

## EFFECTS OF PLANT HORMONES AND EXTERNAL FACTORS IN PROPAGATION OF YELLOW FLOWER TEA BY CUTTINGS

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### SUMMARY

Yellow flower tea (*Camellia* sp.) are multiple purposes of high economic values, used as a nutritious beverage, medicine and ornamental plants. This paper shows results of the propagation of three Yellow flower tea species by cuttings. After microbial treatment by Benlat 0.5% for about 15 minutes, cuttings continued to be treated with plant growth regulators for 5 minutes, evaluating the results after 75 days. In this study, we investigated effects of plant hormone  $\beta$ - indol butyric acid (IBA) and external factors on the cutting propagation of *C. tamdaoensis*, *C. flava* and *C. chrysantha*. The results showed IBA at 150 ppm presents the best performance of cuttings for all three tested Camellias. Moreover, the position of cutting on a mother affected rooting of Yellow flower tea cuttings. The top and middle cuttings reached the survival rate of 75.33 - 80.67%, the root formation rate of 73.33 - 77.67%. In addition, light is a primary energy source necessary for photosynthesis, growth and development of plants. Shade light 50 percent as the survival rate of 87.45 - 93.34% evaluate the results after 60 days. The plant cutting method is suitable to propagating Yellow flower tea for breeding purpose serving conservation and pharmaceutical marketing.

**Keywords:** *C. chrysantha*, *C. flava*, *C. tamdaoensis*, cutting position, light intensity, plant cuttings, Yellow flower tea.

### 1. INTRODUC.DTION

Yellow flower tea (*Camellia* sp.) is a plant belonging to the tea family (*Theaceae*), tea genus (*Camellia*). In the world, the number of species in *Camellia* genus was estimated to be from 120 to 300. A total number of Vietnamese yellow *Camellia* species recorded until now is about 40 species (Dung L.V, 2016). Mostly, the Yellow flower tea of Vietnam are rare and highly endemic, however, they are not so many in individual numbers and narrowly distributed (such as Tam Dao national park, Cuc Phuong national park, Ba Vi national park, Bac Giang, Quang Ninh, and some other provinces of Central and South Vietnam) (Manh T.D *et al.*, 2019).

Many Yellow flower tea species in Northern Vietnam have high economic value and being exploited as nutritious beverages, medicines because they are containing some contains trace elements such as Selenium (Se), Germanium (Ge), Zinc (Zn), Vanadium (V), Molybden (Mo), Mangan (Mn), Kalium (K) (Karak *et al.*, 2017) and some other elements that are effective in protecting health, prevent cancer, strengthen vascular elasticity, regulate cholesterol, lower blood fat, strengthen

immune system (Hyejin J *et al.*, 2018). Among the Yellow flower tea species distributed in Vietnam, there are 3 species are more often be exploited including *C. chrysantha*, *C. flava* and *C. tamdaoensis*. Unfortunately, due to habitat loss, indiscriminate exploitation, climate change, along with low natural regeneration capacity and excessive collecting of seedlings in recent decades, its natural populations have declined dramatically (Manh T.D *et al.*, 2019). Therefore, research for more effective methods in propagation of these *Camellia* sp. plays important roles in conservation and development in Vietnam.

Recently, there are some published papers of propagation of Yellow flower tea (*Camellia* sp.) in Vietnam showed that the most popular propagation method is cuttings which are affected by many internal and external conditions (Viet N.V *et al.*, 2016, 2017; Hoang P.V *et al.*, 2016). Propagation research of *C. chrysantha* by cuttings using growth regulators was conted (Viet N.V *et al.*, 2016). The result showed that IBA gave better results than ABT and NAA; however, the rate of root and shoot intion is still relatively low. Interestingly, when applied the same regulators to another Yellow

flower tea (*C. flava*) (Viet N.V *et al.*, 2017) indicated that the IBA, NAA had similar effects on rooting and shooting induction in middle-cuttings. Another example of plant hormone factor affected to vegetative propagation of *Camellia* sp. was reported by Hoang P.V *et al.* (2016). The authors presented a good influence of ABT on the induction of root and shoot at both top and middle cuttings of *C. tamdaoensis*. Rooting medium and propagation season also had effect on rate of rooting, which indicated that using rooting medium of sterilized sand and propagating in spring were the best for Yellow flower tea (Thach D.V, 2017). These evidences suggest the propagation efficiency of Yellow flower tea (*Camellia* sp.) by cuttings affected by many factors such as position of the cuttings on the shoot (Hoang P.V *et al.*, 2016; Duc T.D *et al.*, 2019), rooting medium (Viet N.V *et al.*, 2016, 2017), season when the cutting were made as well as physical and environmental factors (Thach D.V, 2017). Therefore, it is very practical to research and optimize conditions to get highest propagation efficiency. This study was conducted on evaluate, compare and optimize the effect of IBA along with others such as nutritional conditions, physical conditions to effectively propagate three Yellow flower tea species (*C. chrysantha*, *C. flava* and *C. tamdaoensis*) in propagation by cuttings.

## **2. RESEARCH METHODOLOGY**

### **2.1. Materials**

*Plant samples:* The cutting (not too young and not too old) of three *Camellia* sp. (*C. tamdaoensis*; *C. chrysantha*; *C. flava*) were collected from well-grown, disease-free stock plants in Tam Dao national park, Ba Vi national park (Hanoi city) and Ba Che, (Quang Ninh province), respectively. Mother plants were 1 - 1.4 meter tall, 2 - 7 cm diameter at the stump, and 0.8 - 1.4 meter crown diameter at the time of cutting collection in 11/2018. The climate conditions, where cuttings were collected, include annual precipitation of 1,500

- 1,700 mm, the temperature of 21 - 23<sup>0</sup> C, and air humidity of 85 - 90%. The cuttings were collected from the first branches, which were 8 - 12 months old. After cutting, branches were protected in buckets with ordinary water covering 2 - 3 cm branch bases. It was then transported to the nursery. Total time required from cutting branches to fishing work in a nursery in Hanoi was less than 24 hours.

*Planting substrates:* a mixture of topsoil, burnt husk and clean sand (ratio is 2:1:1).

*Chemicals:* Plant Growth regulators (IBA); fungicide chemicals (Benlat 0.5%).

### **2.2. Methods**

Rooting medium and plant growth regulators have been reported playing important effect on rate of rooting (Teshome *et al.*, 2016; Viet N.V *et al.*, 2016, 2017). Here, we studied the effect of plant hormone to root induction of Yellow flower tea as described by Teshome *et al.*, (2016) and Viet N.V *et al.*, (2017). The cuttings were cut from healthy branches in the morning by using a sharp knife (an angle between the cut and the cutting base should be 45<sup>0</sup>). The cuttings were 10 - 13 cm long and had at least 2 sleeping shoots. Then, leaves on cuttings were cut about 2/3 of the area. After that, the cuttings were soaked into water before being treated with Benlat 0.5% solution for 15 minutes to kill fungi. After fungi treatment, the cuttings were treated separately by one type of plant hormone with different concentrations of IBA (0 (control), 100, 150, 200 ppm) in 15 minutes. Then these treated cuttings were planted into polythene sleeves containing prepared soil mixture (topsoil: burnt husk: sand in the ratio of 2: 1: 1) (Viet N.V *et al.*, 2017) as rooting media at evening. After plugging, the cuttings were covered entirely with white plastic and were irrigated 2 - 3 times/day by spraying to keep moisture of topsoil approximately 90%. Notably, the mixtures were treated with fungicide solution at least 2 days before planting and was watered prior plugging the cuttings. The base of the cuttings was submerged in a substrate of 2.5 - 3.0 cm and in control of hormone treatment cuttings dipped into distilled water.

To investigate the influence of cutting position on propagation of Yellow flower tea, cuttings with a minimum diameter of 9.0 mm were obtained from basal, middle and distal locations on the *Camellia* sp. shoot (adapted from Hoang P.V *et al.*, 2016; Rakibuzzaman *et al.*, 2018). The cuttings were then treated in 15 minutes with optimum IBA concentration determined from above experiments and planted as described above.

We also investigated the effect of light to the development of cuttings. The experiments were carried out under 4 different conditions: S<sub>1</sub>: cover 25%; S<sub>2</sub>: cover 50%; S<sub>3</sub>: cover 75%; S<sub>4</sub>: not cover light (Adapted from Thuoc N.H *et al.*, 1966; Chai *et al.*, 2018).

All experiments were carried out under nursery conditions in spring at Nursery Garden, College of Forestry Biotechnology, Vietnam National University of Forestry, Vietnam. The experiments were randomly designed with 3 replicates and more than 30 cuttings per each replicate.

*Data collection:* 75 days after planting, the cuttings were visually observed and recorded every 20 days with following parameters: number of dead and cutting survival, number of shoot- inducing cuttings, number of root-inducing cuttings, number of roots per sample, length of root.

*Data analysis:* Data were processed and analyse by SPSS and Excel

### 3. RERULTS AND DISCUSSION

#### 3.1. Effect of plant growth regulators to characterization of cuttings

Considering the problem of poor establishment of tea stem cuttings in the nursery the present study was carried out to determine the effect of IBA to propagation by cutting of *Camellia* sp and to select the optimal concentration of IBA for raising tea plants in the nursery.

##### 3.1.1. Effect of plant growth regulators on survival rate of *Camelliae* sp. cuttings

Survival rate of cuttings is a key parameter for a successful vegetative propagation and be depended by many factors such as climate conditions, care regime (Teshome *et al.*, 2016; Thach D.V, 2017), fertilizer (Manh T.D *et al.*, 2019), internal factors, especially types and growth regulators, named auxin group (Viet N.V *et al.*, 2016, 2017). Among those, auxin play most important because auxin (i) has the ability to stimulate rooting induction from the cutting site of the cuttings and (ii) directly affects to quantity and quality of the roots produced by cuttings. In this experiment, the effects of a growth regulator, IBA, were studied. Treatments and experimental results are presented in table 1.

**Table 1. Effect of plant growth regulator on survial rate of cutting**

Treatment	Spieces	IBA (ppm)	Temporal (days) and Survival rate (%)			
			15	35	55	75
Control	<i>C. tamdaoensis</i>	0	72.89±2.2	70.76±2.3	61.73±2.5	55.61±2.1
C <sub>1</sub>		100	88.89±2.2	86.76±3.1	79.73±2.5	77.61±2.3
C <sub>2</sub>		150	89.89±3.1	88.79±3.2	82.79±3.1	81.76±2.5
C <sub>3</sub>		200	91.93±3.1	87.88±2.1	80.83±4.1	78.79±3.4
Control	<i>C. flava</i>	0	73.67±2.3	70.33±2.4	62.71±2.4	54.33±3.1
C <sub>4</sub>		100	83.67±2.3	80.33±2.4	72.71±2.4	68.33±3.1
C <sub>5</sub>		150	86.90±3.2	86.82±3.2	73.78±3.2	72.24±2.5
C <sub>6</sub>		200	84.87±3.0	81.76±3.3	71.73±4.3	69.33±1.6
Control	<i>C. chrysantha</i>	0	71.89±2.2	69.76±3.1	62.73±2.5	56.61±2.3
C <sub>7</sub>		100	94.44±2.5	91.00±3.2	78.89±2.5	73.33±2.3
C <sub>8</sub>		150	94.44±2.4	92.00±2.4	82.56±2.6	83.33±3.1
C <sub>9</sub>		200	93.67±3.5	91.00±2.1	77.78±2.1	77.67±2.0

*Note:* Test results obtained by statistical methods according to Pearson's  $X^2_n$  standard. All experimental formulas give  $X^2_n$  value  $> X^2_{0.05}$ . Data represents mean values  $\pm$  SE of three replicates with 30 explants in each treatment and the experiment was repeated three times.

Results showed that in the presence of IBA, survival rate of all three tested *Camellia* sp. was higher than wildtype but decreased during time frame and was dependent upon tea species. During the root induction phase, (0 to 35 days), survival somewhat decreased, but the effect was not significant ( $P < 0.05$ ). In contrast, survival rate decreased rapidly and significantly during the growth phase. After 75 days of culture (the development phase), the survival rate stabilized for all three species and was highest at 150 ppm of IBA with 81.76%, 72.24% and 83.33% for *C. tamdaoensis*, *C. flava* and *C. chrysantha*, respectively. These results are consistent with other studies in which IBA showed different effects depend on *Camellia* sp. (Viet N.V *et al.*, 2016, 2017). Moreover, the result also indicated that dipping of cuttings with higher concentration of IBA (200 ppm) reduced the survival rate of cuttings. This similar phenomenon had been

observed and reported by some authors (Hoque, 2016, Viet N.V *et al.*, 2017). The reduction of survival rate of cuttings dipped with high auxin concentration could be auxin with high concentrations may lead to growth abnormalities such as inhibiting shoot growth, stimulating ethylene biosynthesis, producing abscisic acid, causing premature aging, necrosis and die. All in all, these results would strengthen positive effects of IBA and different *Camellia* sp. species on their ability of propagation by cuttings and suggest that IBA would be used at 150 ppm for further experiments.

**3.1.2. Plant growth regulator, IBA, promote shoot induction of *Camellia* sp. cuttings**

Bud regeneration is one of important criteria for evaluating the cuttings efficiency. Hence, together with observation of survival rate, the bud regeneration also be monitored (20 ÷ 40 days/time). The results were presented in table 2.

**Table 2. Effect of plant growth regulator on bud generation of *Camelliae* sp. cuttings**

Treatment	Species	Temporal (day)					
		15		35		75	
		Average of bud generation rate (%)	Average of shoot numbers per cuttings	Average of bud generation rate (%)	Average of shoot numbers per cuttings	Average of bud generation rate (%)	Average of shoot numbers per cuttings
Control	<i>C. tamdaoensis</i>	40.23±1.6	0.38±0.2	46.42±1.6	0.43±0.2	50.17±1.5	0.49±0.2
C <sub>1</sub>		59.68±1.6	0.72±0.1	62.80±2.6	0.92±0.1	70.68±1.7	1.27±0.1
C <sub>2</sub>		65.74±2.2	0.74±0.2	72.85±2.0	0.97±0.2	75.74±2.2	1.54±0.2
C <sub>3</sub>		62.71±1.1	0.69±0.1	70.80±2.5	0.88±0.2	72.71±2.1	1.29±0.1
Control	<i>C. flava</i>	42.04±1.6	0.36±0.2	46.02±1.6	0.39±0.2	51.12±1.3	0.45±0.2
C <sub>4</sub>		57.68±2.5	0.68±0.2	61.80±3.1	0.91±0.2	67.68±2.7	1.27±0.1
C <sub>5</sub>		63.74±4.2	0.72±0.1	65.85±1.6	0.96±0.1	69.74±2.2	1.62±0.2
C <sub>6</sub>		61.71±2.5	0.67±0.1	63.80±2.1	0.87±0.2	66.71±2.1	1.21±0.1
Control	<i>C. chrysantha</i>	41.21±1.6	0.33±0.2	46.03±1.3	0.43±0.2	50.24±1.5	0.49±0.2
C <sub>7</sub>		63.74±1.4	0.84±0.2	70.85±3.0	1.17±0.2	73.74±2.5	1.20±0.2
C <sub>8</sub>		65.33±2.6	0.87±0.1	76.00±4.1	1.18±0.2	75.33±2.2	1.57±0.1
C <sub>9</sub>		60.71±3.2	0.79±0.2	69.80±3.1	1.08±0.2	70.71±3.0	1.23±0.2

Note: Test results obtained by statistical methods according to Pearson's  $X^2_n$  standard. All experimental formulas give  $X^2_n$  value  $> X^2_{0.05}$ . Data represents mean values ± SE of three replicates with 30 explants in each treatment and the experiment was repeated three times.

As can be seen from table 2, the shoot induction rate in the present of IBA is higher than the one in the control and when increased growing time led to the higher rate of shoot induction at all experiment. The buds on cuttings sprouted out into lateral shoots after 15 days of planting and one to several new shoots continuously developed from each bud eye region causing increase of shoot numbers. The maximum number of shoots were observed at 75 days in all treatments and varied among tested plants. For example, the shoot inducing rate of *C. chrysantha* at 150 ppm grew up from 65.33% at 15 days to 75.33% after 75 days of planting, while it raised up from 65.74% to 75.74% for *C. tamdaoensis* at the time point is 15 and 75 day after planting. This suggest the dependence of shoot regeneration of *Camellia* sp. on the species using for vegetative regeneration.

It also can be seen from table 2 that the more concentration of IBA were the higher rate of cutting inducing shoots for three *Camellia* sp. For example, the cuttings of *C. tamdaoensis* after 75 days of planting had smallest regeneration rate at 50.17% for control and highest value (75.74%) was observed for IBA treatment dose at 150 ppm. A similar observation was reported for *C. flava* and *C. chrysantha* that took up maximum shoot regeneration rates at 150 ppm with 69.74% and 75.33%, respectively. However, our results also indicated that the IBA at 200 ppm decreased the shoot generation rate of three testing *Camellia* sp. In addition, the average number of shoots per cuttings of three species of Yellow flower tea was low in all treatments, the highest value of the average of shoot numbers per cuttings was 1.54, 1.62 and 1.57 for *C. tamdaoensis*, *C. flava* and *C. chrysantha*, respectively. Thus, it can be said that Yellow flower tea had a relatively slow ability of shoot regeneration.

Bud dormancy in plants is an adaptive strategy for the survival of drought, high and low temperatures and freeze dehydration stress that limit the range of cultivar adaptation.

Therefore, development of a comprehensive understanding of the biological mechanisms and external factors involved in bud dormancy is needed to promote advances in selection and breeding, and to develop improved cultural practices for existing plant cultivars (Hao *et al.*, 2019). Auxin are well known to stimulate plant cell division, release of bud dormancy, induce adventitious bud formation and growth of lateral buds (Hao *et al.*, 2019). In this study, breaking bud dormancy in all three *Camellia* sp was observed for IBA at 150 ppm. These results are consistent with other studies on *Camellia* sp. (Viet N.V *et al.*, 2016, 2017). In a nutshell, these results validate the important role of auxin in tea plant dormancy regulation and provide useful information for further functional studies.

### **3.1.3. Plant growth regulator, IBA, enhances root induction of *Camellia* sp. cuttings**

On cuttings being treated with auxin solution, the largest auxin uptake occurs in the cut surface, so auxin acts to stimulate uncertainly root formation at these sites. After 75 days of planting, roots of the cuttings were collected and measured. However, most of the treated cuttings had secondary root and the number of roots on cuttings was high, so only the primary roots were counted. The results are shown in table 3 and figure 1A, B, C.

Rooting of cuttings were influenced significantly ( $P < 0.05$ ) by the application of IBA (Table 3). As can be seen from table 3, all results of treated samples were higher than the one of control. Rooting percentage of cuttings varied from 46.24% to 80.33% and the maximum (80.33%, 71.67% and 78.33%) rooting was recorded when the respective cuttings of *C. tamdaoensis*, *C. flava* and *C. chrysantha* were treated with 150 ppm IBA. The minimum rooting percentage of 46.24% was recorded in control of *C. flava*. In addition, data presented in table 3 showed that root number, root length and root index were significantly affected ( $P < 0.05$ ) by IBA.

Table 3. Effect of IBA to root induction of *Camellia* sp. cuttings

Treatment	Species	Average of Rooting rate (%)	Average of root numbers/cutting	Average of root length (cm)	Root index
Control	<i>C. tamdaoensis</i>	56.43±2.1	1.37±0.6	1.29±0.1	5.23
C <sub>1</sub>		78.67±1.7	3.17±0.6	2.29±0.3	7.26
C <sub>2</sub>		80.33±2.1	3.76±0.3	2.33±0.3	8.76
C <sub>3</sub>		77.33±2.5	3.33±0.4	2.28±0.4	7.59
Control	<i>C. flava</i>	46.24±1.1	1.12±0.5	1.25±0.3	4.73
C <sub>4</sub>		67.73±2.2	3.34±0.6	2.17±0.6	7.25
C <sub>5</sub>		71.67±3.1	3.63±0.5	2.31±0.2	8.39
C <sub>6</sub>		68.33±2.0	3.35±1.2	2.26±0.2	7.57
Control	<i>C. chrysantha</i>	58.33±2.1	2.17±0.6	1.22±0.1	5.63
C <sub>7</sub>		74.78±2.3	3.95±1.1	2.08±0.4	8.22
C <sub>8</sub>		78.33±2.5	4.61±0.7	2.12±0.6	9.77
C <sub>9</sub>		72.67±2.4	4.34±1.2	2.00±0.2	8.68

Note: Test results obtained by statistical methods according to Pearson's  $X^2_n$  standard. All experimental formulas give  $X^2_n$  value >  $X^2_{0.05}$ . Data represents mean values ± SE of three replicates with 30 explants in each treatment and the experiment was repeated three times.



Figure 1. Effect of IBA at 150 ppm to root and shoot induction of Yellow flower tea cuttings (A): *C. tamdaoensis*; (B): *C. flava*; (C): *C. Chrysantha*

The average of root number per cutting ranged from 1.12 to 4.61 recorded in control of *C. flava* and treatment of IBA at 150 ppm for *C. chrysantha* respectively. In all three tested *Camellia* sp., the average of root number gradually increased with the increased of IBA concentration. Maximum average of root number (3.76, 3.63 and 4.61) was observed

when the cuttings of *C. tamdaoensis*, *C. flava* and *C. chrysantha* treated with 150 ppm IBA.

The root length of the cuttings was responded significantly to the treatments. The root length ranged from 1.22 cm to 2.33 cm in different treatments. The maximum root length (2.33; 2.31; 2.12 cm) was observed in treatment of respective cuttings of *C.*

*tamdaoensis*, *C. flava* and *C. chrysantha* at 150 ppm IBA.

Root index is the general criteria for root quality of three tested *Camellia* sp. The root index was ranged from 4.73 - 9.77. The highest value of the root index for cuttings of *C. tamdaoensis*, *C. flava* and *C. chrysantha* at 150 ppm IBA is 8.76; 8.39 and 9.77, respectively. IBA itself can be used for single node cutting of tea plant as plant regulator and the experimental results of 8000 ppm IBA showed the best result with regard to root number (9.83) of tea cutting. Viet N. V *et al.*, (2018) mentioned that stem cuttings pretreated with IBA in long-dip method got the best rooting traits (rooting 71.67%, root number 3.43 and root length 2.31 cm) in *Camellia* cuttings. The supported findings were reported by Hoque *et al* (2016) who found that maximum cumulative shoot length was recorded in application of auxin at 4000 ppm in *Camellia* (*C. sinensis*) cuttings.

All in all, the results indicated that IBA treated cuttings demonstrated better results as

compared to control in all agronomic parameters. The vegetative propagation of tea through stem cuttings with IBA treatment can be applied on commercial scale for rapid multiplication. IBA application in *Camellia* cutting at 150 ppm produced the highest rooting rate (80.33%), survival rate (83.33%) and root length (2.33 cm). Hence, it can be inferred that IBA had a positive effect on the establishment of stem cuttings of *C. tamdaoensis*, *C. flava* and *C. chrysantha* and 150 ppm IBA can be applied for raising plants from stem cuttings in the tea nursery.

**3.2. Cutting position affected rooting induction of *Camellia* sp. cuttings**

Rooting is a complex developmental process that can be affected by internal and external factors (Peterson *et al.*, 2018). In *Camellia* sp, position of cutting base significantly affected rooting percentage (Hoang P.V *et al.*, 2016; Viet N.V *et al.*, 2017). In this study, we investigated the effect of different cutting position on a *Camellia* sp. shoot. The results were presented in table 4.

**Table 4. Effects of cutting position on rooting of *Camellia* sp.**

Type of cuttings	Species	Average of survival rate (%)	Average of rooting rate (%)	Average of root numbers/cutting	Root length (cm)
Distal	<i>C. tamdaoensis</i>	78.67±2.2	77.67±2.6	3.42±0.2	1.97±0.6
	<i>C. flava</i>	75.33±1.1	75.33±2.0	3.45±0.7	1.98±0.3
	<i>C. chrysantha</i>	77.33±2.5	75.11±1.1	3.53±0.4	2.12±0.6
Middle	<i>C. tamdaoensis</i>	80.67±1.0	75.67±2.0	3.23±0.5	1.95±0.3
	<i>C. flava</i>	77.33±1.6	73.33±1.1	3.26±0.3	1.91±0.3
	<i>C. chrysantha</i>	76.00±1.0	74.78±1.5	3.39±0.1	1.85±0.3
Basal	<i>C. tamdaoensis</i>	73.33±1.5	65.33±1.4	2.87±0.3	1.94±0.4
	<i>C. flava</i>	72.83±0.3	63.33±1.6	2.90±0.5	1.90±0.3
	<i>C. chrysantha</i>	71.67±1.2	61.33±1.2	2.97±0.7	1.93±0.2

Note: Test results obtained by statistical methods according to Pearson's  $X^2_n$  standard. All experimental formulas give  $X^2_n$  value  $> X^2_{0.05}$ . Data represents mean values  $\pm$  SE of three replicates with 30 explants in each treatment and the experiment was repeated three times.

The results showed that the position of the stem cuttings in the branches significantly affected to rooting of *C. tamdaoensis*, *C. flava* and *C. chrysantha*. Maximum survive rate were found in apical portion whereas the lowest survive rate count was in basal portion (Table 4). Similar observation was observed on other rooting criteria such as rooting percent, number of roots per cutting, root length and root index.

In addition to effect of cutting location, the *C. tamdaoensis* presented highest value of survival rate (80.67%) and rooting percent (77.67%) while *C. flava* showed highest value (3.45) of number of roots per sample and *C. chrysantha* presented highest number (2.12 cm) for root length. These results are consistent with other reports for *Camellia* sp (Hoang P.V *et al.*, 2016; Viet N.V *et al.*, 2016). The position of cutting in the shoot affected to rooting since it interferes with hormonal and juvenile issues (Rakibuzzaman *et al.*, 2018). Distal parts are more juvenile and have higher rates of auxin synthesis and basal stem cuttings,

in spite of the lower levels of endogenous auxin (Cunha *et al.*, 2015). It was observed that cutting position had significant effect on *Camellia* sp. propagation and distal portion can be suggested to be used.

**3.4. Shading influenced on survival rate of *Camellia* sp. cuttings**

Because stem cuttings of *Camellia* sp are used for artificial propagation it is necessary to test their growth response to various light conditions. It is also necessary to measure additional physiological parameters to determine what level of light is appropriate for optimal growth and photosynthetic activity of *Camellia* sp. cuttings. This data will help devise effective methods for horticultural propagation using appropriate shade treatment above the stem cuttings. Four different light regimes were applied during the development period. The results were shown in table 6.

**Table 6. Light intensity affects to survival rate of *Camellia* sp. cuttings**

Treatment	Species	Survival rate (%)
S <sub>1</sub>	<i>C. tamdaoensis</i>	83.31±1.2
	<i>C. flava</i>	81.10±0.1
	<i>C. chrysantha</i>	78.90±1.3
S <sub>2</sub>	<i>C. tamdaoensis</i>	93.34±1.0
	<i>C. flava</i>	92.21±0.5
	<i>C. chrysantha</i>	87.45±0.4
S <sub>3</sub>	<i>C. tamdaoensis</i>	75.57±1.6
	<i>C. flava</i>	75.00±1.4
	<i>C. chrysantha</i>	74.43±1.1
S <sub>4</sub>	<i>C. tamdaoensis</i>	6.33±0.3
	<i>C. flava</i>	4.26±0.2
	<i>C. chrysantha</i>	3.57±0.4

Environmental conditions in the rooting bench where leafy cuttings are rooted are crucial for successful rooting. In particular, leaf temperature and air vapor pressure deficit (VPD) play a pivotal role in leaf survival and activity (Chai S *et al.*, 2018). In our experiment, the rooting bench was equipped with a mist apparatus that cooled leaves and

decreased air VPD. All treatments were subjected to the same frequency of mist spray and as consequence of the different light regimes at which leafy cuttings were exposed, air temperature, relative humidity (RH), and air VPD varied depending on the treatment. Particularly, in S<sub>4</sub> treatment, temperature increased and RH decreased in comparison

with other three treatments resulting in higher values of air VPD resulting lowest value of survival rate compared to the other three treatments in three *Camellia* sp. (Table 6). On the other three treatment, the survival rate of S<sub>2</sub> treatment showed highest value (93.34%, 92.21%, 87.45%) for cutting of *C. tamdaoensis*, *C. flava* and *C. chrysantha*, respectively. These results are consistent with other studies on *Camellia* sp. (Viet N.V *et al.*, 2017; Chai *et al.*, 2017). These results contribute to explain the contrasting effect of light intensities observed in many species in previous studies. High light intensity is considered detrimental for cutting rooting because it increases leaf temperature and transpiration and facilitates leaf and cutting dehydration. However, high light regimes decreased the rooting of cutting in many woody and herbaceous species. On the other hand, in some other specie daily light irradiation is positively correlated with cutting rooting and development (Viet N.V *et al.*, 2017). If carbohydrate pool of cuttings can influence cutting rooting, then sustained leaf photosynthesis could contribute to replenish or to increase cutting carbohydrate content. In greenhouses, light intensity, the main driver of leaf photosynthesis, is generally kept low below the light saturation point for photosynthesis) to limit leaf transpiration and heating. Moderate light regimes (below of light saturation point) of leafy cuttings can contribute to improve photosynthetic activity of leafy cuttings. Our study suggests using 50% of shading for propagation of *C. tamdaoensis*, *C. flava* and *C. chrysantha* by cuttings.

#### 4. CONCLUSIONS

Yellow flower tea is evergreen and shadetolerant shrubs and small-sized trees. It has been used as drink, traditional medicines for health improvement. In recent years, the natural propagation of Yellow flower tea is low, hence more researches on vegetative propagation of Yellow flower tea have been widely conducted. However, this method relies

on many internal and external factors. Our results showed that IBA at 150 ppm gave the best effect for vegetative propagation of *C. tamdaoensis*, *C. flava* and *C. chrysantha* by cuttings. In addition, the result also presented collection of cuttings from different part of the mother shoots influenced rooting percentage. Finally, cutting survival percentage and quality can be enhanced by optimization of light environment during rooting.

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## **ẢNH HƯỞNG CỦA HORMONES THỰC VẬT VÀ MỘT SỐ YẾU TỐ MÔI TRƯỜNG ĐẾN NHÂN GIỐNG TRÀ HOA VÀNG BẰNG PHƯƠNG PHÁP GIÂM HƠM**

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### **TÓM TẮT**

Trà hoa vàng (*Camellia* sp.) là loài cây đa tác dụng có giá trị kinh tế cao, dùng làm đồ uống bổ dưỡng, dược liệu và trang trí cảnh quan. Bài báo này, công bố kết quả nhân giống 3 loài Trà hoa vàng bằng phương pháp giâm hom. Hom được xử lý nấm bằng Benlat 0,5% trong 15 phút, tiếp tục xử lý hom với chất điều hòa sinh trưởng thực vật trong 5 phút, đánh giá kết quả sau 75 ngày. Trong nghiên cứu này, chúng tôi đã đánh giá ảnh hưởng của hoóc môn thực vật (IBA) và các yếu tố bên ngoài đối với nhân giống bằng hom của *C. tamdaoensis*, *C. flava* và *C. chrysantha*. Kết quả nghiên cứu cho thấy IBA 150 ppm thể hiện hiệu suất giâm hom cao cho cả ba loại trà hoa vàng được thử nghiệm. Hơn nữa, vị trí cắt trên một mẫu chồi đã có ảnh hưởng đến việc ra rễ của hom. Giâm hom ngọn và hom giữa đạt tỷ lệ sống 75,33 - 80,67%, tỷ lệ hình thành rễ là 73,33 - 77,67%. Ngoài ra, ánh sáng là nguồn năng lượng chính cần thiết cho quá trình quang hợp, sinh trưởng và phát triển của thực vật. Che sáng 50%, tỷ lệ sống đạt 87,45 đến 93,34%. Nhân giống Trà hoa vàng bằng phương pháp giâm hom có thể áp dụng để sản xuất cây giống phục vụ công tác bảo tồn và phát triển nguồn gen quý và đáp ứng cho thị trường nguồn giống cây dược liệu.

**Từ khóa:** *C. chrysantha*, *C. flava*, *C. tamdaoensis*, che sáng, giâm hom, trà hoa vàng, vị trí cắt hom.

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