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Enhancement of IBA, Urea-Phosphate, Paclobutrazol and Their Combinations on Rooting of Royal Poinciana (*Delonix regia*) Stem Cuttings

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Abstract: Royal Poinciana is an important tropical tree species with many uses. To improve the rooting responce of royal Poinciana stem cuttings, urea-phosphate (UP) and paclobutrazol (PBZ) were tested alone or in combination with IBA. UP alone did not stimulate rooting of royal poinciana cuttings; however, when applied together with IBA it significantly enhanced the rooting of cuttings. PBZ alone had a weak effect on rooting of cuttings but in combination with IBA it improved the rooting of cuttings. A triple combination of IBA, UP and PBZ provided the most effective treatment for the improvement of rooting percentage. IBA treatments increased the number of roots per cutting in comparison with the control, but decreased the length of the roots of cuttings. IBA plus UP or PBZ further increased the number of newly formed roots. IBA plus UP increased the average root length. The three compound treatments did not differ from IBA plus PBZ regarding root number per cutting, but the roots were longer than in IBA treated and control cuttings. The survival of rooted cuttings treated with IBA was relatively low. IBA plus UP plus PBZ improved the survival of the rooted Royal Poinciana plants compared with IBA alone. Thus UP and PBZ were shown to enhance the effect of IBA in stimulation of rooting and survival of Royal Poinciana cuttings.

Key words: Cuttings · Royal Poinciana · Rooting · Paclobutrazol · Urea-phosphate

INTRODUCTION

Delonix regia (Boger.) Raf. (Royal poinciana, flamboyant) is an ornamental flowering tree related to the mimosa tree family (*Leguminosae*, subfamily *Caesalpinioideae*), native and endemic to Madagascar [1]. Royal Poinciana can reach up to 40 feet tall, usually with a canopy wider than its height [2]. This species is considered one of the five most beautiful flowering trees in the world, blooming between spring and early summer. The flower is very showy and its color varies from orange to orange-red, including yellow and a rare white [3]. In Iran, specifically in Southern area, the ornamental and landscape characteristics of Poinciana add commercial value for nursery production. Poinciana trees grow best from seed and flowering can be achieved within five to seven years [3]. However, propagation of Poinciana by seeds has several limitations and rooting of cuttings is not an efficient alternative. The seeds hard coating imply in reduced germination percentage [4], the seed position in the pod influences germination and quality of seed product [5] and unfavorable changes in climate during flowering and pollination periods result in low seed production [6]. Studies on the physiology of auxin action showed that auxin was involved in such varied plant activities as stem growth, adventitious root formation [8] and lateral bud inhibition, abscission of leaves and fruits and activation of cambial cells. Indole-3-acetic acid (IAA) was identified as a naturally occurring compound having considerable auxin activity [7]. IAA was subsequently tested for its activity in promoting roots on stem segments and in 1935, investigators demonstrated the practical use of this material in stimulating root formation on cuttings [9]. About the same time it was shown [10] that two synthetic materials, indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA), were even more effective than the naturally occurring or synthetic IAA for rooting. Today, IBA and NAA are still the most widely used auxins for rooting stem cuttings and for rooting tissue-culture-produced micro cuttings. It has been repeatedly confirmed that auxin is required for initiation of adventitious roots on stems and indeed, it has been

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shown that divisions of the first root initial cells are dependent upon either applied or endogenous auxins [1112]. IBA has been found to occur naturally. The formation of root primordium cells depends on the endogenous auxins in the cutting and on a synergic compound such as a diphenol. These substances lead to the synthesis of ribonucleic acid (RNA), which act upon root primordium initiation [13]. The application of some plant growth retardants, together with auxin, has been used to improve the rooting capacity of cuttings in some species [14, 15]. Triazoles have been reported to affect some physiological processes during rooting [14, 16]. Paclobutrazol (triazol), a gibberellin synthesis inhibitor [17], keeps the gibberellin levels in the cuttings at low values; gibberellins are considered substances that inhibit rooting [13]. Paclobutrazol (PBZ), a well-studied chemical that is a member of the triazole family, has been reported as an antigibberellin agent [17]. I has been hypothesized that PBZ is involved in maintaining low levels of the rooting inhibitor gibberellin, in the cuttings [18,19] and in increasing the sink capacity at the base of the cuttings for carbohydrate and/or hormones [19]. It has also been suggested that PBZ reduces water loss [20] and interacts with auxin [15, 21]. Urea-phosphate (UP) is a common fertilizer that can supply nitrogen and phosphate to plants. However, in addition, UP has also been shown to increase the uptake and activity of plant hormones such as gibberellin [22] and auxin [23]. Recently, UP has been found to increase the effect of PBZ in reducing fruit abscission (unpublished data) and has been reported to improve the effect of IBA on rooting of softwood peach cuttings [23]. The mechanism by which UP works is not yet known, but its effect of reducing cell pH also seems to increase the uptake of growth regulators and/or enhance their activity. In the present study, we tried to increase the rooting ability and quality of Royal Poinciana cuttings under the action of different concentrations of plant growth regulator (IBA) and PBZ and UP as cofactors to IBA. Both the rooting ability of the cuttings and the survival of the rooted plants in the nursery were tested.

MATERIALS AND METHODS

Two experiments were conducted in the horticultural science department greenhouse of Persian Gulf University. In first experiment, Hard wood cutting were selected from a tree in university landscapes and treated with different concentration of IBA and NAA (0, 500, 1000, 1500, 2000. 3000, 4000 and 6000 mg l^{-1}). In second experiment, after selection best plant growth regulators and best concentration the influence of UP, PBZ and their interactions with IBA to improve rooting response, root quality and survival of rooted cutting were tested.

Rooting Experiments: The cuttings were treated routinely with different concentration of IBA and NAA talc powder and placed on a rooting box on a mixture of peat, cocopeat and sand (1.1.1 vv) maintained at 25°C. For the UP experiment cuttings were dipped in water for 30 mm and there after dipped in increasing concentrations of UP ($NH_2CONH_2PO_4$. $2H_3PO_4$), (0, 1, 2 and 5 g 1^- UP powder was applied alone or mixed with 3000 IBA (w/w). For the PBZ ((®-[(4-chlorphenyl)methyl]〈(1,1 dimethylethyl)-1H-1,2,4-triazole-1-ethanol) in the Bonzi™ formulation (Uniroyal Chemical Co., Middlebury, CT) containing 0.4% active ingredient) experiment, cuttings were dipped for 30 min in aqueous solutions containing 0, 0.1, 1 and 5 g1^{-1} PBZ. PBZ was applied alone or followed by dipping the cuttings in 3000 IBA in talc powder. The effect of the triple treatment with IBA+UP+PBZ was examined. The cuttings were treated with IBA, UP, PBZ, IBA + UP or IBA +PBZ as described above and untreated cuttings served as control. For the triple treatment cuttings were first dipped for 30 min in 5 g 1^{-1}aqueous solution of PBZ and then in a mixture of 3000 IBA talc powder plus 5 g 1^- UP powder (w/w). The following was recorded: Rooting percentage, Root length and number of root per cuttings in selected time were measured. This experiment was conducted as a complete randomized design in factorial arrangement and complete randomized design with four replicate and each replicate contain 10 cuttings. All data were analyzed with MSTAT (Michigan State Univ., 1988) to detect differences among treatment effects. Means were compared with Duncan New Multiple Range Test (DNMRT) test at a 95% probability level.

RESULTS

IBA and NAA Effects on Rooting of Royal Poinciana Cuttings: A significant difference in NAA concentrations was observed for cuttings. The rooting percentage in the NAA 2000 mg L^{-1} treatment (54.2%) was higher than in other treatments (Table 1), therefore, indicating that this plant growth regulator was effective in promoting rooting in cuttings of this species. NAA at 4000 and 6000 mg L^{-1} showed lower rooting percentages in cuttings. The increase of NAA concentration was accompanied by the rooting percentage decrease, suggesting that high NAA concentrations were

Table 1: Affects of various concentrations of IBA and NAA on Rooting percentage, Number of root/cutting and Mean root length of Royal Poinciana cuttings after 10 weeks

Type of plant growth regulators	Concentration (mgL^{-1})	Rooting percentage	Number of root/cutting	Mean root length(mm)
IBA	0	9f	3.2e	88.2a
	500	24.4e	4.32e	71.21b
	1000	33.5d	8.21d	63.2c
	1500	44.2d	16.41b	52.2d
	2000	64.24b	20.31a	51.55d
	3000	71.1a	23.11a	43.11e
	4000	68.62a	23.1a	34.1f
	6000	66.25a	23.08a	21.2h
NAA	0	9f	3.8e	88a
	500	26.42e	6.81e	82.1a
	1000	42.4d	10.38d	63.2c
	1500	51.44c	13.71c	58.2cd
	2000	54.2c	22.1a	55.55cd
	3000	38.1d	17.32b	39.11e
	4000	25e	13.2c	29.1g
	6000	11f	3.21e	15.11k

Means followed by the same letter in a column are not significantly different ($p<0.05$) by New Duncan Multiple Range Test (DNMRT)

Table 2: The effect of PBZ and UPon enhancement of IBA on rooting percentage, Number of root/cutting, Root length (mm) and survival percentage of Royal poinciana cuttings. The concentrations of the compound were: IBA (3000 mgl^{-1}), UP 5 gl^{-1} and PBZ 5 gl^{-1}

Treatments	Rooting percentage	Number of root/cutting	Root length (mm)	Survival Percentage
Control	9d	3.3c	85.11a	84b
IBA	70.1c	22.18b	43.11d	75c
IBA+UP	89b	23.89b	65.3b	87ab
IBA+PBZ	93b	26.01a	39.44d	89ab
IBA+PBZ+UP	100a	26.11a	55.2c	94a

Means followed by the same letter in a column are not significantly different ($p<0.05$) by New Duncan Multiple Range Test (DNMRT)

phototoxic for the root formation process. Thus, it could be suggested that the concentration that promoted the highest rooting percentage was 2000 mg L^{-1}. Through statistical analysis, it was observed that a significant difference occurred between treatments containing IBA and the control, but no difference was observed between the 3 different high IBA concentrations used (3000, 4000 and 6000 mg L^{-1}). A value of 71.1% rooted cuttings was observed at the concentration of 3000 mg L^{-1} IBA (Table 1). The percentage results of rooted cuttings treated with NAA or IBA showed that cuttings treated with IBA presented, significantly, a higher root formation response in comparison with NAA, suggesting that this auxin was more effective in initiating roots at these concentrations. IBA may also enhance rooting via increased internal-free IBA, or may synergistically modify the action of IAA or the endogenous synthesis of IAA; IBA can enhance tissue sensitivity for IAA and increase rooting [24]. Evaluation the effect of NAA and IBA on number of root per cutting and root length indicated that by application of NAA and IBA root number/cutting were increased significantly. The root length of cuttings was decreased with increasing both plant growth regulators (Table 1). According to Hartmann *et al.* [13], IBA is the best auxin for general use because it is nontoxic to plants over a wide concentration range than NAA and is effective in promoting rooting of a large number of plant species. According to the results in first experiment, IBA (3000 mg L^{-1}) were selected for evaluation the effect of UP and PBZ of rooting response of cuttings.

Interaction of Up and PBZ with IBA: After preliminary experiments between different concentration of UP and PBZ that used in this study 5 mgL^{-1} of UP and PBZ were selected to study interaction of Up and PBZ with IBA. Application of UP and PBZ alone did not increase rooting response (Data not shown). IBA plus UP increased the rooting response than IBA alone. Also, PBZ plus IBA markedly increased rooting response. PBZ+UP+IBA treatment was most effective in promoting rooting response. Paclobutrazol (PBZ) only slightly stimulated the rooting of Royal Poinciana cuttings but, interestingly, the number of adventitious roots formed was dramatically increased when PBZ was used together with indole-3-butyric acid (IBA). Application of PBZ in the first phase of root formation caused the greatest enhancement of the promotive effect of IBA on rooting. In UP treated cuttings the average numbers of root/cuttings were increased, while IBA+ PBZ decreased root length. In cuttings treated with PBZ+IBA+UP the highest number of root per cuttings were observed. All tree treatments (PBZ+IBA+UP, IBA+PBZ, IBA+UP) increased the survival percentage in comparison with IBA alone. Also, the highest survival percentage was observed in combined treatment (Table 2).

Evaluation the effect of PBZ and UP and their combination on survival rate indicated that PBZ+IBA, PBZ+UP+IBA and UP+IBA increased significantly the survival percentage compared to IBA alone. Also, the survival percentage of control treatment was higher than IBA alone (Table 2).

DISCUSSION

Auxin is well known to stimulate the rooting of cuttings [13]. The most widely used auxin for commercial rooting is IBA [25]. Royal Poinciana propagation by cuttings was reported in some study. However, it has been found in various studies rooting percentage and rooting time was poor. Of the two auxins tried for root regeneration IBA was more responsive than NAA. It could be due to sustainable structure of IBA against IAA oxidase.

Successful rooting of cuttings is determined both by the number of roots formed and by root elongation and growth [13]. In royal poinciana cuttings treated with IBA, there is an enhancement of root number and reduction of root length in comparison with untreated cuttings. As a result, the survival of cuttings treated with IBA is much often lower than that of untreated cuttings [16]. Based on these observations, we aimed in the present study to look for ways to increase the rooting of Royal Poinciana cuttings that respond weakly or moderately to IBA and to try to improve the root system development and survival of rooted cuttings. It is well documented that a delicate balance between endogenous stimulatory and inhibitory factors controls the rooting of cuttings [26]. It is generally accepted that while auxin stimulates rooting, cytokinins and gibberellin inhibit it [14, 13]. Nutritional factors have also been reported to promote the rooting of cuttings [27. In the present study, we tested two such chemicals: PBZ, which has been reported to inhibit gibberellin biosynthesis [17] and UP, a common fertilizer that has been reported to enhance hormone uptake and activity [22]. These two compounds have recently been shown to interact with IBA and improve rooting and survival of mung bean and peach cuttings [15, 21 23].

Both UP and PBZ showed a very weak or no stimulatory effect on rooting of cuttings (Table 2). These findings are in agreement with previous reports on these two compounds [19, 2314]. However, when they were applied together with IBA, rooting ability was increased, with PBZ appearing to be more effective than UP for enhancement of IBA induction of root formation. The strong effect of PBZ applied together with IBA on root formation was studied systematically in a model plant system using mung bean cuttings. It was suggested that PBZ may counter the inhibitory effect of gibberellin on root primordia formation [15, 19]. PBZ was found to affect the metabolism of IBA [21] and increase sink capacity of the base of the cuttings [18]. free auxins, provided by conjugates of auxin, is required during all the stages of the rooting process [28] and since it takes about 12 weeks for Royal Poinciana cutting to root, we suggest that both the direct and indirect effect of PBZ on IBA metabolism and indirect effects on the partitioning of assimilates water status [20] may enhance the stimulatory effect of IBA on the rooting of Royal Poinciana cuttings. In agreement with various reports about the effect of fertilizers on rooting of cutting [13, 27] we demonstrated that UP plus IBA promoted root elongation and thus overcome the inhibitory effect of IBA alone. PBZ+UP+IBA treatment showed additive effect on rooting response. Our result demonstrated that the triple treatment had simulation effect on rooting of Royal Poinciana cuttings and also had beneficial effect on survival rate of rooted cuttings compared with IBA alone treatment.

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