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Effect of exogenous hormone and rooting medium on cutting propagation of golden Camellia (*Camellia impressinervis*)

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Abstract

Vegetative propagation method such as cutting, provides uniform plant materials from mother plants. The effect of medium and exogenous hormone pretreatment on rooting were studied for golden camellia - *Camellia impressinervis*, a tree species which is used for healthcare. The results showed that different concentrations of exogenous hormones IBA (Indole-3-butyric acid), IAA (Indole-3-acetic acid), significantly affected rooting efficiency. Cuttings treated with IBA and IAA had significantly higher rooting percentage than control (54.2 %). Higher rooting efficiency was obtained with IBA, which resulted in highest rooting percentage of 91.4 % at 4 months of growth by using concentration of 0.5 % (by weight). Hormone types and their concentrations did not affect mean root number (MRN) and mean root length (MRL). Rooting media significantly affected rooting efficiency, MRN per cutting, and MRL. Using medium of 100 % sand resulted in highest rooting percentage (91.4 %), roots per cutting (4.2) and MRL (5.4 cm) at 4 months of growth. It is concluded that to produce seedlings for *C. impressinervis* by cuttings, rooting medium of 100 % sand should be used and cuttings should be pretreated by 0.5 % IBA. In addition, cutting management is also important for rooting efficiency. It must be controlled with humidity of > 95 % and temperature of < 27 ^{o}C.

Key words: Auxin, environment condition, healthcare value, indole-3-butyric acid, rooting efficiency

Introduction

The vegetative propagation of superior genotypes is the basis to create intensive plantations (Seth and Panigrahi, 2019). Cutting propagation has been given priority in vegetative propagation, as it is easy to apply and usually results in high rooting percentage for many plants (Eed and Burgoyne, 2014). The physiological conditions of the stock plants (Mitchell *et al.*, 2004), time and place of cutting collection (Crawford *et al.*, 2016), and environmental conditions significantly affect the efficiency of cutting propagation. Adventitious rooting is a complex developmental process that can be affected by internal and external factors (Leakey, 2004). Auxins play a critical role in the formation of adventitious root by increasing initiation of the root primordium and growth via cell division (Ren *et al.*, 2019). Auxins promote starch hydrolysis and mobilize sugars and nutrients to the cutting base (Das *et al.*, 1997). During cell division and auxin transport, auxins act primarily through selective proteolysis and cell wall loosening (da Costa *et al.*, 2013).

Camellia impressinervis Hung T. Chang & S. Ye Liang is known as a golden camellia (Hung and Le, 1979; Manh *et al.*, 2019), which has natural distribution in Southern China and North Vietnam and is classified as a critically endangered species (Wheeler and Rivers, 2015). Leaves and flowers of *C. impressinervis* have been used to make tea by soaking in hot water (Tran, 2018). Several researches indicated that the extracts from golden camellias have antioxidant activities, superoxide anions, and hydroxyl free radicals scavenging assays (Wan *et al.*, 2011; Wei *et al.*, 2015). Golden camellias can be used to treat sore throat, diarrhea, irregular menstruation, and cancer prevention (Guangxi Institute of Botany, 1991). Currently, market price (600-700 US$/ 1 kg) of dry flowers and leaves of golden camellias is quite high (Tran, 2018), much higher than green tea leaves. In a golden camellia population there is much difference of flower productivity among plants; a 1.2 m tall tree of *C. impressinervis* can bloom up to 130 flowers in a year, equaling 0.3 kg dry flowers (Tran, 2018).

In plantations derived by sexual propagation, seedlings are genetically variable, which affects growth uniformity, physiological characteristics, yield, and early bearing (Mohassea *et al.*, 2009). On other hand, vegetative propagations (*e.g.*, cutting) provide genetically uniform plant materials from mother plants, which can fruit early. The objectives of this study were to evaluate the effects of exogenous hormones (auxin types and concentrations) and medium on rooting efficiency to establish an optimum cutting propagation scheme for *Camellia impressinervis*.

Materials and method

Experimental site, plant material, rooting pot, and cutting management: The experiment was conducted in a nursery at Silviculture Research Institute, Vietnamese Academy of Forest Sciences in Hanoi capital, Vietnam (21°04'20.8'', 105°46'34.7'').

The cuttings of *C. impressinervis* were collected from garden of local people in Cao Bang province (22°24'56.8'', 106°25'23.9''), 300 km far from Hanoi capital. Trees were dug up from natural forest and transplanted in garden in 2013-2014. After planting trees were generally not tended. Trees were 1-1.4 m tall, 2-7 cm diameter at stump, and 0.8-1.4 m crown diameter at a time of cutting collection in October 2018. Most of the trees started blooming in 2017. When cuttings were collected, there appeared some flower buds. The climate conditions, where cuttings were collected, include annual precipitation of 1,500-1,700 mm, temperature of 21-23°C, and air humidity of 85-90 % (Tuan *et al.*, 2019). 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 nursery in Hanoi. Total time required from cutting branches to fishing work in nursery was with in 24 hours. Each branch was cut into two segments from top to create two cuttings. Cutting was cut at leaf base perpendicularly. Cutting was 8-12 cm long and contained 2-4 leaves. Only 15-20 % area of each leaves was remained (Fig. 1a). Cuttings were then treated by soaking in fungicide for 10 minutes and treated again every seven days by spraying to seedbed until 4 months of growth.

Rooting pots were 4 cm in diameter and 8 cm long, made of non-woven plastic (bottom open), and set on the seedbed in the nursery (Fig. 1a).

Seedbed was covered by transparent nylon to avoid water evaporation and then a layer of nursery shade cloth with shading level of 47-53 % (Fig. 1b). A meter was set inside seedbed for observing humidity and temperature. The cuttings were watered twice a day in the morning and afternoon by spraying system (Fig. 1a). Generally, the seedbed had controlled humidity of > 95 % and temperature of < 27 °C by additional watering and/or openning of the cover.

Experimental design-Rooting medium experiment: Three media were tested including (1) 100 % sand, (2) 100 % soil collected from 0-40 cm depth of forested soil, and (3) 50 % sand + 50 % % soil by volume. Exogenous hormone IBA (Indole-3-butyric acid) 2 % by weight in powder type was used. A randomized complete block design was set up with three replications and each replication contained 30 cuttings. Totally, 270 cuttings were used.

Exogenous hormone type and concentration: Two hormone types were tested including IBA and IAA (Indole-3-acetic acid). In each hormone type, six concentrations were tested including 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 % by weight in powder type. There was one control (no exogenous hormone treatment), leading to total 13 treatments. Rooting medium was 100 % sand. A randomized complete block design was set up with three replications and each replication contained 30 cuttings. Totally, 1,170 cuttings were used.

Survival rate and rooting measurements: Survival rates of cuttings were calculated at 15 days, 1 month, 2 months, 3 months, and 4 months of growth by counting alive cuttings for each treatment replication and experiment. Measurements of rooting efficiency were assessed at 4 months of growth. The measured indices included the number of roots (NR, roots >0.3 cm long), length of three longest roots, and mean root length (MRL) for each plant. Rooting success was expressed as the percentage of cuttings that rooted in each replication, treatment, and experiment.

Statistical analysis: Differences in rooting percentage, mean RN (MNR), and MRL in each experiment were assessed by univariate analysis of variance (ANOVA) and post-hoc test at $P = 0.05$. All analyses were conducted using SAS 9.2 (SAS Institute Inc., Cary, NC, USA).

Results

Rooting medium experiment: Rooting media significantly affected alive percentage at 2 and 3 months and rooting percentage at 4 months of growth (Table 1). The highest alive percentage at 2 months of growth belonged to medium of 100 % sand (96.4 %), followed by medium of 50 % sand + 50 % soil (93.6 %), and by medium of 100 % soil (91.4 %). The similar pattern was observed at 3 months of growth (Table 1). Rooting percentage at 4 months of growth was highest in medium of 100 % sand (91.4 %), followed by medium of 50 % sand + 50 % soil (82.9 %), and by medium of 100 % soil (78.6 %).

Rooting media significantly affected MRN and MRL at 4 months of growth (Fig. 2). Both MRN (4.2 roots/cutting) and MRL (5.4 cm) were highest in medium of 100 % sand, followed by medium of 50 % sand + 50 % soil (3.8 roots/cutting and 5.1 cm), and by medium of 100 % soil (3.6 roots/cutting and 4.7 cm).

The medium of 100 % sand was best for cutting propagation of *C. impressinervis*, which can result up to 91.4 % rotting percentage

Fig. 1. Cuttings in seedbed with spraying system (a), shaded seedbed (b), and rooted cuttings at 4 months of growth in different media (c) [100 % sand (c-1), 50 % sand + 50 % soil (c-2), and 100 % soil (c-3)].

Table 1. Effect of rooting media on alive cutting percentage and rooting percentage (±SE)

Rooting media	Time of growth				
	15 days	1 month	2 months	3 months	4 months
	Alive cuttings (%)				Rooting (%)
100 % sand	100	100	96.4 ±1.01a	93.2 ±1.15a	91.4 ±1.21a
50 % sand + 50 % soil	100	100	93.6 ±0.8b	88.7±1.09b	82.9 ±1.18b
100 % soil	100	100	91.4 ±0.91c	84.5±0.98c	78.6 ±1.05c

Means followed by different alphabets in the same column indicate significant differences by Tukey's test ($P < 0.05$).

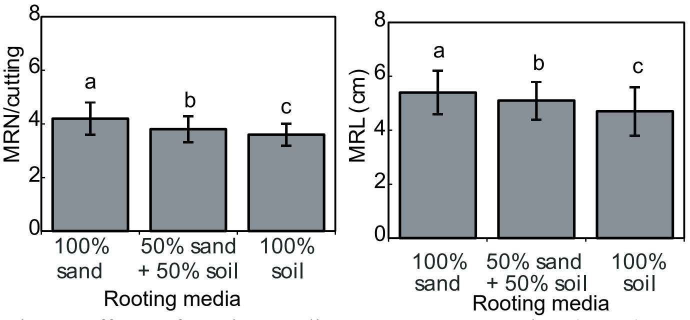

Fig. 2. Effects of rooting media on mean root number (MRN) per cutting and mean root length (MRL) at 4 months of growth. Letters indicate significant differences by Tukey's test ($P < 0.05$) and vertical bars indicate SE.

at 4 months of growth with highest MRN and MRL by pretreating the cuttings with 2 % IBA.

Exogenous hormone type and concentration: Both exogenous hormones IBA and IAA had higher rooting percentage than control (54.2 %; Fig. 3). Between two hormones, IBA showed significantly better effect on rotting percentage than IAA. Except at concentration of 2.5 %, rooting percentage in other IBA concentrations was significantly higher than that in IAA concentrations (Fig. 3). The difference was highest (11.5 %) at hormone concentration of 3 % and lowest (1 %) at hormone concentration of 2 %. There were no significant differences of MRN and MRL between IBA and IAA at 4 months of growth (Table 2 and 3; Fig. 4).

At 1 month of growth, there were no dead cuttings in IBA treatment (Table 2). At 2 months of growth, the dead cuttings appeared only at treatments of 1.0, 2.0, and 3.0 % IBA. Alive cutting percentages were significantly different among IBA concentrations at 2 and 3 months of growth, which was highest at 0.5 % IBA and lowest at 3.0 % IBA (Table 2). At 4 months of growth, rooting percentage was highest at 0.5 % IBA (91.4 %) and lowest at 3.0 % IBA (82.9 %). While, MRN and MRL were not significantly different among IBA concentrations, which ranged from 4.0 to 4.2 roots/cutting and 5.1 to 5.3 cm, respectively (Table 2).

At 1 month of growth, there were no dead cuttings appeared in IAA treatment (Table 3). At 2 months of growth, the dead cutting appeared in treatments of 1.0, 1.5, 2.0, 2.5 and 3.0 % IBA, except 0.5 %. Alive cutting percentages were significantly different among IAA concentrations at 2 and 3 months of growth, which was highest at 0.5 % IAA (Table 3). At 4 months of growth, rooting percentage was highest at 0.5 % IAA (88.6 %) and lowest at 3.0 % IAA (71.4 %). While MRN and MRL were not different among IAA concentrations, which ranged from 4.0 to 4.2 roots/cutting and 5.2 to 5.4 cm/root (Table 3).

Discussion

Many plants belong to the callus rooting species as their roots initiate from calluses (Yang *et al.*, 2015). Rooting in such species is relatively more difficult than species that root from the phloem side of the fascicular cambium (Izhaki *et al.*, 2018). As *C. impressinervis* roots from both callus and the phloem side of the fascicular cambium (Fig. 1c and 4), it could be considered as an easy rooting plant, similar to other species in genus *Camellia* (Yongjun and Ming, 2012). In this study, cuttings were collected from five unknown-age individuals of *C. impressinervis*, which originated from seeds, and they were not tended properly. Thissuggest that genetic variability (Nasri *et al.*, 2019) and difference of internal and external conditions among individuals may cause variation in rooting potential of cuttings. However, rooting efficiency was high (Table 1, 2, 3), indicating

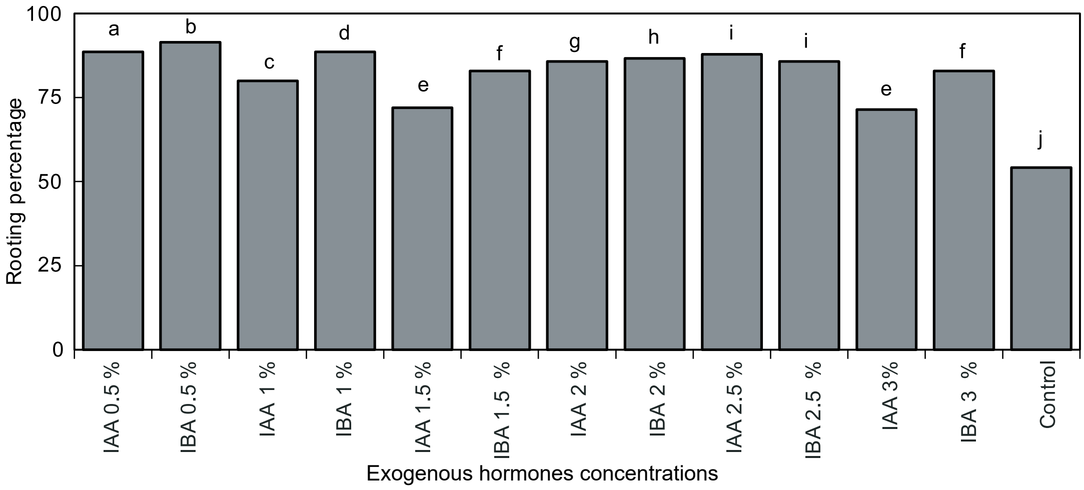

Fig. 3. Effects of exogenous hormones and their concentrations on rooting percentage at 4 months of growth. Different letters indicate significant differences by Tukey's test ($P < 0.05$)

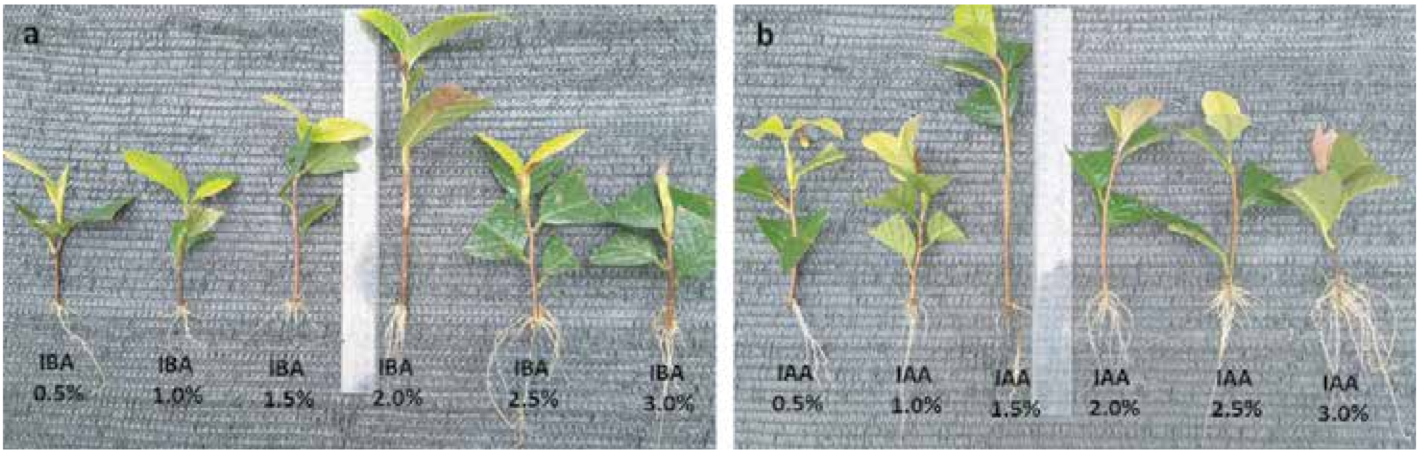

Fig. 4. Rooted cuttings at 4 months of growth in different exogenous hormones and their concentrations, IBA (a) and IAA (b).

Table 2. Effect of IBA concentrations on alive cutting percentage and rooting percentage (±SE)

Concentration (%)	Time of growth						
	15 days	1 month	2 months	3 months	4 months		
	Alive cuttings (%)				Rooting (%)	MRN/ cutting	MRL (cm)
0.5	100	100	100a	95.8 ±0.9a	91.4 ±0.9a	4.0 ±0.7	5.1 ±1.3
1.0	100	100	94.3 ±0.9b	90.1 ±0.8b	88.6 ±1.1b	4.1 ±0.8	5.3 ±1.3
1.5	100	100	100a	87.8 ±1.1c	82.9 ±1.2c	4.2 ±0.7	5.2 ±1.2
2.0	100	100	98.1 ±0.8c	87.8 ±1.0c	86.7 ±1.1d	4.0 ±0.8	5.1 ±1.1
2.5	100	100	100a	86.5 ±1.0d	85.7 ±1.2e	4.1 ±0.8	5.3 ±1.2
3.0	100	100	97.1 ±0.8d	86.9 ±1.1d	82.9 ±1.2c	4.2 ±0.7	5.2 ±1.2

Means followed by different alphabets in the same column indicate significant differences by Tukey's test ($P < 0.05$). MRN is mean root number and MRL is mean root length.

Table 3. Effect of IAA concentrations on alive cutting percentage and rooting percentage (±SE)

Concentration (%)	Time of growth						
	15 days	1 month	2 months	3 months	4 months		
	Alive cuttings (%)				Rooting (%)	MRN/ cutting	MRL (cm)
0.5	100	100	100^{a}	94.6 ±1.0^{a}	88.6 ±0.6^{a}	4.2 ±0.9	5.4 ±1.2
1.0	100	100	97.1 ±1.0^{b}	85.7 ±0.8^{b}	80.0 ±0.6^{b}	4.1 ±0.8	5.3 ±1.1
1.5	100	100	92.0 ±0.9^{c}	88.1 ±0.8^{c}	72.0 ±0.5^{c}	4.1 ±0.7	5.4 ±1.3
2.0	100	100	94.3 ±0.9^{d}	89.6 ±0.7^{c}	85.7 ±0.5^{d}	4.2 ±0.8	5.2 ±1.3
2.5	100	100	97.0 ±1.1^{b}	91.2 ±0.9^{d}	87.9 ±0.6^{e}	4.0 ±0.9	5.3 ±1.2
3.0	100	100	94.3 ±1.0^{d}	90.1 ±0.9^{d}	71.4 ±0.5^{c}	4.1 ±0.8	5.2 ±1.2

Means followed by different alphabets in the same column indicate significant differences by Tukey's test ($P < 0.05$). MRN is mean root number and MRL is mean root length.

high rootability of *C. impressinervis* in cutting propagation. If cuttings are collected from a superior genotype and plants are tended properly, rooting percentage could be higher.

Applying IBA may have an indirect influence through enhancing translocation and movement of carbohydrates to the cutting base and consequently stimulate rooting (Aminah *et al.*, 1995). Therefore, rooting efficiency was higher in IBA treatments than that in IAA treatments (Fig. 3). Similar findings were reported in other species (Eed and Burgoyne, 2014). Such result may also be related to total phenolic content and peroxidase activity, which are higher in IBA treated cuttings, particularly during the initiation and expression phases (Rout, 2006).

Effects of media on rooting efficiency were reported in many plants (Eed and Burgoyne, 2014). Air content and oxygen diffusion rate in media are important for rooting (Ercisli *et al.*, 2002). In the present study, medium of 100 % soil with high ratio of loam and silt particles indicate low air soil, which inhibited forming calluses and roots. Meanwhile, medium of 100 % sand contains 100 % particles >0.02 mm. Therefore, air content and oxygen diffusion rate are higher and suitable for rooting of *C. impressinervis*. It is reported that *C. impressinervis* grows well in high moisture but drainage soil (Tran, 2018). Soil with high ratio of loam and silt particles has high water holding capacity and is not drainage soil. Therefore, medium of 100 % soil should not be used in cutting propagation for *C. impressinervis*.

C. impressinervis is known as a shrub or small-sized tree, which is shorter than 7 m at maturity. This is a shade-tolerant species, growing well under shade of others in evergreen broadleaved forests (Tuan *et al.*, 2019). Therefore, shading seedbed in cutting propagation is required, which is not a case in other species (Yang *et al.*, 2015), especially for light demanding plants. Effects of light intensity on rooting were reported (Fuerncranz *et al.*, 1990). The shading level of 47-53 % was used in the present study, which resulted in 91.4 % rooting percentage of cuttings treated with 0.5 % IBA in medium of 100 % sand. The rooting efficiency could be improved if different shading levels are considered. Therefore, further study should be conducted by testing the effects of different shadings on rootability.

Effects of rooting media, and exogenous hormone types and their concentrations on MRN and MRL of cuttings were reported in many other species (Eed and Burgoyne, 2014; Yang *et al.*, 2015). The similar findings were observed in the medium experiment (Fig. 2) but not by exogenous hormone types and their concentrations in the present study (Table 2 and 3). Highest MRN and MRL in medium of 100 % sand could be resulted from higher air content and oxygen diffusion rate compared to that in 100 % soil (Ercisli *et al.*, 2002). Even fertility in medium of 100 % sand is much lower than that in 100 % soil and may be zero. For initial stage, survival and rootability of cuttings are supported by internal energy itself as new shoots and leaves appeared in cuttings (Fig.1 and 4). Therefore, fertility from surrounding environment/ medium is generally not necessary. After transplanting rooted cuttings to pots with soil, it is necessary to support their growths through better root anchoring and nutrient supply. Transplanting may cause death of rooted cuttings, finally leading to low rate of plants produced. Therefore, additional technique should be considered such as making rooting pots with outer part by soil and inner part by sand. Cuttings will be set in sand portion for higher rooting efficiency and when roots grow long enough, they can anchor better and absorb nutrients from soil portion. With such technique, transplanting is not required. Therefore, it can reduce manpower and increase final rate of plants produced.

Media significantly affected rooting efficiency of *C. impressinervis*. Using 100 % sand for cutting propagation resulted in highest rooting percentage of 91.4 %, number of roots per cutting (4.2), and root length (5.4 cm) at 4 months of growth. Exogenous hormone IBA promoted rooting better than IAA. In addition, IBA concentrations significantly affected rooting efficiency. The best concentration is 0.5 % by weight in powder type. Therefore, we recommended the use of medium of 100 % sand and 0.5 % IBA in cutting propagation of *C. impressinervis* with the control of air humidity of > 95 % and temperature of < 27 ^{o}C.

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