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Research Paper

Effect of Hydrogen Peroxide, Ascorbic Acid and Indolic-3-Butyric Acid on Root Induction and Development in Cuttings of *Bougainvillea spectabilis*

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Abstract

Bougainvillea (*Bougainvillea spectabilis*) is used for planting in the landscape because of their beautiful and colorful flowers. The easiest and cheapest technique to propagate this plant is by cuttings; however the cuttings of this plant are not easily rooted and require the special treatments, including the use of auxin. For this purpose, an experiment was performed in Completely Randomized Design with 15 treatments and four replications and the characteristics such as root and shoot length, root and shoot number, root fresh and dry weight, and the rooted cuttings percent were evaluated. The results showed a statistically significant difference among the used treatments. The use of 400 mg/L indolic-3-butyric acid (IBA) for 24 hrs dipping led to improve rooting parameters particularly the rooted cuttings percent. However, the use of 1000 mg/L IBA for 20 s quick dipping also had satisfactory results, which can be introduced as a suitable treatment due to the reduction of application time and labor costs. Adding substances similar to ascorbic acid (AsAc) and hydrogen peroxide (H_2O_2) also improved some traits such as the root length, root fresh weight and the number of shoots. In general, the use of high concentrations of IBA (2000 mg/L), which are used by quick dipping with the use of pre-treatment with AsAc and H_2O_2 can lead to improve rooting traits in bougainvillea cuttings. When the cost of the plant growth regulators is important, the use of the low-concentration of IBA (400 mg/L) by long-term dipping (24 hrs) can be effective for Bougainvillea cuttings.

Keywords: H_2O_2, IBA, Long-term and quick dipping, Rooting, Vitamin C.

INTRODUCTION

In the horticultural industry most of the perennial ornamental plants are propagated vegetative through cuttings, grafting, budding and layering techniques. Generally, the use of cuttings from stems, roots, terminal buds or leaves is considered the most commonly applied technique, due to its practicability and simplicity. Bougainvillea (*Bougainvillea spectabilis* Willd.), because of its special characteristics, such as high variation in foliage type, production of many flowering inflorescence on one plant and continuous blooming of flowers with short production cycle has been very useful in the ornamental industry (Ibironke, 2019). The genus *Bougainvillea* is a very widespread group throughout the world. It belongs to Nyctaginaceae family and contains approximately 18 species, but only four species (*B. buttiana, B. glabra, B. spectabilis,* and *B. peruviana*) are commercially utilized. However, there are also more than 100 cultivars and three hybrids, the latter not yet recognized. The genus *Bougainvillea* is endemic to South America and was firstly reported in Brazil in 1778 before being introduced to Europe, by French military commander Louis Antoine de Bougainville. They are bushes spread in vines or small trees. They also possess stems with internodes and with straight or slightly curved thorns. The leaves are petiolate, elliptical, or wider towards the base. The bracts and flowers are presented in different colors, depending on the species, cultivars, or hybrid. They bloom throughout the year (Abarca-Vargas and Petricevich, 2018).

Propagation by cutting is very popular method of multiplication of several ornamental plants. But it has a limited success in the propagation of bougainvillea because most of its cultivars are hard-to-root. A considerable work has been done to improve rooting of cuttings in bougainvillea and other ornamental plants. The main sources of plant growth hormones widely used in the promotion of rooting cuttings are the indolic-3-butyric acid (IBA), 1-naphthalene acetic acid (NAA) and indole-3-acetic acid (IAA) (Singh, 2018).

The best length for semi-hard wood cuttings of bougainvillea is 8-7 nodes (Moalemi and Chehrazi, 2003). It is recommended to separate the juicy end of cutting, the use of rooting hormones and a well-drained culture medium with a 24 °C temperature as well as the use of a mist system to increase rooting (Tariq-Shah *et al.,* 2006). The cuttings of bougainvillea usually are treated with 3000-6000 mg/L IBA. Some researchers suggested that the thicker cuttings with higher auxin concentrations will have better rooting however, high levels of auxin may prevent the branching (Khoshkhui, 2003; Xiao, 2003). In bougainvillea, cuttings below the end of the shoot are suitable for rooting. This type of cuttings is more vigorous and more resistant against the diseases (Moalemi and Chehrazi, 2003).

The various chemical compounds, especially the plant hormones and growth regulators, have a significant effect on root formation (Jalilvand, 2003). Auxins play the important role in the formation of adventitious roots. These hormones are involved in some physiological process such as stem growth, preventing the growth of lateral buds, falling of the leaves and fruits, and activating the cells of the vascular cambium (Danehloueipour *et al*., 2006; Jalili-Marandi, 2003). Singh *et al*. (2011) found the best rooting performance of bougainvillea in the use of 4000 mg/L IBA under mist system. Ahmad *et al*. (2002) recommended the use of 1000 mg/L IBA or IAA to improve rooting of the five bougainvillea cultivars cuttings and they reported the bougainvillea cultivar type affect the rooting parameters.

Commercial root-promoting chemicals are normally applied to the basal portion of cuttings using a liquid or talc formulation of auxin. The quick-dip method is often preferred by commercial propagators for application of liquid auxin formulations for reasons of economy, speed, ease, and uniformity of application and results. An extended basal soak may be utilized for some difficult-to-root species (Hartmann *et al*., 2002). Plant damage occurs when the capacity of antioxidant processes and detoxification mechanisms are lower than the amount of reactive oxygen species production. Aerobic organisms have developed complex systems protecting them from reactive oxygen species, consisting of several enzymes and antioxidants. Those mechanisms can slow down or even stop the oxidation of bio-molecules and block the process of oxidative chain reactions (Sgherri *et al*., 2003). The most important are low-molecular antioxidants such as ascorbic acid (AsAc), glutathione, thiols, α-tocopherol and protective pigments such as carotenoids (Tausz *et al*., 2003). Application of AsAc in combination with an auxin (IBA) promotes rooting in terms of number of roots/cutting in various plant species. Bosila *et al*. (2010) recorded the highest values in rooting percentage of bougainvillea cuttings in two successive years by using 5000 mg/L IBA + 50 mg/L AsAc.

Ibironke (2019) to stimulate of rooting in the six *Bougainvillea* species using three different rooting hormones found that the IBA and the coconut water had significant effect on the root emergence and root growth of *Bougainvillea species* compared to the other hormones used and the hard-wood cuttings enhanced the rooting of the *Bougainvillea* compared to the semi-hard wood. Mehraj *et al*. (2013) reported that IBA plays an important role for successful asexual propagation of bougainvillea through cutting. They found that the better performance when cut stems were soaked for 24 hrs in 1000 mg/L IBA, in addition, 100% rooting was also observed in 500 and 2000 mg/L IBA but only 1000 mg/L was resulted 100% survival of the rooted cuttings. Fathi *et al*., (2017) to assess the impact of natural carbohydrate compounds and chemical hormonal compounds on the rooting traits of bougainvillea found that the treatments of 4000 mg/L IBA and 10% grape syrup displayed the best quantity and quality of the rooting.

Adventitious root formation is mediated by multiple changes in plant metabolism and it is controlled by successive and interdependent physiological phases. Adventitious root formation can be stimulated by auxins, but their role in rooting is not exclusive and others compounds are involved. In some plants such as olive exogenous application of IBA and hydrogen peroxide (H_2O_2) has been shown to promote early rooting. Moreover, in real nursery propagation condition H_2O_2 in combination with IBA treatments significantly has been improved rooting (Sebastiani *et al*., 2004). Sebastiani *et al.* (2004) reported that the olive cuttings treated with 4000 mg/L IBA + H_2O_2 had significantly higher root number in comparison with those treated with 4000 mg/L IBA alone. In the easy-rooted species, the preparation of the cuttings from flower branches without flowers does not have a significant effect on rooting, but in the difficult-rooted cultivars, cuttings should be prepared from the shoots that contain leaf buds, because the flowering stimulants are not compatible with rooting, and at the same time there is competition for food uptake between buds and rooting (Satisha *et al*., 2008).

The present experiment was conducted with aim to achieve the best IBA concentration and application time for rooting bougainvillea cuttings, as well as to investigate the effects of cuttings pre-treatment with ascorbic acid and hydrogen peroxide.

MATERIALS AND METHODS

The required semi-woody cuttings were prepared from the 7-years-old common bougainvillea plants (*Bougainvillea spectabilis* Willd.), which had a uniform vegetative growth, with a thickness of about 4 to 5 mm and a length of 15 cm in the mid. of January. The cut of bottom of the cuttings was diagonally performed just below a bud and the cut of top of the cuttings 1 to 2 cm above the first bud. All cuttings had one leaf (Fig. 1A). The cuttings were disinfected using 1% sodium hypo-chloride and then exposed to the open air for 10 minutes. This study was conducted in completely randomized design with 15 treatments and four replications (100 cuttings in each replicate) in the greenhouse conditions with 20±1 °C temperature for day and 15±1 °C temperature for night. The treatments were presented in table 1. After treatment, the cuttings were planted in plastic pots containing the washed sand at a depth of 10 cm. The mist system was adjusted with an interval of one hour per day and every 4 hours at night with a spray time of 20 seconds.

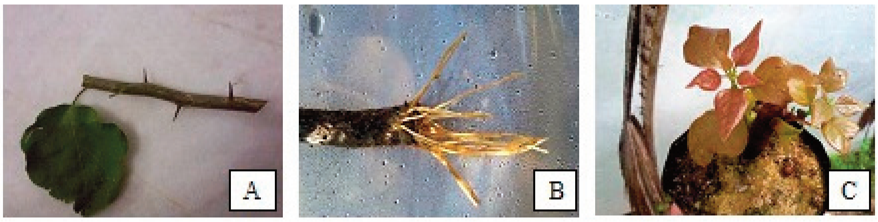

Fig. 1. The leafy cuttings ready for planting (A); The produced root at the end of cutting (B); The grown shoots on the planted cutting (C).

Table 1. List of the used treatments.

Treatment abbreviation	Treatment details
IBA 200-12 hrs	IBA 200 mg/L for 12 hours
IBA 200- 24 hrs	IBA 200 mg/L for 24 hours
IBA 400-12 hrs	IBA 400 mg/L for 12 hours
IBA 400- 24 hrs	IBA 400 mg/L for 24 hours
IBA 1000-10 s	IBA 1000 mg/L for 10 seconds
IBA 1000- 20 s	IBA 1000 mg/L for 20 seconds
IBA 2000-10 s	IBA 2000 mg/L for 10 seconds
IBA 2000- 20 s	IBA 2000 mg/L for 20 seconds
AsAc + IBA 1000	Ascorbic acid 15000 mg/L for 5 min (pre-treatment) + IBA 1000 mg/L for 10s
AsAc + IBA 2000	Ascorbic acid 15000 mg/L for 5 min (pre-treatment) + IBA 2000 mg/L for 10s
H_2O_2 + IBA 1000	H_2O_2 1.5% for 10 seconds (pre-treatment) + IBA 1000 mg/L for 10s
H_2O_2 + IBA 2000	H_2O_2 1.5% for 10 seconds (pre-treatment) + IBA 2000 mg/L for 10s
AsAc + H_2O_2 + IBA 1000	Ascorbic acid for 5 min + H_2O_2 for 10s + IBA 1000 mg/L for 10s
AsAc + H_2O_2 + IBA 2000	Ascorbic acid for 5 min + H_2O_2 for 10s + IBA 2000 mg/L for 10s
Control	Without treatment

In April (about 85 days after cutting) the rooting parameters were measured including the length of root and the new shoot length with a metal ruler; root fresh weight using digital scale with accuracy 0.001; root dry weight by placing the roots in a 75°C oven for 48 hours, and the number of root and the new shoot by counting the roots and shoot grown as well as the percentage of the rooted cuttings. To assess normality of the obtained data, Kolmogorov-Smirnov and Shapiro-Wilk tests were used by using SPSS 16.0 software and after making sure the data were normal, analysis of variance was performed by using SAS 9.1 software and the means were compared using protected least significant difference (PLSD) test. Spearman correlation coefficients between the attributes were calculated using SPSS 16.0 software.

RESULTS

According to the results of analysis of variance the used treatments had significant effect on the assessed attributes at $P<0.01$ except about shoot number ($P<0.05$) (Table 2).

Root length

The use of treatment significantly caused to increase the root length in the bougainvillea cuttings compared to control treatment. The longest roots was observed in the use of AsAc + 2000 mg/L IBA (85.3 mm), which it had significant difference with the control treatment and 2000 mg/L

IBA for 10 or 20 s treatments. The use of 2000 mg/L IBA for 10 or 20 s had the lower effect on the increasing root length, so that 2000 mg/L IBA for 20 s did not differ significantly with control treatment. Overall, the concentration and dipping time of IBA did not have a significant effect on the root length. Cuttings pre-treatment also did not have a significant effect on root length, but in-significantly led to improved root length. However, the use of AsAc in combination with 2000 mg/L IBA was able to significantly increase the root length (Table 3).

Table 2. Analysis of variance concerning the effect of different treatments on the studied attributes.

S.o.V	MS	Error	CV (%)
Root length	1472.2**	560.8	17.5
Root number	506.9**	49.6	12.9
Root fresh weight	8.67**	3.03	8.0
Root dry weight	0.69**	0.27	2.5
The rooted cuttings percent	1055.3**	137.8	19.9
Shoot length	1249.0**	145.0	16.5
Shoot number	23.2*	11.1	14.2
Freedom degree	14	45	

* and ** Significant at $P < 0.05$ and $P < 0.01$, respectively.

Table 3. Effect of the treatments on the studied attributes about root.

Treatments	Root length (mm)	Root No.	Root fresh weight (g)	Root dry weight (g)	The rooted cuttings (%)	Shoot length (mm)	Shoot No.
IBA 200-12 hrs	52.6^{ab}	5.9^{cd}	1.56^{bc}	0.75^{a-d}	18.2^{ef}	20.0^{cd}	3.5^{b}
IBA 200- 24 hrs	52.6^{ab}	17.3^{bc}	2.75^{bc}	1.01^{ab}	44.8^{bc}	48.0^{b}	3.2^{b}
IBA 400-12 hrs	69.5^{ab}	17.3^{bc}	3.25^{b}	0.69^{a-d}	33.9^{b-e}	35.2^{bc}	3.1^{b}
IBA 400- 24 hrs	50.5^{ab}	53.2^{a}	3.31^{b}	1.20^{a}	64.2^{a}	68.0^{a}	2.5^{b}
IBA 1000-10 s	67.4^{ab}	20.0^{b}	1.88^{bc}	0.60^{a-d}	38.2^{bcd}	51.2^{ab}	2.4^{b}
IBA 1000- 20 s	60.0^{ab}	9.1^{bcd}	2.12^{bc}	1.01^{ab}	47.9^{ab}	25.6^{cd}	3.2^{b}
IBA 2000-10 s	42.1^{b}	21.8^{bcd}	2.19^{bc}	0.87^{abc}	32.7^{b-e}	36.0^{bc}	4.1^{b}
IBA 2000- 20 s	31.6^{bc}	0.7^{d}	0.25^{c}	0.03^{cd}	3.0^{f}	12.8^{d}	2.0^{b}
AsAc + IBA 1000	47.4^{ab}	11.8^{bcd}	0.31^{c}	0.05^{cd}	19.4^{def}	23.2^{cd}	2.4^{b}
AsAc + IBA 2000	85.3^{a}	10.0^{bcd}	1.25^{bc}	0.63^{a-d}	27.9^{cde}	14.4^{d}	11.6^{a}
H_2O_2 + IBA 1000	56.8^{ab}	3.2^{d}	0.69^{c}	0.15^{cd}	29.7^{b-e}	19.2^{cd}	3.7^{b}
H_2O_2 + IBA 2000	65.3^{ab}	5.9^{cd}	1.50^{bc}	0.43^{a-d}	20.6^{def}	12.8^{d}	1.9^{b}
AsAc + H_2O_2 + IBA 1000	68.4^{ab}	3.6^{d}	1.62^{bc}	0.32^{bcd}	20.6^{def}	8.0^{d}	2.7^{b}
AsAc + H_2O_2 + IBA 2000	68.4^{ab}	5.5^{cd}	5.94^{a}	1.01^{ab}	26.1^{cde}	10.4^{d}	2.7^{b}
Control	5.3^{c}	0.5^{d}	0.10^{c}	0.01^{d}	3.3^{f}	4.8^{d}	1.5^{b}

*In each column, means with the similar letters are not significantly different ($P < 0.05$) using the LSD test.

Based on the correlation coefficient, a positive significant correlation (0.516*) was observed between root length and shoot number parameters (Table 4).

Table 4. Correlation coefficient among the rooting parameters.

	Root length	Root No.	Root fresh weight	Root dry weight	The rooted cuttings	Shoot length	Shoot No.
Root length	1						
Root No.	0.100	1					
Root fresh weight	0.434	0.373	1				
Root dry weight	0.401	0.619*	0.788**	1			
The rooted cuttings	0.407	0.805**	0.519*	0.800**	1		
Shoot length	0.108	0.901**	0.318	0.604*	0.830**	1	
Shoot No.	0.516*	-0.005	-0.042	0.163	0.109	-0.092	1

* and ** Significant at $P < 0.05$ and $P < 0.01$, respectively.

Root number

The use of some treatments significantly caused to increase the number of root in the bougainvillea cuttings compared to control treatment. The highest root number was recorded in the use of 400 mg/L IBA for 24 hrs (53.2), which it had significant difference with the all treatments. The treatments of 200 mg/L IBA for 24 hrs, 400 mg/L IBA for 12 hrs and 1000 mg/L IBA for 10 s were ranked next, respectively (17.3, 17.3 and 20.0, respectively). Cuttings pre-treatment did not have a significant effect on the number of root, so that all treatments containing AsAc and H_2O_2 had no significant difference with the control treatment. The increase in dipping time at 200, 1000 and 2000 mg/L concentrations of IBA resulted in a significant reduction in the number of the roots in the cuttings. At 400 mg/L IBA, the number of roots increased significantly with the increasing dipping time. On the other hand, the use of pre-treatment reduced the number of the roots, so that the use of H_2O_2 or the simultaneous use of H_2O_2 and AsAc in combination with 1000 mg/L IBA significantly reduced the number of the roots (Table 3). According to the correlation coefficient, a positive significant correlation was observed between root number with root dry weight (0.619*), the rooted cuttings percent (0.805**) and the shoot length (0.901**) parameters (Table 4).

Root fresh and dry weight

The use of some treatments significantly caused to increase the root fresh weight in the bougainvillea cuttings compared to control treatment. The greatest root fresh weight was observed in the use of AsAc + H_2O_2 + 2000 mg/L IBA (5.94 g), which it had significant difference with the all treatments. The treatments of 200 mg/L IBA for 24 hrs, 400 mg/L IBA for 12 or 24 hrs were ranked next, respectively (2.75, 3.25 and 3.31 g, respectively). In general, only the differences between treatments 400 mg/L IBA for 12 or 24 hrs and AsAc + H_2O_2 + 2000 mg/L IBA were significant with the control treatment. Except at a concentration of 400 mg/L IBA, the concentration and duration of use of IBA had no significant effect on root fresh weight (Table 3).

The use of some treatments significantly caused to increase the root dry weight in the bougainvillea cuttings compared to control treatment. The highest root dry weight obtained in the use of 400 mg/L IBA for 24 hrs (1.20 g), which it had significant difference only with the treatments including 2000 mg/L IBA for 20 s, AsAc + 1000 mg/L IBA, H_2O_2 + 1000 mg/L IBA and AsAc + H_2O_2 + 1000 mg/L IBA. In general, there was significant difference between some treatments such as 200 or 400 mg/L IBA for 24 hrs, 1000 mg/L IBA for 20 s, 2000 mg/L IBA for 10 s, and AsAc + H_2O_2 + 2000 mg/L IBA with the control treatment (Table 3). The increase in dipping time at 200, 400 and 1000 mg/L concentrations of IBA resulted in an insignificant increase in the root fresh and dry weight. At 2000 mg/L IBA, the root fresh and dry weight decreased insignificantly with the increasing dipping time. Except in the integrated treatment among AsAc + H_2O_2 + 2000 mg/L IBA, the use of cutting pre-treatment did not have significant effect on the increasing root fresh weight (Table 3). A positive significant correlation was observed between root fresh weight with root dry weight (0.788*) and the rooted cuttings percent (519*) as well as between root dry weight with root number, root fresh weight, the rooted cuttings percent (0.800**) and the shoot length (0.604*) parameters (Table 4).

The rooted cuttings percentage

The highest rooted cuttings percentage was observed in the use of 400 mg/L IBA for 24 hrs (64.2%), which it had significant difference with the all treatments except 1000 mg/L IBA for 20 s treatment. The lowest rooted cuttings percent was recorded in the use of 2000 mg/L IBA for 20 s that it did not have significant difference with the control treatment as well as some treatments such as 200 mg/L IBA for 12 hrs, AsAc + 1000 mg/L IBA, H_2O_2 + 2000 mg/L IBA, and AsAc + H_2O_2 + 1000 mg/L IBA treatments. The increase in dipping time at 200, 400 and 1000 mg/L concentrations of IBA resulted in an increase in the rooted cuttings percent, but at 2000 mg/L IBA, the rooted cuttings percent significantly decreased with the increasing dipping time. The use of cutting pre-treatment did not have significant effect on the rooted cutting percentage (Table 3). A positive significant correlation was observed between the rooted cuttings percentage with the root number, root fresh and dry weight and the shoot length (0.830**) parameters (Table 4).

Shoot length

The use of treatments did significant effect on the shoot length. The longest shoot was observed in the use of 400 mg/L IBA for 24 hrs and 1000 mg/L IBA for 10 s, respectively (68.0 and 51.2 mm, respectively), which they had significant difference with the all treatments. The shortest shoot was recorded in the use of control treatment, which it had significant difference with the some treatments including 200 mg/L IBA for 24 hrs, 400 mg/L IBA for 12 or 24 hrs, and 1000 or 2000 mg/L IBA for 10 s. The increase of dipping time significantly increased the shoot length at 200 and 400 mg/L IBA treatments. In contrast, the increase of dipping time significantly decreased the shoot length at 1000 and 2000 mg/L IBA treatments. The application of cutting pre-treatment did not have significant effect on the length of the shoots, so that the use of cutting pre-treatment significantly decreased the shoot length (Table 3).

Shoot number

The highest shoot number was observed in the use of AsAc + 2000 mg/L IBA (11.6), which it had significant difference with the all treatments. In the use of other treatments was no observed significant effect on the shoot number. However, the use of the treatments insignificantly led to increase the number of shoot on the cuttings (Table 3).

DISCUSSION

In regards the effect of the treatments on the root length of bougainvillea cuttings, it has been determined that the use of 15000 mg/L AsAc for 5 min as pre-treatment and then 2000 mg/L IBA for 10 s dipping was the more effective compared to other treatments. The positive effect of IBA on the root length can be attributed to the effect of auxins on stimulation of root cells division. Although, the use of AsAc + 1000 mg/L IBA did not have significant difference with AsAc + 2000 mg/L IBA treatment, but the root length in the treatment containing lower concentration of IBA (1000 mg/L) was lower. Therefore, the higher IBA concentrations have been effective in achieving this result. Moalemi and Chehrazi (2003) found that the increasing IBA concentration enhances the root length of the cuttings of bougainvillea.

In the present study, the use of the integrated treatments such as AsAc + IBA, H_2O_2 + IBA and AsAc + H_2O_2 + IBA caused to increase root length of the bougainvillea cuttings compared to the control treatments. Many studies have also shown that the mixing rooting facilitators is more effective than using each alone (Khoshkhui, 2003; Moalemi and Chehrazi, 2003; Blythe *et al*., 2004). It seems H_2O_2 in addition to disinfecting the cuttings, increases the activity of starch hydrolysis enzymes by increasing the enzymatic activity of peroxidase and cytochrome oxidase, thus providing the required carbohydrates for the roots and thus can it plays an important role in rooting and increasing the root length of cuttings. It has been reported that the presence of AsAc during rooting prevents the oxidation of phenolic compounds and also prevents the breakdown of auxins, thus it lead to increase the root length (Yilmaz *et al.,* 2003). Similar to our results, Eed *et al*. (2015) obtained the best performance in the use of 2000 ppm IBA for rooting the *Bougainvillea* cuttings.

One of the benefits of the use of auxin is increasing the number of roots per cuttings (Fathi and Esmaeilpour, 2000). It is believed that by increasing the IBA concentration to 2000 mg/L, in addition to increasing the rooting percentage, the number of roots will significantly increase in the bougainvillea cuttings (Moalemi and Chehrazi, 2003). However in our experiment, the use of 400 mg/L IBA for 24 hrs has significantly increased the number of root. The reason for this may be that the cuttings are exposed to IBA for 24 hrs and placing cuttings for a longer period of time has been more effective than increasing the IBA concentration to 1000 and 2000 mg/L. Sultana (2006) also reported that the highest number of root was obtained with 400 mg/L IBA in bougainvillea, which is according to our findings.

In the present experiment, the increase of root fresh weight was observed due to high concentrations of IBA with pre-treatments using AsAc and H_2O_2. Khaled Mousa (2003) in his experiment on olive cuttings found that the use of high concentration of IBA had positive effect on the root quality. In our study, pre-treatment of the cuttings with AsAc led to increase the dry weight of the roots. AsAc plays an important role in the synthesis of the sugars and carbohydrates. On the other hand, the significant role of the sugars and carbohydrates in rooting of the cuttings has been proven (Khoshkhui, 2003). Therefore, it can be stated that AsAc indirectly affects the root weight of the cuttings. The increase of the root weight using pre-treatment of the cutting by AsAc in the present study could be due to the effective role of AsAc as a catalyst in the metabolism of the carbohydrates, fats and proteins, as well as the role of the carbohydrates on the increase of root weight. The use of H_2O_2 in combination with IBA and AsAc also increased root fresh weight, which was consistent with the findings of Sebastiani *et al*. (2004) regarding the positive effect of the combined use of H_2O_2 and IBA in increasing root weight of semi-woody cuttings of different olive cultivars. Obviously, given the effective role of each of the auxiliary factors in increasing the root weight, the combination of these factors can also increase the root weight in each cutting.

In the present study, the highest growth of new shoots is related to semi-woody cuttings that have been treated with a concentration of 400 mg/L IBA for 24 hrs and the treatment with higher IBA concentrations or treatment of the cuttings with the mixed treatments reduced the growth rate of the new shoots. The use of 15000 mg/L AsAc for 5 min and then 2000 mg/L IBA for 10 s led to increase the number of the new shoots. AsAc plays an important role in the development of the shoots in terms of its effect on sugar and carbohydrate levels (Yilmaz *et al.,* 2003). High concentrations of IBA have also been shown to be effective. The increasing effect of the combination of AsAc and IBA in increasing the number of the shoots and roots has also been reported by other researchers. Perhaps one of the reasons for the lack of rooting in the use of the high concentrations of IBA is due to the growth of a large number of the shoots and the consumption of the carbohydrates (Khan *et al*., 2006).

CONCLUSION

According to the results of the present study, the use of 400 mg/L indolic-3-butyric acid (IBA) for 24 hrs dipping caused to improve rooting parameters particularly the rooted cuttings percentage. However, the use of 1000 mg/L IBA for 20 s quick dipping also had satisfactory results, which can be introduced as an appropriate treatment due to the reduction of application time and labor costs. The application of some substances similar to ascorbic acid (AsAc) and hydrogen peroxide (H_2O_2) as pre-treatments of the cuttings also improved some traits such as the root length, root fresh weight and the number of shoots. In general, the use of the high concentrations of IBA (2000 mg/L), which are used by quick dipping with the application of the pre-treatment with AsAc and H_2O_2 can lead to improve the rooting traits in bougainvillea cuttings. When the cost of the plant growth regulators is important, the use of the low-concentration IBA (400 mg/L) by long-term dipping (24 hrs) can be effective for bougainvillea cuttings.

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