Adv. Hort. Sci., 2019 33(1): 3-11 DOI: 10.13128/ahs-22935

AHS
Advances in Horticultural Science

The effect of cutting type, leaf area, leaf number, putrescine and indole-3-Butyric acid on the rooting of Ficus cuttings (*Ficus elastica* Roxb. ex Hornem.)

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Key words: auxin, cutting, plant propagation, polyamine, rubber fig.

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Citation:
GHASEMI GHEHSAREH M., KOSH-KHUI M., 2019 - *The effect of cutting type, leaf area, leaf number, putrescine and indole-3-Butyric acid on the rooting of Ficus cuttings (Ficus elastica Roxb. ex Hornem.)*. - Adv. Hort. Sci., 33(1): 3-11

Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

Competing Interests:
The authors declare no competing interests.

Received for publication 23 March 2018
Accepted for publication 7 August 2018

Abstract: In order to study the importance of lateral or apical buds and also the possibility of replacing the role of bud and leaf with Putrescine (Put) and auxin, three experiments were conducted using leaf-bud cuttings with intact leaf blade (full blade) or halved-blade, and terminal cuttings having 1 to 4 leaves of *Ficus elastica*. Treatments included IBA (0, 2000 mg/l) and Put (0, 1000, 2000, 4000 mg/l). Comparing the lateral cuttings with intact or halved-blade showed that the rooting of the cuttings with intact leaf blade was better. The longest root length and the best rooting index were observed in cuttings treated with 1000 mg/l Put + 2000 mg/l IBA. The highest root number resulted from the IBA treatment. In cuttings with halved-leaf blade, Put along with IBA improved the indices of rooting in comparison with the control. The rooting of leaf-bud cutting was better in comparison with apical cuttings and the application of Put with IBA increased rooting indices in both types of cuttings and there was no significant difference between the different concentrations of Put. Results showed that terminal cuttings with three and four leaves had the longest, heaviest and most abundant roots, and that the rooting index resulted in the highest value. The rooting of leaf-bud cutting with intact leaf blade was better than that of one-leaf apical cutting. In general, the experiment showed that in the one-leaf terminal cutting, the apical bud has a negative effect on rooting and the increase in the leaf area or the application of Put with Auxin improves rooting.

1. Introduction

Rooting of cuttings is a method of vegetative propagation and is one of the most important methods of clonal propagation for many plants. Stimulating the formation of adventitious roots in stem cuttings with the application of Auxin is well-known (Altman, 1972; Bolat, 1995). However, other factors do also play a role and may be restrictive under certain circumstances. Inhibitors (Hess, 1969), rooting co-factors (Hess, 1969; Heaser and Hess, 1972), Auxin antagonists (Heaser and Hess, 1972; Batten and Goodwin, 1981) and nutrients (Nanda *et al.*, 1971; Heaser and Hess, 1972) are specified to have a role in regulating the process of rooting. It has been found that cuttings without buds do not produce roots even when they are treated with Auxin. This suggests that another factor other than Auxin which is likely to be produced in the bud is essential for root formation (Hartmann *et al.*, 2011). It has also been specified that the presence of leaves on cuttings has a stimulatory effect on rooting (Reuveni and Raviv, 1980). The stimulatory effect of the leaves on rooting in stem cuttings has been well-specified in an experiment on Avocado (Reuveni and Raviv, 1980). The cuttings of hard rooting cultivars soon lost their leaves under the mist system, while the leaves remained on rooted cuttings for a long time (9 months). After five weeks, it was observed that the amount of starch found in the planting bed of easy-rooting Avocado cuttings was five times more than that of the beginning of the experiment. In Hibiscus, the maintenance of leaves on cuttings increased rooting (Van Overbeek *et al.*, 1946). Carbohydrates that are transmitted from the leaves are important for root development. But the effects of improving leaves and bud on rooting are likely to be related to other factors (Hartmann *et al*., 2011). It has been reported that carbohydrates and nitrogen affect the onset of adventitious roots. Using tomato cuttings, researchers explained the interactions between carbohydrates and nitrogen in regulating root and shoot growth and adventitious root formation (Kraus and Kraybill, 1918; Starring, 1923; Reid, 1924 a, b). Cuttings from plants grown under conditions of high carbohydrates availability (i.e., high light intensity) relative to nitrogen availability produced roots that grew vigorously (Reid, 1924 a, b; Schrader, 1924). Van Overbeek *et al*. (1946) reported that the rooting stimulation effect of leaves in Red hibiscus cuttings could be replaced with sucrose and nitrogen. In the study of the effect of lateral and apical buds on rooting of Avocados under *in vitro* conditions, it was reported that the apical buds had the highest rooting percentage, root number and root length, but lateral buds had no significant effect on these indices (Zulfiqar *et al.*, 2009).

The effect of Polyamines on the rooting of woody plants varies. Put, Spermidine (Spd), and Spermine (Spm), along with IBA, improved the rooting of hazel cuttings (Rey *et al.*, 1994). On the contrary, higher levels of endogenous Put, Spd and Spm were observed at maturity stage (difficult to root) compared to the juvenile stage (easy to root) (Ballester *et al.*, 1999). The rooting of micro cuttings of olives was increased with the use of Polyamines and NAA, but did not increase in almonds, pistachios, Chestnut, Jujuba, apricots and walnuts (Reuveni and Raviv, 1980). *In vitro* studies showed an increase in Polyamine levels, especially Put, with root formation by culturing leaf explants of the *Datura innoxia* (Chriqui *et al.*, 1986) and *Passiflora alta-caerulea* (Desai and Mehta, 1985). Investigating the role of Polyamines during adventitious root formation with the evaluation of the de-bladed petiole rooting in juvenile stage (easy-to-root) and maturity stage (difficult-to-root) in *Hedera helix* showed that Auxin stimulated the root formation at the juvenile stage (easy to root), but did not have a positive effect on the mature phase. Adding Put, Spd and Spm with or without NAA, did not affect the rooting in juvenile or mature phase. But a significant increase was observed in endogenous levels of Put and Spd in cuttings treated with Auxin (Geneve and Kester, 1991). Thus, the Polyamines may have the role of a secondary messenger for rooting (Hartmann *et al*., 2011). Ghasemi Ghehsareh and Khosh-Khui (2016) showed that Put along with Auxin improved rooting and increased the quality of roots in Ficus leaf-bud cuttings.

According to previous studies regarding the positive role of buds, leaves, glucose, nitrogen, auxins and polyamines in rooting of cuttings, this experiment was conducted to examine the importance of lateral and apical buds, as well as the possibility of replacing the role of buds and leaves with Put and auxin. For this purpose, three separate experiments were carried out using leaf-bud cuttings with intact leaf blade or leaf blade trimmed to 50%, and terminal cuttings of the rubber fig (*Ficus elastica* Roxb. ex Hornem.) having 1 to 4 leaves with IBA and Put treatments.

2. Materials and Methods

Experiment conditions

The experiments were carried out using ficus cuttings in the greenhouse of the Department of Horticultural sciences of Shiraz University. The medium containing washed sand was equipped with a bottom heat system at a temperature of about 22°C.

An intermittent mist system was employed to provide moisture, and the misting was carried out daily at 10, 13 and 16 hours, and each session lasting 1 minute. The greenhouse temperature was between 21 to 25°C during daytime and 15 to 18°C at night.

First experiment

Effect of leaf size, auxin and put. Healthy and uniform leaf-bud cuttings included leaf blade, petiole and a part of the stem with a length of 3 to 4 cm with lateral buds from the mid-section of the annual stems were taken in January. In half of the cuttings, the leaf blade was halved, and the rest remained intact. The cuttings were kept in a Benomyl fungicide solution with a concentration of 2000 ppm for five minutes and then were washed with distilled water. After removing the surface moisture, the cuttings were treated with Put [zero (distilled water), 1000, 2000, and 4000 mg/l)], Indole-3-butyric acid (IBA) (zero and 2000 mg/l, the best concentrations resulting from the previous experiment) (Ghasemi Ghehsareh and Khosh-Khui, 2016) and their combinations by dipping the stems in the solutions for 10 seconds and then planted in the bed.

Second experiment

Effect of leaf-bud or terminal cutting, Auxin and Put. In this experiment, leaf-bud and terminal cuttings with one leaf were used. Growth regulators were used and other conditions were similar to the first experiment.

Third experiment

Effect of leaf-bud or terminal cutting, leaf area and leaf number. In this experiment, the rooting of different cuttings including leaf-bud cuttings with halved blade, leaf-bud cuttings with intact blade and terminal cuttings with 1, 2, 3 and 4 leaves were compared. All cuttings were treated with IBA at a concentration of 2000 mg/l. Other conditions were similar to the first experiment.

In all three experiments, after 2 months, the cuttings were removed from the bed and after isolating the medium form the roots, root fresh weight, root length (length of the longest root), root number were measured. To determine the quality of the roots, rooting index was calculated. For this purpose the cuttings were visually grouped into five groups including cuttings with heavy, medium and weak rooting, rootless but alive cuttings and dead cuttings. For each group, a coefficient was considered as the weight of rooting. Number 5 was for heavy rooting, 4 for medium rooting and 3 for weak rooting. A coefficient value of 2 was used for rootless but alive cuttings, and a coefficient value of 1 was used for dead cuttings (Criley, 2011; Ghasemi Ghehsareh and Khosh-Khui, 2016).

Statistical design and data analysis

The first and second experiments were conducted as factorial in a completely randomized design with three replications. Each replication included 10 cuttings. The third experiment was conducted in a completely randomized design with three replications, each with 6 cuttings. Statistical analysis of the data was performed using SAS 9.13 software and the mean values were compared using LSD ($P \leq 0.05$).

3. Results

First experiment

The results of analysis of data variance showed that the treatments had a significant effect on the root length, root number, root fresh weight and root quality. Maximum root length (8.9 cm) was caused by IBA 2000 mg/l + Put 1000 mg/l treatment in full-blade cuttings, but did not show any significant difference with IBA 2000 mg/l + Put 2000 or 4000 mg/l on full-blade cuttings. Furthermore, no significant difference was observed between the mentioned results and the result of treatment with IBA 2000 mg/l + Put 1000 or 2000 mg/l on halved blade leaves. The lowest root length was observed in cuttings with halved leaf blade, and generally, root length decreased with a decrease in leaf area. In terms of the number of main roots, the highest number (13.3) was obtained by IBA 2000 mg/l on full- blade, and the lowest number of leaves was obtained with halved blade cuttings, without growth regulators. The application of Put did not significantly affect the number of main roots. The highest root fresh weight was observed by the application of Put 4000 mg/l with IBA treatment in full-blade cuttings, which did not have significant difference with other concentrations in full leaves and Put 4000 mg/l with auxin in cuttings with halved blade leaves. In fact, by increasing Put concentration, the root fresh weight increased in both types of cuttings and the amount of increase was more in cuttings with halved-blade leaves (Table 1).

In terms of root quality, the highest rooting index (4.7) was observed by treatment with Put (1000 mg/l) + IBA (2000 mg/l) in full-blade cuttings. The highest rooting index in halved-blade cuttings (3.8) was observed by Put 1000 mg/l + IBA treatment. The lowest rooting index (2.3) was observed in control treatment with halved-blade cuttings. In general, cuttings with leaf blade trimmed to 50% had roots with lower quality compared to cuttings with full leaf. With increasing the Put concentration, the quality of the roots first increased and then decreased (Table 2).

Second experiment

The results showed that the interaction of cutting types (leaf-bud or terminal cutting) with growth regulators had a significant effect on rooting characteristics. The terminal cuttings were not rooted without the application of growth regulators, but rooting improved with the application of rooting growth regulators. The longest root length (9.7 cm) was found in leaf-bud cuttings, and there was not a significant difference between different treatments in this cutting. In the terminal cuttings, the use of Put + auxin in comparison with the control treatment and auxin alone significantly increased the root length, but there were no significant differences between different concentrations of Put. The highest main root number (14.8) was observed in the leaf-bud cutting treated with Put 4000 mg/l + IBA 2000 mg/l. The application of IBA as well as increased Put concentration increased the number of roots. In the terminal cuttings, the highest number of roots (6.4) was observed by treatment with 2000 mg/l of Put along with IBA, which did not differ significantly in different concentrations (Table 3).

The highest root fresh weight was observed in leaf-bud cuttings. The highest fresh weight (2.9 g) was obtained by using auxin alone, and by Put at 2000 mg/ml + IBA, which did not differ significantly with other concentrations of Put. In the terminal cuttings, the application of Put and its increased concentration increased the fresh weight of the root.

The best root quality (4.0) was observed in the leaf-bud cuttings treated with Put (2000 mg/l) +IBA. In the terminal cuttings, the application and increase of the concentration of Put improved the quality of the roots as compared with other treatments (Table 4).

Table 1 - Effect of leaf size, Auxin and Put on root length, root number and root fresh weight of Ficus leaf bud cutting

Leaf size	Treatments					Mean
	control	aux2000	aux2000+put1000	aux2000+put2000	aux2000+put4000	
Root length						
Intact leaf blade	3.6 c *	6.5 ab	8.917 a	8.417 ab	6.917 ab	6.87 A
Halved-blade	0.833 d	5.833 bc	6.75 ab	8.333 ab	5.917 bc	5.5333 B
Mean	2.2167 C	6.1667 B	7.8333 AB	8.375 A	6.416 7 B	
Root number						
Intact leaf blade	1.333 e	13.333 a	8.167 b-d	8.5 bc	10.333 b	8.3333 A
Halved-blade	0.5 e	7.167 cd	5.833 d	6.0 d	6.667 cd	5.2333 B
Mean	0.9167 C	10.25 A	7.0 B	7.25 B	8.5 AB	
Root fresh weight						
Intact leaf blade	0.475 d	1.6533 a	1.5233 ab	1.7533 a	1.93 a	1.467 A
Halved-blade	0.4833 d	0.7033 cd	0.83 b-d	0.9067 b-d	1.2167 a-c	0.828 B
Mean	0.4792 B	1.1783 A	1.1767 A	1.33 A	1.5733 A	

* Means with similar letters (lowercase letters for whole means and capital letters for means of rows and columns) are not significant at 5% level of probability using LSD.

Table 2 - Effect of leaf size, Auxin and Put on root quality (rooting index) of Ficus leaf bud cutting

Leaf size	Treatments	High (×5)	Medium (×4)	Low (×3)	Alive (×2)	Sum of weights	Rooting index
Intact leaf blade	control	0	0	20	10	80	2.7
	aux2000	13	13	4	0	129	4.3
	aux2000+put1000	20	10	0	0	140	4.7
	aux2000+put2000	20	10	0	0	140	4.7
	aux2000+put4000	20	5	5	0	135	4.5
Halved-blade	control	0	0	10	20	70	2.3
	aux2000	0	17	13	0	107	3.6
	aux2000+put1000	0	25	5	0	115	3.8
	aux2000+put2000	5	10	15	0	110	3.7
	aux2000+put4000	5	7	18	0	107	3.6

Third experiment

By comparing the rooting of leaf-bud cuttings having either full or halved-leaf blade, and terminal cuttings having 1, 2, 3 or 4 leaves, it was observed that by increasing the leaf area, the rooting was also increased. Accordingly, the longest roots were obtained in the terminal cuttings with 4 leaves (13.1 cm), followed by terminal cuttings with 3 leaves (9.75 cm). The shortest roots (1.47 cm) were obtained in leaf-bud cuttings with halved-blade. Terminal cuttings with 4 leaves produced the highest main root number (6.4) which did not have a significant difference with two and three leaf cuttings. The highest root fresh weight (1.72 g) was observed in four leaf cuttings. The lowest root number, root fresh weight and rooting index were observed in one-leaf terminal cuttings (Table 5). In terms of rooting index, the highest rate (3.6) was observed in three and four leaf cuttings (Table 6). Generally, the experiment showed that the best rooting was obtained in the three or four leaf terminal cuttings and it was weaker in one-leaf terminal cuttings compared to other cuttings.

Table 5 - Effect of cutting type, leaf area and leaf number on root indices of *Ficus elastica*

Leaf area/cutting type	Root length	Root No.	Root fresh weight
Half leaf/leaf bud	1.47 c *	3.83 bc	0.49 cd
Full leaf/leaf bud	2.25 c	4.6 ab	0.83 bc
1 leaf/terminal	1.92 c	2.33 c	0.22 d
2 leaf/terminal	6.92 b	3.33 ab	0.68 bc
3 leaf/terminal	9.75 a	4.25 ab	0.93 b
4 leaf/terminal	13.0 a	6.42 a	1.72 a

* Means with similar letters (lowercase letters for whole means and capital letters for means of rows and columns) are not significant at 5% level of probability using LSD.

Table 3 - Effect of leaf bud or terminal cutting, Auxin and Put on root indices of Ficus cutting

Cutting type	Treatments					Mean
	control	aux2000	aux2000+put1000	aux2000+put2000	aux2000+put4000	
Root length						
Terminal	0.000 c *	0.900 c	4.200 b	4.300 b	4.170 b	2.7140 B
Leaf bud	9.718 a	8.518 a	8.532 a	9.582 a	8.750 a	9.0200 A
Mean	4.8590 BC	4.7090 C	6.3660 AB	6.9410 A	6.4600 A	
Root number						
Terminal	0.000 c	1.200 c	4.000 b	6.400 b	5.600 b	3.4400 B
Leaf bud	4.167 b	10.833 a	11.667 a	13.333 a	14.833 a	10.9666 A
Mean	2.083 C	6.017 B	7.833 AB	9.867 A	10.217 A	
Root fresh weight						
Terminal	0.0000 c	0.2300 c	0.9460 b	1.3120 b	1.4790 b	0.7934 B
Leaf bud	1.2000 b	2.9000 a	2.4550 a	2.9032 a	2.4318 a	2.3780 A
Mean	0.6000 B	1.5650 A	1.7005 A	2.1076 A	1.9554 A	

* Means with similar letters (lowercase letters for whole means and capital letters for means of rows and columns) are not significant at 5% level of probability using LSD.

Table 4 - Effect of leaf bud or terminal cutting, Auxin and Put on root quality (rooting index) of Ficus cutting

Cutting type	Treatments	High (×5)	Medium (×4)	Low (×3)	Alive (x2)	Sum of weights	Rooting index
Terminal	0	0	0	0	30	60	2.0
	aux2000	2	3	4	21	76	2.5
	aux2000+put1000	1	2	24	3	91	3.0
	aux2000+put2000	3	7	18	2	101	3.4
	aux2000+put4000	2	6	19	3	97	3.2
Leaf bud	0	0	2	24	4	88	2.9
	aux2000	1	18	11	0	110	3.7
	aux2000+put1000	2	22	6	0	116	3.9
	aux2000+put2000	6	17	7	0	119	4.0
	aux2000+put4000	5	14	11	0	114	3.8

Table 6 - Effect of cutting type, leaf area and leaf number on root quality (rooting index) of *Ficus elastica*

Leaf area/cutting type	High (×5)	Medium (×4)	Low (×3)	Alive (×2)	Sum of weights	Rooting index
Half leaf /leaf bud	0	5	8	5	54	3
Full leaf/leaf bud	2	6	5	5	59	3.44
1 leaf/terminal	0	5	4	8	48	2.89
2 leaf/terminal	0	9	6	3	60	3.33
3 leaf/terminal	2	9	5	2	65	3.61
4 leaf/terminal	3	12	1	0	66	3.67

4. Discussion and Conclusions

First experiment

Our test results showed that the application of Put along with auxin increased the main root length and root fresh weight, which is consistent with the results of other researchers (Cristofori *et al*., 2010; Birlanga *et al.*, 2015; Ghasemi Ghehsareh and Khosh-Khui, 2016). The studies of root formation in the mung bean showed that in the rooting phase stimulated with auxin, the endogenous polyamines increase (Friedman *et al.*, 1982; Jarvis *et al.*, 1983). Desai and Mehta (1985) and Chriqui *et al*. (1986) studied the rooting of leaf explants *in vitro* which showed that at the time of root formation the amount of polyamines, especially Put, increases. They showed that the activity of Ornithine Decarboxylase as a key enzyme in Put biosynthesis increased in auxin-treated explants, but the use of polyamines in an auxin-free environment did not lead to rooting. The increase in endogenous polyamines during root formation by *in-vitro* auxin is reported to stimulate the formation of callus, leaf discs and de-bladed petiole explants (Malfatti *et al.*, 1983; Desai and Mehta, 1985; Chriqui *et al*., 1986; Tiburcio *et al.*, 1989; Geneve and Kester, 1991). This increase in Put in the formation and extension of the roots shows that Put is a biochemical marker for root differentiation (Tiburcio *et al*., 1989).

Researchers have shown that Put, Spm and Spd inhibit root formation (Friedman *et al*., 1982; Jarvis *et al*., 1983; Palavan-Ünsal, 1987). Also, Schwartz *et al.* (1986) reported that the activities of Ornithine Decarboxylase (ODC) and Arginine de-carboxylase (ADC) enzymes of Put biosynthesis pathway increase during lateral root formation in *Zea mays*. The biosynthesis of the polyamines is associated with cell division in the organogenesis process. In most cases, polyamines have not been able to stimulate root formation in the absence of auxin (Birlanga *et al.*, 2015), and numerous studies have shown that the simultaneous application of auxins and polyamines increases rooting (Sankhla and Upadhyaya, 1988; Birlanga *et al*., 2015). Biondi *et al.* (1990) showed that the polyamines do not directly influence the induction of adventitious root in the *Prunus avium* micro-cuttings, but do play a role in the later stages of root development and elongation. However, Put improved the quality of roots in our experiment.

In this experiment, the decrease in leaf area caused a decrease in rooting, which is consistent with Akinyele (2010) results in *Bachholzia coriaria*. It was reported that leaf size had a very significant effect on rooting, and that the rooting of full-leaf cuttings was better than half-leaf ones.

Our results showed that the highest number of main roots was obtained by auxin treatments alone and the combination of Put along with auxin reduced the number of main roots, but increased root length and weight. On the other hand, rooting index increased in treatments containing both auxin and putrescine in full-blade cuttings. It seems that Put, along with auxin, increases root weight by increasing the number of root branches and also the root length, and in comparison with using auxin alone, it reduced the number of thick roots, thereby improving the quality of the roots. Based on our results, the simultaneous application of auxin and Put has been shown to improve rooting, and to some extent it has been able to compensate for the decrease in leaf area in cuttings with halved-blades. This was observed in a manner that no significant difference existed among the indices of root length and root weight, even when comparing cuttings with full and half blades treated with Put 2000 or 4000 mg/l. This suggests that perhaps a part of the positive role of the leaf blade in rooting is related to polyamines and their derivatives.

Second experiment

Results of comparisons between one-leaf terminal and leaf-bud cuttings showed that rooting rate in terminal cuttings was zero without using growth regulators and treatment of cuttings with Put and IBA significantly increased rooting in comparison to the use of auxin alone, thereby confirming the results of the first experiment and indicating that part of the positive role of leaf in rooting is related to Put. On the other hand, the rooting conditions of the leaf-bud cuttings is better than that of the terminal cuttings, which indicates that the apical buds have had a negative effect on rooting. The results of this experiment are consistent with previous reports by Al-Zebari and Al-Brifkany (2015), where it was observed that the rooting of Citron (*Citrus medica*) cuttings was mostly successful in cuttings obtained from the middle of the stem and the least rate of rooting was in the apical cuttings. This indicates that the apical bud is not only a strong source of auxin, compared to the lateral bud (Thimann and Skoog, 1933; Thimann *et al.*, 1934), but also a strong consumer which cannot play a positive role in rooting without the presence of a leaf. Concerning the inhibitory effect of the apical buds on the growth of lateral buds, it has been reported that the apical bud is a strong consumer of auxin and sucrose. In this regard, Thimann (1937) suggested that the optimal auxin concentration for stem elongation is more than the concentration necessary for the development of lateral buds. There is also evidence that the apical bud is a strong consumer which limits access to sugars for lateral buds (Taiz *et al.*, 2015). This strong sink effect of the apical bud can also have a negative effect on rooting.

A study on *in vitro* rooting of avocado showed that the apical buds had a better rooting than the lateral buds (Zulfiqar *et al.*, 2009). On the other hand, rooting is a highly critical and energy-demanding process that is influenced by the complex interaction between sucrose and hormonal levels (Birlanga *et al.*, 2015). Roberts and Fuchigami (1973) studied the effect of seasonal changes on auxin and rooting of the Douglas-Fir stem cuttings. They showed that at the time of removing cuttings from the beds, rooted cuttings showed a certain activity in buds, but most of the cuttings with very active buds had not produced roots. Lanphear and Meahl (1963) observed that rooting occurred more actively at or before the bud break, but it decreased sharply with increasing bud activity. The decline was probably due to the competition for growth factors necessary for rooting.

Third experiment

By comparing the leaf-bud cuttings with half and full blade, and terminal cuttings with 1, 2, 3 and 4 leaves, all of which were treated with IBA 2000 mg/l, was observed showed that increasing the leaf area, improves rooting. However, by comparing leaf-bud cuttings and one-leaf terminal cuttings, leaf-bud cuttings had a better rooting, which confirmed the results of the second experiment, indicating that the apical bud probably acted as a strong consumer and reduced rooting. The increase in the number of leaves improved rooting so that the highest rooting was observed in terminal cuttings with 3 and 4 leaves. This suggests that, although the apical bud is a strong source of auxin, it was not able to compensate for the lack of factors necessary for rooting. Here, the importance of other roles of the leaf becomes clear. The results of this experiment are consistent with reports by other researchers (Leakey *et al.*, 1982; Badji *et al.*, 1991; Tchoundjeu and Leakey, 1996; Tchoundjeu *et al.*, 2002; Atangana *et al.*, 2006; Opuni-Frimpong *et al.*, 2008). In an experiment by Leakey (Leakey, 2004), stem cuttings of the *Khaya senegalensis* showed that rooting was limited to leafy cuttings. The inability of rooting in leafless cuttings was associated with the rapid evacuation of carbohydrates in stem tissues, but their concentration in leafy cuttings increased (Leakey *et al.*, 1982). This shows that rooting depends on the formation and consumption of carbohydrates after the cuttings are separated from the mother plant (Leakey and Coutts, 1989). Van Overbeek *et al*. (1946) reported that rooting stimulation by the presence of leaves on Red hibiscus cutting could be replaced with sucrose and organic or inorganic nitrogen. Welander (1976) showed that *in vitro* rooting of Sugar Beet Hypocotyls was stimulated by an increase in sucrose and inorganic nitrogen in the presence of high concentrations of IAA, whereas low concentrations of auxin were ineffective. Gabryszewska (2011) studied *Syringa vulgaris* under *in vitro* conditions and showed that increasing the amount of sucrose in the medium causes spontaneous root formation on cultivated plantlets in the presence of low levels of nitrogen salts. The planting of Rosa 'Improved Blaze' shoots for the purpose of investigating the effect of sucrose and inorganic nitrogen on adventitious root formation showed that high concentrations of sucrose led to the production of higher and more elongated roots (Hyndman *et al.*, 1981). Therefore, the role of the leaf in helping rooting can occur by the presence of carbohydrates through photosynthesis and also by polyamines as a source of nitrogen.

In general, this study shows that the application of Put along with auxin improves rooting and root quality in *ficus* cuttings. Moreover, in terminal cuttings, due to the competition for nutritional and hormonal factors necessary for the simultaneous growth of buds and the occurrence of rooting, a certain amount of leaf area is necessary to provide these factors. The application of Put along with auxin can partly satisfy this requirement in cuttings with smaller leaf areas.

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