Effects of Indole butyric acid (IBA), Wounding, Cutting Position and Rooting Medium on Rooting of Giant Crape Myrtle (Lagerstroemia flos-reginae Retz) Stem Cuttings

تأثير IBA والتجريح وموضع العقلة و وسط التجذير على تجذير العقل الساقية لنبات لاجسترومیا فلوسرجني

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Abstract:

The objective of this work was to study the effect of Indole butyric acid (IBA) concentration, wounding, cutting position and rooting medium on rooting of *lagerstroemia flos-reginae* stem cuttings. The research was conducted in the form of four experiments. In the four experiments treatments were arranged in a completely randomized design with four replications. Sixteen cuttings represented an experimental unit. Statistical analysis was carried out using Genstat Statistical package (Genstat 2010). Mean separation was done using Duncan's multiple range tests at 5% level of significance. Parameters measured were rooting percentage, number of roots/cutting and root length. Rooting percentage, number of roots per cutting and root length were highest with IBA at higher concentrations (2000 and 3000 ppm). Wounding plus IBA solution scored the highest rooting percentage, number of roots and root length. Basal cuttings recorded the highest rooting percentage, number of roots and root length. Rooting percentage was 100% in the media coarse sand, fine sand, fine sand+silt and fine sand+coarse sand. The highest number of roots and root length were recorded by coarse sand. The study recommends the use of wounded basal cuttings treated with IBA solution at 1000 ppm and inserted in coarse sand for propagation of *Lagerstroemia flos-reginae.*

Key words: *Lagerstroemia flos-reginae*, Stem cuttings, Indole butyric acid (IBA), Wounding, Cutting position, Rooting medium.

المستخلص:

كان الهدف من هذا العمل دراسة تاثير تركيز اندول حمض البيوتريك (IBA) والتجريح وموضع العقلة و وسط التجذير على تجذير العقل الساقية لنبات لاجستروميا فلوسرجني. أجري البحث في شكل اربع تجارب. تم توزيع المعاملات في تصميم كامل العشوائية بأربعة مكررات في الأربع تجارب. مثلت ستة عشر عقلة الوحدة التجريبية. أجري التحليل الاحصائي باستخدام برنامج الجينستات(٢٠١٠). تم فصل المتوسطات بإستخدام إختبار دنكن عند مستوى المعنوية ٥%. القياسات التي اخذت كانت نسبة التجذير وعدد الجذور بالعقلة وطول الجذر. كان أعلى نسبة تجذير و عدد الجذور بالعقلة و طول الجذر مع اندول حمض البيوتريك (IBA) عند تراكيزه العالية (٢٠٠٠ و ٣٠٠٠ جزء بالمليون). التجريح + محلول اندول حمض البيوتريك أحرز أعلي نسبة تجذير وأكبر عدد للجذور و أطول جذر. العقل القاعدية سجلت أعلي نسبة تجذير وأكبر عدد للجذور

و أطول جذر. نسبة التجذير كانت ١٠٠ % في الأوساط رمل خشن، رمل ناعم، رمل ناعم + سلت (١:١) و رمل خشن + رمل ناعم (١:١). أعلى عدد للجذور و أعلى طول للجذر سجلا بواسطة الرمل الخشن. توصي الدراسة باستخدام العقل القاعدية المجرحة و معاملة باندول حمض البيوتريك بتركيز ١٠٠٠ جزء بالمليون مغروسة في الرمل الخشن في اكثار نبات لاجسترروميا فلوسرجني.

كلمات مفتاحية: لاجسترروميا فلوسرجني، العقل الساقية، اندول حمض البيوتريك (IBA)، التجريح، موضع العقلة، وسط التجذير.

Introduction

Giant Crape Myrtle (*Lagerstroemia flos-reginae* Retz / *Lagerstroemia speciosa* (L) Pers / *Lagerstroemia reginae* Roxb.) is a tropical plant found in many parts of Southeast Asia including the Philippines, Vietnam, Malaysia and southern China. It is a tree that can grow as tall as 20 m. It is an important medicinal and ornamental tree (Vijayan *et al* 2015). In landscaping it is used for different purposes such as a screen and lawn specimen. It makes a useful timber tree. The leaves of this tree, have been traditionally consumed in various forms by Philippinos for treatment of diabetes and kidney related diseases. In the 1990s, the popularity of this herbal medicine began to attract the attention of scientists worldwide. Since then, researchers have conducted numerous *in vitro* and *in vivo* studies that consistently confirmed its antidiabetic activity. Scientists have identified different components to be responsible for its activity (Klein *et al* 2007). The discovery of auxins as plant growth regulating chemicals in the 1930s and their ability to stimulate adventitious rooting in stem cuttings marked a major milestone in the modern history of plant propagation (Blythe, *et al* 2007). Application of indolebutyric acid (IBA) is generally considered to promote adventitious root formation in cuttings, the response to IBA may vary from species to species. Auxin treatments are commonly used in commercial plant propagation to increase overall rooting percentages, hasten root initiation, increase the number and quality of roots, and encourage uniformity of rooting (Hartmann *et al*., 2002). Ghosh *et al*, (1988) studied the effect of NAA and IBA on adventitious root formation in stem cuttings of pomegranate (*Punica granatum* L.) under intermittent mist. They found that, IBA was more effective than NAA in inducing rooting of hard wood, semi-hard wood and soft-wood cuttings. IBA at 5000 ppm resulted in maximum rooting success (83.33%) but at higher concentration (10,000 ppm) a greater number of roots and a higher root length were recorded. Sabbah *et al* (1991) treated stem cuttings of Citrus, related genera and intergeneric somatic hybrids with 1000 and 3000 ppm of both IBA and NAA and rootone powder (containing 2000 ppm NAA and 1000 ppm IBA). Cuttings of the different Citrus clones responded significantly in root production to IBA and NAA treatments. The lowest concentration of NAA (1000 ppm) and the highest concentration of IBA (3000 ppm) yielded the maximum rooting percentage (75%) across all selections. Cuttings treated with NAA and IBA, both at 1000 and 3000 ppm, produced greater number of roots that were longer and thicker than those of the control. In a work conducted by Razvi *et al* (2018), juvenile cuttings of *Lagerstroemia speciosa* were treated with IBA 2000 (0.2% IBA) and 4000 ppm (0.4% IBA) in the form of powder and one group of cuttings were treated only with telicam powder (control). Highly significant variation in rooting percentage was observed among treatments.The maximum (93.33%) rooting was discernible in untreated cuttings (treated with telicam powder only) followed by cuttings treated with IBA 2000 ppm (80.7) while minimum (74.7%) rooting was observed in cuttings treated with IBA 4000 ppm. The mean number of roots among the different treatments showed significant differences. Maximum (4.87) number of roots was noticed in the cuttings treated with IBA 4000 ppm followed by (4.17) in the cuttings treated with IBA 2000 ppm, while minimum (3.50) number of roots was observed in untreated cuttings. There was no significant difference in root length among treatments. Maximum (16.6 cm) root length was noticed in cuttings treated with IBA 4000 ppm followed by (14.4 cm) in those treated with IBA 2000 ppm and minimum (14.3 cm) root length was recorded in untreated cuttings. In a study carried out by Ingle and Venugopal (2018), stevia (*Stevia rebaudiana* Bertoni) cuttings were treated with IBA at zero (control), 50, 100, 200, 300, 400, 500 ppm. The rooting percentage was reported highest (92%) with the treatment IBA 500 ppm followed by IBA 400 ppm (90%). Number of roots per cutting increased with the increasing concentration of IBA. The number of roots was highest (49.96) in the cuttings treated with IBA 500 ppm followed by IBA 400 ppm (38.20). Root length differed significantly among different growth regulator treatments and it was found to be superior over control. The length of root was highest (14.05 cm) in the cutting treated with IBA 500 ppm followed by IBA 400 ppm (11.55 cm). Best rooting is usually obtained from cuttings taken from the basal portion of the shoots (Hartmann and Kester, 1983). This may be attributed higher accumulation of carbohydrates and concentration of endogenous root-promoting substance that are produced in buds and leaves. According to Thompson (1986), tip cuttings of *Epacris impressa* derived from 12 cm of the stem had less rooting percentage than the cuttings derived from 12-24 cm below the apex. This was attributed to high carbohydrates level or to higher endogenous level of anxins in the basal cuttings. Mahgoub (1990) and Hansen (1986) studied the effect of position of cutting on the branch of some ornamental plants on rooting the cuttings. Basal cuttings taken from Russelia, bougainvillea and schefflera resulted in higher number of roots and root length than median or terminal cuttings. They attributed that to high carbohydrate and endogenous auxins levels and the presence of preformed root initials in the basal parts. Rooting substrates serve a number of purposes, including anchoring the cutting in place, holding water for the cutting, supplying sufficient aeration for adventitious rooting, and reducing the amount of irradiance that reaches the base of the cutting (Hartmann et al., 2011). The uptake of water in cuttings is proportional to the content of water, by volume, in the rooting substrate (Grange and Loach, 1983). Water in excess, however, prevents proper aeration (Erstad and Gislerod, 1994). Geczi (1974) used perlite, hot bed soil and sand as rooting media in grapes. He found that perlite was the best rooting media followed by sand. Vander Vossen and Op. DE. Laak (1976) reported that the best rooting media for rooting soft-wood cuttings of coffee was a combination of equal parts of loamy soil, coarse sand acid swamp soil. Not all plants root easily and some plants such as *Ilex*, *Camellia* and *Rhododendron*, require wounding to encourage root production. It is thought that wounding creates a root promoting stimulus, perhaps of a chemical nature (Anonymous 2021). Wounding can influence rooting success of woody cuttings through either physiological or mechanical mechanisms or both. Physiological factors include changes such as natural accumulation of auxins, ethylene, and carbohydrates in the wounded area that will stimulate cell division resulting in the formation of callus and root primordia along the margins of the wood. Mechanical factors include an increase in the surface area through which water or auxins may be absorbed and a mechanical separation of the layer of sclerenchyma tissues, or fiber cells, present in some species, which acts as a physical barrier through which developing root primordia cannot penetrate

(Hartmann *et al*. 1997). *Lagerstroemia flos-reginae* can be propagated sexually through seeds or vegetatively through tissue culture and by stem cuttings. Sexual propagation by seeds results in genetic variation among seedlings, due to segregation resulting in heterogeneous offspring with considerable variation in vigor. For tissue culture propagation, high infrastructure and technical knowledge is needed. In the Sudan no research work has been carried out regarding propagation of *Lagerstroemia flos-reginae* by stem cuttings and hence The objective of this work was to study the effect of rooting hormone (IBA), wounding of cuttings, position of shoot from which cuttings are taken and rooting medium on rooting of *lagerstroemia flos-reginae* stem cuttings.

MATERIALS AND METHODS

This study was carried out at Elmogran nursery (Latitudes 15-36 N, Longitudes, 32-30 E and altitude 380 m. A.M.S, L.) that belongs to the Horticultural sector adminsrtation of the Fedral ministry of agriculture and irrigation, Sudan during the period December 2015 to February 2016. About 3 years old *lagerstroemia flos-reginae* trees growing at Elmogran nursery were the source of stem cuttings.

Experiment one: Effect of different concentrations of IBA on rooting ability of *lagerstroemia flos-reginae* hardwood cuttings:

The basal 1cm of the cuttings were dipped for about 5 seconds in IBA solutions that had the following concentrations before being inserted into coarse sand as rooting medium contained into polyethylene bags: 1000 ppm, 1500 ppm, 2000 ppm, 3000 ppm and control.

Experiment two: Effect of wounding and IBA on rooting ability of *lagerstroemia flos-reginae* hardwood cuttings:

Treatments were: Wounding, Wounding + IBA solution at 1000 ppm, Wounding + IBA in a powder (Seradix 3 for hardwood cuttings) and control.Wounding was done by a sharp razor blade making tow vertical cuts down each side of the base of the cutting penetrating through the bark and into the wood tissue. For application of the rooting powder the bases of the cuttings were dipped in water then dipped in the powder to a depth of about 2 cm, lightly tapped against the edge of the powder container to remove excess powder and inserted into coarse sand contained into polyethylene bags immediately after treatment.

Experiment three: Effect of position of shoot from which cuttings are taken on rooting ability of *lagerstroemia flos-reginae*:

Treatments were: terminal cuttings, median cuttings and basal cuttings. The cuttings were inserted into coarse sand contained into polyethylene bags.

Experiment four: Effect of rooting medium on rooting ability of *lagerstroemia flos-reginae* hardwood cuttings:

Cuttings were inserted into the following rooting media that were contained into polyethylene bags**:** Coarse sand, fine sand, silt, coarse sand + fine sand (1:1), coarse sand + silt (1:1) and fine sand + silt (1:1). In the four experiments cuttings were covered by a polyethylene cover and left in the rooting medium for 45 days.

The experimental design for the four experiments was a completely randomized design with four replications. Sixteen cuttings represented an experimental unit. Statistical analysis was carried out using Genstat Statistical Package (Genstat 2010) and differences between means were compared using Duncan's multiple range tests at 5% level of significance. The following parameters were measured 45 days after insertion of the cuttings into the rooting medium: Rooting percentage, number of roots/cutting and root length.

RESULTS AND DISCUSSION

Effect of different concentrations of IBA on rooting percentage, number of roots/cutting and root length in *lagerstroemia flos-reginae* hardwood cuttings:

There were significant differences ($P \leq 0.05$) in rooting percentage between treatments In the four experiments . As shown in table 1, rooting percentage was highest (90%) with the treatment IBA at both 2000 and 3000 ppm followed by IBA at 1500 ppm (55%), 1000 ppm (50%) and control (35%) in that order. Number of roots per cutting differed significantly among different IBA treatmens and followed the same trend as the rooting percentage being highest (3.6) at 3000 ppm IBA and lowest (2.1) at zero ppm IBA (control). Root length differed significantly among different IBA treatments. All IBA concentrations were superior over control. The length of root was highest (3.9 cm) at 2000 ppm IBA treatment and lowest (2.1cm) at the control. These results are in line with the results of Ingle and Venugopal (2018) in stevia (*Stevia rebaudiana* Bertoni) cuttings and those of Sabbah *et al.* (1991) in stem cuttings of Citrus. Regarding number of roots per cutting and root length, Razvi *et al.* (2018) reported similar results in *Lagerstroemia speciosa* cuttings. The increase in length of the roots might be due to an early initiation of roots at higher concentrations of IBA and more utilization of the food materials due to early formation of the roots. Similar trend has been reported by Chalapathi *et al* (2001) and Debnath (2008) in stevia._Similar results were also obtained by other researchers in different plants (Fouda and Schmidt,1995 in *Rosa canina* and Kumar (1962) in *Ficus elastica L.*).

Table1: Effect of different concentrations of IBA on rooting of hardwood cuttings of *Lagerstroemia flosreginae* L. 45 days after cutting insertion in the rooting medium.

IBA concentration (ppm)	Rooting percentage (%)	No. of roots/cutting	Root length (cm)
1000	50 ab	2.5^{c}	2.8^{b}
1500	55 ab	3.1^{ab}	3.2^{ab}
2000	90 a	3.4^{ab}	3.9^{a}
3000	90 a	3.6^{a}	2.9^{b}
Control	35 c	2.1^{bc}	2.1^{c}

Means followed by the same letter (s) in a column are not significantly different at p=0.05 according to Duncan's multiple range test.

Effect of wounding plus IBA as solution at 1000 ppm or powder (Seradix 3) on rooting percentage, number of roots and root length in *lagerstroemia flos-reginae* hardwood cuttings:

Table 2 demonstrates that wounding plus IBA solution scored the highest rooting percentage (90%) followed by wounding (89%) while minimum rooting percentage (35%) was recorded by the control. The mean number of roots among the different treatments showed significant differences. Maximum number of roots (3.5) was recorded by the treatment wounding plus IBA solution followed by the treatment wounding + IBA powder while minimum number of roots (2.4) was observed in untreated cuttings (control). Root length differed non significantly among different treatmens and followed the same trend as the number of roots/cutting. The result is in line with that reported by Rodríguez-Pérez, *et al* (2003) who obtained highest rooting percentage in cuttings of *Leucospermum* *cordifolioum x L. lineare* when using wounded cuttings treated with 2000 ppm of IBA. The result is also in conformity with that of Heike de Silva *et al* (2003) in Leyland cypress (x *Cupressocyparis leylandii*) where wounded cuttings treated with 10000 ppm IBA resulted in the highest percentage of rooted cuttings Wounding may enhance rooting through natural accumulation of auxins, ethylene, and carbohydrates in the wounded area that will stimulate cell division resulting in the formation of callus and root primordia along the margins of the wood. Wounding increases the surface area through which water or auxins may be absorbed. Wounding may lead to mechanical separation of a layer of sclerenchyma tissues, or fiber cells, present in some species, which acts as a physical barrier through which developing root primordia cannot penetrate (Hartmann *et al* 1997).

Table 2: Effect of wounding plus IBA as solution at 1000 ppm or powder (Seradix 3 for hardwood cuttings) on rooting of hardwood cuttings of *Lagerstroemia flosreginae* L. 45 days after cutting insertion in the rooting medium.

Treatments	Rooting percentage (%)	No. of roots/cutting	Root length (cm)
Wounding	89 ab	2.6^{ab}	1.8^{a}
Wounding + IBA solution	90 ab	3.5^{a}	1.9^{a}
Wounding + IBA powder	55 b	3.1^{ab}	1.8^{a}
Control	35 c	2.4^{b}	1.7^{a}

Means followed by the same letter (s) in a column are not significantly different at p=0.05 according to Duncan's multiple range test.

Effect of cutting position on rooting percentage, number of roots/cutting and root length in *lagerstroemia flos-reginae* hardwood cuttings:

The effect of cutting position on rooting of *lagerstroemia flos-reginae* is shown in table 3. The highest rooting percentage (100%) was recorded by basal cuttings followed by terminal cuttings (66.6%) while minimum rooting percentage (50%) was recorded by medium cuttings. There were significant differences in number of roots/cutting and root length. The highest values of both (11.7 roots, 8 cm respectively) were obtained with basal cuttings followed by terminal (9 roots, 6.1cm respectively) and medium cuttings (7 roots, 2.7 cm respectively) in order. These results are in agreement with the result reported by other research workers (Hansen, 1986 in *Schefflera arboricola,* Mahgoub, 1990 in Russelia and bougainvillea,Thompson,1986, in *Epacris impressa*). They attributed the better rooting of basal cuttings to the presence of high concentration of carbohydrates and endogenous auxins required for root initiation, existence of root initials in the basal part and presence of natural rooting inhibitors such as gibberellins in the terminal parts.

Table 3: Effect of cutting position on rooting of cuttings of *Lagerstroemia flosreginae* L. 45 days after cutting insertion in the rooting medium.

Treatments	Rooting percentage (%)	No. of roots/cutting	Root length (cm)
Basal cuttings	100 a	11.7[a]	8[a]
Medium cuttings	50 b	7[b]	2.7[b]
Terminal cuttings	66.6 c	9[ab]	6.1[a]

Means followed by the same letter (s) in a column are not significantly different at p=0.05 according to Duncan's multiple range test.

Effect of rooting media on rooting percentage, number of roots/cutting and root length in *lagerstroemia flos-reginae* hardwood cuttings:

As shown in table 4, rooting percentage was 100% in the media coarse sand, fine sand, fine sand+silt and fine sand+coarse sand followed by silt + coarse sand (50%) and lowest in silt (41.6). There was a significant difference in number of roots/cutting. The highest number of roots (14) was recorded by coarse sand while minimum number of roots (8) was shown by silt. Coarse sand recorded the highest root length (3.3 cm) differing significantly from all the other treatments between which there was no significant difference. The result is comparable with that reported by various researchers. Geczi (1974) used perlite, hot bed soil and sand as rooting media in grapes. He found that perlite was the best rooting media followed by sand. Vander Vossen and Op. DE. Laak (1976) reported that the best rooting media for rooting soft-wood cuttings of coffee was a combination of equal parts of loamy soil, coarse sand and acid swamp soil.

Palzkill and Feldman (1993) compared the three media [perlite/vermiculite, 1:1 (vol/vol); peat/perlite/vermiculite, 1:1:1 and peat/perlite, 1:1] in rooting of jojoba cuttings. Rooting averaged 74% for perlite/vermiculite, 78% for peat/perlite/vermiculite and 64% for peat/perlite. It is worth mentioning that an ideal rooting medium provides sufficient porosity to allow good aeration and has a high water holding capacity, yet is well drained (Hartmann *et al*.1997). It can be concluded that the use of wounded basal stem cuttings treated with IBA solution at 1000 ppm and inserted into coarse sand is a suitable method for propagation of *Lagerstroemia flos-reginae.*

Table 4: Effect of various rooting media on rooting of hardwood cuttings of *Lagerstroemia flosreginae* L. 45 days after cutting insertion in the rooting medium.

Rooting media	Rooting percentage (%)	No. of roots/cutting	Root length (cm)
Coarse sand	100 a	14^{a}	3.3^{a}
Fine sand	100 a	9.3^{ab}	2.5^{b}
Silt	41.6 b	8^{b}	2.6^{b}
Fine sand + Silt	100 a	12.6^{ab}	2.4^{b}
Fine sand + Coarse sand	100 a	12.3^{ab}	2.3^{b}
Silt + coarse sand	50 b	13.3^{a}	2.2^{b}

Means followed by the same letter (s) in a column are not significantly different at p=0.05 according to Duncan’s multiple range test.

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