horticultura brasileira

Research

HOU, PC; LIN, KH; HUANG, YJ; WU, CW; CHANG, YS. 2020. Evaluation of vegetation indices and plant growth regulator use on the rooting of azalea cuttings. *Horticultura Brasileira*. 38: 153-159. DOI - http://doi.org/10.1590/S0102-053620200207

Evaluation of vegetation indices and plant growth regulator use on the rooting of azalea cuttings

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ABSTRACT

The objective of our study was to develop a protocol enabling the use of vegetation indices to evaluate the rooting of Azalea (*Rhododendron pulchrum* cv. Sweet) cuttings. Six root growth parameters were recorded after exposing those cuttings to rooting media for 47 days. Among plants with different soil-plant analysis development (SPAD) and normalized difference vegetation index (NDVI) values, those with higher values exhibited significantly higher number of roots, root length, and root dry weight, suggesting that reflectance indices were useful in measuring the root growth parameters of the cuttings. Another aim of this work was to study the effects of plant growth regulators (PGRs) on rooting for cutting propagation. Azalea cuttings were soaked in the treatments with indole-3-butyric acid (IBA) at 2,000 mg L^{-1} or combined with 1-naphthaleneacetic acid (NAA; 2,000 mg L^{-1}), salicylic acid (SA; 10^{-4} M), and thiamine (TA; 800 mg L^{-1}). The same observations as SPAD and NDVI on six different rooting parameters were recorded and analyzed after cutting's exposure to rooting media for 99 days for the auxin test, and 62 days for the SA and TA tests. Compared to NAA alone, IBA enhanced root growth and development as determined by increases in all parameters, and therefore it was used thereafter. Successful results for the number of roots and root dry weight were achieved using azalea cuttings with a combination of IBA and SA. In addition, the mix of IBA and TA resulted in higher number of roots and length of root. These combined treatments are recommended for establishing stem cuttings to produce nursery plants of azalea.

Keywords: *Rhododendron pulchrum*, indole-3-butyric acid, naphthaleneacetic acid, plant growth regulator, salicylic acid, thiamine.

RESUMO

Índices vegetativos e uso de reguladores de crescimento para enraizamento de estacas de azaléia

O objetivo do nosso estudo foi desenvolver um protocolo que permita o uso de índices vegetativos para avaliar o enraizamento de estacas de azáleia (*Rhododendron pulchrum*). Seis parâmetros de crescimento radicular foram registrados depois que essas estacas foram expostas ao meio de enraizamento por 47 dias. Entre as plantas com diferentes valores de desenvolvimento solo-planta (SPAD) e índice de vegetação com diferença normalizada (NDVI), aquelas com valores mais altos exibiram significativamente, maior número de raízes, comprimento e peso seco das raízes, sugerindo que os índices de refletância foram úteis na mensuração dos parâmetros de crescimento radicular de estacas de azaléia. Outro objetivo deste trabalho foi estudar os efeitos de reguladores de crescimento de plantas (PGRs) no enraizamento para propagação das estacas. As estacas foram embebidas em ácido indol-3-butírico (AIB) a 2.000 mg L^{-1} ou combinado com ácido 1-naftalenacético (NAA; 2.000 mg L^{-1}), ácido salicílico (SA; 10-4 M) e tiamina (TA; 800 mg L^{-1}). As observações dos valores SPAD e NDVI em seis parâmetros diferentes de enraizamento foram analisadas após as estacas terem sido expostas ao meio de enraizamento por 99 dias para o teste de auxina e 62 dias para os testes de SA e TA. Comparado ao NAA sozinho, AIB melhorou o crescimento e desenvolvimento radicular, conforme determinado pelo aumento em todos os parâmetros e, portanto, foi utilizado posteriormente. Resultados bem-sucedidos para o número de raízes e seu peso seco foram obtidos com estacas tratadas com uma combinação de AIB e SA. Além disso, a mistura de AIB e TA resultou em maior número de raízes e comprimento de raiz. Esses tratamentos combinados são recomendados para o estabelecimento de estacas visando a produção de plantas de azáleia em viveiro.

Palavras-chave: *Rhododendron pulchrum*, ácido indol-3-butírico, ácido naftalenoacético, regulador de cresciment, ácido salicílico, tiamina.

Received on November 6, 2019; accepted on April 20, 2020

The *Rhododendron* genus comprises approximately 1,000 species and is the largest genus within the Ericaceae family (Dampc & Luczkiewicz, 2013). Among them, azalea (*Rhododendron pulchrum*) is one of the most popular ornamental plants worldwide, and is frequently cultivated in gardens and pots due to its vigorous growth and hardiness (Cheon *et al*., 2011). It is a compact plant, with purple flowers and an early, long flowering period. Although azalea can be raised from seeds, it suffers from a loss of desirable characteristics from the mother plant;

therefore, commercial propagation is done vegetatively by means of cuttings. However, *Rhododendron* species being propagated by cuttings was found to be difficult or ineffective; the poor establishment of azalea stem cuttings has been a major problem with rooting, and rooting also takes a longer time (Almeida *et al*., 2005; Singh *et al*., 2016). Therefore, there is an urgent need for an alternative method by using plant growth regulator (PGR) for the large-scale multiplication of this plant.

The most desired and important factor in the vegetative propagation of ornamental plants is an early rooting ability. Consequently, methods used to improve the propagation efficiency are important for nursery owners. Physiological indices such as the photosynthetic performance, spectral reflectance, water potential, electrical conductivity of cell tissues, and chlorophyll content greatly influence seedling survival (Wu *et al*., 2015). Visual observations frequently cause experimental errors, whereas destructive measurements damage plants and make further experiments impossible, or are time-consuming and destructive to plants. Reflectance spectroscopy is an under-exploited noninvasive technique that can be used in physiological studies because of its simplicity, rapid, and nondestructive nature (Levizou *et al*., 2005). Various reflectance spectra from leaves have been employed to calculate a series of vegetation indices used to monitor plant growth. 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 a plant growth (Devitt *et al*., 2005; Wu *et al*., 2016).

Several factors can contribute to the development and differentiation of roots, including PGRs, genetic characteristics, juvenile or adult stage propagating materials, and concentrations of nutrients (Bañados *et al*., 2012). Root induction is one of the most common and exploited physiological effects of auxins, and occurs through stimulation of cellular dedifferentiation (Mauad *et al*., 2004). Among auxins used for this purpose, indole-3-butyric acid (IBA) is the most effective and least toxic for plant tissues (Mazzini-Guedes *et al*., 2017). PGRs have been used as plant growth substances for enhancing the rooting of cuttings. Singh *et al*. (2013) reported that Anderson medium (Anderson, 1975) supplemented with IBA at different concentrations was essential for the *in vitro* rooting of *Rhododendron* plants, and the non-auxinic chemical, salicylic acid (SA), can stimulate root formation in young shoots of the ornamental plants. SA is a phenolic compound of hormonal nature, and has been studied for its effects on various physiological processes related to various abiotic stresses (Shen *et al*., 2016), growth and development of plants, and stimulation of root development (Bhupinder & Usha, 2003).

Using PGR is one of the strategies aimed at improving rooting efficiency and reducing the duration of the use of exogenous auxins (Montero-Calasanz *et al*., 2013). In most plant species application of auxins facilitates root production from branch cuttings, however, some do not root at all even with auxin application (Nautiyal *et al*., 1992). The effectiveness of auxins in rooting branch cuttings varies with season and concentration level, and the seasonal changes in the effectiveness of exogenously applied auxins relate with the level of exogenous auxin (Davies, 1996; Dhillon *et al*., 2011). B-vitamins promote roots from cutting, and different forms of vitamin-B are known to stimulate cell division (Aberg, 1961). The vitamin-B1 (thiamine, TA) is considered to be a growth factor for the plant root, and has beneficial effect on root formation (Singh *et al*., 2005). Thiamine acts as an enzyme cofactor and has important effect on metabolically reactions such as glycolysis or in pentose phosphate and tricarboxilic acid cycle. In addition to thiamine value as a nutritional compound, it is also secondary messenger in activation of proteins with low molecular weight (Sepahvand *et al*., 2012). There is no report on the thiamine, being used to stimulate the root-induction process in cuttings of azalea plants. Action mechanisms of PGRs and TA on physiological and biochemical processes in cuttings of azalea plants remain unknown. The objectives of this work were to study the effects of reflectance spectroscopic indices on the rooting of azalea cuttings, and also to evaluate the effects of PGRs and TA on the rooting of azalea cuttings for propagation. In order to maximize the propagation of cuttings, the effects of IBA supplemented with optimal concentrations of TA, SA, and 1-naphthaleneacetic acid (NAA) in the nursery were determined, which offer insights into the mechanism in plant propagation for the future.

MATERIAL AND METHODS

Azalea (*Rhododendron pulchrum* cv. Sweet) was grown on the National Taiwan University (NTU) campus (Taipei, Taiwan), and plants were observed from April to June 2013. Plants of similar stature, with approximately 120 cm height and a canopy of 150 cm in diameter were selected, and these plants were not pruned or subjected to any chemical treatment during the experimental period. The experiment was carried out in a greenhouse of the Horticulture Department at NTU between September 22 and December 30, 2013. Previously, we found that uniform top single-node cuttings, 5-cm long with two or three leaves from vigorous young shoots and cuttings produced higher rooting scores, quantity of root, maximum root lengths, rooting percentages, and survival rates, compared to those 10 cm long for several *Rhododendron* species, including *R. asabrum. R. mucronatum, R. indicum,* and *R. kanehirai* (data not shown). Therefore, 5-cm long healthy apical shoot cuttings of azalea were used in the present study.

The first experiment was based on the effects of reflectance spectroscopic indices on the rooting of azalea cuttings. We attempted to determine whether rapid and nondestructive measurements of reflectance spectroscopy can be used as sensitive metrics to develop precise, integrated, quantitative measurements of rooting parameters of cuttings. Three healthy, fully expanded leaves per single-nodal cuttings were used for spectral reflectance with an NDVI 300 spectrophotometer (Plant Pen, Photon System Instruments, Brno, Czech Republic). Spectral wavelengths of 740 nm (R740) and 660 nm (R660) were used to calculate the vegetation index and to determine useful information related to rooting development, such as the NDVI, which is calculated as (R740 – R660) / (R740 + R660) (Devitt *et al.*, 2005). Chlorophyll contents of leaves per single-nodal cuttings were determined using a SPAD analyzer (SPAD-502 Chlorophyll Meter, Konica Minolta, Tokyo, Japan). All NDVI and SPAD readings were visually separated into 'high' and 'low' groups, where 0.730 and 41.3 were the respective cutoff points for NDVI and SPAD, respectively. In the evening, the basal portion (4~5 cm) of a cutting was placed in a Styrofoam flat (28 × 55 × 6 cm) filled with a mixture of 5 parts soil: 2 parts peat moss: 2 parts vermiculite: 1 part perlite (by volume) as rooting medium. One cutting was placed in each hole (5 × 5 × 6 cm), and 50 holes per flat were arranged on beds under shed net tunnels to protect the cuttings from direct sunlight. The temperature and relative humidity in the greenhouse were respectively maintained at 25~28°C and 90%~100% during rooting. The second experiment was based on the influence of IBA combined with PGRs and TA on the rooting of *R. pulchrum* cuttings. The above-mentioned prepared two- or three-leaf wound stem cuttings of 4~5 cm were soaked in a solution of IBA (2,000 mg L^{-1}) supplemented with (2,000 mg L^{-1}), SA (10^{-2}, 10^{-4}, and 10^{-6} M), and TA (400, 800, and 1,000 mg L^{-1}) for 15 min, each stem was planted in the flats filled with soil medium. Four replicates of each treatment were established the first day, with each containing seven cuttings. Control cuttings were only sprayed with water. All treatments with IBA and/or NAA, SA, and TA were performed once at the beginning of the experiment.

Different growth parameters of established cuttings were evaluated. Soil-filled flats with cuttings were lightly irrigated every day for the first week of dibbling, and then irrigation was maintained at alternate days up to the experimental period. Cuttings were exposed to rooting media for 99 days for the auxin tests (September 22 to December 30, 2012) and for 62 days for the SA (September 24 to November 25, 2012) and TA (September 30 to December 1, 2012) tests. Thereafter, cuttings were carefully uprooted, medium particles adhering to the roots were carefully removed by hand, and the following six root morphological parameters were determined for all treatments:

a) The stage of rooting was evaluated on a five-point (0~4) scale that rated the development of root length: 0= cutting had no 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. Scores for the rooting stage represent the mean of three independent observations by trained personnel.

b) Number of roots 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.

c) Maximum root length: The maximum root length (cm) of axillary randomly obtained plants for all treatments in each replicate was measured with the help of a measuring tape, and then the average was calculated.

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

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

f) Survival rate of cuttings: The percentage of surviving cuttings was calculated.

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 $p \leq 0.05$.

RESULTS AND DISCUSSION

SPAD and NDVI readings vs. rooting success of azalea cuttings

Different responses and levels among rooting parameters of azalea cuttings were detected during SPAD and NDVI testing (Table 1). Significant increases were observed in the higher NDVI group (0.732~0.782) compared to the lower group (0.642~0.721) in all parameters except for the percentage of survival cuttings. In addition, higher values for the number of roots (25.1), root length (3.45 cm), and root dry weight (13.19 mg) were detected in the higher SPAD group (41.5~47.5) compared to the lower group (32.5~41.2). However, SPAD values did not significantly reflect the rooting stage, rooting percentage, or percentage of survival cuttings.

When SPAD and NDVI values were >41.3 and >0.730, respectively, they became useful for measuring the rooting stage and rooting percentage of azalea when developing indices for nondestructive chlorophyll estimation. The NDVI is used to assess chlorophyll contents and can indicate the photosynthetic capacity. In this study, SPAD and NDVI values of cuttings in the lower group had lower chlorophyll concentrations compared to the higher group, which is consistent with visual observations (photos not shown). In general, cuttings with higher SPAD and NDVI values also had higher rooting parameter values. Therefore, we suggest that when the vegetation index reaches values of 41.5~47.5 for SPAD or 0.732~0.782 for NVDI, shoot cuttings of azalea 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 potential for rapid productivity gains.

Effects of the PGRs and TA on the rooting of azalea cuttings

We attempted to study the effect of auxin application on pre-conditioned azalea stem cuttings. Various PGR combinations were tested to optimize root growth. According Table 2, both IBA and NAA resulted in different root morphological responses by cuttings. A significant difference in the rooting stage was detected only in the control (3.1), but there were no significant differences in any treatments (2.2~2.6) containing IBA and NAA alone or mixed together. Cuttings of azalea had significantly higher number of roots (13.5) with IBA (2,000 mg L^{-1}) mixed with NAA (2,000 mg L^{-1}) compared to IBA (8.6) and NAA (4.3) alone and the control (5.1). In addition, 2,000 mg L^{-1} IBA used alone produced the maximum level for inducing root length (2.67 cm) and increasing the root dry weight (5.22 mg), respectively, compared to other treatments. Hence, IBA 2,000 mgL^{-1} was selected to root azalea cuttings in subsequent SA and TA treatments (Tables 3 and 4). In addition, the root percentage decreased in all treatments (52.38%~61.9%), but the control resulted in a significantly higher percentage of roots (76.19%). Nevertheless, treatment devoid of auxin gave the highest rooting stage (3.1) and percentage (76.19%), suggesting that the use of auxin was ineffective, and its influence led to root stage and percentage arrest. Initially, roots were produced as a cluster from the basal cut end and gradually developed into thick, healthy roots (photo not shown). The highest (100%) and lowest (66.7%) survival percentages of cuttings were respectively found for the treatment containing 2,000 mg L^{-1} NAA+IBA and the control. Furthermore, Xian *et al*. (2009) mentioned that stem cuttings pretreated with 2,000 mg L^{-1} IBA had the best rooting traits (86.7% rooting, root number of 23.1, and 6.4 cm root length) in *Paeonia* cuttings, indicating that auxin plays a key role in several root developmental processes, as it acts as an indicator for the division, elongation, and differentiation of root cells. However, no studies had previously been conducted on the effects of IBA combined with SA and TA on the rooting of azalea stem cuttings. In addition, because of the costs of these chemical compounds, improvements in rooting parameters of this cultivar will reduce the cost per plant and per acre of new plantings.

Table 3 shows the influence of SA treatments, applied alone and in combination with IBA (2,000 mg L^{-1}), on azalea as assessed by observing changes in six rooting morphological characteristics of stem cuttings. All treatments and the control were successfully rooted up to stage 4, with 100% rooting and survival of cuttings. Significant higher number of roots (28.8~30) were observed with 10^{-4}

Table 1. Effects of soil-plant analysis development (SPAD) and normalized difference vegetation index (NDVI) values on different parameters in the rooting of azalea cv. Sweet. Taipei, National Taiwan University, 2013.

Analysis	Rooting stage	Number of roots	Root length (cm)	Root dry weight (mg)	Rooting (%)	Survival (%)
NDVI values						
0.732-0.782	3.2 a[1]	8.0 a	1.00 a	0.70 a	75.00 a	100.00 a
0.642-0.721	2.7 b	3.0 b	0.45 b	0.10 b	55.00 b	100.00 a
$LSD_{0.05}$	0.42	0.42	0.13	0.06	7.74	--
Significance	*	***	***	***	*	NS
SPAD values						
41.5-47.5	4.0 a	25.1 a	3.45 a	13.19 a	100.00 a	100.00 a
32.5-41.2	4.0 a	16.2 b	2.55 b	5.74 b	100.00 a	100.00 a
$LSD_{0.05}$	--	1.12	0.52	3.39	--	--
Significance	NS	***	**	**	NS	NS

Rooting stage values varied from 0 to 4, that rated the development of root length: 0= cutting had no 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. [1]Values followed by same letters in the column do not differ significantly, p≤0.05 level of least significant difference (LSD) test (*n*= 4); ***= p≦0.001, **= p≦0.01, *= p≦0.05; NS and - = no significant difference; All values are averages of 20 cuttings.

Table 2. Effects of 2,000 mg·L^{-1} auxin (IBA and NAA) or mixed on different parameters in the rooting of azalea cv. Sweet. Taipei, National Taiwan University, 2013.

Treatments	Rooting stage	Number of roots	Root length (cm)	Root dry weight (mg)	Rooting (%)	Survival (%)
Control	3.1 a[1]	5.1 c	1.62 b	1.01 c	76.19 a	90.48 b
IBA (2,000 mg L^{-1})	2.6 b	8.6 b	2.67 a	5.22 a	61.00 b	76.19 c
NAA (2,000 mg L^{-1})	2.2 b	4.3c	2.59 ab	4.17 ab	52.83 c	66.67 d
IBA (2,000 mg L^{-1}) + NAA (2,000 mg L^{-1})	2.4 b	13.5 a	2.03 ab	3.05 b	57.14 b	100.00 a
$LSD_{0.05}$	0.45	1.11	0.90	1.67	10.66	20.13
Significance	**	***	*	**	*	*

Rooting stage values varied from 0 to 4, that rated the development of root length: 0= cutting had no 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. [1]Mean values followed by same letters in the column do not differ significantly, p≤0.05 level of least significant difference (LSD) test (*n*= 4); ***= p≦0.001, **= p≦0.01, *= p≦0.05; NS and - = no significant difference; All values are averages of 20 cuttings. IBA = indole-3-butyric acid; NAA= 1-naphthaleneacetic acid.

M of SA alone and combined with IBA compared to other treatments. In contrast, the root length of cuttings significantly decreased (3.09 cm) when 10^{-2} M of SA was applied alone compared to other treatments. Furthermore, significantly higher (17.33 mg) and lower (6.42 mg) root dry weights were detected in IBA (2,000 mg L^{-1}) + SA (10^{-4} M) and SA (10^{-2} M) alone, respectively. In *Phaseolus aureus*, *Acer saccharinum,* and *Acer griseum* plants, SA highly promoted the *in vivo* rooting of cuttings when applied particularly with auxin (Kling & Meyer, 1983). Li (1995) illustrated that SA had positively and synergistically acted with IAA and promoted the root formation in mung bean cuttings. The use of SA caused a positive effect on rooting of *Henna* cuttings soaked in 2,000 mL L^{-1} NAA + 200 mL L^{-1} SA (Salehi-Sardoei *et al.*, 2013). Karimi *et al.* (2012) reported that the response of Pomegranate (*Punica granatum*) to IBA and SA treatments significantly increased rooting in 'Gorj-e-Shahvar' compared to 'Gorj-e-Dadashi', and this different response between cultivars may be due to differences in endogenous auxin concentration in the cultivars. Furthermore, optimal levels of SA might be biologically significant, perhaps in conditioning interactions with other organisms, similar to the role for SA in host-pathogen signaling (Morse *et al.*, 2007). Basically, the most effective concentration of SA for rooting was 10^{-4} M, and the combination with IBA seemed to promote the number of roots, root elongation, and root dry weight. A high concentration of SA (10^{-2} M) seemed to inhibit the number of roots, root length, and root dry weight since significantly lower values for these parameters were recorded after treatment with SA (10^{-2} M) alone. Although our study did not illuminate a specific role for elevated SA in azalea, it would appear that 10^{-4} M of SA may be suitable.

Table 3. Effects of indole-3-butyric acid (IBA, 2,000 mg L^{-1}) and different salicylic acid (SA) concentrations on different parameters in the rooting of azalea cv. Sweet. Taipei, National Taiwan University, 2013.

Treatments	Rooting stage	Number of roots	Root length (cm)	Root dry weight (mg)	Rooting (%)	Survival (%)
Control	4.0 a[1]	23.9 b	3.83 a	13.36 c	100.00 a	100.00 a
SA (10^{-6} M)	4.0 a	25.6 b	3.80 a	13.80 bc	100.00 a	100.00 a
SA (10^{-4} M)	4.0 a	28.8 a	3.95 a	14.97 b	100.00 a	100.00 a
SA (10^{-2} M)	4.0 a	17.1 c	3.09 b	6.42 d	100.00 a	100.00 a
IBA (2,000 mg L^{-1}) + SA (10^{-4} M)	4.0 a	30.0 a	4.01 a	17.33 a	100.00 a	100.00 a
$LSD_{0.05}$	--	2.85	0.54	1.46	--	--
Significance	NS	***	*	***	NS	NS

Rooting stage values varied from 0 to 4, that rated the development of root length: 0= cutting had no 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. [1]Mean values followed by same letters in the column do not differ significantly, p≤0.05 level of least significant difference (LSD) test (*n*= 4); ***= p≦0.001, **= p≦0.01, *= p≦0.05; NS and - = no significant difference; All values are averages of 20 cuttings.

Table 4. Effects of indole-3-butyric acid (IBA, 2,000 mg L^{-1}) and different thiamine (TA) concentrations on different parameters in the rooting of azalea cv. Sweet. Taipei, National Taiwan University, 2013.

Treatments	Rooting stage	Number of roots	Root length (cm)	Root dry weight (mg)	Rooting (%)	Survival (%)
Control	4.0 a[1]	19.3 b	4.00 b	11.99 a	100.00 a	100.00 a
TA (400 mg L^{-1})	4.0 a	20.0 b	3.71 b	7.14 d	100.00 a	100.00 a
TA (800 mg L^{-1})	4.0 a	19.9 b	3.71 b	8.85 c	100.00 a	100.00 a
TA (1,000 mg L^{-1})	4.0 a	18.7 b	3.60 c	11.00 b	100.00 a	100.00 a
IBA (2,000 mg L^{-1}) + TA (800 mg L^{-1})	4.0 a	24.6 a	4.46 a	10.39 b	100.00 a	100.00 a
$LSD_{0.05}$	--	2.5	0.40	1.50	--	--
Significance	NS	***	*	***	NS	NS

Rooting stage values varied from 0 to 4, that rated the development of root length: 0= cutting had no 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. [1]Mean values followed by same letters in the column do not differ significantly, p≤0.05 level of least significant difference (LSD) test (*n*= 4); ***= p≦0.001, **= p≦0.01, *= p≦0.05; NS and - = no significant difference; All values are averages of 20 cuttings.

There were differences in root characteristic of azalea cuttings developed with TA concentrations and combinations. In Table 4 it appears that neither the rooting stage (4), rooting percentage (100%), nor survival percentage (100%) of cuttings significantly responded to any treatment. The number of roots (24.6) and root length (4.46 cm) were significantly influenced by application of IBA (1,000 mg L^{-1}) combined with 800 mg·L^{-1} TA compared to other treatments. However, the maximum dry weight (11.99 mg) was observed in the control which was significantly higher than those of other treatments. The number of roots and length in all shoot cuttings were promoted by all IBA+SA and IBA+TA treatments compared to IBA alone (Table 2), suggesting that both SA and TA seemed to be important for inducing the root number, and the addition of SA (10^{-4} M) and TA (800 mg L^{-1}) proved synergistic with IBA. In fact, increased root growth by application of TA to auxin-treated cuttings of various horticultural species has been documented. Singh *et al.* (2005) reported a strong synergistic interaction between TA and auxins for adventitious rhizogenesis in *Tectona grandis* cuttings, and concluded better rooting in the interaction treatments auxin-TA synergism compared to TA (800 ppm) alone or auxin (250 ppm IBA + 125 ppm IAA + 125 ppm NAA). They further explained that auxins are essential for the induction of adventitious roots while the subsequent growth of induced root primordial depends upon TA supply. Girouard (1969) postulated that possibly TA acts as a mobilizing factor in one or the other way to synthesize rhizocaline complex in light exposed leaves and transported basipetally to promote adventitious rhizogenesis as auxin synergist. TA is very important for *in vitro* growth and development of excised roots in many plants, and has a direct or indirect role in promotion of adventitious rhizogenesis in some woody plants (Aberg, 1961; Palanisamy *et al*., 1998). Exogenous application of various auxins stimulates adventitious rhizogenesis in shoot cuttings of many species, and the additional application of auxins stimulates the activity of the cambium, consequently, mobilization of reserve food material occurs at faster rate for the site of root initiation (Blaskesley *et al.*, 1991). Bojarczuk & Jankiewicz (1975) demonstrated that the stimulatory effect on rooting of *Syringa vulgaris* differed very little following treatment by different auxins: NAA, IAA, and IBA at optimal concentration; however, substances such as TA could promote rooting of cuttings when added separately. Mahdi & Sallam (1978) reported that *Populus alba* cuttings treated with rooting solution containing NAA, IBA, and TA yielded a higher percentage of rooted cuttings than those treated with hormex rooting powder which contains only IBA as the active ingredient. In the case of *Prosopis alba*, the number of roots and maximum root length per cutting increased with the concentration of 0.3% IBA and 0.1% TA compare to 0.3% IBA alone (Klass *et al.*, 1987). Furthermore, Ansari & Kumar (1994) pointed out that IAA induced adventitious roots in branch cuttings of *Dalbergia sissoo* whereas, TA promoted growth of roots. In *Taxus cuspidata* and *Taxus brevifolia*, Chee (1995) found that the cuttings treated with supplemented TA consistently produced better rooting response.

The rooting stage, percentage, and survival rate of cuttings were promoted in the control devoid of IBA, SA, and TA, and the reason for the adverse effects of these chemicals might have been due to endogenous levels of IBA within the cuttings which altered the growth responses. IAA is the main auxin in most plants, while IBA is the most common exogenously applied plant growth regulator. IBA is relatively stable and insensitive to the auxin degrading enzyme systems. It has even greater ability to promote adventitious root formation than IAA (Štefančič *et al.*, 2005). Considering the problem of the poor establishment of azalea stem cuttings in the nursery, the present study was carried out to determine the effect of IBA and to select the optimal concentrations of SA and TA for application to stem cuttings for the rapid multiplication and increasing uniformity of rootings of azalea plants in the nursery on a commercial scale. This has the advantage of clonal or true-to-type propagation of elite trees.

In conclusions, root development and morphology of azalea cuttings were demonstrated to depend on IBA combined with SA or TA, which were key factors in root formation. IBA alone produced higher root parameters in cuttings than did NAA. Different concentrations of SA and TA proved to be excellent for root growth and development, in which SA at higher concentrations induced greater values for the number of roots, root length, and root dry weight. These results can be used to develop management practices for using stem-node cultivation of azalea in gardens, reduce the time for rooting, and offer hope for IBA combined with SA or TA for propagating ornamental plants. Furthermore, the SPAD and NDVI are nondestructive measurements that can be applied on a large-scale to assess the rooting stage and percentage of bedded ornamental plants grown in open fields.

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