf-derived cell suspension cultures of *Pinellia tripartita* (Blume) Schott. Plant Cell Tiss Org Cult 80: 267-270

Kitamura S, Murata G, Koyama T (1980) Colored illustrations of herbaceous plants of Japan (Monocotyledon). Hoikusa Pub. Co., Tokyo pp196

Kurata K, Tai T, Yang Y, Kinoshita K, Koyama K, Takahashi K, Watanabe K, Nunoura Y (1998) Quantitative analysis of anti-emetic principle in the tubers of *Pinellia ternata* by enzyme immunoassay. Planta Medica 64:645-648

Lee BK, Cho TS (1987) Experimental studies on pharmalogical action of Banhahubagtang, a combined preparation of oriental medicine. Kor J Pharmacogn 18:14-25

Murashge T, Skoog F (1962) A reversed medium for rapid growth and bioassays with tabacco culture. Physiol Plant 15: 473-497

Tae KH, Kim JH (2005) *Pinellia koreana* (Araceae), a new species from Korea. Novon 15:484-487

Tsay HS, Gau TG, Chen CC (1989) Rapid clonal propagation of *Pinellia ternata* by tissue culture. Plant Cell Reports 8:450-454

Yoo CY, Lim HT (1997) Effect of thidiazuron on the formation of micro-tubers and plantlet regeneration of *Pinellia ternata*. Korean J Medicinal Crop 5:21-27

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