Journal of Phytology 2024, 16: 226-230
doi: 10.25081/jp.2024.v16.9346
https://updatepublishing.com/journal/index.php/jp

Research Article

ISSN: 2075-6240

Shoot organogenesis and plant regeneration of *Achyranthes japonica*

Ji Hyun Yoo*

Department of Oriental Medicine and Pharmaceutical Science, Joongbu University, 201 Daehak-ro, Chubu-myeon, Geumsan-gun, Chungcheongnam-do, 32713, Republic of Korea

ABSTRACT

This study focused on the *in vitro* plant regeneration and micropropagation of *Achyranthes japonica Nakai* by employing various cytokinins and auxins to determine their effects on shoot and root elongation. Seed sterilization was conducted utilizing a 70% solution of ethanol and subsequent sodium hypochlorite treatment. Seeds were germinated on Murashige and Skoog medium. Shoot regeneration was assessed by placing stem nodes, which were excised from the *in vitro* plants, on the supplemented medium. The effects of various cytokinin concentrations, BAP, kinetin, TDZ, and zeatin were analyzed. According to the results, TDZ at 2.0 mg/L demonstrated the highest number of effects of regeneration of shoots with minimal growth of 3.6±0.4 mm. At the same time, supplementation of kinetin without auxins leads to longer shoot length (17.2±2.1 mm). The addition of auxins such as IAA and IBA along with kinetin showed that the shoot length was increased than that of the control. The effects of $AgNO_3$ and putrescine on shoot regeneration were also analyzed. It was stated that $AgNO_3$ at 10 mg/L provided the most suitable shoot induction (3.8±0.49 shoots/explant). In addition, it is shown that among the various auxin concentrations, 0.1 mg/L IBA is the most suitable concentration for root regeneration (11.4±2.5 roots/shoot). The results imply that hormonal concentrations have significant effects on plant regeneration. Overall, these results identify the potential need for further research concerning the micro propagational techniques of *A. japinca* and similar plants.

Received: September 18, 2024
Revised: December 03, 2024
Accepted: December 09, 2024
Published: December 13, 2024

***Corresponding author:**
Ji Hyun Yoo
E-mail: jhyoo@joongbu.ac.kr

KEYWORDS: *Achyranthes japonica*, Growth media, Auxins, Shoot organogenesis, Plant regeneration

INTRODUCTION

Achyranthes japonica is a perennial member of Achyranthes genus in Amaranthaceae family which is widely distributed in East Asian countries including Korea, China, and Japan. In these countries, *A. japonica* is mainly used in traditional medicines or folk remedies (Park & Kim, 2020). It is used in Korea's traditional medicine to treat hypertension, rheumatism, osteoarthritis, and as an analgesic and diuretic. Moreover, both *in vitro* and *in vivo* experiments have demonstrated that the *A. japonica* extract has various physiological properties, including anti-allergic, anti-inflammatory, antioxidant, arthritis alleviation, hepato-protective, anti-osteoporosis and anti-cancer (Jung *et al.*, 2007; Kim & Park, 2010; Bang *et al.*, 2012; Jang *et al.*, 2012; Lee *et al.*, 2020, 2024; Eun *et al.*, 2021).

Organogenesis is a process by which shoots or roots are induced to differentiate from a cell or group of cells. In organogenesis, as it is generally practiced, a shoot is induced and developed in explant tissue and the shoot is then transferred to a new medium in which the induction and development of roots can occur (Kim *et al.*, 2023). They demonstrated that the ratio of the two kinds of hormones determines the plant organ formed from the classic experiments of (Skoog & Miller, 1957). They initiated shoot and root organogenesis in callus cultures of tobacco by varying the ratio of auxin and cytokinin supplied in the growth medium. Thus, high cytokinin-to-auxin ratios favor shoots while low ratios of cytokinin-to-auxin favor roots (Sathasivam *et al.*, 2021). In contrast, in another study, it has been reported that an equal concentration of the two phytohormones causes callus to proliferate (Pierik, 1997). Despite, the experiments conducted by Skoog and Miller, it becomes evident that the successful transformation of organs in many plant species is attributable to the establishment of the medium components, an appropriate explant, and the control of physical conditions (Brown & Thorpe, 1986).

Previous studies reported that accumulation of ethylene improved the efficiency of regeneration as well as production of plants having lower gene transfer capability when using *in vitro* micropropagation (Seong *et al.*, 2005). Shoot regeneration frequency can be enhanced when ethylene inhibitors are added to the shoot regeneration medium. In the study conducted by (Kumar *et al.*, 1998), the effect of ethylene inhibitors on shoot organogenesis was studied. Ethylene inhibitors have been added to the shoot regeneration medium in several studies (Chae *et al.*,

2012) that have added the inhibitors of ethylene to enhance the shoot organogenesis of *Gloxinia* (*Sinningia speciosa* Baill). It has been reported from other studies as well that ethylene inhibitors increase the efficiency of shoot organogenesis and plant regeneration (Kim *et al.*, 2016a, b; Hassan & Islam, 2021; Park *et al.*, 2022).

Rooting is the final culture stage before the acclimatization, is a central concept in the micropropagation system (Ismail *et al.*, 2011; Millán-Orozco *et al.*, 2011; Choi *et al.*, 2024). The survival of *in vitro* dependent plantlets in the field is dependent on a well-developed root system, which is useful to the plant when it comes to the absorption of water and nutrients from the soil (Benková & Bielach, 2010). Roots could be initiated through the exogenous application of natural or synthetic auxins (Osterc & Štampar, 2011).

This experiment was conducted to find a highly efficient method of shoot organogenesis from stem node explants of A. *japonica*. We established the most effective medium composition and application techniques of plant hormones, $AgNO_3$, and putrescine, as well as studied the rooting capacity of the regenerated shoots of A. *japonica* in the presence of different concentrations of auxins.

MATERIALS AND METHODS

Seed Sterilization and Germination

The seeds of *Achyranthes japonica* were purchased from an experimental farm at Chungnam National University (Daejeon, Korea). Seeds were then surface-sterilized for 30 sec in 70% (v/v) ethanol and for 15 min in 4.5% (v/v) sodium hypochlorite with a few drops of Tween 20. After that, they were thoroughly washed with sterilized distilled water several times under aseptic conditions. The seeds were then cultured on a solid basal medium for germination. The basal medium was a Murashige and Skoog's (Murashige & Skoog, 1962) medium including 3% sucrose and solidified using 0.7% plant agar. Before 0.7% plant agar was added the medium was titrated to pH 5.8. Make up the volume by adding distilled water and then vigorously stirring. The medium was then sterilized by autoclaving for 20 min at 121 °C. Ten seeds were placed on each Petri dish. The seeds were placed into a growth chamber at 25±1 °C under standard cool white, fluorescent tubes with a flux rate of 35 μmol s^{-1} m^{-2} for a 16 h photoperiod. The seeds germinated for 1 week and seedlings were transferred to magenta boxes containing the same MS medium for the next 3 weeks for an establishing plant material.

In Vitro Plant Regeneration

Segments of A. *japonica* stems, 2.0 cm in length with one node, were aseptically cut from plants, and cultivated *in vitro*. Explants were cultivated on a 20 mL appropriate medium in a petri dish. For shoot regeneration from stem internodes, MS medium was supplemented with 0, 0.5, 1, and 2 mg/L BAP, kinetin, thidiazuron (TDZ), and zeatin. For shoot regeneration, different concentrations of indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), and naphthaleneacetic Acid (NAA) (0, 0.1, 0.5, and 1.0 mg/L) in combination with 1 mg/L of kinetin were tested. Shoot organogenesis was also improved with differently concentrated ethylene inhibitor $AgNO_3$ in appropriate media. Cultures of all tested plants were maintained at 25±1 °C in a growth cabinet with a 16-h photoperiod under cool white, fluorescent tubes (35 μmol s^{-1} m^{-2}) for 6 weeks.

Rooting of Regenerated Shoots

The regenerated shoots were cultured on the respective medium in a magenta box. The medium was composed of the MS medium with 0, 0.1, 0.5, and 1.0 mg/L of IAA, IBA, and NAA for root regeneration from the regenerated shoot. The medium was solidified with 8 g/L of plant agar and dispensed 50 mL/culture vessel; four shoots were cultured at each culture vessel. The regenerated shoots were incubated at 25±1 °C in the growth chamber with a 16-h photoperiod under standard cool white, fluorescent tubes 35 μmol s^{-1} m^{-2} for four weeks. After four weeks the plants with shoots that were fully rooted were washed with sterile water to eliminate plant agar, transferred to pots, and prepared in high humidity by covering them with polyethylene bags for one week. They were then transferred to the soil and further incubated in the growth chamber for two weeks under a 16-h photoperiod, 18-20 °C night/day temperature. Thereafter the well-developed plants were allowed for hardening and maintained in the greenhouse for further works.

Statistical Analysis

The experimental data derived from the 50 tested stem nodes were expressed as the mean±standard deviation. The data were investigated by using Duncan's multiple range test (DMRT) at a significance threshold of $p<0.05$. The analysis was performed using SPSS software.

RESULTS

Figure 1a represents a developmental process of plant regeneration. The stem node explant of A. *japonica* was

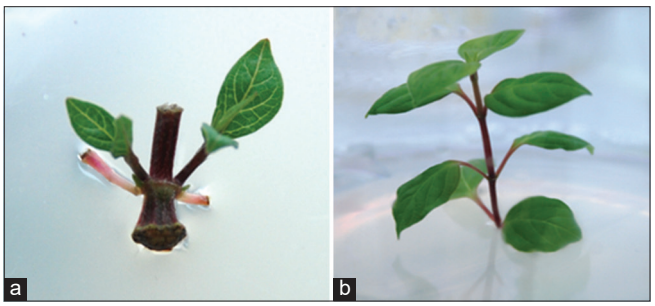

Figure 1: *In vitro* plant regeneration and micropropagation of *Achyranthes japonica*. a) Shoot primordia emerging from a stem node explant of *A. japonica* 3 weeks after cultivation on MS solid media supplemented with 1 mg/L Kinetin and after 6 weeks of culture, fully developed shoots were produced from the stem node culture and b) The rooted plant are in a culture vessel

cultured on Murashige and Skoog solid media with 1 mg/L of Kinetin, a cytokinin-promoting cell division, after 3 weeks of cultivation the shoot primordia arising from the explant. The initial organogenesis is where the cells start to differentiate into shoots under *in vitro* conditions. The formation of shoots indicates the successful sign of the stimuli of growth from the media and hormonal condition.

Figure 1b depicts the fully developed shoots that have been rooted into plantlets after 6 weeks of culture. Those shoots developed from the stem node explant that was cultured onto the media. The shoot appears to be well-rooted in the culture vessel with growing healthy leaves, showing successful rejuvenation and rooting to be able to grow independently.

Effect of Different Concentrations of Cytokinin on Shoot Regeneration and Growth

Six weeks after culturing the stem node explant of A. *japonica* in MS medium with different concentrations of BAP, Kinetin, TDZ, and zeatin, the shoots and growth were as follows shown in Table 1. The result showed that the control (without cytokinin) showed the lowest shoot induction, even the shoots were very stunted and restricted. TDZ appeared to have induced the highest shoot regeneration, 2.0 mg/L TDZ proved 3.5±0.3 shoots/explant, and the shoot length was 3.6±0.4 mm. Kinetin 1.0 mg/L also appeared to have induced the longest shoot 17.20±2.1 mm, but the shoots were very minimal 1.8±0.2/explant. Zeatin also showed the longest shoots, and it proved effective at 1 mg/L to 16.5±1.4 mm. But BAP induced moderate formation of the shoot but a much higher number of shoots 3.0±0.3/explant at 0.5 mg/L and the shoot length is less same in all the concentrations. But 0.5 and 1.0 mg/L of TDZ is more appropriate, since the shoots are more in number 8.6±0.8 mm and 6.2±0.7 mm, respectively, which indicates that it is also good for *rhizogenesis*.

Table 2 shows the combined effect of 1.0 mg/L kinetin with different concentrations of auxins, such as IAA, IBA, and NAA on shoot regeneration and growth from the stem node explants of A. *japonica* after 6 weeks of culture. In a control group, the presence of kinetin without any auxin showed that the shoot regeneration was 1.80±0.20/explant with a shoot length of 17.20±2.12 mm. With IAA, it was noticeable that the shoot number and shoot length increased gradually. The highest shoot length of 25.40±3.98 mm was observed with a moderate number of shoots per explant (2.20±0.21). In contrast, in IBA treatment the shoot length was highest only at 0.5 mg/L (29.40±4.84 mm) with a reasonable shoot per explant (2.00±0.22). The highest shoot regeneration was observed with NAA at 0.5 mg/L (2.90±0.33), whereas the shoot length was considerably decreased (18.80±3.12 mm). However, at 1.0 mg/L NAA concentration the explant showed reduced shoot regeneration (1.90±0.25) and shoot length (14.80±2.82 mm). Overall, the result showed that IAA and IBA were highly effective alternatives for increasing the shoot elongation, although IBA was slightly effective with the percentage shoot length. NAA is effective in shoot regeneration but with a moderate concentration, which is at 0.5 mg/L. From this result, it is shown that the choice of auxin and its concentration is effective in determining the number of shoots and the quality of shoots in terms of the short length.

Table 1: Effect of different concentrations of cytokinins on shoot regeneration and growth from stem node explant cultures of *A. japonica* after 6 weeks of culture

Cytokinin (mg/L)		Shoots per explants	Shoot length (mm)
BAP	0.0	1.3±0.1^{d}	4.8±0.4^{b}
	0.5	3.0±0.3^{a}	5.3±0.5^{b}
	1.0	2.3±0.2^{b}	5.6±0.6^{b}
	2.0	1.8±0.2^{c}	6.9±0.7^{a}
Kinetin	0.0	1.3±0.1^{b}	4.8±0.4^{b}
	0.5	1.8±0.2^{a}	16.9±1.5^{a}
	1.0	1.8±0.2^{a}	17.20±2.1^{a}
	2.0	1.5±0.2^{ab}	16.5±1.7^{a}
TDZ	0.0	1.3±0.1^{b}	4.8±0.4^{c}
	0.5	3.3±0.3^{a}	8.6±0.8^{a}
	1.0	3.3±0.4^{a}	6.2±0.7^{b}
	2.0	3.5±0.3^{a}	3.6±0.4^{d}
Zeatin	0.0	1.3±0.1^{b}	4.8±0.4^{d}
	0.5	2.8±0.3^{a}	12.5±1.1^{b}
	1.0	2.5±0.2^{a}	16.5±1.4^{a}
	2.0	2.8±0.3^{a}	9.8±1.1^{c}

Table 2: The combined effect of 1.0 mg/L kinetin with different concentrations of auxins on shoot regeneration and growth from stem node explant cultures of *A. japonica* after 6 weeks of culture

Kinetin 1.0	Auxin (mg/L)		Shoots per explant	Shoot length (mm)
	IAA	0.0	1.80±0.20^{b}	17.20±2.12^{b}
		0.1	1.80±0.14^{b}	20.10±3.41^{ab}
		0.5	2.10±0.19^{ab}	22.80±2.15^{ab}
		1.0	2.20±0.21^{a}	25.40±3.98^{a}
	IBA	0.0	1.80±0.20^{b}	17.20±2.12^{d}
		0.1	1.90±0.19^{b}	23.90±1.37^{c}
		0.5	2.00±0.22^{ab}	29.40±4.84^{a}
		1.0	2.30±0.16^{a}	26.60±4.25^{b}
	NAA	0.0	1.80±0.20^{b}	17.20±2.12^{b}
		0.1	2.30±0.32^{b}	26.30±4.11^{a}
		0.5	2.90±0.33^{a}	18.80±3.12^{b}
		1.0	1.90±0.25^{b}	14.80±2.82^{b}

The addition of $AgNO_3$ to the culture medium had a concentration-dependent effect on shoot regeneration and shoot length. Without $AgNO_3$ addition (control), only 2.90±0.33 shoots per explant and 18.80±3.12 mm shoot length formed (Table 3). The highest shoot regeneration (3.80±0.49 shoots per explant) was recorded at 10 mg/L $AgNO_3$ with 28.20±2.78 mm length. In addition, in 20 mg/L $AgNO_3$ treatment, the result showed that the lowest shoots per explant (2.40±0.17) and shoot length (17.20±3.22 mm) were observed.

Similarly, putrescine also had a positive effect on shoot regeneration and growth. The regeneration was only 2.90±0.33 shoots per explant and the shoot length was 18.80±3.12 mm. The best result was recorded at 50 mg/L putrescine from the callus, the regeneration was 3.50±0.36 shoots per explant with a shoot length of 23.90±3.00 mm. The shoots per explants (3.30±0.42) and shoot length (25.10±5.30 mm) were slightly lower at 100 mg/L putrescene. At the highest concentration (200 mg/L putrescine),

Table 3: The combined effect of Kinetin 1 mg/L, NAA 0.5 mg/L, and different concentrations of $AgNO_3$ and putrescine on the shoot regeneration and growth from stem node explant cultures of *A. japonica* after 6 weeks of culture

Hormone (mg/L)		Shoots per explants	Shoot length (mm)
$AgNO_3$	0	2.90±0.33^{c}	18.80±3.12^{b}
	1	2.70±0.27^{c}	19.10±3.07^{b}
	3	3.10±0.43^{bc}	22.60±2.12^{ab}
	5	3.60±0.42^{ab}	28.20±2.78^{a}
	10	3.80±0.49^{a}	27.20±4.37^{b}
	20	2.40±0.17^{c}	17.20±3.22^{b}
Putrescine	0	2.90±0.33^{ab}	18.80±3.12^{a}
	10	3.00±0.27^{ab}	18.60±2.41^{a}
	30	2.80±0.26^{b}	19.50±3.75^{a}
	50	3.50±0.36^{a}	23.90±3.00^{a}
	100	3.30±0.42^{ab}	25.10±5.30^{a}
	200	3.10±0.33^{ab}	20.60±2.12^{a}

Table 4: Effect of different concentrations of auxins on root regeneration and growth from the regenerated shoot of *A. japonica* after 4 weeks of culture

Cytokinin (mg/L)		No. of root/shoot	Root length (mm)
IAA	0.0	4.3±0.4^{b}	3.1±0.2^{b}
	0.1	10.4±1.1^{a}	4.1±0.5^{a}
	0.5	3.5±0.4^{b}	4.3±0.4^{a}
	1.0	3.1±0.3^{b}	3.2±0.2^{b}
IBA	0.0	4.3±0.4^{b}	3.1±0.2^{b}
	0.1	11.4±2.5^{a}	4.5±1.2^{a}
	0.5	6.7±1.9^{b}	2.1±0.6^{bc}
	1.0	4.5±1.1^{b}	1.2±0.4^{c}
NAA	0.0	4.3±0.4^{a}	3.1±0.2^{a}
	0.1	3.2±0.2^{b}	1.1±0.2^{b}
	0.5	1.4±0.1^{c}	0.5±0.1^{c}
	1.0	ND	ND

ND = Not Detected

shoot per explant and shoot length showed a marked decrease with the value of 3.10±0.33 and 20.60±2.12 mm, respectively. In general, $AgNO_3$ had a more definite effect on shoot number and length with its optimum concentrations (5 and 10 mg/L) yielding higher values than putrescine. Though putrescine also enhanced shoot regeneration and growth at 50-100 mg/L, it was slightly lower than $AgNO_3$ in terms of shoot length and shoot number. Both have an adverse effect in higher concentrations.

Table 4 presents the effects of different concentrations of the auxins: IAA, IBA, and NAA on root regeneration and growth of regenerated shoots of A. *japonica* after 4 weeks of culture. The control or the treatment without auxin had the lowest root formation, with 4.3±0.4 roots per shoot and the shortest root length of 3.1±0.2 mm. Among the auxins, IBA at 0.1 mg/L had the highest or most effective root induction, with 11.4±2.5 roots per shoot and a relatively long root length of 4.5±1.2 mm, which indicates that this concentration is most effective for root regeneration. Similarly, at 0.1 mg/L IAA, also showed the highest number of roots, with 10.4±1.1 number of root/shoot with the root length of 4.1±0.5 mm, which was slightly lower than that of the 0.1 mg/L IBA. However, at higher concentrations, both IAA and IBA showed a reduced number of root/shoot and root length, with 0.5 mg/L IAA producing only 2.3±0.1 roots/shoot with a root length of 2.5±0.1 mm. Among the different concentrations of IBA treatment, 1.0 mg/L produced the lowest numbers of roots and root length. However, in all NAA concentrations, root length was the shortest, and the number of roots decreased. At 0.1 mg/L NAA produced a 3.2±0.2 roots/shoot with a root length of only 1.1±0.2 mm. In the case of 0.5 mg/L of NAA, the number and length of roots significantly decreased. At the highest NAA concentration, we observed none of the root production, which indicates that a higher concentration of NAA might inhibit the root regeneration in A. *japonica*. From this result, it is shown that 0.1 mg/L IBA was the most effective auxin concentration for inducing root formation in A. *japonica*.

DISCUSSION

The results of this research confirmed knowledge that was already known, for instance, the need for plant growth regulators in the *in vitro* regeneration of A. *japonica*. In particular, thidiazuron was identified as the most effective cytokinin for the generation of shoots, and its optimal concentration was found to be 2.0 mg/L. The mean value was determined to be 3.5±0.3 shoots per explant, however, the callescences also led to reduced shoot length, which, in this case, a total of 3.6±0.4 (Chae & Park, 2012). The trend of decreasing average shoot length with high TDZ concentrations is consistent with the findings of a previous study, in which it was found that though TDZ enhances the number of shoots, it, however, inhibits elongation. When the amounts of concentration were decreased to 0.5 mg/L, the average shoot length increased by approximately 2, signifying a better minimum limit for shoot elongation (Park *et al.*, 2015).

Consistent with previous works, the research has shown the crucial role of $AgNO_3$ which is the ethylene inhibitor. The concentration of 10 mg/L was optimal, as it allowed these shoots per explant, and the effectiveness of the ethylene inhibitor in such concentrations was estimated at 3.8±0.49. Overall, $AgNO_3$ can be viewed as an effective substance to facilitate growth disregarding the inhibitory potential of ethylene (Chae & Park, 2012; Park *et al.*, 2022). $AgNO_3$'s ethylene-inhibiting properties have been well-documented in similar studies involving *Polygonum multiflorum* and *Aloe saponaria*, which showed improved shoot regeneration efficiency when $AgNO_3$ was applied (Kim *et al.*, 2016b; Park *et al.*, 2022). This result was consistent with our study result that $AgNO_3$ increased the shoot regeneration in A. *japonica*.

IBA was the best auxin at the rooting phase, it gave on average 11.4±2.5 roots per shoot at a concentration of 0.1 mg/L. This finding is in agreement with the results of other research efforts, where IBA was one of the most effective auxins in terms of stimulating root induction in various plant species, especially at lower concentrations (Chae *et al.*, 2012; Kim *et al.*, 2023). In addition, the data indicated that when the concentration of IAA-IBA was above optimal, there were fewer roots. This implies that the increased level of auxins becomes inhibitory to the formation of roots (Kim *et al.*, 2023). There is a consensus in the literature that rooting is a delicate balance and also with regard to the effect of auxins (Chae *et al.*, 2012). This finding

was expected because the appropriate cytokinin to auxin ratio is imperative for abundant shoots and developing a proper root system. It is believed that more research will concentrate on the elucidation of the mechanisms that lie in the interaction between the plant growth regulators with many interesting outcomes for other widely used plants (Park *et al*., 2022).

CONCLUSION

It can be concluded that the optimization of cytokinins, auxins, $AgNO_3$, and putrescine was essential for the effective *in vitro* regeneration of A. *japonica*. In particular, TDZ was the most potent cytokinin responsible for shoot generation, whereas kinetin was the maximum efficient agent to support the elongation of the shoots. In addition, IAA and IBA should be considered as auxins that promote the formation of the roots, while IBA was more efficient in this case. Both shoot number and its length were better promoted by $AgNO_3$ compared to putrescine. In such a way, the efficiency of micropropagation in A. *japonica* was determined by the combination and homework of hormones, and this work can support further studies in the area.

REFERENCES

Bang, S. Y., Kim, J.-H., Kim, H.-Y., Lee, Y. J., Park, S. Y., Lee, S. J., & ssssseH. (2012). *Achyranthes japonica* exhibits anti-inflammatory effect via NF-κB suppression and HO-1 induction in macrophages. *Journal of Ethnopharmacology, 144*(1), 109-117. https://doi.org/10.1016/j.jep.2012.08.037

Benková, E., & Bielach, A. (2010). Lateral root organogenesis—from cell to organ. *Current Opinion in Plant Biology, 13*(6), 677-683. https://doi.org/10.1016/j.pbi.2010.09.006

Brown, D. C., & Thorpe, T. A. (1986). Plant regeneration by organogenesis. In I. K. Vasil (Eds.), *Cell Culture and Somatic Cell Genetics of Plants* (Vol. 3, pp. 49-65), Orlando, Florida: Academic Press.

Chae, S. C., & Park, S. U. (2012). Improved shoot organogenesis of *Echinacea angustifolia* DC treated with ethylene inhibitors. *Life Science Journal, 9*(4), 1725-1728.

Chae, S. C., Kim, H. H., & Park, S. U. (2012). Ethylene inhibitors enhance shoot organogenesis of gloxinia (*Sinningia speciosa*). *The Scientific World Journal, 2012*, 859381. https://doi.org/10.1100/2012/859381

Choi, M., Kim, H.-H., Seo, H., Sathasivam, R., Kim, J. K., & Park, S. U. (2024). Influence of medium and gelling agents concentration on *in vitro* rooting of *Polygonum tinctorium*. *Journal of Phytology, 16*, 8-12. https://doi.org/10.25081/jp.2024.v16.8793

Eun, S. Y., Cheon, Y.-H., Park, G. D., Chung, C. H., Lee, C. H., Kim, J.-Y., & Lee, M. S. (2021). Anti-osteoporosis effects of the *Eleutherococcus senticosus*, *Achyranthes japonica*, and *Atractylodes japonica* mixed extract fermented with nuruk. *Nutrients, 13*(11), 3904. https://doi.org/10.3390/nu13113904

Hassan, M. F., & Islam, S. M. S. (2021). Effect of silver nitrate and growth regulators to enhance anther culture response in wheat (*Triticum aestivum* L.). *Heliyon, 7*(5), e07075. https://doi.org/10.1016/j.heliyon.2021.e07075

Ismail, R. M., Elazab, H. E. M., Hussein, G. M. H., & Metry, E. A. (2011). *In vitro* root induction of faba bean (*Vicia faba* L.). *GM crops, 2*(3), 176-181. https://doi.org/10.4161/gmcr.2.3.18003

Jang, G. Y., Kim, H. Y., Lee, S. H., Kang, Y. R., Hwang, I. G., Woo, K. S., Kang, T. S., Lee, J. S., & Jeong, H. S. (2012). Effects of heat treatment and extraction method on antioxidant activity of several medicinal plants. *Journal of the Korean Society of Food Science and Nutrition, 41*(7), 914-920. https://doi.org/10.3746/jkfn.2012.41.7.914

Jung, S.-M., Choi, S.-I., Park, S.-M., & Heo, T.-R. (2007). Antimicrobial effect of *Achyranthes japonica* Nakai extracts against *Clostridium difficile*. *Korean Journal of Food Science and Technology, 39*(5), 564-568.

Kim, C.-S., & Park, Y.-K. (2010). The therapeutic effect of *Achyranthis radix* on the collagen-induced arthritis in mice. *The Korea Journal of Herbology, 25*(4), 129-135.

Kim, J. K., Baskar, T. B., & Park, S. U. (2016a). Effect of carbon sources and sucrose concentrations on shoot organogenesis of *Aloe saponaria*. *Biosciences Biotechnology Research Asia, 13*(2), 925-930. https://doi.org/10.13005/bbra/2116

Kim, J. K., Baskar, T. B., & Park, S. U. (2016b). Silver nitrate and putrescine enhance invitro shoot organogenesis in *Polygonum tinctorium*. *Biosciences Biotechnology Research Asia, 13*(1), 53-58. https://doi.org/10.13005/bbra/2002

Kim, Y. C., Park, W. T., Sathasivam, R., Kim, H.-H., Kim, J. K., & Park, S. U. (2023). Effect of media and gelling agents on shoot organogenesis of *Liriope platyphylla*. *Journal of Phytology, 15*, 52-56. https://doi.org/10.25081/jp.2023.v15.8319

Kumar, P. P., Lakshmanan, P., & Thorpe, T. A. (1998). Regulation of morphogenesis in plant tissue culture by ethylene. *In Vitro Cellular and Developmental Biology-Plant, 34*, 94-103. https://doi.org/10.1007/BF02822771

Lee, D.-H., Han, J. W., Park, H., Hong, S. J., Kim, C.-S., Kim, Y. S., Lee, I. S., & Kim, G. J. (2024). *Achyranthis radix* extract enhances antioxidant effect of placenta-derived mesenchymal stem cell on injured human ocular cells. *Cells, 13*(14), 1229. https://doi.org/10.3390/cells13141229

Lee, G.-H., Hwang, K.-A., Kang, J.-H., & Choi, K.-C. (2020). Effect of *Achyranthes japonica* Nakai extract on immunity and anti-inflammation in dogs. *Canadian Journal of Veterinary Research, 84*(4), 294-301.

Millán-Orozco, L., Corredoira, E., & San José, M. d. C. (2011). *In vitro* rhizogenesis: histoanatomy of *Cedrela odorata* (Meliaceae) microcuttings. *Revista de Biología Tropical, 59*(1), 447-453. https://doi.org/10.15517/rbt.v59i1.3211

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum, 15*(3), 473-497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

Osterc, G., & Štampar, F. (2011). Differences in endo/exogenous auxin profile in cuttings of different physiological ages. *Journal of Plant Physiology, 168*(17), 2088-2092. https://doi.org/10.1016/j.jplph.2011.06.016

Park, J. H., & Kim, I. H. (2020). Effects of dietary *Achyranthes japonica* extract supplementation on the growth performance, total tract digestibility, cecal microflora, excreta noxious gas emission, and meat quality of broiler chickens. *Poultry Science, 99*(1), 463-470. https://doi.org/10.3382/ps/pez533

Park, W. T., Kim, Y. B., Sathasivam, R., Kim, H.-H., & Park, S. U. (2022). Effect of silver nitrate and putrescine on *in vitro* shoot organogenesis of *Polygonum multiflorum*. *Journal of Phytology, 14*, 36-40. https://doi.org/10.25081/jp.2022.v14.7593

Park, W. T., Park, S. U., Yeo, S. K., Uddin, M. R., & Kim, H. H. (2015). An efficient protocol for shoot organogenesis and plant regeneration of *Polygonum multiflorum*. *Biosciences Biotechnology Research Asia, 12*(2), 1075-1079.

Pierik, R. L. M. (1997). *In vitro culture of higher plants*. Dordrecht, Netherlands: Springer Publishing. https://doi.org/10.1007/978-94-011-5750-6

Sathasivam, R., Kim, M. C., Chung, Y. S., & Park, S. U. (2021). Effect of cytokinins on growth and phenylpropanoid accumulation in Tartary buckwheat sprouts (*Fagopyrum esculentum* Moench). *Journal of Aridland Agriculture, 7*, 89-94. https://doi.org/10.25081/jaa.2021.v7.7198

Seong, E. S., Song, K. J., Jegal, S., Yu, C. Y., & Chung, I. M. (2005). Silver nitrate and aminoethoxyvinylglycine affect *Agrobacterium*-mediated apple transformation. *Plant Growth Regulation, 45*, 75-82. https://doi.org/10.1007/s10725-004-6126-y

Skoog, F., & Miller, C. (1957). Chemical regulation of growth and organ formation in plant tissue cultured *in vitro*. *Symposia of the Society for Experimental Biology, 11*, 118-131.