

**A STUDY ON VEGETATIVE PROPAGATION OF *Huperzia serrata*
BY CUTTINGS IN SA PA, LAO CAI**

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ABSTRACT

Huperzia serrata is a precious medicinal plant used in medicine to support and treat Alzheimer's disease. Currently, this species is in danger of extinction due to indiscriminate exploitation for commercial purposes. Furthermore, this species reproduces slowly and is difficult to cultivate artificially. The reproduction by spores of this species is very long and takes 15–20 years from the spore germination to mature plant, whereas asexual reproduction by tissue culture is also difficult. Therefore, cutting is an effective method to propagate this species on a large scale. In this study, we surveyed the factors affecting the survival rate, the rooting and the number of new leaves of the stem cuttings, including cutting length, growing substrate, and effects of growth-regulating hormones IBA (0 ppm, 500 ppm, 1000 ppm, 2000 ppm and 3000 ppm) and α -NAA (0 ppm, 10 ppm, 20 ppm, 30 ppm and 40 ppm). The experimental results showed that using cuttings of 6 cm in length treated with IBA at a concentration of 1000 ppm for 30 mins and cultured on the substrate mixture of soil, decomposed animal manure and rice husk at a ratio of 3:1:1 was the best for cuttings of *H. serrata* in Sa Pa. The results of this study will contribute to the conservation and development of genetic source of *H. serrata* in Vietnam.

Keywords: *H. serrata*, cutting, IBA, regulating hormones, NAA, propagation.

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INTRODUCTION

H. serrata is a precious medicinal plant in the red list of the Program of research on conservation and development of rare and precious gene sources for medicinal plants (Nong Van Duy, 2015). This plant has many medical effects, especially dementia (Alzheimer) of the elderly. At present, this plant is being indiscriminately procured and exploited, leading to a decline in this precious medicinal resource in nature. Hence, studies on *H. serrata* are receiving the concern of many countries in the world, including Vietnam.

The reproduction of *H. serrata* is a combination of sexual and asexual reproduction (Whittier et al., 2007). Similar to other species of genera *Huperzia* and *Lycopodium*, the spore germination process of *H. serrata* is poor under controlled conditions. The gametophytes and sporophytes of this species are often long-lived and grow slowly in an environment having high relative humidity and abundant organic matter; therefore, it is difficult to propagate (Ma et al., 2006). Studies show that spores of species belonging to the genus *Huperzia* take 2–5 years to develop into sporophytes (Whittier et al., 2007). This development process in *H. serrata* is much slower and usually requires 15–20 years of growth since spore germination to maturity (Ferreira et al., 2016). Thus, the spore germination methods of *H. serrata* in soil environment or sterile culture media are not-yet performed. Although vegetative propagation of *H. serrata* by tissue culture has been successful in the world, it is difficult to obtain large biomass and requires a long time (Ma et al., 2008; Ma et al., 2015; Manpreet et al., 2016). Moreover, the process of transferring tissue culture plants to natural habitats is difficult; therefore, the propagation by cuttings is a suitable option for rapid propagation of this plant.

In Vietnam, *H. serrata* is scattered in the midland provinces and high mountains in North West and Central High Land; however, this precious genetic resource is being lost. Until now, there have been only surveys on

distribution areas and growth of this plant (Nguyen Quang Hieu et al., 2017), but there have not been any specific studies on the propagation of this plant in natural conditions. Therefore, we investigated the effect of several factors (substrate, cutting length, growth-regulating hormones) on propagating this plant by cuttings.

MATERIALS AND METHODS

The samples of *H. serrata* used in the present study were healthy and not infected with pests and diseases, evenly sized, and branched. The plant samples were collected at Ta Phin forest, Sa Pa. The study was conducted in Sa Pa from April 2015 to November 2016.

Plant materials were identified following Nong Van Duy (2015). The stem cuttings were collected on 7:00 - 9:00 am, the lower of cuttings was bevel cut to increase the contact area of cuttings to soil and enhance the growth of roots.

The nursery was designed in the forest (Figure 1d). The cuttings were treated with IBA and α -NAA, and then immediately plugged into the substrate (Hoang Minh Tan, 1993).

The experiments were designed into complete randomized blocks in triplicate. Each experiment included 120 cuttings with a potting soil size of 8 cm x 10 cm per replication.

Three experiments were designed from 1 to 3 respectively and each of the following experiments was designed based on the results of the previous experiment.

Experiment 1: study on each of the length of cuttings from 4 cm to 10 cm (Fig. 1a).

Experiment 2: using 4 substrate mixtures of forest soil: decomposed animal manure: rice husk: vermicompost with ratios as follows: CT1 ratio: 3: 1: 0: 0; CT2 ratio: 3: 1: 1: 0; CT3 ratio: 3: 1: 1: 1; CT4 ratio: 3: 0: 1: 1 (Fig. 1b).

Experiment 3: cuttings were treated with α - NAA at concentrations of 0 ppm, 10 ppm, 20 ppm, 30 ppm and 40 ppm for 5 mins, and

with IBA at concentrations of 0 ppm, 500 ppm; 1000 ppm, 2000 ppm and 3000 ppm for 30 mins (Fig. 1c).

Monitoring parameters: survival rate (%); rooting rate (%); rate of cutting with new leaves (%); number of new leaves; number of

roots. Each experiment was monitored for 4 months since cuttings were planted into the substrate (Fig. 1d).

Experimental data were processed by Microsoft Excel 2007 and IRRISTAT version 5.0.

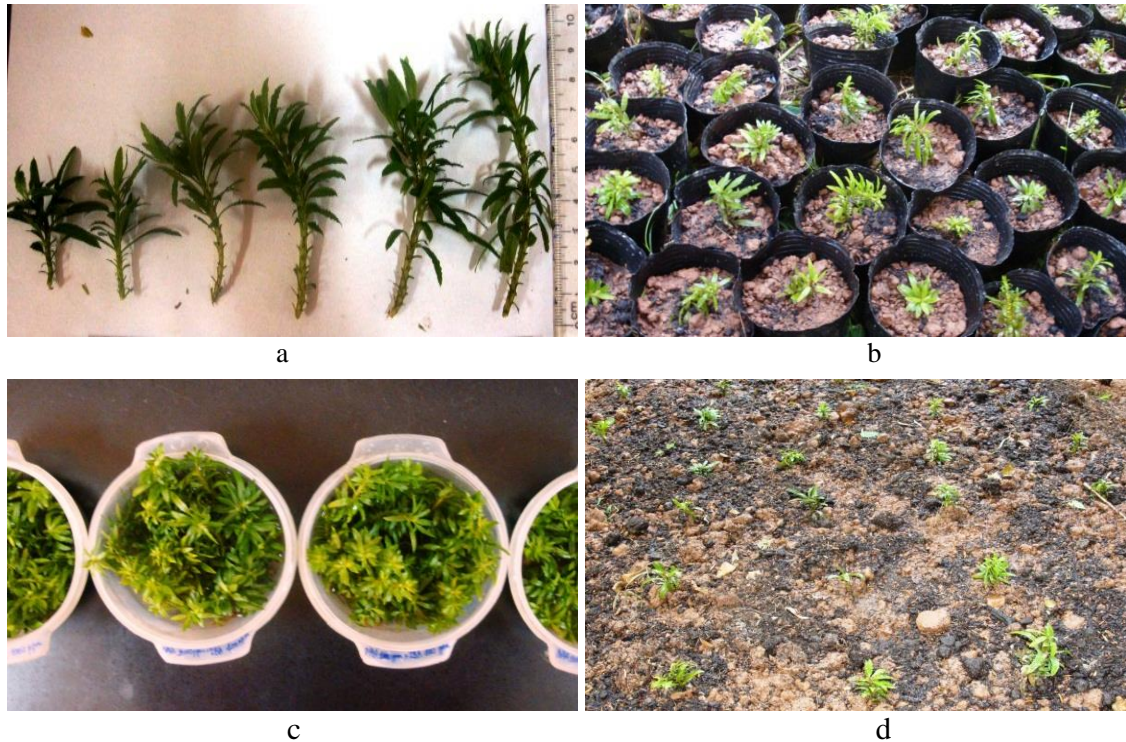


Figure 1. Vegetative propagation of *Huperzia serrata* by cutting: a. Cuttings with different lengths used in vegetative propagation of *Huperzia serrata*; b. *Huperzia serrata* was planted on the CT2 substrates; c. Cuttings were treated with growth-regulating hormones IBA and α -NAA; d. The nursery of *Huperzia serrata*

RESULTS AND DISCUSSION

Effect of cutting length on survival rate, rooting and growth of new leaves of *H. serrata*

In the original ecological environment, the cuttings of *H. serrata* grew easily and healthy. After 4 experimental months, the height of the cuttings almost unchanged but a few new leaves were grown. We found that the cutting length affected the growth of *H. serrata*. Experimental results are presented in table 1.

The results showed that the use of the cuttings of 4 cm and 5 cm in lengths had

significantly lower survival, rooting and the rate of cutting with new leaves than the cuttings with longer lengths. The cuttings of 7 cm in length showed the highest survival rate of 88.33% and not significantly different compared with the survival rate of the cuttings with the length of 6 cm to 10 cm. Thus, the use of the cuttings with the length of 6 cm would save the number of *H. serrata* used but still gave a high survival rate (86.39%). Moreover, the cuttings of 6 cm in length showed highest rooting rate (23.47%) and the rate of cutting with new leaves (68.49%), and significantly differed from the cuttings of

other lengths at the statistical significance of 95% and high reliability of 2.3% and 2.4%, respectively. These results revealed that the use of the cuttings of 6 cm in length gave the best efficiency for the propagation of *H. serrata*. This result is consistent with the previous study described by Long et al.

(2014). Authors propagated *H. serrata* by cuttings method in the Yantuozhai region's forest farm and showed that the cuttings of 6 cm in length gave the best efficiency. Therefore, the cuttings with the length of 6 cm were used for further experiments.

Table 1. Effect of cutting length on the growth of *H. serrata* after 4 months

Cutting length	Survival rate (%)	Rooting rate (%)	Rate of cuttings with new leaves (%)
4 cm	74.72 ^c	10.04 ^c	32.74 ^d
5 cm	78.06 ^b	17.79 ^d	47.71 ^c
6 cm	86.39 ^a	23.47 ^a	68.49 ^a
7 cm	88.33 ^a	21.39 ^c	65.41 ^b
8 cm	86.94 ^a	22.36 ^b	67.73 ^{ab}
9 cm	87.22 ^a	21.98 ^{bc}	65.29 ^b
10 cm	86.62 ^a	21.47 ^c	67.64 ^{ab}
CV %	1.5%	2.3%	2.4%
5% LSD	2.284	0.799	2.55

Note: The different letters in a column indicated significantly difference at $P < 0.05$.

Effect of substrates on the propagation of *H. serrata*

According to Ninh Thi Phip (2013), planting substrates have a great influence on the ability to propagate plants. In propagation by cuttings, the substrates have the function of holding the cuttings in fixed positions and provide sources of water and nutrients for the cuttings. Furthermore, the substrates allow air to penetrate the base of cuttings. A good substrate must have sufficient porosity, good aeration, water retention and drainage capacity, no pests and weeds. The previous studies have shown that the most favorite environment for cuttings of *H. serrata* is the ecological forest environment (Zhang et al., 2009; Long et al., 2014). Therefore, in this study, we used forest soil as the main substrate and supplemented with manure, rice husk and vermicompost at different ratios to find the best substrate mixture for cuttings of *H. serrata*. At the same time, on the basis of previous experiment result, the cuttings of 6 cm in length were used. The experimental results are presented in table 2.

The growth parameters of the cuttings using substrate mixtures supplemented with manure were higher than those of the control substrate. This suggests that increasing the porosity and nutrients of the substrates can enhance the growth ability of *H. serrata*. The survival rate of substrate mixtures CT2 (88.89%) and CT3 (89.72%) were significantly higher than that of other substrate mixtures. Although the survival rate of CT3 was higher than that of CT2, this difference was not statistically significant. Moreover, rooting rate (29.07%) and rate of cuttings with new leaves (83.44%) of CT2 substrate mixture were significantly higher than those of other substrate mixtures. It was observed that rooting rate was positively correlated to the rate of cuttings with new leaves, indicating a two-way relationship between root and leaf growth. The root growth supported for taking nutrients and water from the habitat to the plant and enhanced the growth of leaves. At the same time, the growth of leaves helped the plant grow and synthesize vegetative nutrients and promoted root growth. The CT2 mixture with

a combination of forest soil, decomposed animal manure and rice husk that made the substrates soft, nutritious, and moist shows the most favorable for cuttings of *H. serrata*.

Table 2. Effect of substrates on the growth of *H. serrata* cuttings after 4 months

Substrate mixture	Survival rate (%)	Rooting rate (%)	Rate of cuttings with new leaves (%)
Control (forest soil)	86.39 ^b	23.47 ^c	68.49 ^{cd}
CT1	87.5 ^b	25.71 ^b	69.85 ^c
CT2	88.89 ^a	29.07 ^a	83.44 ^a
CT3	89.72 ^a	24.77 ^b	70.90 ^b
CT4	86.94 ^b	24.92 ^b	67.41 ^d
CV%	0.7	3.2	1.6
5% LSD	1.18	1.552	2.187

Note: The different letters in a column indicated significantly difference at $P < 0.05$.

Effect of the growth-regulating hormones IBA and α -NAA on the growth of *H. serrata* cuttings

The addition of growth-regulating hormones plays an important role in increasing survival, root and shoot growth rates. Ninh Thi Phip (2013) suggested that using auxin to stimulate the growth of cutting root would improve the rate of plant growth. The effective auxin concentration used for herbaceous and softwood was less than 1000 ppm, and for half-hard woody plants was 2000 - 3000 ppm. On the other hand, Zhang et al. (2009) reported that IBA concentration of 2000 ppm gave the best effect for *H. serrata* cuttings. According to Qin et al. (2010), shoots of *H. serrata* before cuttings were soaked in IBA solution of 1000 ppm for 30 mins gave a high survival rate up to 93–98% and rooting rate reached 98%. In addition, Long et al. (2014) using *H. serrata* cuttings of 6 cm length soaked in α -NAA solution of 20 ppm for 5 mins gave a high survival rate up to 90% and rootlets of cuttings appeared only after 60 days of planting. In this study, we used cuttings of 6 cm in length treated with two growth-regulating hormones IBA and α -NAA with different concentrations and treating times, then planted in the CT2 substrate. The experimental results are presented in table 3.

The experimental results showed that *H. serrata* grew better in the forest soil environment; therefore, the growth of roots and new leaves of the cuttings were also observed when no growth-regulating hormones added. However, when IBA was used, number of roots and new leaves significantly increased. Especially, number of roots (2.38 ± 0.06) and new leaves (2.61 ± 0.06) of the cuttings were the best when the cuttings were treated with IBA at a concentration of 1000 ppm. At a concentration of 2000 ppm, number of roots (2.07 ± 0.05) and new leaves (2.43 ± 0.07) significantly decreased. As a consequence, treatment of the cuttings with IBA at a concentration of 1000 ppm for 30 mins before cutting was most suitable.

For the cuttings treated with α -NAA, the growth of cuttings significantly increased compared to the cuttings without treatment of α -NAA. In particular, cuttings treated with α -NAA at a concentration of 20 ppm for 5 mins gave the highest number of roots (1.97 ± 0.06) and new leaves (2.33 ± 0.07). However, when comparing the promoting efficiency of α -NAA and IBA on the growth of *H. serrata* roots and leaves, α -NAA was less effective than IBA. Therefore, the treatment of cuttings with IBA at a concentration of 1000 ppm for 30 mins was proven to be the best for *H. serrata* cutting propagation.

Table 3. Effect of the growth-regulating hormones IBA and α -NAA on the growth of *H. serrata* cuttings after 4 months

Hormones	Concentration (ppm)	Number of roots	Number of new leaves
IBA	0 (water)	1.36 \pm 0.05 ^a	1.58 \pm 0.08 ^a
	500	1.42 \pm 0.05 ^b	1.64 \pm 0.08 ^b
	1,000	2.38 \pm 0.06 ^d	2.61 \pm 0.06 ^d
	2,000	2.07 \pm 0.05 ^e	2.43 \pm 0.07 ^c
	3,000	1.97 \pm 0.06 ^c	2.18 \pm 0.09 ^c
CV%		1.9	1.4
5% LSD		0.0494	0.0430
α -NAA	0 (water)	1.49 \pm 0.05 ^a	1.98 \pm 0.08 ^a
	10	1.69 \pm 0.06 ^c	2.04 \pm 0.08 ^a
	20	1.97 \pm 0.06 ^d	2.33 \pm 0.07 ^c
	30	1.91 \pm 0.06 ^e	2.29 \pm 0.08 ^c
	40	1.8 \pm 0.07 ^b	2.19 \pm 0.08 ^b
CV%		1.5	2.2
5% LSD		0.0505	0.0853

Note: the different letters in a column indicated significantly difference at $P < 0.05$.

CONCLUSIONS

The results of our study showed that the cuttings of 6 cm in length treated with IBA at a concentration of 1,000 ppm for 30 mins and the substrate mixture of soil, decomposed animal manure and rice husk at a ratio of 3:1:1 are the best materials for vegetative propagation of *H. serrata* by cutting in Sa Pa. The results of this study will contribute to the conservation and development of genetic source of *H. serrata* in Vietnam.

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