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► To cite this version:

L. H. Tien, L. D. Chac, L. T. L. Oanh, P. T. Ly, H. T. Sau, et al.. Effect of auxins (IAA, IBA and NAA) on clonal propagation of *Solanum procumbens* stem cuttings. *Plant Cell Biotechnology and Molecular Biology*, 2020, 21 (55-56), pp.113-120. hal-03045585

HAL Id: hal-03045585

<https://hal.science/hal-03045585>

Submitted on 7 Jan 2021

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EFFECT OF AUXINS (IAA, IBA AND NAA) ON CLONAL PROPAGATION OF *Solanum procumbens* STEM CUTTINGS

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Article Information

Editor(s):

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Reviewers:

(1) Guță Ionela-Cătălin, Institute for Biotechnology in Horticulture Stefanesti, Arges, Romania.

(2) M. M. Sharma, Manipal University, India.

Received: 02 September 2020

Accepted: 07 November 2020

Published: 28 November 2020

Original Research Article

ABSTRACT

Solanum procumbens Lour. is a precious medicinal plant with plenty applications in medicine and pharmacy. Auxin is a plant hormone which is critical to the induction and growth of roots of cuttings. In this study, an experiment was conducted to evaluate the effects of indole acetic acid (IAA), indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA) on the rooting and sprouting of *S. procumbens* stem cuttings. The experiment involved four distinctive concentrations of IAA (500, 1000, 1500 and 2000 ppm), IBA (500, 1000, 1500 and 2000 ppm) and IBA + NAA (250 + 250, 500 + 500, 750 + 750 and 1000 + 1000 ppm) along with the control group used to treat *S. procumbens* cuttings. The experiment applied the randomized complete block (RCB) method with 3 replications. The results showed that IAA, IBA and NAA had a remarkable influence on rooting and sprouting abilities of *S. procumbens* cuttings. The best records of sprouting rate (92.34%), root number (32.25), root length (6.8 cm), root weight (0.574 g), sprout length (11.7 cm) and leaf-pair number (7.5) were obtained in cuttings treated with IBA at 500 ppm. Furthermore, the beneficial effects of auxins wore off as their concentrations increased. In general, this study emphasizes that IBA at 500 ppm is the most effective auxin in terms of stimulating rooting and sprouting in *S. procumbens* cuttings. The results of the study also promise to create a supply of high-quality *S. procumbens* seedlings for gardeners.

Keywords: *Solanum procumbens*; auxin; propagation; stem cuttings; rooting; sprouting; seedling.

INTRODUCTION

Solanum procumbens Lour. (the synonym name is *Solanum hainanense* Hance) is a precious and narrowly distributed medicinal plant. It is only found in Vietnam and some other countries such as Cambodia, Thailand, China. *S. procumbens* is comprised of many compounds, for example, polyphenols, terpenoids, steroids and alkaloids [1-4]. *S. procumbens* is proved to have anti-inflammatory, liver protection and anti-cancer effects [3-6]. In the past, *S. procumbens* were harvested mainly from wild plants. However, the wild populations of the species are under rapid decline due to habitat loss, clearing of forest edges, soil erosion, and other biological bottlenecks. Currently, the demand for *S. procumbens* to produce health supplement products is on the increase. While the natural supply of *S. procumbens* is on the verge of depletion, the cultivated area in Vietnam remains small, with both productivity and quality failing to meet the requirements of GACP–WHO. That leads to a huge demand for high-quality seedlings which can well grow and develop.

Vegetative propagation through stem cuttings is a method which offers various advantages: characteristics of the mother plant are preserved; plants grown from cuttings are quick to produce flower and fruit; the rate of multiplication is high and it takes little time to get the seedlings [7-9]. Auxin is a plant hormone that exerts positive impact on root formation and cutting quality [10]. Indole-3-butyric acid (IBA) is a plant growth hormone in the auxin family which is widely used for root stimulation [11]. Naphthalene acetic acid (NAA) and indole acetic acid (IAA) also belong to the auxin family, and they are commonly used for root initiating [12]. Studies have shown that auxin is essential to plant propagation using cuttings [12-24]. However, too high auxin concentration will lead to reverse inhibition, and auxin will become inhibitors then [23-26]. In this study, we conducted experiments to evaluate the effects of auxins (IAA, IBA and NAA) and determine the optimal concentration of these hormones on the clonal propagation of *Solanum procumbens* stem cuttings.

MATERIALS AND METHODS

Research Materials

The *Solanum procumbens* stem cuttings were collected at the Northern Central Center of Medicinal Materials Research (Thanh Hoa, Vietnam). These cuttings (18–20 cm in length, 0.3–0.4 cm in diameter with 3–4 buds) were selected from well-grown and healthy 6-month-old mother plants. Up to 50% of the leaves of the selected cuttings were removed. This study used auxins (IAA, IBA and NAA) that were purchased from Thanh Hoa Chemical Company, Vietnam.

Experimental Design and Hormone Treatments

The experiment was conducted at the Northern Central Center of Medicinal Materials Research (Thanh Hoa, Vietnam) from October 2018 to January 2019. It was established with the randomized complete block (RCB) method. Each formula was replicated 3 times, each time involved 300 cuttings, and the area of each experimental plot was 10 m².

There were 13 treatment formulas in total, including 12 groups with hormone application (T₁ – T₁₂) and 1 control group (T₀) (Table 1). Selected cuttings were washed with distilled water twice. The lower portions of the cuttings were dipped up to 3–4 cm for about 15 minutes according to the treatment formulas in Table 1. Cuttings treated with distilled water were considered as control (T₀). After that, the cuttings were brought to the nursery which had been prepared in advance, placed at a 45-degree angle and covered with soil. Watering the cuttings with the solution which had been used for cuttings treatment as per the formulas.

Caring for the Cuttings

During the first 15–20 days after being put in the nursery, the cuttings required sufficient watering, high air humidity and the soil moisture of 80%. To limit water vapor in leaves, it was essential that the nursery have a roof. In case there was no rain, it was advisable to water 1–2 times a day. After

this period, the required soil moisture was 60-70%, and the watering frequency was once every 2 days. *S. procumbens* could not tolerate waterlogging. In case of heavy rain, water must be promptly drained. Weeds are regularly removed to reduce pests and diseases. Soil treatment was carried out in advance, so during this stage, the cuttings were under no significant influence of pests and diseases.

Data Collection

From the 2nd week onwards, data on callus formation and root initiation were recorded weekly. The sprouting rate, sprout length, leaf-pair number, sprout diameter, root number, root length and root weight of each cutting were recorded after 70 days.

Statistical Analysis

Each experiment was repeated 3 times, 30 samples were observed in each formula. The data were analyzed using IRRISTAT 5.0 for analysis of variance and means separated using Duncan's multiple range test ($p < 0.05$).

RESULTS

Early rooting and shooting developed in most auxin-treated cuttings compared with the untreated ones. Effects of auxins (IAA, IBA and NAA) at different concentrations on rooting and sprouting behaviors of *S. procumbens* cuttings showed differences in sprouting rate, leaf-pair number, sprout length, sprout diameter, root number, root length and root weight (Table 2). Sprouting rate is

one of the important criteria for evaluating the effect of plant growth regulators on cuttings. The highest sprouting rate (92.34%) was observed in cuttings treated with T₅ (IBA 500 ppm), followed by cuttings treated with T₁ (IAA 500 ppm) at 85.34% (Table 2). The combination treatment giving the highest sprouting rate (65.31%) was T₉ (IBA 250 ppm + NAA 250 ppm). Application of T₄ (IAA 2000 ppm), T₈ (IBA 2000 ppm), T₁₁ (IBA 750 ppm + NAA 750 ppm) and T₁₂ (IBA 1000 ppm + NAA 1000 ppm) gave lower sprouting rates compared to the control (Table 2). These were treatment formulas with high concentrations; therefore, application of these treatments exerted a remarkable inhibition on the sprouting rates of the cuttings. This indicated that *S. procumbens* cuttings best responded to IBA at 500 ppm for better sprouting.

In addition to sprouting rate, root indicators are also important criteria for evaluating the cuttings. Auxin plays an important role in stimulating the rooting of cuttings. Records on root number, root length and root weight of *S. procumbens* cuttings are shown in Table 2. Among the treatment formulas with different IAA concentrations, the maximum of root number, root length, and root weight were obtained in T₁ (IAA 500 ppm). Among the treatment formulas with different IBA concentrations, the maximum of root number, root length, and root weight were obtained in T₅ (IBA 500 ppm). Furthermore, cuttings treated in T₅ (IBA 500 ppm) gave the highest root number (32.25), root length (6.80 cm) and root weight (0.547 g) compared to the other treatments (Table 2). In combination treatments, T₉ (IBA 250 ppm + NAA 250 ppm) showed the strongest synergistic

Table 1. Auxin concentrations of the experimental treatments

Treatments	Auxin (ppm)		
	IAA	IBA	NAA
T ₀	–	–	–
T ₁	500	–	–
T ₂	1000	–	–
T ₃	1500	–	–
T ₄	2000	–	–
T ₅	–	500	–
T ₆	–	1000	–
T ₇	–	1500	–
T ₈	–	2000	–
T ₉	–	250	250
T ₁₀	–	500	500
T ₁₁	–	750	750
T ₁₂	–	1000	1000

effect with the root number of 28.07, root length of 6.39 cm and root weight of 0.475 g while T₁₂ (IBA 1000 ppm + NAA 1000 ppm) resulted in the weakest synergistic effect (Table 2). It should be noted that in treatments with higher doses of the three auxin types, remarkable inhibition effects on root number, root length and root weight were obtained among the cuttings.

Sprout length, leaf-pair number and sprout diameter are the criteria for evaluating the growth of cuttings, thereby determining the quality of cuttings under the influence of auxin. The records on these indicators are shown in Table 2. The highest sprout length (11.7 cm) was observed in cuttings treated with T₅ (IBA 500 ppm), followed by cuttings treated with T₁ (IAA 500 ppm) and T₉ (IBA 250 ppm+ NAA 250 ppm) at 9.8 cm and 9.9 cm respectively (Table 2). The greatest sprout diameter (0.43 cm) was recorded in cuttings treated with T₁ (IAA 500 ppm), and the sprout diameter of 0.42 cm was obtained in application of T₅ (IBA 500 ppm) (Table 2). Among the combination treatments, the greatest sprout diameter (0.40 cm) was observed in cuttings treated with T₉ (IBA 250 ppm + NAA 250 ppm) (Table 2). Cuttings treated with T₅ (IBA 500 ppm) and T₉ (IBA 250 ppm + NAA 250 ppm) gave the highest leaf-pair number (7.5), followed by cuttings in application of T₁ (IAA 500 ppm). As the auxin concentration increased, the records on sprout length, leaf-pair number and sprout diameter decreased. Cuttings treated with distilled water (T₀, control group) gave the lowest records on sprout length, sprout diameter and leaf-pair number (Table 2).

DISCUSSION

Vegetative propagation is an integral process to improve the supply of the best cultivars with the highest genetic quality, which is not always recorded among sexually propagated seedlings [17]. Studies have demonstrated that vegetative propagation through stem cuttings is a popular tool for large-scale propagation of threatened plant species which have suffered from abnormal flowering and fruiting behaviors, poor abilities to germinate, formulate seedlings or regenerate [20,27]. Auxin is known to play an important role in stimulating rapid root formation from cuttings of plants [12-24]. In this study, the auxins-treated

cuttings (IAA, IBA and NAA) with different concentrations showed different rooting and sprouting behaviors of *S. procumbens* (Table 2).

It can be indicated from the results of this experiment that the roots of *S. procumbens* cuttings could be effectively formulated in application of auxin (Table 2). The effects of the hormone auxin group on rooting behaviours and development of different plant species have also been discussed in several studies. Shahzad et al. conducted a study on the impacts of IBA and NAA on guava cuttings [12]. Sevik and Guney analyzed how IAA, IBA, NAA and GA₃ affected *Melissa officinalis* [13]. Yusnita et al. studied the effects of IBA and NAA on Malay apple [14]. Kontoh conducted a study on the effectiveness of IBA and NAA in *Voacanga africana* [16]. Kesari et al. analyzed how IBA, IAA and NAA affected *Pongamia pinnata* [17]. Husen et al. conducted a study on the impacts of IBA on mulberry [18]. Kamila et al. analyzed how IBA, IAA and NAA affected *Hypericum gaitii* [22]. Doungous et al. analyzed how IBA, IAA and NAA affected *G. africanum* and *G. buchholzianum* [23]. Yan et al. studied the impacts of IAA, IBA and NAA on hybrid aspen [24]. Studies show that, in general, the group of auxin hormone do have an effect on the rooting process. Our research results also are consistent with these results.

In addition, auxin also has a positive effect on the sprouting rate, sprout length, sprout diameter and leaf-pair number of *S. procumbens* cuttings (Table 2). Previous research suggested that it was the cumulation of carbohydrate in cuttings that promoted the sprouting [12,19]. Application of auxin influenced cuttings in some ways such as increasing the root number, root length; trigger or initiate the production of root-promoting chemicals such as radiocarbon in the roots [12]. Root development in cuttings may require an increase in photosynthetic activity and other activities performed on leaves [19]. Therefore, the application of auxin positively affected the sprouting and leaf development of *S. procumbens* cuttings, as auxin helped to establish a stronger root system that was effective in nutrient absorption. The results in this study are consistent with the role of auxin in the sprouting and leaf development of cuttings reported by other researchers [12,16,17,19].

Table 2. Effects of indole acetic acid (IAA), indole-3-butyric acid (IBA), and naphthalene acetic acid (NAA) treatments on rooting and sprouting of *Solanum procumbens* stem cuttings

Treatments	Sprouting (%)	Root number	Root length (cm)	Root weight (g)	Sprout length (cm)	Sprout diameter (cm)	Leaf-pair number
T ₀	41.75 ± 1.31 ^f	14.70 ± 2.10 ^f	4.74 ± 0.62 ^{dc}	0.180 ± 0.030 ^{f-i}	4.9 ± 1.12 ^c	0.32 ± 0.03 ^c	5.2 ± 0.4 ^f
T ₁	85.34 ± 2.02 ^b	29.92 ± 3.59 ^b	6.51 ± 0.76 ^{ab}	0.518 ± 0.069 ^b	9.8 ± 2.05 ^b	0.43 ± 0.02 ^a	7.3 ± 0.5 ^{ab}
T ₂	72.79 ± 1.86 ^c	26.33 ± 2.30 ^{bc}	5.36 ± 0.52 ^{dc}	0.430 ± 0.035 ^{cd}	8.5 ± 1.92 ^{b-d}	0.40 ± 0.01 ^{a-d}	6.8 ± 0.3 ^{b-d}
T ₃	45.30 ± 1.38 ^f	16.55 ± 2.60 ^f	4.86 ± 0.48 ^{dc}	0.227 ± 0.050 ^f	7.9 ± 1.47 ^{b-d}	0.38 ± 0.03 ^{b-d}	6.2 ± 0.4 ^{dc}
T ₄	30.06 ± 1.18 ^g	15.44 ± 2.60 ^f	4.55 ± 0.64 ^{dc}	0.175 ± 0.019 ^{g-i}	6.4 ± 1.25 ^{c-c}	0.35 ± 0.02 ^{dc}	6.5 ± 0.3 ^{cd}
T ₅	92.34 ± 2.10 ^a	32.25 ± 4.28 ^a	6.80 ± 0.85 ^a	0.547 ± 0.068 ^a	11.7 ± 2.13 ^a	0.42 ± 0.02 ^{ab}	7.5 ± 0.4 ^a
T ₆	75.05 ± 1.80 ^c	26.44 ± 4.46 ^{bc}	5.23 ± 0.63 ^{dc}	0.409 ± 0.049 ^d	8.5 ± 1.81 ^{b-d}	0.41 ± 0.02 ^{a-c}	6.8 ± 0.5 ^{b-d}
T ₇	55.77 ± 1.50 ^c	21.11 ± 3.30 ^d	4.94 ± 0.80 ^{dc}	0.223 ± 0.050 ^{fg}	6.5 ± 1.46 ^{c-c}	0.39 ± 0.03 ^{a-d}	6.9 ± 0.4 ^{a-c}
T ₈	28.09 ± 1.15 ^g	15.63 ± 2.46 ^f	4.50 ± 0.65 ^e	0.136 ± 0.047 ⁱ	6.0 ± 1.57 ^{dc}	0.38 ± 0.02 ^{b-d}	5.8 ± 0.3 ^{ef}
T ₉	65.31 ± 1.57 ^d	28.07 ± 4.20 ^b	6.39 ± 0.71 ^{a-c}	0.475 ± 0.058 ^{bc}	9.9 ± 2.08 ^b	0.40 ± 0.03 ^{a-c}	7.5 ± 0.4 ^a
T ₁₀	56.03 ± 1.51 ^e	22.55 ± 2.90 ^{cd}	5.55 ± 0.59 ^{b-d}	0.326 ± 0.073 ^c	9.1 ± 1.75 ^{a-c}	0.38 ± 0.03 ^{b-d}	6.5 ± 0.5 ^{b-d}
T ₁₁	32.06 ± 1.21 ^g	20.55 ± 3.20 ^d	5.39 ± 0.56 ^{c-c}	0.193 ± 0.048 ^{f-h}	7.8 ± 1.81 ^{b-d}	0.36 ± 0.02 ^{c-c}	6.8 ± 0.3 ^{a-d}
T ₁₂	21.79 ± 1.07 ^h	16.59 ± 2.70 ^{ef}	4.59 ± 0.47 ^{dc}	0.154 ± 0.045 ^{hi}	6.5 ± 1.19 ^{c-c}	0.35 ± 0.02 ^{dc}	6.5 ± 0.5 ^{b-d}

T₀ = distill water, T₁ = IAA 500 ppm, T₂ = IAA 1000 ppm, T₃ = IAA 1500 ppm, T₄ = IAA 2000 ppm, T₅ = IBA 500 ppm, T₆ = IBA 1000 ppm, T₇ = IBA 1500 ppm, T₈ = IBA 2000 ppm, T₉ = IBA 250 ppm + NAA 250 ppm, T₁₀ = IBA 500 ppm + NAA 500 ppm, T₁₁ = IBA 750 ppm + NAA 750 ppm, T₁₂ = IBA 1000 ppm + NAA 1000 ppm. Values are given as means of three replicates ± SD. Within a column, means followed by similar superscripts not different as determined by Duncan's new multiple range test (p < 0.05)

In particular, the treatment of *S. procumbens* stem cuttings with IBA at 500 ppm was found to be superior in rooting and sprouting behaviors compared to other treatments (Table 2). Previous studies have proven IBA to be the best auxin for general use as it is not plant-toxic over a broader concentration range than NAA or IAA [11,17]. Optimal concentrations of IBA could induce the mobilization and utilization of carbohydrates, nitrogen fraction, water and mineral nutrient absorption [11,12]. Better rooting and sprouting records with appropriate IBA concentrations have been reported in many different plant species [12,17,18,20,28].

Although application of auxin improved the rooting and sprouting processes, it is worth noting that a higher concentration of auxin did not essentially give better rooting and sprouting results. The stem cuttings in *S. procumbens* did not significantly increase the rooting and sprouting capacities, which might suggest that at certain levels, auxins became toxic to plants. Similar results were found in other plants such as hybrid aspen [24], *Hemarthria poura* [25] and peach [26]. Results of the current survey are consistent with those by Doungous et al. [23] and Hartmann et al. [27], who have shown that application of auxin at high concentrations can ineffectively promote or even inhibit the rooting and sprouting of cuttings.

CONCLUSION

The seedling shortage is one of the main factors restricting the development of *S. procumbens* material supply, thus restricting the creation of medicinal products with medicinal effects. With the advantages of vegetative propagation, propagating *S. procumbens* by stem cuttings technique was conducted in our research by promoting rooting and sprouting using auxin. In treatments with three auxin types (IAA, IBA and NAA) at different concentrations, *S. procumbens* stem cuttings treated with IBA at 500 ppm were proved to give the best sprouting rate, root number, root length, root weight, sprout length and leaf-pair number. However, it should be noted that the application of higher doses of all three auxin types inhibited the rooting and sprouting behaviors. The results of this study provide an

opportunity for nurseries to easily mass produce *S. procumbens* seedlings, thereby increasing their cultivated areas in Vietnam.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ACKNOWLEDGEMENTS

The authors would like to thank the handling editor and anonymous referees for valuable comments which helped to improve the manuscript. The authors also thank the Northern Central Center of Medicinal Materials Research (Vietnam), for supporting this research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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