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Research Article

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Effect of silver nitrate and putrescine on *in vitro* shoot organogenesis of *Polygonum multiflorum*

Woo Tae Park[1†], Yeon Bok Kim[2†], Ramaraj Sathasivam[3], Haeng-Hoon Kim[4*], Sang Un Park[3*]

[1]Department of Herbal Crop Research, National Institute of Horticultural and Herbal Science, RDA, Eumseong 27709, Republic of Korea, [2]Department of Medicinal and Industrial Crops Korea National College of Agriculture and Fisheries, Jeonju 54874, Republic of Korea, [3]Department of Crop Science, Chungnam National University, 99 Daehak-Ro, Yuseong-Gu, Daejeon 34134, Korea, [4]Department of Agricultural Life Science, Sunchon National University, Suncheon, 57922, Korea
[†]contributed equally to this work

ABSTRACT

Polygonum multiflorum is a flowering plant that belongs to the family Polygonaceae and it is commonly used for medicinal and ornamental purposes. Few studies have been studied about the regeneration of this species. Therefore, we aimed to develop a suitable protocol for regeneration and subsequent growth of shoots by comparing the silver nitrate ($AgNO_3$) (ethylene inhibitor) and the putrescine (polyamine). Internode explants were cultured on shoot regeneration media (Murashige and Skoog (MS) media containing 2 mg L^{-1} of 6-benzylaminopurine). To analysis, the effect of $AgNO_3$ and putrescine on shoot regeneration and length, different concentrations (mg L^{-1}) of $AgNO_3$ (0, 1, 5, 7, 10, and 20) and putrescine (0, 10, 30, 50, 100, and 200) were added to the MS media. The result showed that at the highest concentration (20 mg L^{-1}) of $AgNO_3$ treatment decreased number of shoots (NOS) (1.4 ± 0.2 mm) and shoot length (9.7 ± 1.6 mm) was observed. Putrescine considerably increased the regeneration efficiency, NOS per explant, and shoot length in all the concentrations when compared to $AgNO_3$ treatment. Among the different concentrations, the highest NOS (2.52 ± 0.2 mm) was obtained in cultures supplemented with 30 mg L^{-1} putrescine, whereas the further increase in putrescine concentration reduced shoot regeneration. The longest shoots (20.5 ± 1.7 mm) were achieved in cultures supplemented with 200 mg L^{-1} putrescine. The findings of this study indicate that the addition of putrescine to the media could be suitable for *P. multiflorum* micropropagation and plant transformation.

KEYWORDS: *Polygonum multiflorum*, plant regeneration, silver nitrate, putrescine

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***Corresponding Authors:**
Sang Un Park
E-mail: supark@cnu.ac.kr
Haeng-Hoon Kim
E-mail: cryohkim@scnu.ac.kr

INTRODUCTION

The tuberous roots of *Polygonum multiflorum* are commonly used as a tonic, and also as a source of many traditional Chinese medicinal remedies (Bounda & Feng, 2015; Lei *et al*., 2015; Lin *et al*., 2015). This species also has a wide range of biological and medicinal properties, and is particularly used for its anti-hyperlipidemia (Xian *et al*., 2017), anti-inflammatory (Park *et al*., 2017), antidiabetic (Tang *et al*., 2017), neuro-protective (Lee *et al*., 2017), anti-aging (Ling & Xu, 2016), anti-metastatic (Lin *et al*., 2016), and antitumor (Zhu *et al*., 2016) properties. Moreover, in some Asian countries, this plant has been used as the source of a drug used to treat the premature graying of hair. In this regard, some published findings have indicated that optimal doses of *P. multiflorum* could be used as a potential agent for the treatment of premature graying and other pigmentation loss-related conditions (Sun *et al*., 2013; Han *et al*., 2015; Sextius *et al*., 2017; Thang *et al*., 2017).

The basis for the varied medicinal and therapeutic properties of *P. multiflorum*is the presence in this plant of many secondary metabolites, such as aloe-emodin, anthraquinones, chrysophanol, emodin, flavonoids, phenolic compounds, physcion, rhein, stilbenes, tannins, and derivatives (Lin *et al*., 2003; Yao *et al*., 2006; Yi *et al*., 2007; Kim *et al*., 2008).

From ancient times, the propagation of *P. multiflorum* has been practiced either by tuberous roots division or by sowing seeds. Most plant species are typically propagated through seed; however, for some species, including *P. multiflorum*, propagation by seed is hard due to the low germination rate and the delay in root harvesting. Taken these into consideration, this species is

conventionally propagated through the roots division (Shinju *et al.*, 1994).

Previously, there have been a few attempts to perform *in vitro* micropropagation of *P. multiflorum via* shoot organogenesis (Lin *et al.*, 2003). Considering the importance of this species, an efficient method for regeneration is essential, particularly with respect to establishing a micropropagation system and a genetic transformation procedure. In this study, we aimed to develop an improved method for plant regeneration and micropropagation of *P. multiflorum* from stem node cultures by using different concentrations of $AgNO_3$ and putrescine.

MATERIALS AND METHODS

Seed Sterilization and Germination

Seeds of *P. multiflorum* were purchased from Aram Seed Company, Seoul, Korea. Seeds were sterilized with ethanol (70%) for 30 s and then followed by sodium hypochlorite (2%) for 10 min. Then the seeds were further rinsed with sterilized water three times. Seven seeds were placed on the Petri dishes containing an agar-solidified culture medium. The MS medium consisted of (MS (Murashige & Skoog, 1962), basal salt, vitamin medium), the pH was adjusted to 5.8, and then 0.7% (w/v) agar was added. After that, the medium was autoclaved at 121°C for 20 min. All the chemicals used in this study were purchased from Sigma-Aldrich, St. Louis, MO, USA. In this study, all the experiment cultures were kept in a growth chamber at 25°C, under a 16-h photoperiod in a white fluorescent standard cool lamp with 35 µmol photons m^{-2} s^{-1} for 2 weeks.

In vitro Regeneration

Stem nodes of *P. multiflorum* were aseptically cut into pieces (~1 cm in size). After that, the explants were placed on Petri dishes containing MS medium. For regeneration of shoot from stem internodes, 0.5 mg L^{-1} of 6-benzylaminopurine (BAP) was added to the MS medium before sterilization. To analysis, the effect of $AgNO_3$ and putrescine on shoot regeneration, different concentrations (mg L^{-1}) of $AgNO_3$ (0, 1, 5, 7, 10, and 20) and putrescine (0, 10, 30, 70, 100, and 200) were added and grow it according to the above-mentioned conditions for 5 weeks.

Rooting of Regenerated Shoots

Regenerated shoots (~1 cm long) were placed in MS medium containing 3 g L^{-1} Gelrite and incubated in a growth chamber for 5 weeks as described above. The rooted plants were washed with sterile distilled water and then transfer to the plastic pots containing sterile vermiculite for 1 week. Then the plants were transferred to soil and maintained according to the above-mentioned conditions for 2 weeks and then transferred to the greenhouse.

Statistical Analysis

Data were expressed as the means ± standard deviation of 50 examined meristems. All data analysis was done by using Statistical Analysis System version 9.2 (SAS Institute Inc., Cary, NC, USA, 2009)

RESULTS

Effect of $AgNO_3$ and Putrescine on Shoot Regeneration and Length

In this study, we analyze the effect of various concentrations of $AgNO_3$ on the regeneration of *P. multiflorum*. Regeneration of explants from the internode initiated after 6 weeks of culture (WOC). It was observed that both shoot regeneration and growth (in terms of shoot length (SL)) were not enhanced, instead, it was inhibited. The number of shoots/explant and SL decreased with an increasing concentration of $AgNO_3$. The highest NOS (2.0) per explant and the highest SL (14.1 mm) were obtained under the control conditions (without $AgNO_3$). Within the treatment range of $AgNO_3$ concentrations, the lowest shoot number (1.4) and the shortest SL (9.7 mm) were observed at the highest concentration (20 mg L^{-1}) of $AgNO_3$ (Figure 1).

The response of *P. multiflorum* shoot regeneration on different concentrations of the putrescine was analyzed in the explants grown after 6 WOC. Significant increases in shoot regeneration and length were detected in the of the explant grown in media

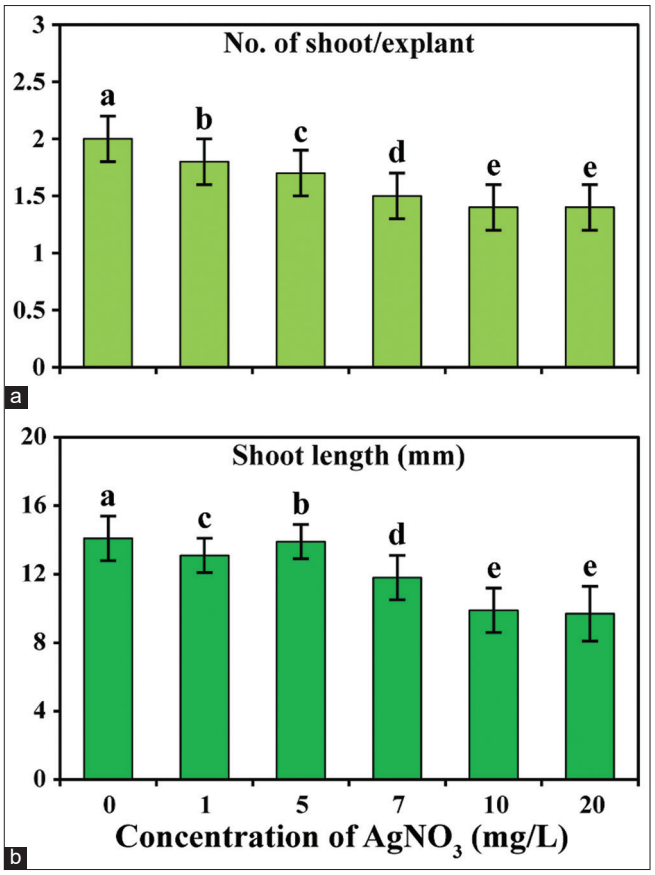

Figure 1: Effect of $AgNO_3$ on shoot regeneration and growth from stem internode explant of *Polygonum multiflorum* after 6 weeks culture on MS medium. Values represent the mean ± standard deviation of 50 shoots

supplemented with various concentrations of putrescine. With an increase in the concentration of putrescine, shoot regeneration increased up to a concentration of 30 mg L^{-1}, whereas at higher concentrations the shoot regeneration was decreased. The number of regenerated shoots initiated by the treatment ranged from 1.6 to 2.5, whereas in the control treatment it was 2.0. The highest shoot regeneration (2.5 shoots/explant) was achieved with the application of 30 mg L^{-1} putrescine, whereas the lowest shoot regeneration (1.6 shoots/explant) was obtained with the 200 mg L^{-1} putrescine treatment (Figure 2). Shoot growth in terms of SL was highly influenced by the concentrations of putrescine. An increasing linear trend was observed up to the highest concentration of putrescine (200 mg L^{-1}). The length of regenerated shoots ranged from 14.1 to 20.5 mm within the treatments. The highest SL (20.5 mm) was found in the 200 mg L^{-1} putrescine treatment, being 1.45 times longer than that observed in the control treatment.

DISCUSSION

Plant tissue culture is an alternative and most useful tool for the regeneration and micropropagation of many plants because of their specific requirements. To enhance the plant regeneration system, we examined the effects of different concentrations of $AgNO_3$ and putrescine on the shoot organogenesis efficiency in *P. multiforum*. In this study, treatment with $AgNO_3$ did not enhance the shoot regeneration or shoot growth, whereas putrescine treatment both regeneration and shoot growth were enhanced. In *Persicaria tinctoria* the shoot regeneration significantly increases in the MS medium containing 2 mg L^{-1} BAP, these results support the previous study result reported by (Park *et al.*, 2016). However, using the same concentration of BAP, does not significantly enhance the shoot development in *P. multiforum*, whereas together addition of the $AgNO_3$ improved the shoot regeneration and elongation.

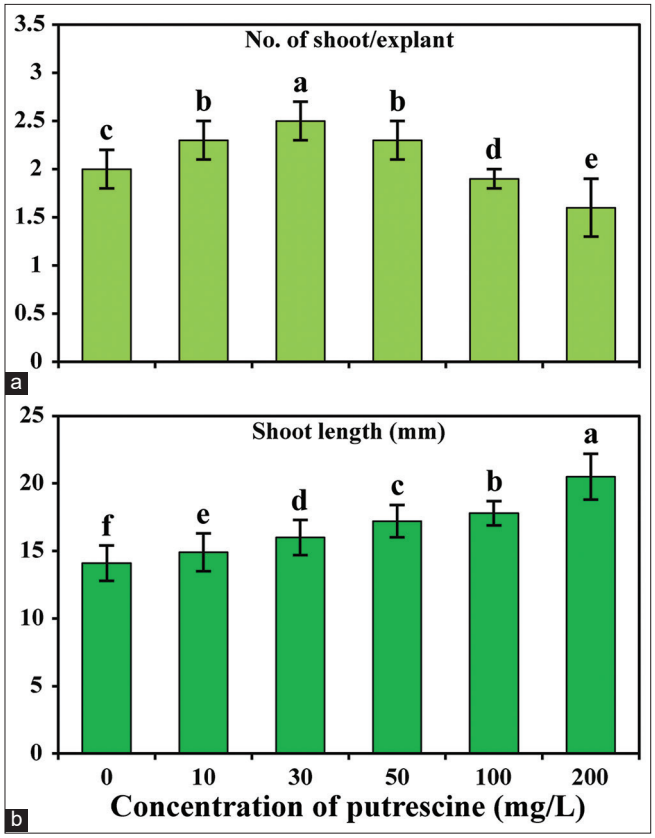

Figure 2: Effect of putrescine on shoot regeneration and growth from stem internode explant of *Polygonum multiflorum* after 6 weeks culture on MS medium. Values represent the mean ± standard deviation of 50 shoots

Previously, it has been shown that certain concentrations of $AgNO_3$ and BAP promote the growth of embryonic callus developed from root segments of date palm (Roshanfekrrad *et al.*, 2017). In addition, the $AgNO_3$ has been demonstrated to influence shoot bud formation and subsequent proliferation in *Vigna mungo* (Mookkan & Andy, 2014). In sesame, the transgenic shoots were recovered by using shoot induction MS medium containing 5.0 mg L^{-1} of $AgNO_3$. In addition, they have reported that in sesame, several factors have been found to be essential for regeneration and transformation, however, the most effective successful recovery of sesame shoots is based on the plant genotype and addition of $AgNO_3$ (Al-Shafeay *et al.*, 2011). The optimum medium for regeneration of rapeseed (*Brassica napus*) was found to be medium supplemented with 3, 0.15, and 5 mg L^{-1} of 6-BAP, 1-naphthaleneacetic acid, and $AgNO_3$, respectively, which leads to a considerable increase in the shoot regeneration (Uliaie *et al.*, 2008). The synergistic effect of 1 mg L^{-1} kinetin and 2 mg L^{-1} benzyl adenine (BA) has been shown to promote higher shoot regeneration efficiency (80.6%) than either kinetin or BA treatment alone in bottle gourd cotyledon explants without the addition of polyamines (PAs) or $AgNO_3$. In terms of regeneration, it has been observed that sensitivity to PAs and $AgNO_3$ is hormonal-dependent when shoots were rooted in ½ MS media containing 0.1 mg L^{-1} IAA (Shyamali & Hattori, 2007). An examination of the factors influencing consistent regeneration of shoot from leaf explants of *B. napus* L., showed that the addition of $AgNO_3$ to callus induction medium had a significant effect on shoot regeneration (Akasaka-Kennedy *et al.*, 2005). Arun *et al.* 2016 demonstrated that the inclusion of PAs in culture medium along with optimal concentrations of plant growth regulators has been shown to enhance shoot induction and elongation in soybean. The putrescine (62.08 µM) alone has been found to substantially enhance root induction (96.3%). Furthermore, rapid and efficient *in vitro* mass propagation of *Hybanthus enneaspermus* plants from leaf and node explants has been established for commercial utilization by employing different combinations and concentrations of plant growth regulators and PAs. After 8 WOC, the maximum NOS/leaf explant was obtained on MS medium containing 20, 4, and 1.5 mg L^{-1} of spermidine, BA, and IAA, respectively. After 5 WOC, the extended shoots were rooted (16 roots/shoot) in MS medium containing 1.5 mg L^{-1} IBA in combination with 20mg L^{-1} putrescine (Sivanandhan *et al.*, 2015).

CONCLUSION

Micropropagation techniques is one of the most vital methods in plant tissue culture techniques used for plant growth

development and induction of genetic transformation. Currently, shoot organogenesis is one of the most extensively used approaches for *in vitro* plant regeneration and transformation techniques. From this study, we found that this protocol can be effectively used to enhance the regeneration of a large number of plants, especially *P. multiflorum*. Although the $AgNO_3$ did not enhance shoot regeneration and subsequent shoot growth, the putrescine was found to promote both the shoot organogenesis and elongation frequency in this species. This finding can potentially provide basis information for the genetic improvement of *P. multiflorum*.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

WTP, YBK, RS, and HHK wrote the manuscript, performed the experiments, and analyzed the data. SUP designed the experiments and coordinated the implementation of research work. All authors read and approved the final version.

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