DOI: https://doi.org/10.59294/HIUJS.VOL.4.2023.391

Effects of plant growth regulators and basal media on *Atractylodes macrocephala* Koidz.'s shoot multiplication

Nguyen Thanh Tai*, Phan Thuy Quyen, Pham Thu Nhi, Pham Quang Thang and Le Thanh Hung
Research and Development Center for High Technology Agriculture

ABSTRACT
Atractylodes macrocephala Koidz. belonging to the genus Atractylodes is a high-value medical plant with more than 79 phytochemical components. However, few studies about the miropropagation protocol of this species are conducted in Vietnam. The purpose of this study is to investigate the effects of some PGRs and basal media on Atractylodes macrocephala Koidz's in vitro shoot multiplication viewed as the most critical stage in the miropropagation protocol. In this study, stem nodes with dorminant shoots were cultured on different media after selecting an optimized medium from experiments supplemented with BA in combination with IBA with different concentrations. After four weeks of culture, the highest number of shoots on MS medium containing 1 mg/L BA in combination with 0.3 mg/L IBA was 2.18 shoots/explant. Interestingly, the optimized medium for AM's in vitro shoot proliferation was $^{1/2}$MS medium supplemented with 1 mg/L BA in combination with 0.3 mg/L IBA. The highest number of shoots reached at 14.5 shoots/explant being more than 4 times compared to the results of the previous studies. The result significantly contributes to the efficient micropropagation of Atractylodes macrocephala Koidz. for comercial production.

***Keywords**: Atractylodes macrocephala* Koidz., plant growth regulators, shoot multiplication

1. INTRODUCTION

Atractylodes macrocephala Koidz. is a valuable medicinal plant widely distributed in Southeast Asia, including China, Korea and Japan. Its rhizome called “Baizhu” in traditional herbal medicine contains more than 79 chemical compounds like sesquiterpenoids, triterpenoids, polyacetylenes, and flavonoids with a range of biological activities [1]. Especially, volatile oil such as atractylon and atractylodin accounts for about 1.4% [2]. The role of *Atractylodes macrocephala* (AM) in improving gastrointestinal function is investigated thanks to the accumulation of abundant polysaccharides and atractylenolides in AM rhizomes [3]. In addition, other various pharmacological activities including anti-tumor activity, immunomodulatory effects, anti-inflammatory activity and anti-oxidative activity are assessed in numerous studies [1, 4, 5]. In Vietnam, AM rhizomes are mainly imported from China, and cultivating this species is partly limited since *Atractylodes macrocephala* only grows rapidly in the cool climate regions. As a result, the demand for commercial production of AM is enormously increasing.

Practically, the AM's conventional propagation is rather low because of the poor germination of the seeds. Therefore, the establishment of an *in vitro* micropropagation protocol is viewed as an efficient approach to propagate the rapid mass propagation of *Atractylodes macrocephala* varieties for commercial production. Although there are some studies conducted all over the world [6 - 8], few studies are carried out in Vietnam, except for the study of Nguyen Manh Dung [9]. However, the above studies focus on the influences of PGRs on AM's shoot multiplication in only MS (Murashige & Skoog) medium. Other basal media such as Gamborg's B5, VW (Vacin and Went) and SH (Schenk & Hildebrandt) have not investigated yet so far. Chemical ingredients in basal media play a critical role in the development of cells in plant tissue culture, so selecting an adequate medium in each stage of *in vitro* micropropagation protocol is absolutely necessary [10]. Following the above

Corresponding Author: Nguyen Thanh Tai
Email: thanhtai2407@gmail.com

reasons, this study is carried out to explore the influences of PGRs and basal media on *Atractylodes macrocephala*'s shoot proliferation to increase a large number of *in vitro* shoots for the *in vitro* micropropagation protocol.

2. MATERIALS AND METHODS

Plant materials

Explant source

Explants of this study are 3-month-old seedlings bought from Xuyen Viet Corporation Company at Lao Cai Province.

Explant sterilization

Stem nodes in length 2 cm excised from the above seedlings were sterilized with comercial bleach containing 5% sodium hypochlorite diluted in water in 1:1(v/v) within 5 minutes in the first step. And then, these explants were continuously treated in comercial bleach containing 5% sodium hypochlorite diluted in water in 1:5 (v/v) in 10 minutes before rising with sterilized water in 6 times.

Shoot initiation medium

The above aseptic nodes were cultured in MS medium supplemented with 0.5 mg/L 6-benzylaminopurine) (BA) (30 g/L sucrose, and 8 g/L agar (Figure 1).

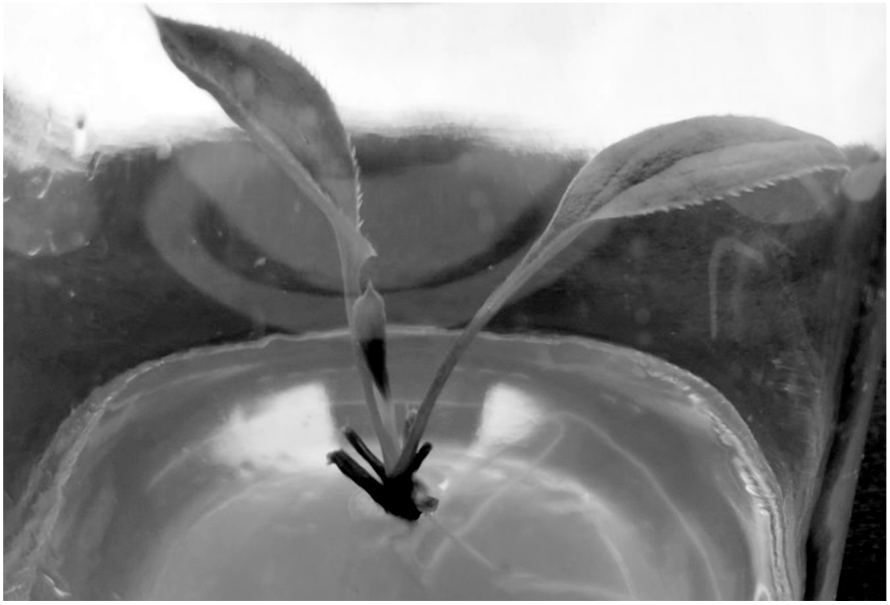

Figure 1. The shoots developing from stem nodes after 4 weeks of culture

Plant material

Nodal segments with the length from 2 to 3 cm are excised from the *in vitro* shoots after 4 weeks of culture.

Cultural conditions

All the explants were incubated in the growth room under conditions, including temperature 25 ± 2^{0}C, relative humidity 60 ± 5%, and a 12-h photoperiod under a photosynthetic photon flux density of 40 ± 5 µmol.m^2 s^1.

Methods

Investigation of the effects of 6-benzylaminopurine (BA) in combination with Indole-3-Butyric Acid (IBA) on *in vitro* shoot multiplication of *Atractylodes macrocephala* Koidz.

4-week-old nodal segments containing dorminant shoots were cultured in MS medium supplemented with BA at different concentrations (0.5; 1; 1.5; 2 mg/L) in combination with IBA at two different concentrations (0.3 and 0,5 mg/L), 30 g/L sucrose, and 8 g/L agar. The experiment design was a completely randomized design (CRD) including 9 treatments; one factor with 3 replications, each repeated 3 flasks, 3 explants/flask.

The number of shoots per explant was recorded after 4 weeks of culture.

Investigation of effects of basal media on *in vitro* shoot multiplication of *Atractylodes macrocephala* Koidz.

Nodal segments containing dorminant shoots excised from single shoots isolated from the shoots in the above experiment after 8 weeks of culture (Figure 2). These explants were cultured in different

basal media, including MS (Musharige Skoog), $^{1/2}$MS (a half of all nutrients), MS$^{1/2}$ (a half of macro-nutrients, VW (Vacin and Went), and Gamborg's B5 supplemented with the optimized concentration of BA in combination with IBA from the above experiment, 30 g/L sucrose, and 8 g/Lagar.

The experiment design was a completely randomized design (CRD) including 5 treatments; one factor with 3 replications, each repeated 3 flasks, 5 explants/flask.

The number of shoots per explant was recorded after 4 weeks of culture.

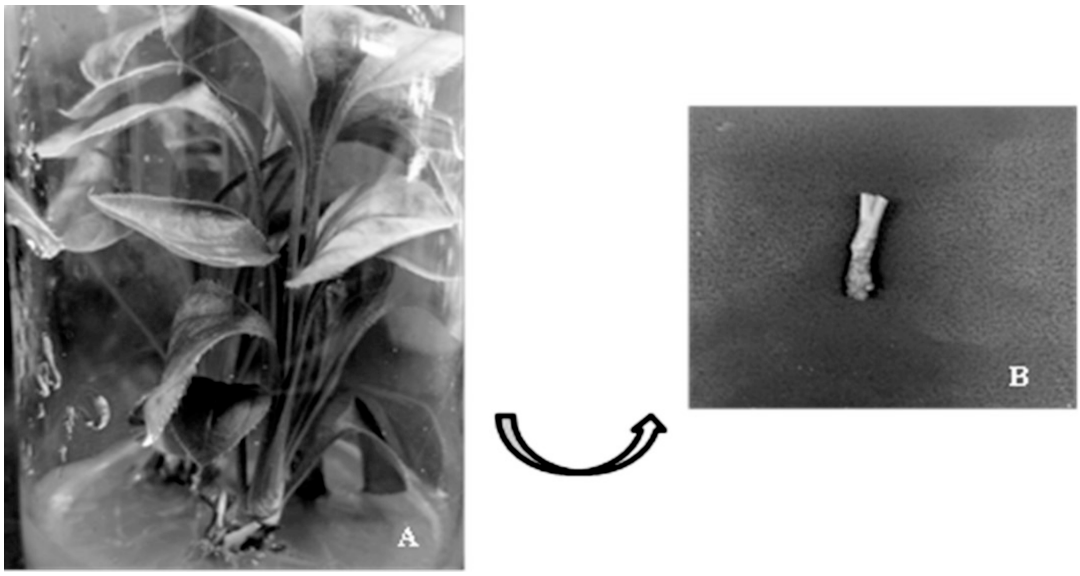

Figure 2. (A) The shoots after 8 weeks of culture; (B) A nodal segment excised from a single shoot

Statistical analysis

All data were tested using a one-way analysis of variance (ANOVA) by SAS version 9.2.

3. RESULTS AND DISCUSSION

Effects of BA in combination with IBA on *in vitro* shoot multiplication of *Atractylodes macrocephala* Koidz.

In the micropropagation protocol for medical plants, cytokinins are used alone or in combination with auxins to induce dorminant shoots through regulating in plants' development and growth [10]. In this experiment, BA are employed in combination with IBA with different concentrations. After four weeks of culture, there are differences from the number of shoots recorded in treatments (Table 1).

Table 1. AM's *in vitro* shoot multiplication after 4 weeks of culture in MS medium supplemented with different PGRs

Treatments	Plant growth regulators (PGRs)		Number of shoots
	BA (mg/L)	IBA (mg/L)	
Control	0	0	1^{e}
NT1	0.5	0.3	1.44^{cd}
NT2	1	0.3	2.18^{a}
NT3	1.5	0.3	1.63^{bc}
NT4	2	0.3	1.22^{de}
NT5	0.5	0.5	1.22^{de}
NT6	1	0.5	1.71^{b}
NT7	1.5	0.5	1.44^{cd}
NT8	2	0.5	1.33^{d}
CV%			8.4

In the same columns, different letters indicate statistically significant difference ($p \leq 0.05$); Significant differences of the treatments using Duncan's multiple range tests; Control: cultured medium without plant growth regulators

According to Table 1, the highest number of shoots (2.18 shoots/explant) was recorded in MS medium supplemented with 1 mg/L BA in combination with 0.3 mg/L IBA. When BA concentrations were higher, the number of shoots decreased. Specifically, BA concentrations in combination 0.3 mg/L IBA went from 1 up to 2 mg/L. As a result, the number of shoots went down from 2.18 to 1.22 shoots/explant. Similarly, whereas BA concentrations in combination 0.5 mg/L IBA rose from 1 to 2 mg/L, the number of shoots decreased from 1.71 to 1.33 shoots/explant.

In the previous studies, MS medium was supplemented BA in combination with NAA rather than with IBA [6 - 7]. Particularly, in the study of Chen et al., MS medium was supplemented with 1 mg/L BA in combination with 0.2 mg/L NAA [6]. The highest number of shoots is 4.55 shoots/explant being higher than the shoot numbers in this study. It can be seen that different auxins in combination with the same cytokinin can differently affect the development of shoots.

Moreover, the results of the study showed that the rate of nodal segments developing shoots was 100 percent. The shoots had two leaves or more and the formation of roots in all treatments (Figure 3). This outcome was similar to the study of Liang *et al*. as well as Tao *et al.* in terms of the rate of nodal segments developing shoots when culturing the explants of *Atractylodes macrocephala* Koidz. in the medium containing the plant growth regulators [7 - 8].

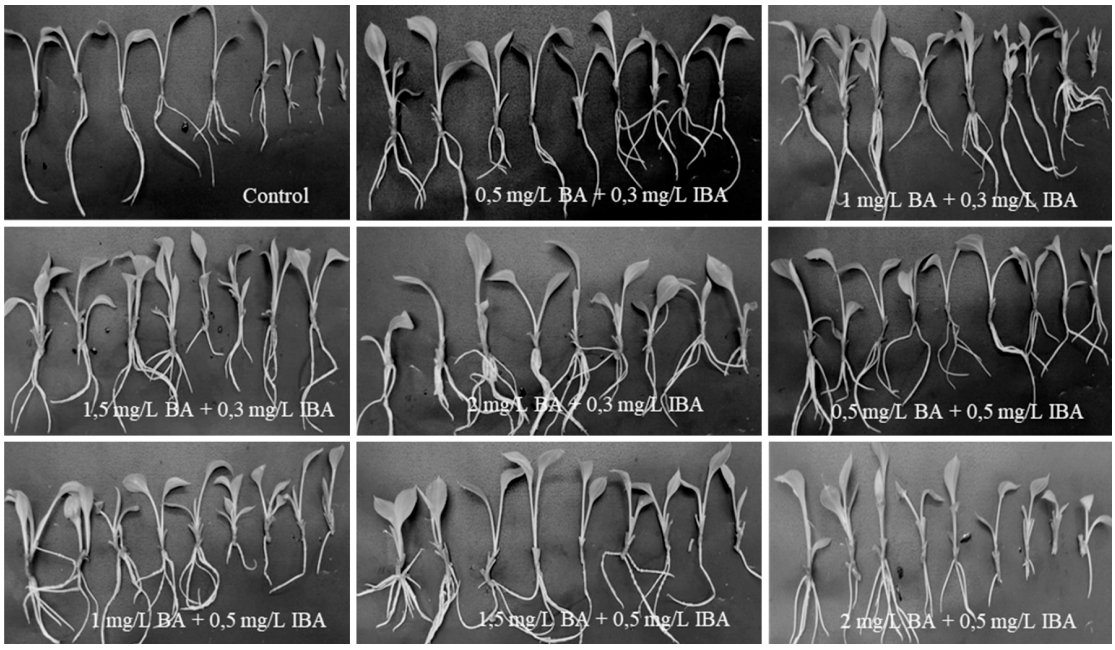

Figure 3. The shoot proliferation of *Atractylodes macrocephala* Koidz. after four weeks of culture

Effects of basal media on *in vitro* shoot multiplication of *Atractylodes macrocephala* Koidz.

Basal media provide essential nutrients for the growth and development of cells during the period of *in vitro* culture [10]. After four weeks of culture, all explants differently created shoots in each treatment (Table 2).

Table 2. The number of shoots of *Atractylodes macrocephala* Koidz. after 4 weeks of culture in different basal media

Treatments	Basal media	Number of shoots
M1	MS	8.67^{d}
M2	$^{1/2}$MS	14.09^{a}
M3	MS$^{1/2}$	10.4^{bc}
M4	VW	9.67^{c}
M5	B5	10.67^{b}
CV%		4.6

In the same columns, different letters indicate statistically significant difference ($p \leq 0.05$); Significant differences of the treatments using LSD's multiple range tests

The Table 2 showed that the highest number of shoots was 14.09 shoots/explant in $^{1/2}$MS medium supplemented with 1 mg/L BA in combination with 0.3 mg/L IBA in comparison with the other media such as MS, VW, and B5. While the number of shoots reaching 8.67 shoots/explant in this experiment was higher than the number of shoots in the previous experiment at 2,18 shoots/explant in the medium MS supplemented with 1 mg/L BA in combination with 0.3 mg/L IBA. There was a difference between the age of the explants in this experiment (nodal segment after 8 weeks of culture) and in the previous experiment (nodal segments after 4 weeks of culture). This led to the larger segments containing many dormant shoots (Figure 4).

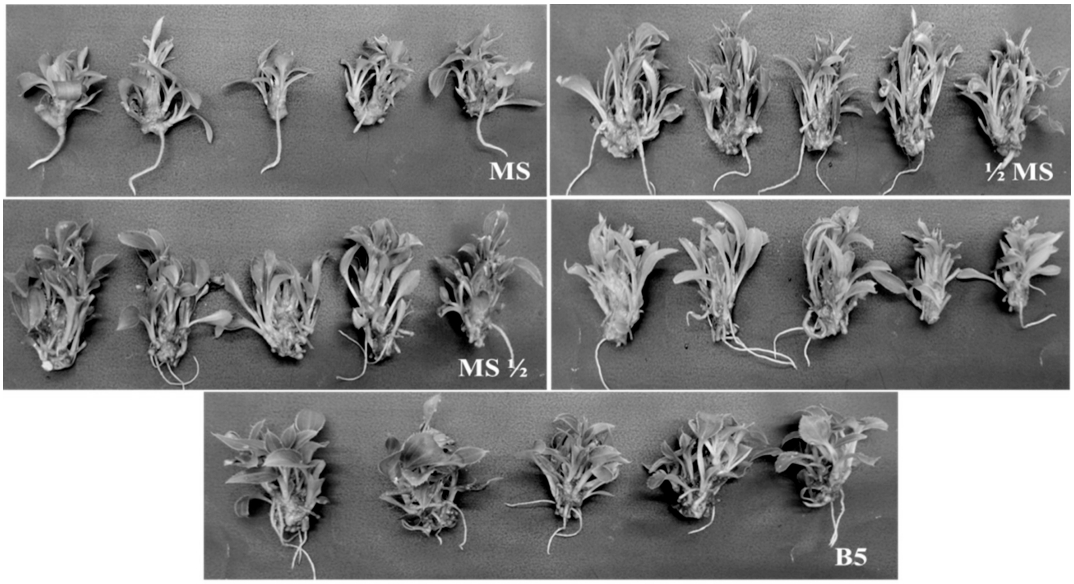

Figure 4. The shoot proliferation of *Atractylodes macrocephala* Koidz. after four weeks of culture

Numerous studies have been conducted to propagate *Atractylodes macrocephala v*arieties from different parts of the world. However, MS medium had been used in all previous studies, with different numbers of shoots produced. In the study of Chen et al., the highest number of shoots (4.55 shoots/explant) was produced in MS medium supplemented with 1 mg/L BA and 0.2 mg/L NAA [6]. In addition, the number of shoots recorded in MS medium supplemented with NAA in combination with TDZ is 5.61 shoots/explant [8]. According to the study of Nguyen Manh Dung, MS medium supplemented with 2 mg/L BA in combination with 1.5 mg/L Kinetin is recorded the highest number of shoots (3.24 shoots/explant) [9]. It can be seen that the number of shoots in this study is much higher than in the previous studies when culturing on ½ MS medium supplemented 1 mg/L BA in combination with 0.3 mg/L IBA. This leads to the efficient micropropagation protocol of *Atractylodes macrocephala* Koidz. for production. The result of the study shows that the basal medium plays a great importance in propagating the shoots. A suitable medium must be chosen at every stage of the *in vitro* micropropagation technique depending on the propagated species rather than using the same basal medium for all the plants.

4. CONCLUSION

The optimized medium for shoot proliferation of *Atractylodes macrocephala* Koidz. is ½ MS medium supplemented with 1 mg/L BA in combination with 0.3 mg/L IBA with shoot number reaching at 14.09 shoots/explant.

REFERENCES

[1] B. Zhu, Zhang, W. J. Hua, L. W. Cheng, and P. L. Qin. “The traditional uses, phytochemistry, and pharmacology of *Atractylodes macrocephala* Koidz: a review.” *Journal of Ethnopharmacology*, Vol. 226, pp. 143-167, 2018. DOI: 10.1016/j.jep.2018.08.023

[2] S. Gu, L. Li, H. Huang, B. Wang, and T. Zhang. "Antitumor, antiviral, and anti-inflammatory efficacy of essential oils from *Atractylodes macrocephala* Koidz. Produced with different processing methods." *Molecules*, Vol. 24, No. 16,

pp. 1-14, 2019. DOI: 10.3390/molecules24162956.

[3] R. Wang, G. Zhou, M. Wang, Y. Peng, and X. Li. "The metabolism of polysaccharide from *Atractylodes macrocephala* Koidz. and its effect on intestinal microflora." *Evidence-based Complementary and Alternative Medicine*, Vol. 2014, No. 3, pp. 1-8, 2014. DOI:10.1155/2014/926381.

[4] G. H. Jin, K. W. Li, Y. D. Lee, T. J. Jeong. and C. Y. Kim. "Anti-inflammatory components isolated from Atractylodes macrocephala in LPS-induced RAW264. 7 macrophages and BV2 microglial cells." *Applied Biological Chemistry*, Vol. 65, No. 1, pp. 1-13, 2022. DOI: 10.1080/14786410801931629.

[5] K. Zhou, J. Chen, J. Wu, Q. Wu, C. Jia, Z. X. Y. Xu, ... and S. Jiang. "Atractylenolide III ameliorates cerebral ischemic injury and neuroinflammation associated with inhibiting JAK2/STAT3/Drp1-dependent mitochondrial fission in microglia."*Phytomedicine*, Vol. 59, pp. 1059 – 1063, 2019. DOI: 10.1111/cns.13839

[6] J. Chen, K. Pan, B. Gu, J. Wang, Y. Wu, and T. Wan. "*Atractylodes macrocephala* rapid propagation by direct shoot and plant regeneration by leaf. Chinese Agricultural Science Bulletin, 2006, Vol.11, pp. 1-12, 2006. DOI: 10.11924/j.issn.1000-6850.061165.

[7] X. Liang. "Study on rapid propagation of *Atractylodes macrocephala* Koidz. Shoot tip." *Journal of Anhui Agriculture Sciences*, Vol. 35, pp. 1-15, Feb. 2009.

[8] J. Y. Tao, L. S. Gao, P. H. Huang, L. Ma. "Tissue culture of shoot tip and the optimization of rapid propagation of *Atractylodes macrocephala* Koidz." *Pharmaceutical Biotechnology,* vol. 6, pp. 1-10, Jan. 2010.

[9] N. M. Dung. "Study on the micropropagation protocol of *Atractylodes macrocephala* Koidz. through tissue culture. M.S. Thesis, Vietnam National University of Agriculture, Vietnam, 2014.

[10] G. C. Phillips and M. Garda. "Plant tissue culture media and practices: an overview." *In Vitro Cellular & Developmental Biology-Plant*, Vol. 55, pp. 242-257, 2019. DOI: 10.1007/s11627-019-09983-5.

Ảnh hưởng của chất điều hòa sinh trưởng và môi trường khoáng lên sự nhân chồi *in vitro* cây Bạch truật

Nguyễn Thanh Tài, Phan Thủy Quyên, Phạm Thu Nhi, Phạm Quang Thắng và Lê Thành Hưng

TÓM TẮT

Bạch truật (Atractylodes macrocephala Koidz.) thuộc chi Thương truật (Atractylodes) là cây có chứa nhiều dược liệu quý với hơn 79 hợp chất hóa học khác nhau. Tuy nhiên, ở Việt Nam, các nghiên cứu về quy trình nhân giống in vitro cây Bạch truật vẫn còn nhiều hạn chế. Mục đích của nghiên cứu này là khảo sát ảnh hưởng của các chất điều hòa sinh trưởng thực vật và môi trường khoáng khác nhau lên sự nhân chồi của cây Bạch truật. Đây là giai đoạn quan trọng trong quy trình nhân giống. Trong nghiên cứu này, đốt thân có chứa chồi ngủ được nuôi cấy trên các môi trường khoáng khác nhau sau khi đã chọn được môi trường có bổ sung nồng độ BA kết hợp IBA thích hợp. Sau 4 tuần nuôi cấy, số chồi cao nhất được ghi nhận trên môi trường MS có bổ sung 1 mg/L BA kết hợp với 0.3 mg/L IBA với số chồi 2.18 (chồi/mẫu). Số chồi đạt được 14.5 chồi/mẫu khi nuôi cấy trên môi trường $^{1/2}$MS có bổ sung 1 mg/L BA kết hợp với 0.3 mg/L IBA, cao gấp 4 lần số chồi đạt được so với các nghiên cứu trước. Kết quả của nghiên cứu này góp phần lớn vào quy trình nhân giống hiệu quả cây Bạch truật phục vụ cho sản xuất giống thương mại.

Từ khóa: *Bạch truật, chất điều hòa sinh trưởng thực vật, nhân chồi*

Received: 16/05/2023
Revised: 10/06/2023
Accepted for publication: 10/06/2023