

# Effects of Indole Butyric Acid (IBA) and Naphthalene Acetic Acid (NAA) on Rooting and Shooting of Jiaogulan (*Gymnanthemum extensum*) Cutting Stem

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Received: May 15, 2020 / Revised: July 7, 2020 / Accepted: August 3, 2020

**Abstract**—The effects of IBA and NAA on rooting and shooting of the Bitter leaf tree (*Gymnanthemum extensum*) cutting stems were investigated. The stems were obtained from a private garden in Chiang Mai Province. The 25 cm long cutting stems were dipped into distilled water (control), IBA, and NAA with 250, 500, 1000, 2000, and 3000 ppm concentrations for 30 minutes and put in plastic cups in the plant nursery (Program of Biotechnology, Faculty of Science, Maejo University, Chiang Mai) from April to May of 2019. The experiment was performed in a completely randomized design (CRD). The data was collected 30 days after treatments. The result showed that the cutting stem treated with 0 ppm (control), 250 ppm of IBA, and 500 ppm of IBA gave rise to the highest percentage of rooting (100%). In contrast, the cutting stems treated with IBA at 1,000 and 2,000 ppm concentrations yielded 80% of rooting. NAA at 250, 500, 1,000, and 2,000 ppm concentrations gave rise to 73, 73, 73, and 66.7% rooting, respectively. On the other hand, 3000 ppm of NAA provided the lowest percentage of rooting at 53.3%, respectively. Two hundred and fifty ppm of NAA gave the highest average number of roots per cutting compared to the other treatments. The average root length and average root width of control and all treatments were not significantly different ( $P < 0.5$ ). The control, IBA, and NAA at 250, 500, 1,000, and 2,000 ppm concentrations gave the highest percentage of shooting (93 - 100%).

**Index Terms**—*Gymnanthemum extensum*, Indole Acetic Acid, Naphthalene Acetic Acid, Cutting, Rooting, Shooting

## I. INTRODUCTION

Stems of the plant grow like wine, and the leaves have five oval-shaped, saw-toothed edges with white hairs. Jiaogulan can be cultivated from seeds and plant cuttings and can be harvested in 4-5 months after planting [1]. In Thailand, Jiaogulan can grow with good quality in the Northern region, which includes Chiang Mai, Chiang Rai, and Mae Hong Son provinces [2]. Jiaogulan is a popular medicine herb. The leaf and stem of the plant contain saponin glycosides and possess antioxidant activity [3]. Many researchers have reported the optimum concentrations of hormones used for root induction on cutting stems. IBA and NAA are plant bio-regulators that belong to the auxin group [4]. IBA that regulates growth and influences various developmental processes, induces early root formation, callus formation, stem elongation, enhance flowering, enzyme induction, and leaf and fruit senescence [5]. Mulberry stem cutting required plant growth regulators such as IBA and NAA to promote rooting and axillary shoot. Sokhuma et al.[6] reported that the using of IBA and NAA (500, 1,000, 2,000, and 3,000 ppm) on the mulberry stem cutting. The result showed that 3,000 ppm of IBA was the best result of concentration showing 3,000 ppm of IBA was 93.33% survival rate, 86.67 % of rooting, 18.8 roots, 10.82 cm of root length. NAA significantly increased the number of root and root length [7]. However, the effects of the plant growth hormones on Jiaogulan have not been previously reported.

The aim of this study was to investigate the effects of IBA and NAA on rooting and shooting of Jiaogulan cutting stems.

## II. MATERIALS AND METHOD

### A. The Plant Material

The cutting stems of Jiaogulan from private garden Chiang Mai province. The cutting stem was long 25 cm long and diameter about 0.8-1.5 cm.

#### The chemicals

The hormone concentrations used are given below:

IBA: 250, 500, 1,000, 2,000, and 3,000 ppm

NAA: 250, 500, 1,000, 2,000, and 3,000 ppm

### B. The Other Materials

Plastic cup size (8 cm in diameter and 15 cm in height) with plastic cup lid and 750 ml of distilled water.

### C. Experimental Design

The experiment was laid out in a complete randomized design (CRD) with 3 replicates. The total of 330 cutting stem samples were used. The sample size was maintained for each treatment with 10 cuttings/treatment. Prior to the treatment of cutting stem, the bottom of each was prepared with sharp knife to avoid the cutting damage and immersed for 30 minutes in the following treatments:

- Treatment 1: 0 ppm (control)
- Treatment 2: 250 ppm IBA
- Treatment 3: 500 ppm IBA
- Treatment 4: 1,000 ppm IBA
- Treatment 5: 2,000 ppm IBA
- Treatment 6: 3,000 ppm IBA
- Treatment 7: 250 ppm NAA
- Treatment 8: 500 ppm NAA
- Treatment 9: 1,000 ppm NAA
- Treatment 10: 2,000 ppm NAA
- Treatment 11: 3,000 ppm NAA

The samples were air dried for 5 minutes, and the cutting stems were dipped in plastic cups and put in the plant nursery (Program of Biotechnology, Faculty of Science, Maejo University, Chiang Mai, Thailand) from April to May 2019. Over 30 days, the data on percentage of rooting, number and length of root, percentage of shooting, number of shoot, number of leaves, and width and length of leaf were collected.

All data were analyzed using Analysis of Variance (ANOVA). Significantly different means were separated according to the method of Duncan's New Multiple Rang Test (DMRT) as described in Steel et.al. [8].

## III. RESULTS

### A. Percentage of Rooting, Number of Root and Length of Root

The results showed that all 11 experimental treatments were significantly different ( $P < 0.05$ ) (Table I). The control, 250 ppm IBA, and 500 ppm IBA gave the highest percentage of rooting (100%) and follow: 1,000, 2,000 and 3,000 ppm IBA were 80.0, 80.0 and 73.3% respectively with significant difference ( $P < 0.05$ ) (Table I, Fig. 1). NAA in the concentrations of 250, 500, 1,000, and 2,000 ppm gave higher percentage of rooting compared to that from the 3,000 ppm NAA treatment. The number of roots of the 250 and 500 ppm NAA treated samples were higher than those of control and others treatments. The 250 ppm NAA treatment gave rise to the highest length of root (10.69 cm), significantly different from the control ( $P < 0.05$ ) (Table I, Fig. 1).

TABLE I  
PERCENTAGE OF ROOT, NUMBER OF ROOTS AND  
LENGTH OF ROOT

Treatments		Rooting (%)	Number of roots	Length of root (cm)
Control	00 ppm	1,100.00±0.00 <sup>a</sup>	99.33±1.52 <sup>b</sup>	66.99±0.85 <sup>c</sup>
IIBA	2,250 ppm	1,100.00±0.00 <sup>a</sup>	8.20±1.42 <sup>b</sup>	77.69±0.82 <sup>b</sup>
	5,500 ppm	1,100.00±0.00 <sup>a</sup>	8.47±1.36 <sup>b</sup>	77.69±0.82 <sup>b</sup>
	11,000 ppm	880.00±0.11 <sup>b</sup>	57.83±1.72 <sup>c</sup>	7.04±1.60 <sup>c</sup>
	22,000 ppm	880.00±0.12 <sup>b</sup>	75.58±0.95 <sup>c</sup>	4.35±0.94 <sup>d</sup>
	33,000 ppm	373.33±0.12 <sup>c</sup>	33.80±0.37 <sup>d</sup>	22.10±0.24 <sup>d</sup>
NNAA	2,250 ppm	773.33±0.12 <sup>c</sup>	111.64±2.33 <sup>a</sup>	110.69±0.54 <sup>a</sup>
	5,500 ppm	773.33±0.12 <sup>c</sup>	110.80±0.92 <sup>a</sup>	77.95±1.18 <sup>b</sup>
	11,000 ppm	773.33±0.12 <sup>c</sup>	9.73±2.16 <sup>a</sup>	77.05±0.77 <sup>c</sup>
	22,000 ppm	673.33±0.12 <sup>c</sup>	9.00±0.73 <sup>b</sup>	66.66±0.43 <sup>c</sup>
	33,000 ppm	553.33±0.13 <sup>d</sup>	77.82±1.82 <sup>c</sup>	55.35±0.80 <sup>d</sup>

\*<sup>a,b,c,d</sup>The different letters in the same column represent statistically significant differences ( $P < 0.05$ ) by DMRT

### B. Percentage of Shooting and Number of Shoot and Leaves

We found that the control, IBA, and NAA at 250, 500, 1,000, and 2,000 ppm yielded the highest percentage of shooting (93-100%). On the other hand, 3,000 ppm of IBA and NAA led to 60% shooting, which was the lowest shooting percentage, significantly different from the other treatments ( $P < 0.05$ ) (Table II).

Compared to the other treatments, the control,

250 ppm IBA, and 250 ppm NAA led to higher number of shoots. The numbers of shoots and leaves significantly decreased with increasing concentrations of IBA and NAA ( $P < 0.05$ ) (Table II).

The 3,000 ppm IBA and NAA treatments led to the lowest numbers of shoots and leaves (1.22 and 1.00, respectively) (Table II). In contrast, the numbers of leaves in the control, IBA, and NAA at 250 and 500 ppm were higher than those in the other treatments (Table II).

TABLE II  
PERCENTAGE OF SHOOTING, NUMBER OF SHOOTS, AND NUMBER OF LEAVES

Treatments		Shooting (%)	Number of shoots	Number of leaves
Control	00 ppm	1,100.00±0.00 <sup>a</sup>	92.00±1.52 <sup>a</sup>	68.77±0.73 <sup>a</sup>
IIBA	2,250 ppm	1,100.00±0.00 <sup>a</sup>	22.13±0.14 <sup>a</sup>	78.83±0.82 <sup>a</sup>
	5,500 ppm	1,100.00±0.00 <sup>a</sup>	11.67±0.13 <sup>b</sup>	78.43±0.66 <sup>a</sup>
	11,000 ppm	8,100.00±0.00 <sup>a</sup>	51.60±0.16 <sup>b</sup>	77.80±0.48 <sup>b</sup>
	22,000 ppm	8,100.00±0.00 <sup>a</sup>	71.40±0.13 <sup>c</sup>	77.73±0.52 <sup>b</sup>
	33,000 ppm	360.00±0.13 <sup>c</sup>	31.22±0.15 <sup>d</sup>	25.11±0.82 <sup>c</sup>
NNAA	2,250 ppm	7,100.00±0.00 <sup>a</sup>	12.00±0.14 <sup>a</sup>	19.21±0.68 <sup>a</sup>
	5,500 ppm	7,100.00±0.00 <sup>a</sup>	11.73±0.12 <sup>b</sup>	88.77±0.73 <sup>a</sup>
	11,000 ppm	793.00±0.07 <sup>b</sup>	11.69±0.13 <sup>b</sup>	77.00±0.62 <sup>b</sup>
	22,000 ppm	693.00±0.07 <sup>b</sup>	11.43±0.13 <sup>c</sup>	67.36±0.54 <sup>b</sup>
	33,000 ppm	560.00±0.13 <sup>c</sup>	71.00±0.00 <sup>d</sup>	56.44±0.93 <sup>c</sup>

\*<sup>a,b,c,d</sup>The different letters in the same column represent statistically significant differences ( $P < 0.05$ ) by DMRT

### IV. DISCUSSION

This result is an indication that control and IBA enhanced rooting in Jiaogulan (*Gymnanthemum extensum*) stem cutting better than NAA. Cutting of control and treated with IBA had the highest mean percentage of rooting (80 - 100%) than cutting treated with NAA having 53.33-73.3%. These were found to be significantly different ( $P < 0.05$ ). IBA was more effective than NAA in enhancing rooting ability in Jiaogulan stem cutting. The result complies with that other tropical tree species like *Prunus africana* [9] and *Juniperus procera* [10] reported that IBA significantly enhanced rooting ability compared to NAA. Further, it has been evident that IBA was more effect in increasing the percentage of rooting as the case in *Jatropha curcas* [11] and in *Pongamia pinnata* [12] compared to NAA. IBA was more effective in inducing plants rooting than NAA, because the former was more stable than the latter, and more important, IBA can be changed into IAA (Indole Acetic Acid) by the mechanism of parallel to fatty acid oxidation [13].

In the present study, higher concentrations of IBA had significantly low rooting ability in *Gymnanthemum extensum* in agreement with studies various on *Juniperus procera*, which demonstrated

significant difference in mean root numbers due to the variations in IBA concentrations [14]. Similar reported have also been reported in other tree species like *Dalbergia sisso* Roxb that have relatively poor rooting response in presence of high concentration of IBA [15]. The decline in rooting percentage with high concentration of IBA is inhibitory to root initiation, as has been recorded in a number of other tree species. Depending on the endogenous level of growth regulating substance, exogenous application of auxin may be promotive, in effective or even inhibitory for the rooting of cuttings [16]. Other results were rooting of cuttings may be influenced more by the other factors such as physiological age of the cuttings and status of rooting media in terms of aeration and drainage properties as stated by Narin and Watna [17].

All treatments were significantly different in percentage of shooting, number of shoot, and number of leaves. The results found that control, IBA and NAA at 250, 500, 1,000, and 2,000 ppm were highest percentage of shooting (93.3 - 100%). In contrast IBA and NAA 3,000 ppm were the lowest percentage of shooting (60.0%). This study was similar to the previously reported effects of auxins (IBA and NAA) on shooting of mulberry (*Morus alba* Linn.) cv. Chiang Mai 60, which was decreased with high

concentrations of hormones (IBA and NAA). The result showed that the control (0 ppm), IBA and NAA at 250, 500, and 1,000 ppm were higher percentage of shooting than IBA and NAA at 2,000 and 3,000 ppm [18]. In This study we found that the high auxins (IBA or NAA) had decreased the percentage of rooting and shooting of Jiaogulan stem cutting. Since Jiaogulan stem cutting have adequate auxins which induce rooting and shooting. Thus the high concentrated of auxins can inhibit growth in term of rooting and shooting [19].

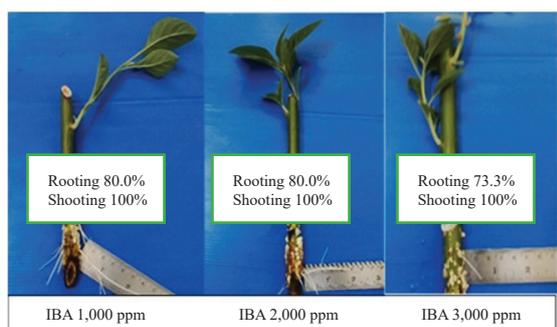
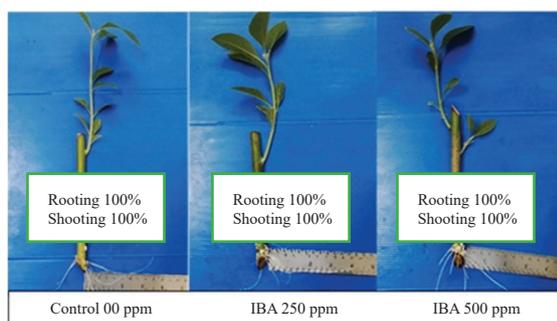


Fig. 1. Effects of IBA on rooting and shooting of Jiaogulan (*Gymnanthemum extensum*) cutting stem.

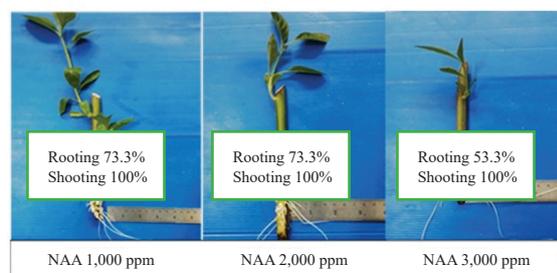
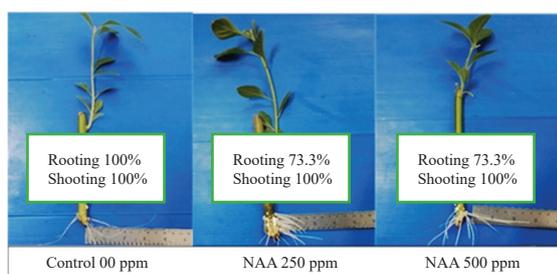


Fig. 2. Effects of NAA on rooting and shooting of Jiaogulan (*Gymnanthemum extensum*) cutting stem.

## V. CONCLUSION

The results showed that there were significant differences on percentage of rooting, number and length of roots, percentage of shoots, number of shoots, number of leaves, and width and length of leaves. Slight selective response to growth hormone was observed in percentage of rooting, number and length of roots: the control and the IBA treated cutting formed roots better than the NAA treated cutting. However, the control (untreated) cutting gave the best performance for all the parameters assessed on roots and shoots of Jiaogulan.

## ACKNOWLEDGEMENT

The authors gratefully acknowledge the financial support from the Program of Agriculture Interdisciplinary, Faculty of Engineering and Agro-Industry, Maejo University, Chiang Mai, Thailand and the nursery and laboratory Program in Biotechnology, Faculty of Science, Maejo University, Chiang Mai, Thailand.

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