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Effect of naphthalene acetic acid on sprouting and rooting of stem cutting in *Mussaenda* species

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Abstract

Mussaendas are increasingly popular for the showy colour they provide during much of the year in garden landscapes. However, root formation in stem cuttings is a major challenge for cultivation of *Mussaenda* species. Therefore, the experiment was carried out at the Landscaping section of the Department of Horticulture, Bangladesh Agricultural University, Mymensingh during the period from August to November 2018 to study the effect of phytohormone naphthalene acetic acid (NAA) on sprouting and rooting of stem cuttings in Mussaenda species. The two-factor experiment consisted of three *Mussaenda* species *viz*., *Mussaenda frondosa* (White flag bush), *Mussaenda philippica* (Pink flag bush) and *Mussaenda erythrophylla* (Red flag bush), and five concentrations of NAA *viz*., 0 (control), 0.1%, 0.2%, 0.3% and 0.4%. The experiment was laid out in randomised complete block design (RCBD) with three replications. The results revealed that all the traits related to sprouting and rooting potential of stem cuttings were significantly influenced by combined effects of Mussaenda species and various NAA concentrations. The maximum sprouted bud per cutting (3.57), highest number of roots per cutting (5.88) and the longest root length (4.32 cm) were recorded from *M. philippica* except number of leaves per stem cutting (4.42) in *M. erythrophylla.* The increasing concentrations of NAA application responded positively on sprouting and rooting characters in all three *Mussaenda* species used for this experiment. The best results obtained from the treatment where NAA was applied at the concentration of 0.3% with 3.92 sprouted buds per cutting, 4.77 leaves per cutting, 7.15 roots per cutting and 5.26 cm length of roots. The treatment combination of *M. philippica* (Pink flag bush) and 0.3% NAA, followed by NAA at 03% with *M. erythrophylla* (Red flag bush) was found to be best in terms of sprouting and rooting of stem cuttings in Mussaenda.

Key words: *Mussaenda*, NAA, sprouting, rooting, stem cutting

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Introduction

The Mussaendas are a group of ornamental evergreen shrubs suited to tropical and subtropical climates with a bright future, both as landscape plants and as potted floral decorations (Ogbu *et al.,* 2017). The genus *Mussaenda* L. belongs to the large family Rubiaceae, which also contains *Gardenia*, *Ixora*, *Pentas* and *Coffea* (coffee). The most distinctive feature of *Mussaenda* is that the floral display is primarily derived from the calyx, with some individual flowers within an inflorescence carrying an enlarged petaloid sepal. Some cultivars have all five sepals enlarged, which are called calycophylls or sometimes semaphylls (also called bracts), a structure, which signals a pollinator. The bracts may have different shades, including red, rose, white, pale pink or some mixtures. The major attractions of *Mussaenda* in the landscape are their extended flowering period. They will loosen their leaves and go dormant through the cooler and drier winter, but put on a spectacular display throughout the warm, wet months. If conditions are suitable, they can flower year-round; there is presumably no daylength requirement. It can blossom from November to May in the southern hemisphere and from May to November in the northern side of the equator, while numerous species can bloom lasting through the year in tropical atmospheres (Mathew and Karikari, 1990; Rosario, 1998). They have poor drought and cold tolerance. The genus *Mussaenda* is also an important source of medicinal natural products, particularly iridoids, triterpenes and flavonoids (Vidyalakshmi *et al*., 2008). Some species of *Mussaenda* have been used in Chinese and Fijian traditional medicine. The *Mussaenda* species are native to West-Africa, Madagascar, Asia and the Pacific (John and Joe, 2014). There are more than 200 known species, but around ten species are used for cultivation (Sheat and Schofield, 1995). The broadly cultivated species in the garden landscape include *Mussaenda frondosa* (White flag bush), *Mussaenda philippica* (Pink flag bush) and *Mussaenda erythrophylla* (Red flag bush) and *Pseudomussaenda flava* (also referred to as *Mussaenda flava*, *M. glabra*, *M. luteola*, *M. lutea* or *M. incana*) (Mabberley, 2008).

The garden Mussaendas are scrambling shrubs and mostly range from 60 to 450 cm in height, depending upon the species, while the wild species can climb up to 9 m in height. Mussaenda leaves are opposite, bright to dark green and rounded elliptic often pubescent (covered with short, fine hairs) and prominently veined (John and Joe, 2014; Sheat and Schofield, 1995). Mussaendas are known for its low pollen fertility, poor fruit production and limited seed production (Rosario, 1998: Steentoft, 1988). *Mussaenda* species are mostly propagated by stem cuttings taken from hard woods, semi-hard woods and sometimes soft woods and rooted in a rooting media under optimum nursery conditions, however, some species/cultivars of potential aesthetic value are difficult to propagate by stem cuttings (Chadha and Choudhury, 2007; Ogbu, 2011). Phytohormones, especially various auxins such as Indole acetic acid (IAA), Indole butyric acid (IBA), Naphthalene acetic acid (NAA) and 2,4-Dichloropheoxy acetic acid (2,4-D) have been reported to promote rooting in cuttings of the most of the plant species (Hartmann *et al.*, 2007). Menon *et al*., (2013) reported that NAA has great sprouting and rooting potential in stem cuttings of Bougainvillea. It was found that naphthalene acetic acid has great influence on adventitious root development and associated physiological changes in stem cutting of *Hemarthria compressa* (Yan *et al.*, 2014). Recently, use of *Mussaenda* species has been increased in the garden landscape in Bangladesh. However, challenges arise for bud sprouting and root formation in the stem cuttings of *Mussaenda*. For quality root production concentrations of phytohormone chemicals are outmost importance, otherwise, it will lead to an undesirable effect. Therefore, the present study was undertaken to find out the effect of phytohormone naphthalene acetic acid on sprouting and rooting of stem cuttings in *Mussaenda* species.

Materials and Methods

In order to investigate the effect of phytohormone naphthalene acetic acid on sprouting and rooting of stem cuttings in *Mussaenda* species, the experiment was carried out at the Landscaping section of the Department of Horticulture, Bangladesh Agricultural University, Mymensingh during the period from August to November 2018. The experimental site was medium high land belonging to the Old Brahmaputra Floodplain under the Agro-Ecological Zone 9 having non-calcareous dark gray floodplain soil (UNDP and FAO, 1988). The two-factor experiment consisted of three Mussaenda species *viz*., *Mussaenda frondosa* (White flag bush; Figure 1a), *Mussaenda philippica* (Pink flag bush; Figure 1b) and *Mussaenda erythrophylla* (Red flag bush; Figure 1c), and five concentrations of NAA *viz*., 0 (control), 0.1%, 0.2%, 0.3% and 0.4%. The stem cuttings of *M. frondosa*, *M.*

philippica and *M. erythrophylla* about 20 cm in length were collected from their respective parent stands at the garden of the Landscaping section of Bangladesh Agricultural University, Mymensingh, Bangladesh.

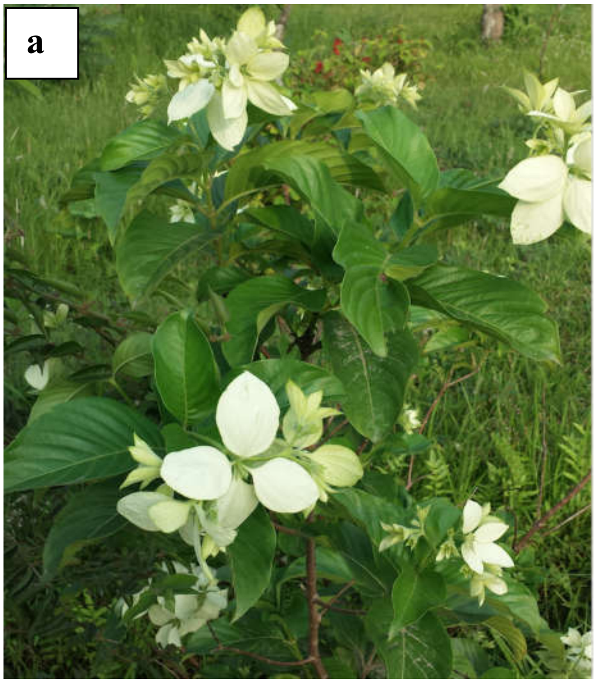

Figure 1. (a) *Mussaenda frondosa* (White flag bush), (b) *Mussaenda philippica* (Pink flag bush) and (c) *Mussaenda erythrophylla* (Red flag bush) plants were used as mother plants for cuttings.

While preparing the cuttings, a smooth cut in each cutting was given on distal end and slanting cut was given at proximal or lower end just below the node. The basal ends of the cuttings were carefully dipped into various NAA solutions *viz*., 0.1%, 0.2%, 0.3%, 0.4% plus control treatment with no NAA for 5 minutes before planting them in the rooting medium. The solutions were prepared by dissolving the NAA compound in 95% ethanol and adding distilled water. The rooting bed used for the growing medium of the cuttings was sandy loam in texture with pH 7.0 and was prepared by mixing equal ratio of sand, well decomposed cowdung and garden soil in the ratio of 1:1:1 (v/v). The stem cuttings were then placed in the rooting bed at a spacing of 30 x 15 cm and depth of 5-6 cm in raised beds of 1m x 1m dimensions in the month of September 2018. Intercultural operations such as weeding, mulching and irrigation were done as and when necessary throughout the experiment. The experiment was conducted in a three replicated randomised complete block design (RCBD) each treatment comprised of 10 cuttings and a total of 150 cuttings of each species were managed. The data on sprouted bud per stem cutting, number of leaves per stem cutting, number of roots per stem cutting and length of roots per cutting (cm) were recorded after two months of plantation. The data obtained were then statistically analyzed using Analysis of Variance (ANOVA) and means were compared according to Least Significant Difference (LSD) at 5% probability level.

Results and Discussion

***Effects of Mussaenda species*:** The results revealed that all the traits related with sprouting and rooting potential of stem cuttings of Mussaenda were significantly influenced by different species (Table 1). The maximum sprouted bud per stem cutting (3.57) was observed in *M. philippica* (S_2), which was statistically different to those of the other species and the minimum sprouted bud per stem cutting (2.09) was recorded with *M. frondosa* (S_1). The highest number of leaves per stem cutting (4.42) was obtained from *M. erythrophylla* (S_3), followed by *M. philippica* (S_2) (3.97), whereas the lowest number of leaves per stem cutting (2.99) was found from *M. frondosa* (S_1). This might be due to the linear increment of the sprouting buds and number of leaves per stem cutting with the increase of vegetative growth stage (Menon *et al.,* 2013).

Table 1. Individual effects of species and phytohormone NAA on sprouting and rooting characters of *Mussaenda.*

Treatments	No. of sprouted bud/cutting	No. of leaves/cutting	No. of roots/cutting	Length of root (cm)
Factor A: Mussaenda species				
S_1: *Mussaenda frondosa* (White)	2.09	2.99	3.84	3.31
S_2: *M. philippica* (Pink)	3.57	3.97	5.88	4.32
S_3: *M. erythrophylla* (Red)	3.36	4.42	4.87	3.95
$LSD_{0.05}$	0.113	0.063	0.123	0.082
Level of significance	*	*	*	*
Factor B: NAA concentrations				
T_0: 0 (Control)	1.92	2.78	2.90	2.55
T_1: 0.1%	2.80	3.41	4.43	3.43
T_2: 0.2%	3.40	4.20	5.51	4.25
T_3: 0.3%	3.92	4.77	7.15	5.26
T_4: 0.4%	3.01	3.80	4.33	3.80
$LSD_{0.05}$	0.146	0.081	0.159	0.106
Level of significance	*	*	*	*

*Significant at 5% level of probability, S_1= *Mussaenda frondosa* (White flag bush), S_2= *Mussaenda. philippica* (Pink flag bush), S_3= *Mussaenda erythrophylla* (Red flag bush), T_0= 0 (Control), T_1= 0.1% NAA, T_2= 0.2% NAA, T_3= 0.3% NAA, T_4= 0.4% NAA.

The highest number of roots per cutting (5.88) and the longest root length (4.32 cm) was recorded from *M. philippica* (S_2) followed by *M. erythrophylla* (S_3) (4.87 and 3.95 cm, respectively), while the lowest number of roots per cutting (3.84) and the shortest root length (3.31 cm) was observed from *M. frondosa* (S_1). The obtained results seemed to complement with those reported by Ogbu *et al*. (2017). This result indicates that the numbers of sprouts and leaves per cutting are not the same for the tree species of *Mussaenda*, which might be genetically controlled. Rooting and sprouting potential of stem cuttings is one of the critical steps in plant propagation of woody plants. It varies from species to species including so many other factors. However vegetative propagation has an advantage in developing true to type, disease free varieties of economically and commercially important plants (Kochhar *et al.*, 2008). Vegetative propagation of *Mussaenda* by stem cuttings has been found to be very effective because of its simplicity and practicability in our developing countries. However, the sprouting and rooting rate of success is low and varies from species to species, hence many researchers tried various auxins for initiation of rooting in cuttings of various horticultural crops as reported by (Leaky *et al*., 1982; Sherer *et al*., 1985).

***Effects of phytohormone NAA*:** The results showed that all the traits related to sprouting and rooting potential of stem cuttings of Mussaenda were significantly influenced by various concentrations of NAA (Table 1). The highest number of sprouted bud (3.92) and leaves (4.77) per stem cutting was observed in cuttings, which were treated with NAA concentration of 0.3% (T_3), followed by cuttings treated with 0.2% NAA (T_2) (3.40 and 4.20, respectively), while the lowest number of sprouted bud (1.92) and leaves (2.78) per stem cutting was recorded from control, where cuttings were not treated with NAA (T_0). This might be due to the increased concentrations of phytohormone NAA. Similar result was also found by Kochhar *et al.* (2008) who observed maximum number of sprouts from stem cuttings of *Jatropha curcas* when treated with auxin i.e. NAA @ 100 ppm as compared to 10 ppm. Jadhav (2007) observed more number of leaves from the cuttings treated with higher concentration of NAA @ 200 ppm. The highest number of roots per cutting (7.15) and the longest root length (5.26 cm) were also found from the stem cuttings treated with NAA at a rate of 0.3% (T_3), followed by the cuttings treated with 0.2% NAA (T_2) (5.51 and 4.25 cm, respectively), while the lowest number of roots per cutting (2.90) and the shortest root length (2.55 cm) were observed from control, where cuttings were not treated with NAA (T_0). Increased number and length of roots per cutting might be due to the increasing concentration of NAA. Ramadayal *et al.* (2001) reported highest percentage of rooting, number of roots per cutting and root length from the hard wood cuttings of Bougainvillea variety Mary Plamer in response to the auxin. The increase in length of roots in cuttings treated with growth regulators may be due to the accumulation of metabolites at the site of application of auxins, cell enlargement, enhanced hydrolysis of carbohydrates, synthesis of new proteins, and cell division induced by the auxins (Asl *et al.*, 2012).

***Combined effects of Mussaenda species and phytohormone NAA*:** The combined effect of species and NAA concentrations had significant influence on all the sprouting and rooting parameters under study (Table 2). The maximum number of sprouted buds per stem cutting (4.66) was obtained from the cuttings of *Mussaenda philippica* treated with phytohormone NAA @ 0.3% solution (S_2T_3), followed by 4.36 from the cuttings of *M. erythrophylla* treated with phytohormone NAA @ 0.3% solution (S_3T_3), while the minimum number of sprouted buds per stem cutting (1.40) was obtained from the cuttings of *M. frondosa*, which were not treated with NAA (S_1T_0). This may be due to the different type of plant, season variability, and thickness of the stem cutting. Wahab *et al.* (2001) also reported that sprouting is mainly attributed to the stored carbohydrates in the cuttings used for sprouting. However, with auxin application to the cutting and subsequent increase in the rooting may result in the increase of sprouting, this indirect effect of auxin on sprouting highlights the role of certain materials produced in the roots, responsible for sprouting. The highest number of leaves per stem cutting (5.76) was recorded from the combination of *M. erythrophylla* treated with phytohormone NAA @ 0.3% solution (S_3T_3), followed by 4.86 from the combination of *M. philippica* treated with phytohormone NAA @ 0.3% solution (S_2T_3), whereas the lowest number of leaves per stem cutting (5.76) was recorded from the combination of *M. frondosa* with control treatment (S_1T_0). The highest number of roots per stem cutting (9.03) and the longest root length (6.23 cm) was found from the combined treatment of *M. philippica* plus NAA @ 0.3% ((S_2T_3), followed by 7.20 and 5.23 cm, respectively, from the combined treatment of *M. erythrophylla* plus NAA @ 0.3% (S_3T_3), while the lowest number of roots per stem cutting (2.60) and the shortest root length (2.30 cm) was observed from *M. frondosa* with control treatment (S_1T_0).

Table 2. Combined effects of species and phytohormone NAA on sprouting and rooting characters of *Mussaenda.*

Treatment combinations	No. of sprouted bud/cutting	No. of leaves/cutting	No. of roots/cutting	Length of root (cm)
S_1T_0	1.40	2.26	2.60	2.30
S_1T_1	1.80	2.70	3.66	2.86
S_1T_2	2.50	3.30	4.23	3.70
S_1T_3	2.73	3.70	5.23	4.33
S_1T_4	2.03	3.00	3.46	3.36
S_2T_0	2.26	2.90	3.20	2.66
S_2T_1	3.20	3.50	5.26	3.76
S_2T_2	4.03	4.50	6.70	4.70
S_2T_3	4.66	4.86	9.03	6.23
S_2T_4	3.70	4.10	5.23	4.23
S_3T_0	2.10	3.20	2.90	2.70
S_3T_1	3.40	4.03	4.36	3.66
S_3T_2	3.66	4.80	5.60	4.36
S_3T_3	4.36	5.76	7.20	5.23
S_3T_4	3.30	4.30	4.30	3.80
$LSD_{0.05}$	0.253	0.140	0.275	0.183
Level of significance	*	*	*	*

*Significant at 5% level of probability, S_1= *Mussaenda frondosa* (White flag bush), S_2= *Mussaenda. philippica* (Pink flag bush), S_3= *Mussaenda erythrophylla* (Red flag bush), T_0= 0 (Control), T_1= 0.1% NAA, T_2= 0.2% NAA, T_3= 0.3% NAA, T_4= 0.4% NAA.

Conclusion

From the above results, it can be concluded that the application of NAA at 0.3% concentration remarkably enhanced sprouting and adventitious root production in the ornamental *Mussaenda philippica* (Pink flag bush), followed by NAA at 03% with *Mussaenda. erythrophylla* (Red flag bush), which was better than the other concentrations and control treatments used with *Mussaenda. frondosa.*

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