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EVALUATION OF PLANT GROWTH REGULATORS ON ROOT FORMATION OF SEMIHARDWOOD AND HARDWOOD CUTTINGS OF *Sarcandra glabra* (Thunb.) Nakai

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ABSTRACT: The aim of this study is to evaluate the effects of three types of plant growth regulators (α-naphthyl acetic acid, indole-3-acetic acid and β-indol butyric acid) on the root formation of the semihardwood and hardwood cuttings of Sarcandra glabra (Thunb.) Nakai. The effects of plant growth regulators on root formation were evaluated based on living percentage, rooting percentage, secondary rooting percentage, and the number and the length of roots. The results showed that the best root growth regulator was β-IBA. The overall effective concentrations of growth regulators were 1.0 and 1.5%. The living percentage of the semihardwood cuttings was lower than that of the hardwood cuttings. However, the root growth parameters of the semihardwood cuttings were better than those of the hardwood cuttings.

Keywords: Sarcandra glabra, α-NAA, IAA, β-IBA, cuttings, plant growth regulators, root formation.

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INTRODUCTION

Sarcandra glabra (Thunb.) Nakai is an evergreen subshrub that grows to the height of 50 - 150 cm. The branches of *Sarcandra glabra* are cylindric, erect, and glabrous with swollen nodes. *Sarcandra glabra* is distributed in various environments such as forest, valleys, ravines, slopes, roadsides, trailsides, grasslands, swamps, streamside, and sandy soil of from near sea level to 2,000 m. *Sarcandra glabra* is distributed wildly from Southeast Asia to India [18]. In Vietnam, this plant has been cultivated in some mountainous provinces such as Lang Son, Bac Can, Thai Nguyen, Ninh Binh, Quang Nam, Kon Tum and Lam Dong [5].

In folklore, *Sarcandra glabra* has been used to treat many diseases such as pancreatic cancer, gastric, colorectal, liver and throat diseases, encephalitis, bacillary dysentery, appendicitis, boils, bone fractures, arthritis, and backache [1, 3, 9]. Several pharmaceutical components such as isofraxidin, fumaric acid, chloranthalactone and sesquiterpene lactones have been identified from this plant [17]. Isofraxidin has anticancer and cholagogic effects, whereas fumaric acid has antibiotic, antalgic and antitumor effects [11]. Yen et al. (2010) [16] reported that, flavonoid compounds (tectoridin) and hexandrin (1β, 28-dihydroxylup-20 (29) en) could be extracted from *S. glabra*. This inceptive finding promoted further studies about *S. glabra* extracts on the inhibiton of cancer cells growth *in vitro*.

Currently an increasing demand for *S. glabra* for medication has been leading to the risk of depletion of pharmaceutical sources. Propagation of *Sarcandra glabra* using tissue culture have been studied [18, 19]. However, propagation by tissue culture requires modern equipments and professional staffs, and the development of seedlings depends on the season. As an alternative, propagation using cuttings should be considered because it is a simple yet effective method, especially for minority communities, to preserve this precious medicinal source. In Anhui Province, China, propagation of *S. glabra* using cuttings was as high as 80% of rooting percentage [8, 12]. However, there were limited studies about factors affecting propagation of *S. glabra* using cutting techniques. Therefore, in this study, we aimed to evaluate the effects of the plant growth regulators on rooting of two types of *Sarcandra glabra* cuttings, semihardwood and hardwood cutting.

MATERIALS AND METHODS

Study location

The experiment was conducted in the greenhouse of the Department of Seedling and Biotechnology, Forest Science Institute of Central Highlands and South of Central Vietnam, located at Da Lat city, Lam Dong province, Vietnam. The average annual temperature ranges from 18 to 25^{o}C. The average annual rainfall of this region is 2,200 mm, where 80-90% of annual rainfall is received during rainy season of May to November.

Samples collection

The branches of *Sarcandra glabra* were collected from Bidoup - Nui Ba Natinal Park during rainy season. Then, they were cut into segments with a length of approximately 15 cm. Two types of cuttings, semihardwood and hardwood cuttings were used. Semi-hardwood cuttings were taken from parts of the tree where the lower portion of the cutting had lignified. They were taken 3 weeks after collection of softwood cuttings, which were parts of the tree where the wood was still soft, succulent and the wood had not yet lignified. Hardwood cuttings were taken from fully matured parts, where the entire stem had lignified.

Plant regulators treatment

Three plant growth regulators: α-NAA (α-naphthyl acetic acid), IAA (indole-3-acetic acid) and β-IBA (β-indol butyric acid) (MERCK®, Germany) were used in this study. The concentrations tested for growth regulators was 0; 0.5; 1.0; 1.5 and 2.0% (w/w). α-NAA, IAA, and β-IBA were dissolved in a small quantity of alcohol, and then were mixed with talc to form a slurry. The slurries were allowed to dry with gentle heat to evaporate the alcohol and then were grounded and passed through a sieve. The collected powders of each plant growth regulators were used for the plant growth experiments.

Experimental design

Factorial arrangement of treatments on the basis of randomised complete block design was used as the experimental design: 2 kinds of cutting types; 3 plant growth regulators (PGRs), each with 5 concentrations with 3 blocks (replications) and 30 cuttings per treatment per block. The experiment was conducted on sand.

Observation recording and statistical analysis

All cuttings were harvested 75 days after treatment and the following data were recorded: the living percentage (%), rooting percentage (%), secondary rooting percentage (%), the number of roots, and the length of roots (cm). The statistical analysis was performed with SPSS 16.0 (Statistical Package for Social Sciences version 16) software using Duncan's range tests.

RESULTS AND DISCUSSION

Effects of plants growth regulators on two types of cuttings of *S. glabra* were determined using various plant growth parameters and are presented in table 1. The results showed that there was significant difference in rooting percentage between the two cutting types. Statistical differences of rooting percentage, secondary root percentage and number of roots were also found between three different types of PGRs. Besides, concentrations of PGRs also caused differences in rooting percentage, secondary root percentage and length of roots. The interactions between the three variables and between PRGs and their concentration only caused significant difference in secondary root percentage. In the other hand, there were interactions of cutting types x PGRs and cutting types x concentration on both rooting percentage and secondary root percentage. Overal, the statistical analysis indicated that cutting types had the most important impact on plant growth parameters. Details of the effects of each variable are presented in table 2, 3 and 4 and further dicussed below.

Cuttings types are classified into softwood, semihardwood and hardwood based on their maturity [4]. Root growth is affected by cuttings types and species of plants. For example, Yeshiwa et al. (2015) [15] reported that, in case of roses, the hardwood cuttings showed better root growth than softwood and semihardwood. In case of Himalayan yew, *Taxus wallichiana*, semihardwood has better growth than softwood and hardwood [13]. In this study, however, softwood cuttings were excluded because of the high frequency of immature death. The present results showed that all root growth parameters of the semihardwood cuttings was significantly (P<0.05) better than the hardwood cuttings (table 2). Thus, semihardwood cuttings were recommended for breeding production. Apart from rootings, hardwood cuttings had higher survival rates and should be further studied to have better conclusion.

Table 1. Tests of between - subjects effects

Variable		Living percentage (%)		Rooting percentage (%)		Secondary root percentage (%)		Number of roots/cuttings		Length of roots/ cuttings (cm)	
Source	df	F	P	F	P	F	P	F	P	F	P
1. Cutting types	1	10.49	*	623.94	*	434.15	*	66.70	*	16.94	*
2. PGRs	2	0.17	ns	75.79	*	70.50	*	11.31	*	1.09	ns
3. Concentrations	3	0.32	ns	16.96	*	29.35	*	1.73	ns	5.64	*
1*2	2	0.03	ns	95.13	*	15.71	*	0.78	ns	0.37	ns
1*3	3	0.12	ns	6.14	*	7.38	*	1.28	ns	1.41	ns
2*3	6	0.27	ns	0.64	ns	10.56	*	1.39	ns	0.81	ns
1*2*3	6	0.09	ns	0.32	ns	4.97	*	1.29	ns	0.23	ns
Error	52										

*Significant at p<0.05, ns: non-significant.

Table 2. Influence of cutting types on root formation

Cuttings	Living percentage (%)	Rooting percentage (%)	Secondary root percentage (%)	Number of roots/cuttings	Length of roots/cuttings (cm)
Semihardwood	81.14	71.35	26.96	4.11	3.38
Hardwood	87.70	39.31	9.70	2.74	2.45

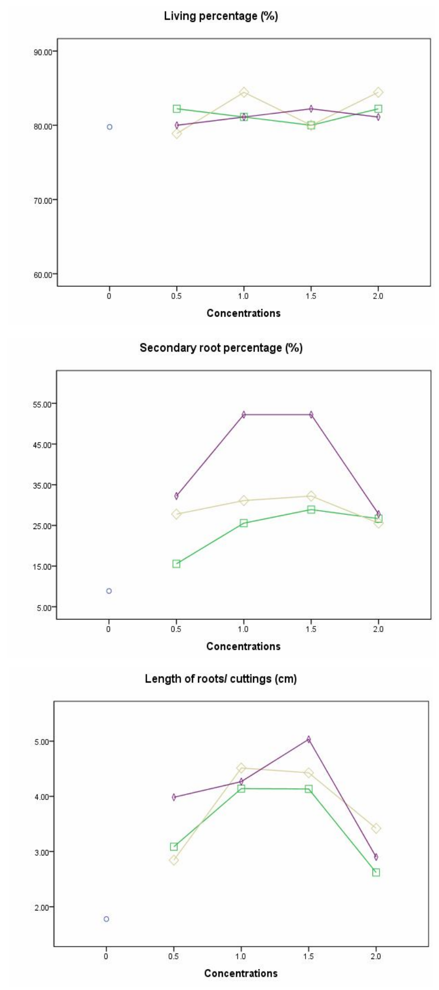
Table 3. Influence of three different plant growth regulators on root formation

Growth regulators	Living percentage (%)	Rooting percentage (%)	Secondary root percentage (%)	Number of roots/cuttings	Length of roots/ cuttings (cm)
Control	83.39	26.89^{a*}	6.67^{a}	1.61^{a}	1.32
α-NAA	84.59	57.47^{b}	16.53^{b}	4.04^{bc}	3.09
IAA	85.42	56.65^{b}	19.31^{bc}	4.26^{c}	3.41
β-IBA	84.03	73.20^{c}	27.91^{c}	3.35^{b}	3.44

Table 4. Influence of plant growth regulator concentrations on root formation

Concentration (%)	Living percentage (%)	Rooting percentage (%)	Secondary root percentage (%)	Number of roots/cuttings	Length of roots/cuttings (cm)
0	83.39	26.89^{a*}	6.67^{a}	1.61	1.32^{a}
0.5	83.89	55.16^{b}	17.59^{b}	3.57	3.07^{bc}
1.0	84.45	63.48^{b}	23.89^{b}	4.03	3.76^{c}
1.5	84.07	67.04^{b}	26.10^{b}	3.95	3.73^{c}
2.0	86.30	64.07^{b}	17.41^{b}	3.91	2.69^{b}

*Means within columns followed by different letter were significantly different at P≤0.05 using Duncan's test.



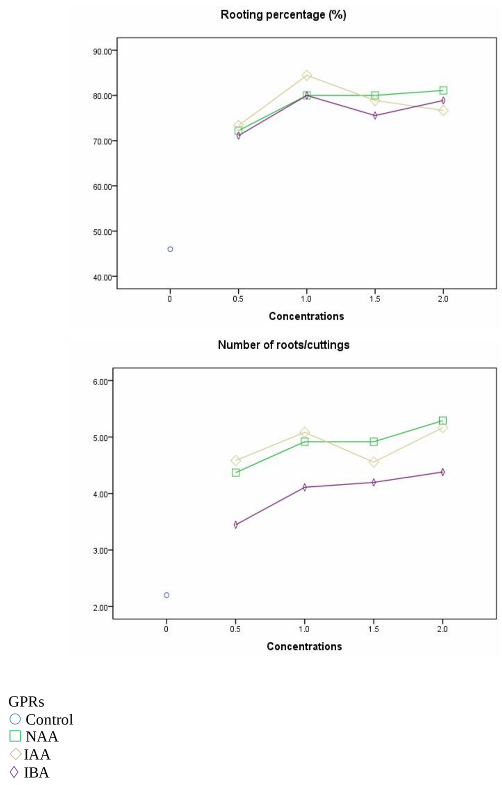


Figure 1. Effects of three plant growth regulators at various concentrations on root formation of semihardwood cuttings

The growth regulators have the ability to stimulate and promote the rooting of cuttings. This was also proven repeatedly. In this study, the percentage of live cuttings and length of roots were not significantly different between the plant gowth regulators and the controls (P>0.05). The other root growth parameters of the cuttings were significantly higher between the treatments and the controls (P<0.05). These results show that the growth regulators used could effectively promote the root growth of cuttings. Cuttings treated with β-IBA had highest rooting and secondary root percentage.

Nelson et al. (1992) [10] examined the effects of four different auxins (IAA, β-IBA, α-NAA, and indole-3-propil) on the growth of *Pinus taeda*, *Pinus elliotti* var. *elliotti*, and *Pinus palustris* and found that IAA and β-IBA were highly effective, while α-NAA and indole-3-propil were less effective on the rooting of the cuttings of these species. When β-IBA was applied to stem cuttings or microcuttings to stimulate rooting, it was partially converted to IAA [6]. β-IBA could also enhance rooting via increasing internal-free β-IBA. Since it could synergistically modify the action of IAA and endogenous synthesis of IAA, β-IBA could enhance tissue sensitivity for IAA and therefore increase rooting [7]. According to Hartmann et al. (2002) [4], β-IBA was the best auxin for general use because it was nontoxic to plants over a wide concentration range than α-NAA, and was effective in promoting rooting of a large number of plant species.

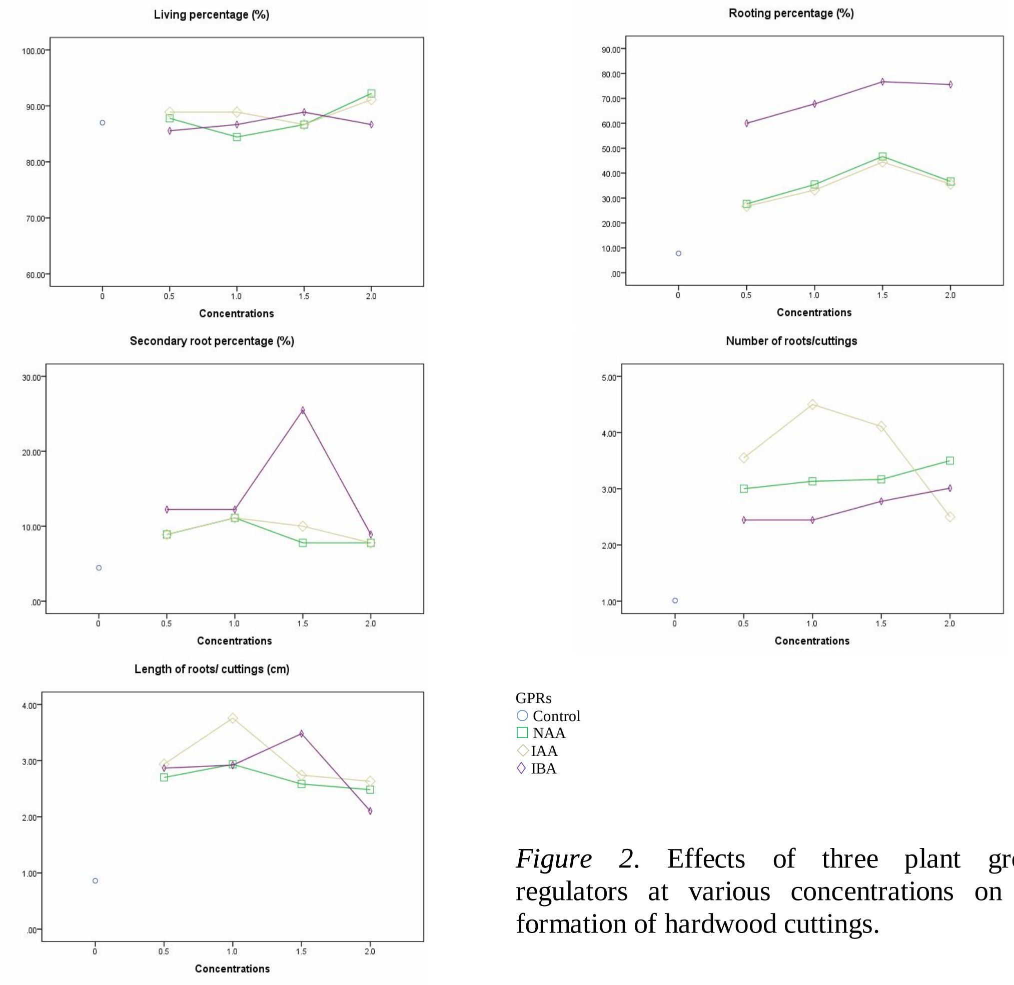


Figure 2. Effects of three plant growth regulators at various concentrations on root formation of hardwood cuttings.

The growth regulator concentration has direct effect on rooting parameters. The effective dose of plant growth regulators could be determined depending on types of growth regulator, types of cuttings, and the species of plants [2]. Regardless of the type of the growth regulators, dose-response analysis revealed that the type and the concentration of plant growth regulators did not affect the living percentage and number of roots ($P>0.05$). The parameters of rooting percentage, secondary roots percentage, and length of roots had differences ($P<0.05$). For the rooting percentage, the percentage of secondary roots and the number of roots, no significant dose-response effects were observed, although every parameters were significantly higher in the treatments compared to those of the control. The best results about the length of roots were obtained at the concentration of 1.0 and 1.5%, followed by 0.5, 2.0%, regardless of the type of plant growth regulators and the types of cuttings. As an overall data in table 2, the concentration range from 1.0 to 1.5% were beneficial for propagating *Sarcandra glabra* by cuttings. At low concentration, growth regulators could not stimulate the roots formation but excess concentration also caused adverse effect due to inhibition of root formation ability. This notion was previously reported by Wen et al. (1991) [14].

CONCLUSION

The results of this study showed that plant growth regulators could promote the root growth for cutting propagation. In three plant growth regulators, β-IBA showed highest efficacy. The appropriate concentration of β-IBA was 1.0-1.5% for semihardwood cuttings. Semihardwood cutting was recommended for breeding production. Since high survival rate, but not the rootings, was associated with hardwood cuttings, further study should be conducted to have better understandings on the type of cuttings.

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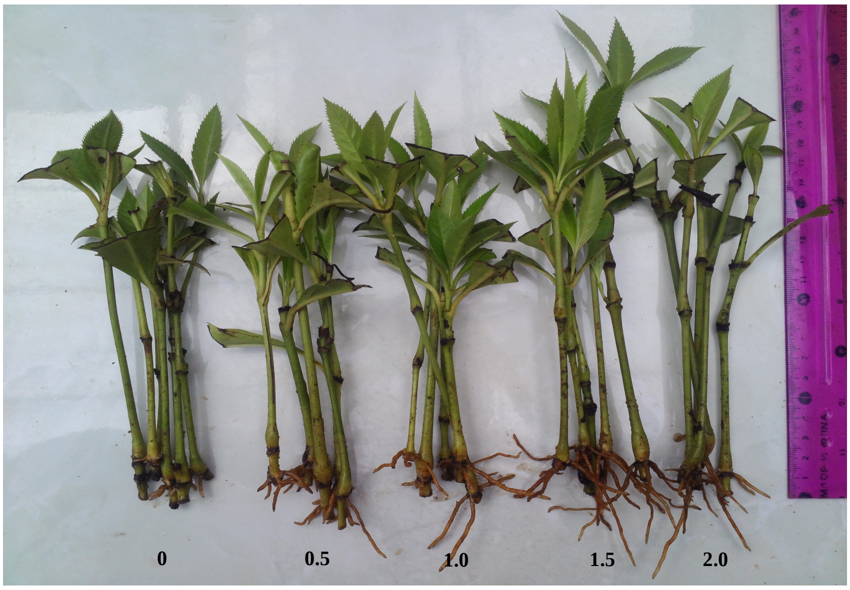

Figure 3. Rooting of cuttings on β-IBA (Concentrations: 0; 0.5; 1.0; 1.5 and 2.0%)

Figure 4. Primary root and secondary root

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ĐÁNH GIÁ ẢNH HƯỞNG CỦA CÁC CHẤT ĐIỀU HÒA SINH TRƯỞNG ĐẾN SỰ RA RỄ CỦA HOM NỬA HÓA GỖ VÀ HÓA GỖ CÂY SÓI RỪNG (*Sarcandra glabra* (Thunb.) Nakai)

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TÓM TẮT

Nghiên cứu này nhằm đánh giá các ảnh hưởng của chất điều hòa sinh trưởng, nồng độ và loại hom đến khả năng ra rễ của cây Sói rừng, *Sarcandra glabra* (Thunb.) Nakai. Ba loại chất điều hòa sinh trưởng (α-naphthyl acetic acid, indole-3-acetic acid và β-indol butyric acid ở các nồng độ: 0; 0,5; 1,0; 1,5 và 2,0% với hai loại hom (hom nửa hóa gỗ và hom hóa gỗ) đã được nghiên cứu. Ảnh hưởng đến sự ra rễ được phân tích dựa trên tỷ lệ hom sống, tỷ lệ hom ra rễ, tỷ lệ hom ra rễ thứ cấp, số lượng rễ và chiều dài rễ trung bình. Các kết quả cho thấy, chất điều hòa sinh trưởng tốt nhất là β-IBA. Nồng độ chất điều hòa sinh trưởng đạt hiệu quả cao ở nồng độ 1,0-1,5%. Hom nửa hóa gỗ có tỷ lệ sống của hom thấp hơn hom hóa gỗ nhưng các thông số khác đều cao hơn hom hóa gỗ.

Từ khóa: Cây sói rừng, α-NAA, β-IBA, hom cây, chất điều hòa sinh trưởng, IAA, ra rễ.

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