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# Applications of vegetation indices and biostimulators to the rooting of camellia cuttings

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## Abstract

There are no reports on biostimulators being used to stimulate the rooting process of camellia (*Camellia japonica*) cuttings. The influences of vegetation indices and biostimulators on the rooting of shoot cuttings of the camellia 'Nine Bends' were determined using the reflectance spectroscopy. Six root growth parameters were recorded, 81 days after sticking cuttings. Different groups of soil-plant analysis development (SPAD) and normalized difference vegetation index (NDVI) values were used. Higher SPAD and NDVI values were found to be most effective in predicting rooting stages and percentages, whereas root number and length and dry weight could not be predicted. Indole-3-butyric acid (IBA) at a concentration of 2000 ppm alone or combined with three concentrations of thiamine (TA), ascorbic acid (AA) and catechol (CAT) were applied to cuttings of camellia. The maximum root number and dry weight were recorded in TA (1000 mg $L^{-1}$) alone and IBA+TA (800 mg $L^{-1}$). However, IBA+AA (1000 mg $L^{-1}$) treated cuttings showed the highest rooting stage, root number, root length, and dry weight compared to other treatments. Cuttings treated with IBA+CAT (50 or 100 mg $L^{-1}$) produced greater root number, length, and dry weight compared to other treatments. The effects of biostimulators applied to tea stem cuttings for raising camellia plants in the nursery would offer insights into the mechanism of its action in plant conservation, and there is a need to improve propagation technology.

**Key words**: *Camellia japonica*, indole butyric acid, normalized difference vegetation index (NDVI), reflectance spectroscopy, stem cutting, soil-plant analysis development (SPAD)

## Introduction

*Camellia japonica* which belongs to Theaceae family, is an ornamental bush with variations in flower color, shape, fragrance, bloom size, etc., and primarily has a subtropical and warm-temperate distribution. Currently, there are more than 22,000 varieties or cultivars cataloged in the International Camellia Society (http: //www.camellia-international.org/). The genus *Camellia* is native to South and East Asia and comprises about 120 large evergreen shrub species worldwide (Ming and Bartholomew, 2007). These species are economically important as a food resource, for ornamental horticulture, and in cosmetics (Vela *et al.*, 2013). *C. japonica* is distributed naturally in some islands in East Asia. Due to over-exploitation after the 1950s, populations and habitats of the species have decreased sharply (Zhou *et al.*, 1994). There are about 82 species in tropical and subtropical Asia, and twelve species in Taiwan (Hsieh *et al.*, 1996). *C. japonica* maintains higher levels of genetic variability within populations than other woody species (Nybom, 2004).

The level of population diversity and differentiation for *C. japonica* has clear conservation and management implications. Human utilization is the best preservation, so the successful artificial propagation of this species in future can not only guarantee its *ex-situ* conservation and sustainable survival but also enhance the *in situ* conservation. Therefore, detailed studies of the reproductive biology of this species should be carried out to yield valuable information for conservation management of *C. japonica*.

Camellia is a self-sterile, cross-pollinated woody perennial plant, and propagation through seeds do not produce true-to-type plants. Therefore vegetative propagation is the only method for producing true-to-type plants. Cultivated camellia is conventionally propagated by single leaf node cuttings (Nasiro, 2017). The most desired and important factor in selecting plant bushes for vegetative propagation is the early rooting ability. However, the establishment of camellia stem cuttings has been a major problem due to poor rooting (Gyana, 2006). As the landscaping demand for camellia is increasing, any methods to improve the propagation efficiency would be important for nurserymen. Physiological indices such as the photosynthetic performance, spectral reflectance, water potential, electrical conductivity of cell tissues, and chlorophyll content greatly influence plant survival (Wu *et al.*, 2015). Visual observations frequently result in experimental errors, whereas destructive measurements damage plants and make further experiments nearly impossible. Reflectance spectroscopy is an under-exploited noninvasive technique that can be used in physiological studies because of its simplicity, rapidity, and nondestructive nature (Levizou *et al.*, 2005). Reflectance spectra are altered when stress occurs, enabling the use of a series of different vegetation indices such as the soil-plant analysis development (SPAD) and leaf normalized difference vegetation index (NDVI) for quantitative yield measurements of plant growth (Devitt *et al.,* 2005; Wu *et al.*, 2016).

Plant growth regulators (PGRs) are important factors determining plant growth and development, and scientists have contributed significantly to horticulture and practical gardeners with plant growth substances to enhance the rooting of cuttings. Biostimulators contain active substances of natural origin, improve the quality of plant materials, hasten processes of plant growth and development, and limit stress effects on plants. They also intensify water uptake and nutrient transport and stimulate photosynthesis (Dobrzanski *et al.,* 2008; Pruszynski, 2008). There are no reports on biostimulators such as thiamine (TA), ascorbic acid (AA), and catechol (CAT) being used to stimulate the process of cuttings, especially in difficult-to-root woody plants. In nurseries, *C. japonica* plant is propagated mainly by cuttings and allowed to grow for at least 2 years before marketing. The mechanisms of these three biostimulators' actions on physiological and biochemical processes of cuttings remain unknown. The objectives of this study were to employ nondestructive quantitative measurements to determine rooting parameters of stem cuttings, and also to evaluate the effects of the biostimulators TA, AA, and CAT on the rooting of stem cuttings of the camellia 'Nine Bends'. The effects of IBA supplemented with an optimal concentration of TA, AA, and CAT applied to tea stem cuttings for raising camellia plants in the nursery were determined, which offer insights into the mechanism of its action in plant propagation for the future.

## Materials and methods

The experiment was carried out in a greenhouse of the Horticulture Department, National Taiwan University between March 10 and June 1, 2016. Uniform single-node cuttings measuring approximately 7.0 to 8.0 cm long with two or three attached leaves from vigorous young shoots were used. These shoots were collected from healthy bushes of *C. japonica* 'Nine Bends' grown in our departmental nursery. 'Nine Bends' is one of the most popular camellia plants in Taiwan, and is frequently cultivated in gardens due to its attractive flowers and excellent adaptability.

**Experiment 1**: **Effects of reflectance spectroscopy indices on the rooting of camellia cuttings**: The basal portion (2-3 cm) of the cuttings was inserted into Styrofoam flats measuring 28 × 55 × 6 cm filled with a commercial soil mixture (Farfard, Agaqam, MA, USA). One cutting was placed in each cell hole (5 × 5 × 6 cm), and 50 holes per flat were arranged on beds under net tunnels which protected against direct sunlight. The temperature and relative humidity in the greenhouse were respectively maintained at 25-28 °C and 90 %-100 % during rooting. Healthy, fully expanded leaves of camellia were used to determine chlorophyll content using a SPAD analyzer (SPAD-502 Chlorophyll Meter, Konica Minolta, Tokyo, Japan) and spectral reflectance with an NDVI 300 spectrophotometer (PlantPen, Photon System Instruments, Brno, Czech Republic). Spectral wavelengths at 740 and 660 nm were used to calculate the vegetation index and determine useful information related to growth status and rooting development. The value of NDVI was calculated as $(R740 – R660) / (R740 + R660)$ (Devitt *et al.,* 2005). All NDVI and SPAD readings were separated into 'high' and 'low' groups, where 70.0 and 0.78 were the respective cutoff points for NDVI and SPAD.

**Experiment 2**: **The influence of IBA combined with biostimulators on the rooting of camellia cuttings**: The above-mentioned prepared three-leaf cuttings of camellia were soaked in a solution of IBA (2000 mg $L^{-1}$) supplemented with TA (400, 800, and 1000 mg $L^{-1}$), AA (100, 1000, and 10, 000 mg $L^{-1}$), and CAT (50, 100, and 200 mg $L^{-1}$). Four replicates of each treatment were used with each containing seven cuttings. Control cuttings were only sprayed with water. Cuttings at 3 cm were carefully (without wounding the basal portion) soaked in these solutions for 15 min, followed by burying in the above-mentioned flats filled with soil medium. All treatments with IBA and/or biostimulators were performed once at the beginning of the experiment. Soil-filled flats with cuttings were lightly irrigated every day for the first week of dibbling, and then irrigation was maintained at a 1-day interval up to the experimental period. Cuttings were carefully uprooted, medium particles adhering to the roots were carefully removed by hand, and the following five growth parameters were determined for all treatments.

**Rooting stage**: The stage of rooting was evaluated on a five-point (0-4) scale that rated the development of root length: 0, cutting without visible roots; 1, cutting had formed a callus; 2, branched roots of 0.1-0.3 cm had formed; 3, a medium-sized root system with branched roots of 0.3-0.5 cm had formed; and 4, a well-developed, branched root system of >0.5 cm had formed. The scores for the rooting stage represent the mean of three independent observations by trained personnel.

**Root number per cutting**: The average number of roots per cutting was recorded for all treatments in each replicate. Only cuttings with a rooting stage system within the scale range of 3-4 were counted.

**Maximum root length**: The maximum root length (cm) of randomly selected plants for all treatments in each replicate was measured with the help of a measuring tape.

**Root dry weight**: The root biomass of each cutting was separately dried in an oven and weighed to obtain the root dry weight.

**Rooting percentage of cuttings**: Only cuttings with a rooting stage greater than scale 2 were counted.

**Statistical analysis**: All experiments were arranged in a completely randomized design. All parameters were subjected to a one-way analysis of variance (ANOVA), with a significance level of $P \leq 0.05$ using CoStat statistical software (Cohort Berkeley, Monterey, CA, USA). For significant values, means were separated by the least significant difference (LSD) test at $P \leq 0.05$ using CoStat.

## Results and discussion

*C. japonica* is an evergreen ornamental shrub. In floriculture, *C. japonica* importance ranks the highest due to its ornamental characteristics, and it is traded worldwide as potted plant. It is also prized for its beautiful flowers and medicinal properties (Mondal *et al.,* 2004). Different responses and levels among rooting parameters of camellia cuttings were detected during SPAD and NDVI testing. Significant increases in the degree of rooting stage (3.2) and rooting percentage (85 %) were observed in the higher SPAD group (71.6-81.3) compared to the lower group (57.4-70.6) (Table 1). However, SPAD values did not significantly reflect the root number, root length or dry weight. The same trend was evident for the NDVI, where these five parameters significantly differed only in the rooting stage (2.0) and rooting percentage (60 %) in the higher NDVI group (0.784-0.826) compared to the lower group (0.740-0.776).

When SPAD and NDVI values were >70.0 and >0.78, respectively, they became useful for measuring the rooting stage and rooting percentage of camellia when developing indices for nondestructive chlorophyll estimation. The SPAD and NDVI are used to assess chlorophyll contents and photosynthetic capacity (Devitt *et al.,* 2005; Bonneville and Fyles, 2006; Wu *et al.,* 2016). In this study, SPAD and NDVI values of cuttings in the lower group (54.4-70.6 and 0.740-0.776, respectively) compared to the higher group (71.6-81.3 and 0.784-0.826, respectively) had lower chlorophyll concentrations, which was consistent with visual observations (photos not shown). Cuttings with higher SPAD and NDVI values also had higher rooting parameter values. Therefore, when the vegetation index reaches values of 71.6-81.3 for SPAD or 0.784-0.826 for NVDI, shoot cuttings of camellia can be propagated. This means that many hundreds of individual cuttings can be screened per day, providing ample opportunity to discover individuals that manifest quality indicators and exhibit greater rooting stages and rooting percentages for the mass vegetative multiplication of selected genotypes, which offers the promise of rapid productivity gains. However, no studies have been conducted on the effects of TA, AA, and CAT on the rooting of stem cuttings from camellia. In addition, because of the cost of these biostimulants used, improvements in rooting parameters of this cultivar will reduce the cost per plant or per acre of new plantings.

*C. japonica* is the most important ornamental species within the genus *Camellia* and is commonly propagated by cuttings and grafting, particularly in nurseries, as at least 3-4 years or more are needed from seed sowing for most cultivars to produce a plant with flowers. In Europe, *C. japonica* does not form seeds, and vegetative propagation by cuttings is limited due to the lack of suitable planting material as a result of winter dormancy and season-dependent rooting ability of the cuttings (Mondal *et al.,* 2004). For deciding upon cutting collection time for vegetative propagation of *C. japonica*, it is essential to recognize the SPAD and NDVI values to determine rooting parameters of stem cuttings, and the higher SPAD and NDVI values were selected for biostimulators on the rooting of camellia cuttings. Compared to the control, higher root numbers of cuttings was induced by application of TA (Table 2). Notably, the application of TA (1,000 mg $L^{-1}$) alone to cuttings significantly produced 12.5 roots on average. Dry weights of roots varied between 10.36-20.97 mg, and the maximum dry weight was recorded for cuttings treated with IBA combined with 800 mg $L^{-1}$ TA. This treatment (IBA+800 mg $L^{-1}$ TA) was used as a reference treatment allowing us to assess the efficiency of the other treatments, including 400, 800, and 1000 mg $L^{-1}$ of TA. No significant differences were shown in rooting stage, root length or rooting percentage in all treatments. Table 3 illustrates that the maximum rooting stages of 3.9 and 4.0, recorded when cuttings were treated with IBA combined with 100, 1,000, or 10,000 mg $L^{-1}$ AA against the minimum rooting stages of 3.7 (IBA treatment only) and 3.6 (control). Higher average root number of 12.7 was recorded with IBA and 1000 mg $L^{-1}$ AA supplementation. The maximum root length (2.41 cm) was observed when cuttings were treated with IBA+1000 mg $L^{-1}$ AA, which was statistically similar to the data of 2.00 cm noted for IBA+100 mg $L^{-1}$ AA, while the minimum root length (1.50 cm) was observed with IBA alone. Dry weights of roots ranged 4.37-9.21 mg in different treatments, and the maximum dry weight was observed for treatment with IBA+AA (1000 mg $L^{-1}$) which was significantly higher than those of the other treatments. Furthermore, CAT treatments showed higher results over control (Table 4).

Table 1. Effects of SPAD and normalized difference vegetation index (NDVI) values on different parameters in the rooting of *C. japonica* cuttings

| | Rooting parameters of *C. japonica* | | | | |
|---|---|---|---|---|---|
| | Rooting stage | Number of roots | Length of root (cm) | Dry weight of roots (mg) | Rooting percentage ( %) |
| SPAD values | | | | | |
| 71.6-81.3 | 3.2 a | 5.7 a | 1.00 a | 3.28 a | 85.00 a |
| 57.4-70.6 | 2.8 b | 5.3 a | 0.96 a | 2.50 a | 60.00 b |
| NDVI values | | | | | |
| 0.784-0.826 | 2.6 a | 2.0 a | 0.40 a | 0.44 a | 60.00 a |
| 0.740-0.776 | 2.2 b | 1.6 a | 0.27 a | 0.40 a | 45.00 a |

Each value is the average of 30 cuttings. Means within a column followed by same letters do not significantly differ according to the LSD test at $P \leq 0.05$ ($n = 4$).
SPAD, soil-plant analysis development.
NDVI, normalized difference vegetation index.
Rooting stage was evaluated on a five-point (0-4) scale that rated the development of root length.

Table 2. Effects of indole-3-butyric acid (IBA; 2000 mg·$L^{-1}$) and different thiamine (TA) concentrations on different parameters in the rooting of *C. japonica* cuttings

| Treatment (mg·$L^{-1}$) | Rooting stage | Number of roots | Length of root (cm) | Dry weight of roots (mg) | Rooting percentage ( %) |
|---|---|---|---|---|---|
| 0 | 3.7 a | 9.1 b | 2.53 a | 10.36 c | 95.00 a |
| TA 400 | 3.7 a | 9.7 b | 3.39 a | 17.50 ab | 90.00 a |
| TA 800 | 4.0 a | 10.1 b | 3.05 a | 11.06 c | 100.00 a |
| TA 1000 | 4.0 a | 12.5 a | 3.17 a | 14.59 b | 100.00 a |
| IBA ×TA 800 | 4.0 a | 9.7 b | 2.53 a | 20.97 a | 100.00 a |

Each value is the average of 30 cuttings. Means within a column followed by same letters do not significantly differ according to the LSD test at $P \leq 0.05$ ($n = 4$).

Table 3. Effects of indole-3-butyric acid (IBA; 2000 mg·$L^{-1}$) and different ascorbic acid (AA) concentrations on different parameters in the rooting of *C. japonica* cuttings

| Treatment (mg·$L^{-1}$) | Rooting stage | Number of roots | Length of root (cm) | Dry weight of roots (mg) | Rooting percentage ( %) |
|---|---|---|---|---|---|
| 0 | 3.6 b | 8.8 c | 1.74 b | 5.51 b | 90.00 a |
| IBA | 3.7 b | 5.1 d | 1.58 b | 5.13 b | 90.00 a |
| IBA×AA 100 | 4.0 a | 10.3 b | 2.00 ab | 6.32 b | 100.00 a |
| IBA×AA 1000 | 4.0 a | 12.7 a | 2.41 a | 9.21 a | 100.00 a |
| IBA×AA 10000 | 3.9 a | 8.1 c | 1.50 b | 4.37 b | 100.00 a |

Each value is the average of 30 cuttings. Means within a column followed by same letters do not significantly differ according to the LSD test at $P \leq 0.05$ ($n = 4$).

Table 4. Effects of indole-3-butyric acid (IBA; 2000 mg·L$^{-1}$) and different catechol (CAT) concentrations on different parameters in the rooting of *C. japonica* cuttings

| Treatment (mg·L$^{-1}$) | Rooting stage | Number of roots | Length of root (cm) | Dry weight of roots (mg) | Rooting percentage ( %) |
|---|---|---|---|---|---|
| 0 | 4.0 a$^z$ | 8.6 b | 1.74 bc | 5.51 bc | 90.00 a |
| IBA | 4.0 a | 5.1 c | 1.58 c | 5.13 c | 90.00 a |
| IBA×CAT 50 | 4.0 a | 11.0 a | 2.68 a | 7.94 a | 100.00 a |
| IBA×CAT 100 | 4.0 a | 11.0 a | 2.28 a | 8.51 a | 100.00 a |
| IBA×CAT 200 | 3.7 b | 8.9 b | 1.91 bc | 6.27 b | 90.00 a |

Each value is the average of 30 cuttings. Means within a column followed by same letters do not significantly differ according to the LSD test at $P \leq 0.05$ ($n$ = 4).

In this study, TA (1000 mg L$^{-1}$) significantly increased the root number (12.5) of single-node leafy stem cuttings of camellia, and the highest dry weight of roots (20.97 mg) was observed with the use of IBA+TA (800 mg L$^{-1}$), indicating that TA is indispensable for developing excised roots with a direct or indirect synergist role in rhizogenesis. Singh *et al.* (2005) reported a strong synergistic interaction between TA (vitamin B1) and auxins for adventitious rhizogenesis in *Tectona grandis* cuttings. Dhillon *et al.* (2011) demonstrated that treatment of stem cuttings of *Jatropha curcas* with 600 and 800 mg L$^{-1}$ TA for 16 h was most effective at promoting sprouting in the spring and monsoon seasons. In addition, in the study, IBA application alone did not exert a significant influence on any rooting parameter compared to other treatments. However, IBA application to cuttings supplemented with 1000 mg L$^{-1}$ AA produced the highest rooting stage (4.0), root number (12.7), root length (2.41 mm), and root dry weight (9.21 mg) compared to the other treatments, indicating that AA (1000 mg L$^{-1}$) had a positive effect on the establishment of stem cuttings and can be applied to raise better plants from stem cuttings in *C. japonica* nurseries. Sarrou *et al.* (2014) noted that rooting of shoot cuttings of *Punica granatum* treated with 1000 mg L$^{-1}$ IBA combined with 352 mg L$^{-1}$ AA produced the greatest number (11) of roots per cutting. Ascorbic acid (vitamin C) is one of the most important factors in $H_2O_2$ detoxification of cells, and at high concentrations, it helps cells maintain a constant redox potential (Noctor and Foyer, 1998). Increasing the ascorbate content in onion roots by adding exogenous ascorbate, the immediate precursor in the ascorbate synthesis pathway, to the culture media led to stimulation of root primordia sprouting in onion bulb bases, and to an enhanced elongation rate in growing roots (Cordoba-Pedregosa *et al.*, 2005).

Treatment with a combination of IBA and CAT (50 or 100 mg L$^{-1}$) exhibited a synergistic effect on the root number, maximum root length, and dry weight of cuttings, which were significantly better than using IBA alone and the control. This can be attributed to the effectiveness of comparative CAT doses on the early induction of callus formation and faster emergence and development of roots in treated cuttings. However, Maynard & Bassuk (1990) showed that CAT acts to reduce the rooting potential. With IAA, all tested orthodiphenols, paradiphenols, and triphenols promoted adventitious root formation from stem slices of *Malus* 'Jork 9' (De Klerk *et al.,* 2011). Our results suggested that vegetative propagation of camellia plants through stem cuttings with IBA+CAT treatments can be applied on a commercial scale for rapid multiplication and increasing uniformity of rootings, and this has the advantage of clonal or true-to-type propagation of elite trees. On the contrary, neither the application of TA, AA, and CAT nor single spraying with IBA stimulated the rooting percentage compared to the control.

In conclusion, our findings indicated that the use of tested biostimulants in suitable concentrations increased the ability to root woody plant cuttings. Application of TA (1000 mg L$^{-1}$) alone or IBA combined with AA (1000 mg L$^{-1}$) or CAT (50 or 100 mg L$^{-1}$) to cuttings demonstrated better results compared to IBA alone and the control in terms of the root number, maximum root length, and dry weight. These results seem promising and offer hope for IBA combined with a biostimulator or a biostimulator alone for propagating ornamental shrubs. Furthermore, the SPAD and NDVI developed for evaluating and pre-screen the rooting parameters of cuttings can be applied as nondestructive measurements for identifying stage for higher rooting percentage of bedded ornamental plants grown in open fields.

For conservation and application, there is a need to improve propagation technology. Thus, Taiwanese *C. japonica* populations can serve as a basis for the floricultural industry as a germplasm resource, and also can be protected *in situ*. Genetic differentiation may be enhanced by selecting and propagating desirable populations with obvious horticultural characteristics.

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