**Effects of indole butyric acid, putrescine and benzyladenine on rooting and lateral bud growth of *Ficus elastica*** Roxb. ex Hornem **leaf-bud cuttings**

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**Abstract**

An investigation was conducted on the effects of indole-3-butyric acid (IBA), putrescine (Put) and benzyladenine (BA) on rooting and bud sprouting of leaf-bud cuttingsof *Ficus elastica* Roxb. ex Hornem. Cuttings were dipped in IBA and Put at concentrations of 0.0 (control) 1000, 2000 and 4000 mg/l. After rooting of the cuttings, indices of rooting percentage, number, length and weight of roots was measured and rooting index was calculated (in 6 cuttings). Then, remain of rooted cuttings were foliar sprayed by BA at concentrations of 0.0, 250, 500 and 1000 mg/l. The results showed that all cuttings were rooted, but the best rooting index was observed in IBA 2000 + Put 4000 mg/l treatment. The highest number of roots per cutting (19.00) was obtained with Put 4000 mg/l + IBA 4000 mg/l and the lowest (4.17) was observed in control. Root length increased with increasing Put concentrations and decreased with increasing IBA concentration. The highest length (12.17 cm) was observed with Put 4000 mg/l and the lowest root length (7.1 cm) with Put 1000 + IBA 4000 mg/l. The highest weight of roots (3.49 g) was obtained with Put 4000 + IBA 1000 mg/l, and the lowest weight (1.2 g) was observed with control. In spite of the positive effects of IBA on rooting, higher concentrations produced thick and brittle roots, but use of Put improved the roots and led to more acceptable roots. The highest shoot length (30.5 cm) was obtained on cuttings treated with IBA 4000 + Put 4000 + BA 1000 mg/l and the lowest shoot length (14.0 cm) was observed at BA 250 mg/l treatment that was not significantly different compared to control. The highest number of leaves (6.0) was obtained with IBA 1000 + BA 4000 mg/l and the lowest (3) obtained with control and BA 250 mg/l. Overall, means showed that BA positively affected the number of leaves but IBA and Put had no significant effects on this parameter.

Key words: *Ficus elastica*, rooting, bud sprouting, polyamine

**1- Introduction**

Asexual propagation of woody plants is an important commercial method of production large quantities of genetically uniform plant materials. A lot of woody plants are commonly propagated by different stem cuttings and therefore adventitious roots must be formed successfully. Adventitious root formation arising from any part of the plant other than normal root development is difficult in some woody plants and easy in some ones. Various plants have the ability of adventitious root formation (Hartmann *et* *al*., 1997, Brickell, 2003).

The growth hormones, the substances which regulate the growth of the species, can play an important role in inducing the roots in the cuttings and influencing their growth. The plant hormones known as auxines, are the most effective in rooting in many plant species (Sadhu 1996).

Polyamines (PAs), including diamine putrescine (Put), triamine spermidine (Spd) and tetramine spermine (Spm), are organic compounds with two or more primary amino groups that exist ubiquitous in plant cells. They play important roles in regulation of DNA replication and cell division, controlling of morphogenesis and senescence, resistance to environmental stresses (Galston and Sawhney, 1990; Martin-Tanguy, 2001; Kaur-Sawhney *et al*., 2003; Couée *et al*., 2004). It is documented that PAs have profound effects on plant growth and development (Farooq *et al*., 2007).

Cytokinins are important plant hormones that regulate various processes of plant growth and development including cell division and differentiation, enhancement of leaf expansion and nutrient mobilization [Hassan, EA and F.M. El-Quesni, 1989, Sliudo, K, 1994]. The response of plants to cytokinins have been also discussed in more papers where Eraki [1994] on *Hibiscus sabdariffa* L. plants mentioned that application of BA significantly increased plant height, number of branches, fresh weight of herb/plant as well as fresh and dry weights of leaves than the control. Hassanein [1985] on *Pelarganium gravealens,* El-Sayed *et al.* [1989] on *Polianthus tuberosa,* Menesi *et al.* [1991] on *Calendula officinalis* and Mazrou *et al.* [1994] on sweet basil, found that foliar application of BA increased fresh and dry weights of different organs.

*Ficus elastica* (Rubber tree) is a broadleaf evergreen shrub or tree that widely grown in the tropics as an ornamental tree. In colder climates, this is an extremely popular houseplant. This plant usually is propagated by stem cuttings or air layering (Brickell, 2003). To increase with cuttings, stem cuttings from 5 to 27 cm in length or leaf- bud cuttings are used in the spring (Hartmann et.al. 2011). One of the problems in propagation with leaf-bud cuttings is no growth or slow growth of lateral buds to produce shoots. The purpose of this study was to investigate the effects of Put, IBA and BA on rooting and growth of lateral buds in *F. elastica* leaf-bud cuttings.

**2- Material and methods**

**2-1-Experiment I: Effects of Put and IBA on rooting**

The Experiment was conducted using *F. elastica* plants in the greenhouse of horticultural science department of Shiraz University. Healthy and uniform leaf-bud cuttings including the lamina, petiole and a segment of stem length of 3 to 4 cm and the lateral buds were taken from middle portion of one year old shoots in January. Cuttings were dipped in 2% Benomyl fungicides for 5 min and subsequently washed in distilled water before giving hormonal treatment.Cuttings were divided into 16 groups of 30 cuttings in each group. Group 1 was treated with distilled water as control. Others were treated with Indole-3-butyric acid (IBA) (1000, 2000 and 4000 mg/l), putrescine (1000, 2000 and 4000 mg/l) and their interaction. Cuttings were treated by submersing the stem portion in each treatment solution for 10 seconds. Then, cuttings were planted on a bed of washed sand equipped with heating under intermittent mist system.

After 1 months of treatment, 6 cuttings of each treatments were removed from the medium and rooting percentage, root fresh weight, root length (length of the longest root) and root number were measured. For determine the quality of the roots, rooting index was calculated using Richard A. criley method. For this purpose sort the Rooted cuttings into categories. The cuttings in these categories are each assigned weights: 5 for heavy rooting, 4 for medium rooting and 3 for light rooting. Alive, but not rooted cuttings receive a score of 2 and dead cuttings a 1 (Criley, R.A. 2008).

**2-2- Experiment п: Effect of indole butyric acid, putrescine and benzyladenine on bud growth.**

After the rooting of all cuttings, they were transplanted, as two cuttings in a plastic pot 30 cm indiameter that were filled with media containing disinfected soil, leaf mold andsand (1:1:1) by volume. After the establishment of the plants in February, different concentrations of BA (0, 250, 500 and 1000 mg/l) applied on each 6 cuttings (three pots) of previous treatment as foliar sprays. Treatments were distributed in a factorial experiment in a completely randomized design with three replicates for each treatment. After 5 months, growth indices of bud growth percentage, developed shoots (branch length), internode length and leaf number were measured.

Statistical analysis of data was performed using SAS software and the means were compared at 5% level using Duncan's range test.

**3- Results and discussion**

**3-1- Root formation**

The results showed that the all cuttings were rooted, but the best rooting index obtained from Aux 2000 + Put 4000 mg/ltreatment (table 1).

High concentrations of auxin led to thick and brittle roots, but putrescine application improves the roots quality and increased the number of secondary thin roots (figure 1). The highest number of roots per cutting (19.00) was obtained with Put 4000 mg/l + IBA 4000 mg/l thatdid not differ with other concentrations of putrescine The lowest root number (4.17 pcs) were obtained in control. Roots increased with increasing concentration of auxin and putrescine. Root length increased with increasing Putrescine concentrations and decreased with increasing auxin concentration so that the maximum length (12.17 cm) obtained with Put 4000 mg/land the lowest root length (7.1 cm) was related to Put 4000 mg/l+ IBA 4000 mg/l. Root fresh weight increased with increasing Auxin and putrescine concentrations. Maximum weight of roots (3.49 g) obtained with Put 4000 mg/l+ IBA 1000 mg/l, which was not significantly different with IBA 4000 mg/l (3.44 g). Minimum root fresh weight (1.2 g) obtained with control (Table 2).

Overally the results indicated that although auxin has the positive effects on root formation, higher concentrations produced thick and brittle roots, but use of Put improved the roots and led to more acceptable roots (Figure 1). So we can be use putrescine with auxin to improve the quality of roots.

Bilgrami *et al*. [1980].attributed the stimulation of root formation and rooting percentage on cuttings treated with IBA to the activation of rhizocaline (a growth cofactor present in the plant tissues), besides the activation of cell division from which the roots are initiated. Auxin levels are closely associated with rooting on stem cuttings **[**Hartmann *et al.* 2011**,** Taiz and Zeiger, 1998**].**

Many studies have hypothesised a role for polyamines in the rooting process, and their relationship with auxins and peroxidases. According to Gaspar *et al*. (1997) IAA and Put, an important polyamine, might be required to initiate cell division at the end of the rooting inductive phase. Polyamines induce rooting in the olive (Rugini and Wang, 1986, Rugini *et al*. 1997), possibly at the very early stages of rooting. It has also been suggested that polyamines might be considered precocious markers of rooting.

The results also showed that Put can be a useful substance in increasing *Ficus elastica* qualitity of roots. In fact, it has been demonstrated that polyamines were involved in a wide range processes including cell division, protein synthesis and DNA replication and play an important role in various morphogenetic responses (Bais and Ravishankar, 2002). polyamines playing an important role in primary, lateral, and adventitious root development (Nag *et al*. 2001, Neves *et al*. 2002; Cou`ee *et al*. 2004; Arena *et al*. 2005; Pastur *et al*. 2007, Naija *et al*. 2009).

The results were aligned to Rugini and Wang (1986), Rugini (1992), Rugini *et al*. (1997) in olive, Cristofori *et al.* (2010) in hazelnut, Hausman *et al*. (1994) in Poplar, Zikah *et al*. (2006) in the cuttings of GF-677 (hybrid of peach and almond).

Cristofori *et al*. (2010) found that, young cuttings collected from hazelnut ‘Tonda Gentile Romana’ in early September rooted poorly when treated with IBA alone, but showed the best rooting (80%) after the application of a combination of 1000 mg/l IBA and 1600 mg/l putrescine.

A positive correlation between polyamine accumulation and the initial stage of adventitious rooting was observed in some woody species like *Prunus avium* [Biondi *et al*. 1990], poplar [Hausman *et al*. 1995], pear [Baraldi *et al*. 1995] and walnut [Heloir et al. 1996], suggesting that polyamines could be used as markers of the rooting process. The accumulation of Put, resulting from the inhibition of its conversion to Spd by cyclohexylamine (CHA), an inhibitor of spermidine synthase, favoured poplar rooting even in the absence of auxin [Hausman et al. 1997]. This led the authors to suggest that the catabolism of Put to γ-aminobutyric acid (GABA) is essential for root formation by poplar shoots *In vitro* [Neves *et al*. 2002].

Friedman *et al*. (1982) suggested a possible regulatory role for PAs in combination with auxins in the early phase of adventitious root formation. Since Spd has been found to increase phenolic compounds in rose cell suspensions (Muhitch and Fletcher, 1986), it might contribute to the formation of the auxin-phenolic complex putatively responsible for rooting (Hess, 1962).

**3-2- Bud growth:**

The highest lateral bud growth percentage (82%) was obtained on cutting treated with 250mg/l BA, that was significantly different from other treatments and the lowest (74%) observed at control (Table 3). The highest shoot length (30.5 cm) was obtained on cuttings treated with IBA 4000 + Put 4000 + BA 1000 mg/l and the lowest shoot length (14 cm) was observed at BA 250 mg/l treatment that was not significantly different compared to control (Table 4).

Means showed that BA has a positive effect on the number of leaves, but showed no significant effect of IBA and Put (data not given). The highest number of leaves (6.6) was obtained with IBA 1000 + BA 4000 mg/l which had not significantly different compared to the other treatments. The lowest number of leaves (3.0) obtained with control and BA 250 mg/l (Table 5). Overall, means showed that BA positively affected the number of leaves but IBA and Put had no significant effects on this parameter. Interaction of different treatments have no significant effect on internode length (data not given) .

In general, all three growth regulators had positive effect on aerial plant growth. Put and BA had a positive effect on shoots length and BA significantly increased number of leaves.

The increase of vegetative growth due to IBA treatments is in agreement with the findings of Panwar *et al.* [1999] on *Bougainvillea peruviana,* Schoellhom [2001] on *Dichorisandra thyrsiflora* and *Cestrum elegans*, Singh [2001] on *Bougoinvilleo peruviona*, Sharma *et al*. [2002] on *Gardenia lucida*, Hussein [2003] on *Beaumontia grandiflora*. They reported that IBA at concentrations of 1000-5000 mg/l improved the plant vegetative growth. The promotive effect of IBA on the vegetative growth may be due to the enhancement of rooting percentage and root growth on the treated cuttings, which leads to more uptake of water and nutrients from the growing medium, resulting in an increase in vegetative growth [Devlin *et al*. 1986]. Also, some growth characteristics are promoted when certain growth factors called calines, present in each part of the plant, can be activated and be more effective in combination with auxins e.g., stem elongation is promoted by the combination of auxin and caulocaline. In this way auxin behaves like a coenzyme for the growth factor caline [Bilgrami *et al*. 1980].

Cytokinins are involved in lateral shoot formation (Hartmann *et al*. 2011, Li and Bangerth, 2003). They act as regulators releasing from apical dominance. Although auxins are required for axillary meristem initiation (McSteen, 2009). Shoot development requires high levels of cytokinins (Doerner, 2007).

Outgrowth of axillary buds is well correlated with the cytokinin level in the buds. It is thought that auxin acts to control the concentration of cytokinin derived from the roots (Bangerth, 1994).Cytokinins increase biosynthesis of nucleic acids and mitotic activity in apices of buds. (Sharpe and Schaeffer 1970). These results may be due to accumulation of greater photosynthates leading to better growth parameters [Manjul *et al*. 2002.].

This increment in plant height may be due to the role of cytokinin (BA) in increasing cell division in apical meristems and cambium [Krishmmoorthy, 1981.]. Our results are comparable with those obtained by Mazrou *et al*. (1988) on sage plant, El-Keltawi and Croteau (1987) on sage and mint plants and Eraki (1994) on *Hibiscus sabdartffa* L.

Significant effects of Put on shoot growth in our study are in agreement with the findings of Rugini *et al.* (1993). They showed that Put increase the number of stem and leaf blade expansion in the pear. Also according to the application time of Auxin and Put (on cuttings), their effects on shoot growth is more related to the rooting of cuttings so that cuttings with stronger root system have better absorbance of water and nutrients and cytokinin production, that increasing shoot growth.

Table 1: Rooting Index of cuttings treated with IBA and Put.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| IBA concentration  (mg/l) | Put concentration  (mg/l) | high (×5) | medium (×4) | low (×3) | Sum of weights | rooting index |
| 0.0 | 0.0 |  |  | 6 | 18 | 3.0 |
| 1000 | 0.0 | 4 | 2 |  | 28 | 4.7 |
| 2000 | 0.0 | 2 | 2 | 2 | 24 | 4.0 |
| 4000 | 0.0 |  | 5 | 1 | 23 | 3.8 |
| 0.0 | 1000 |  | 3 | 3 | 21 | 3.5 |
| 1000 | 1000 | 2 | 3 | 1 | 24 | 4.0 |
| 2000 | 1000 | 3 | 3 |  | 27 | 4.5 |
| 4000 | 1000 | 4 | 1 | 1 | 27 | 4.5 |
| 0.0 | 2000 | 1 | 4 | 1 | 24 | 4.0 |
| 1000 | 2000 | 3 | 2 | 1 | 26 | 4.3 |
| 2000 | 2000 | 4 | 2 |  | 28 | 4.7 |
| 4000 | 2000 | 4 | 2 |  | 28 | 4.7 |
| 0.0 | 4000 | 1 | 4 | 1 | 24 | 4.0 |
| 1000 | 4000 | 4 | 2 |  | 28 | 4.7 |
| 2000 | 4000 | 5 | 1 |  | 29 | 4.8 |
| 4000 | 4000 | 1 | 5 |  | 25 | 4.2 |



Aux 4000 mg/l

Aux 4000 + Put 2000 mg/l

Figure 1: Effect of putrescine on improving the quality of the roots.

Table 2: Interaction between IBA and putrescine on the, length and fresh weight of roots.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | number Root | | | | |  |
| Put  (mg/l) | IBA (mg/l) | | | | | MEANS |
| control | | 1000 | 2000 | 4000 |
| control | 4.167h\* | | 9.833d-f | 10.833d-f | 17.667ab | 10.6250B |
| 1000 | 6.000gh | | 10.833d-f | 11.667c-e | 17.167ab | 11.4167B |
| 2000 | 8.000fg | | 10.333d-f | 13.333cd | 17.167ab | 12.2083B |
| 4000 | 9.500ef | | 13.000c-e | 14.833bc | 19.000a | 14.0833A |
| MEANS | 6.9167D | | 11.0000C | 12.6667B | 17.7500A |  |
| Root length (cm) | | | | | | |
| control | 9.717 ac | | 8.200bc | 8.517bc | 8.583bc | 8.7542AB |
| 1000 | 10.417ab | | 8.167bc | 8.533bc | 7.117c | 8.5583B |
| 2000 | 10.583ab | | 8.850bc | 9.583a-c | 10.583ab | 9.9000A |
| 4000 | 12.167a | | 10.417ab | 8.750bc | 7.500c | 9.7083AB |
| MEANS | 10.7208A | | 8.9083B | 8.8458B | 8.4458B |  |
| root fresh weight (g) | | | | | | |
| Put  (mg/l) | IBA (mg/l) | | | | | MEANS |
| control | 1000 | | 2000 | 4000 |
| control | 1.2000e | 3.1433a-c | | 2.9000a-d | 3.4400ab | 2.6708 AB |
| 1000 | 2.0567d | 2.3550cd | | 2.9033a-d | 2.5483b-d | 2.3538 B |
| 2000 | 2.3350cd | 2.6683a-d | | 2.4550cd | 3.2333a-c | 2.7850 A |
| 4000 | 2.9450a-d | 3.4883a | | 2.4317cd | 3.1217a-c | 2.9967 A |
| MEANS | 2.1342C | 2.9138AB | | 2.6725B | 3.0858A |  |

\* means in each row or column with the same letter are not significantly different at 5% level by Duncan test.

Table 3: Effect of BA on Bud growth percentage.

|  |  |
| --- | --- |
| BA concentration (mg/l) | Bud growth percentage |
| control | 0.74 d\* |
| 250 | 0.82 a |
| 500 | 0.76 c |
| 1000 | 0.79 b |

\* Means with the same letter are not significantly different at 5% level by Duncan test.

Table 4: Effect of indole butyric acid, Put and benzyladenine on shoot length.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| IBA concentration (mg/l) | Put concentration (mg/l) | BA | | | | IBA MEANS | Put  MEANS |
| Control | 250 | 500 | 1000 |
| Control | Control | 16.5 eg\* | 14.0 g | 18.0 d-g | 21.3 a-f | 24.66 A | 21.07 C |
| 1000 | 24.8 a-e | 23.3 a-g | 24.1 a-f | 24.7 a-e | 24.43 B |
| 2000 | 26.3 a-e | 25.2 a-e | 25.2 a-e | 28.2 a-c | 25.89 B |
| 4000 | 27.8 a-d | 27.9 a-d | 28.2 a-c | 29.1 a | 28.14 A |
| 1000 | Control | 14.5 fg | 16.5 e-g | 18.3 b-g | 24.9 a-e | 24.59 A |  |
| 1000 | 24.5 a-e | 22.3 a-g | 24.0 a-f | 29.2 a |
| 2000 | 27.2 a-d | 27.0 a-d | 25.3 a-e | 26.8 a-d |
| 4000 | 26.8 a-d | 27.8 a-d | 28.0 a-d | 27.8 a-d |
| 2000 | Control | 18.2 c-f | 21.8 a-g | 23.2 a-g | 26.6 a-d |
| 1000 | 23.5 a-g | 24.5 a-e | 25.6 a-e | 25.1 a-e | 25.25 A |  |
| 2000 | 23.0 a-g | 22.5 a-g | 23.3 a-g | 29.1 a |
| 4000 | 27.2 a-d | 28.8 a | 29.3 a | 29.0 a |
| 4000 | Control | 23.2 a-f | 25.6 a-e | 26.3 a-e | 27.4 a-d |
| 1000 | 23.0 a-g | 23.3 a-g | 23.8 a-f | 23.8 a-f | 26.19 A |  |
| 2000 | 23.9 a-f | 25.2 a-e | 28.4 ab | 26.8 a-d |
| 4000 | 27.8 a-d | 26.2 a-e | 28.2 a-c | 30.5 a |
| BA MEANS |  | 21.01 B | 24.35 B | 25.19 B | 27.19 A |

\* Means in each row or column with the same letter are not significantly different at 5% level by Duncan test.

Table 5: Effect IBA, Put and BA on leaf number.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| IBA | Put | BA | | | | IBA MEANS | Put  MEANS |
| control | 250 | 500 | 1000 |
| control | control | 3.0 d\* | 3.0 d | 4.0 b-d | 5.3 a-d | 4.8 A | 4.86 A |
| 1000 | 4.5 a-d | 4.5 a-d | 4.8 a-d | 5.0 a-d | 4.71 A |
| 2000 | 4.4 a-d | 5.0 a-d | 5.0 a-d | 5.8 ab | 5.06 A |
| 4000 | 5.0 a-d | 5.4 a-d | 5.2 a-d | 5.5 a-c | 5.18 A |
| 1000 | control | 3.3 cd | 5.0 a-d | 4.8 a-d | 6.6 a | 5.05 A |  |
| 1000 | 4.5 a-d | 4.5 a-d | 4.8 a-d | 4.8 a-d |
| 2000 | 5.0 a-d | 5.2 a-d | 5.0 a-d | 5.8 ab |
| 4000 | 5.2 a-d | 5.3 a-d | 5.2 a-d | 6.0 ab |
| 2000 | control | 4.2 a-d | 5.8 ab | 6.0 ab | 6.3 ab | 5.09 A |  |
| 1000 | 4.5 a-d | 4.5 a-d | 5.0 a-d | 5.3 a-d |
| 2000 | 4.3 a-d | 4.5 a-d | 4.4 a-d | 5.3 a-d |
| 4000 | 5.0 a-d | 5.8 ab | 5.0 a-d | 5.0 a-d |
| 4000 | control | 4.3 a-d | 4.4 a-d | 4.5 a-d | 4.8 a-d | 4.91 A |  |
| 1000 | 4.0 b-d | 5.0 a-d | 5.0 a-d | 5.0 a-d |
| 2000 | 4.5 a-d | 5.5 a-c | 5.4 a-d | 6.0 ab |
| 4000 | 4.3 a-d | 4.8 a-d | 5.2 a-d | 5.2 a-d |
| BA MEANS | | 4.39 C | 4.97 B | 4.98 B | 5.47 A |  | |

\* Means in each row or column with the same letter are not significantly different at 5% level by Duncan test.

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