Three Mussaenda Cultivars Propagated by Stem Cuttings Exhibit Variation in Rooting in Response to Hormone and Rooting Conditions

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SUMMARY. The effects of 1H-indole-3-butyric acid (IBA), cutting position on stock plants, the date of propagation, the type of rooting substrate and temperature on rooting of mussaenda (*Mussaenda erythrophylla* Schumach. & Thonn. 'Ashanti Blood' and 'Rosea', and *Mussaenda philippica* A. Rich 'Aurorae') stem cuttings were determined. Cuttings of 'Ashanti Blood' produced the largest number of roots when treated with 15 mmol (3000 ppm) IBA and rooted in perlite at 29 °C (84 °F). Cutting position on stock plants did not affect rooting in any of the three cultivars. Propagation date and temperature of the rooting medium affected root numbers in 'Aurorae'. With 'Rosea', only the type of rooting substrate affected root number. Rooting percentage was 22%, 48%, and 39% in 'Ashanti Blood', 'Aurorae', and 'Rosea' respectively. After 30 days of propagation average root length was 4, 12, and 4 mm (0.2, 0.5, and 0.2 inch) in 'Ashanti Blood', 'Aurorae', and 'Rosea' respectively.

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Growers must determine precise rooting conditions for each cultivar to obtain consistent rooting of cuttings. This process may not be economically feasible on a commercial basis because rooting percentages are relatively low. We conclude that other methods of clonal propagation need to be

evaluated before uniform rooted stem cuttings of *mussaenda* can be produced economically.

Mussaendas are ornamental shrubs indigenous to the Pacific Islands, Asia and

the tropics of Africa (Sharma et al., 1990), and are the most widely accepted ornamental plants of the Philippines (Rosario, 1987). *Mussaenda*s appeal to gardeners and scientists because of their ornate bracts (Jayaweera, 1963; Sharma et al., 1990).

Table 1. Treatment combinations used to test rooting conditions in three cultivars of *mussaenda*. Treatment combinations follow a Box-Behnken experimental design. The coded design level is given in parenthesis.

Treatment	IBA ^z (mmol)	Cutting position	Date cuttings taken	Medium	
				Porosity (%)	Temp (°C) ^y
1	1.5 (-1)	Terminal (-1)	22 Jan. 1994 (0)	11 (0)	34 (0)
2	15.0 (+1)	Terminal (-1)	22 Jan. 1994 (0)	11 (0)	34 (0)
3	1.5 (-1)	Basal (+1)	22 Jan. 1994 (0)	11 (0)	34 (0)
4	15.0 (+1)	Basal (+1)	22 Jan. 1994 (0)	11 (0)	34 (0)
5	8.0 (0)	Midshoot (0)	7 Jan. 1994 (-1)	6 (-1)	34 (0)
6	8.0 (0)	Midshoot (0)	28 Dec. 1993 (+1)	6 (-1)	34 (0)
7	8.0 (0)	Midshoot (0)	7 Jan. 1994 (-1)	43 (+1)	34 (0)
8	8.0 (0)	Midshoot (0)	28 Dec. 1993 (+1)	43 (+1)	34 (0)
9	8.0 (0)	Terminal (-1)	22 Jan. 1994 (0)	11 (0)	34 (0)
10	8.0 (0)	Basal (+1)	22 Jan. 1994 (0)	11 (0)	29 (-1)
11	8.0 (0)	Terminal (-1)	22 Jan. 1994 (0)	11 (0)	37 (+1)
12	8.0 (0)	Basal (+1)	22 Jan. 1994 (0)	11 (0)	37 (+1)
13	1.5 (-1)	Midshoot (0)	7 Jan. 1994 (-1)	11 (0)	34 (0)
14	15.0 (+1)	Midshoot (0)	7 Jan. 1994 (-1)	11 (0)	34 (0)
15	8.0 (0)	Midshoot (0)	28 Dec. 1993 (+1)	11 (0)	34 (0)
16	15.0 (+1)	Midshoot (0)	28 Dec. 1993 (+1)	11 (0)	34 (0)
17	8.0 (0)	Midshoot (0)	22 Jan. 1994 (0)	6 (-1)	29 (-1)
18	8.0 (0)	Midshoot (0)	22 Jan. 1994 (0)	43 (+1)	29 (-1)
19	8.0 (0)	Midshoot (0)	22 Jan. 1994 (0)	6 (-1)	37 (+1)
20	8.0 (0)	Midshoot (0)	22 Jan. 1994 (0)	43 (+1)	37 (+1)
21	8.0 (0)	Terminal (-1)	7 Jan. 1994 (-1)	11 (0)	34 (0)
22	8.0 (0)	Basal (+1)	7 Jan. 1994 (-1)	11 (0)	34 (0)
23	8.0 (0)	Terminal (-1)	28 Dec. 1993 (+1)	11 (0)	34 (0)
24	8.0 (0)	Basal (+1)	28 Dec. 1993 (+1)	11 (0)	34 (0)
25	1.5 (-1)	Midshoot (0)	22 Jan. 1994 (0)	6 (-1)	34 (0)
26	15.0 (+1)	Midshoot (0)	22 Jan. 1994 (0)	6 (-1)	34 (0)
27	1.5 (-1)	Midshoot (0)	22 Jan. 1994 (0)	43 (+1)	34 (0)
28	15.0 (+1)	Midshoot (0)	22 Jan. 1994 (0)	43 (+1)	34 (0)
29	8.0 (0)	Midshoot (0)	7 Jan. 1994 (-1)	11 (0)	29 (-1)
30	8.0 (0)	Midshoot (0)	28 Dec. 1993 (+1)	11 (0)	29 (-1)
31	8.0 (0)	Midshoot (0)	7 Jan. 1994 (-1)	11 (0)	37 (+1)
32	8.0 (0)	Midshoot (0)	28 Dec. 1993 (+1)	11 (0)	37 (+1)
33	1.5 (-1)	Midshoot (0)	22 Jan. 1994 (0)	11 (0)	29 (-1)
34	15.0 (+1)	Midshoot (0)	22 Jan. 1994 (0)	11 (0)	29 (-1)
35	1.5 (-1)	Midshoot (0)	22 Jan. 1994 (0)	11 (0)	37 (+1)
36	15.0 (+1)	Midshoot (0)	22 Jan. 1994 (0)	11 (0)	37 (+1)
37	8.0 (0)	Terminal (-1)	22 Jan. 1994 (0)	6 (-1)	34 (0)
38	8.0 (0)	Basal (+1)	22 Jan. 1994 (0)	6 (-1)	34 (0)
39	8.0 (0)	Terminal (-1)	22 Jan. 1994 (0)	43 (+1)	34 (0)
40	8.0 (0)	Basal (+1)	22 Jan. 1994 (0)	43 (+1)	34 (0)
41	8.0 (0)	Midshoot (0)	22 Jan. 1994 (0)	11 (0)	34 (0)
42	8.0 (0)	Midshoot (0)	22 Jan. 1994 (0)	11 (0)	34 (0)
43	8.0 (0)	Midshoot (0)	22 Jan. 1994 (0)	11 (0)	34 (0)
44	8.0 (0)	Midshoot (0)	22 Jan. 1994 (0)	11 (0)	34 (0)
45	8.0 (0)	Midshoot (0)	22 Jan. 1994 (0)	11 (0)	34 (0)
46	8.0 (0)	Midshoot (0)	22 Jan. 1994 (0)	11 (0)	34 (0)

^zIBA = 1H-indole-3-butyric acid.

^y°F = 1.8 (°C) + 32.

Heterostyly, low pollen fertility (Rosario et al., 1990) and poor fruit production (Rosario, 1987) limit sexual reproduction. In addition, some potentially important cultivars of *mussaenda* are extremely difficult to propagate by stem cuttings (Kumar and Vijayakumar, 1984; Sharma et al., 1990; St. Hilaire et al., 1996).

Bose et al. (1975) showed that 20-cm (8-inch) long tip cuttings of *M. philippica* 'Pink' produced more roots than semiwoody middle or woody basal cuttings of the same length regardless of the concentration of IBA used to treat those cuttings. All of the 10- and 15-cm (4- and 6-inch) tip cuttings treated with 14.8 mmol (3000 ppm) of IBA rooted, but only 70% of the 20-cm (8-inch) tip cuttings rooted. Kumar and Vijayakumar (1984) showed that 93% of cuttings of *M. philippica* 'Pink' rooted when treated with IBA at 29.5 mmol (6000 ppm), but only 56% of cuttings *M. philippica* 'Pink' rooted when treated with the same concentration of naphthaleneacetic acid (NAA). Very little is known about the effect of rooting substrate temperature and the date of propagation on rooting of *mussaenda*.

The objective of this experiment was to evaluate adventitious root formation in stem cuttings of three cultivars of *mussaenda* in response to IBA concentration, air-filled porosity of rooting media, temperature of rooting media, position cuttings were on stock plants, and date of taking cuttings. Our approach was to use a Box-Behnken experimental design (Box and Draper, 1987; Myers and Montgomery, 1995) to study several factors that affected adventitious rooting in each cultivar because previous experiments focused on testing the effects of one or two factors on root formation.

Materials and methods

Twenty five 1-year-old stock plants each of *M. erythrophylla* 'Ashanti Blood', *M. erythrophylla* 'Rosea', and *M. philippica* 'Aurorae' were obtained from Eileen's Gardens (Cabo Rojo, P.R.) and grown in IEM 600 (IEM plastics, Reidsville, N.C.) plastic pots 20 cm (8 inch) tall, 22 cm (9 inch) wide; volume = 5400 cm³ (2 gal) filled with Pro-Mix BX (Premier Horticulture Inc., New York) horticultural substrate. Stock plants were grown adjacent to the propagating bench. Plants received foliar sprays of 20N-8.8P-

16.6K (Evergreen, Fervill Inc., Cataño, P.R.) at 7 kg·m⁻³ (1 oz/gal⁻¹) every week and granular treatments of 10N-4.3P-8.3K (Green Crop, Ochoa Fertilizers, Cataño, P.R.) at 28 g (2 oz)/plant every 2 weeks.

Cuttings were taken on 28 Dec. 1993, and 7 and 22 Jan. 1994. Shoots were divided into 15-cm (6-inch) terminal, middle-shoot, and basal cuttings so that each cutting had two or three nodes. Approximately two-thirds of the lamina were removed to reduce transpiration. Proximal cutting ends were dipped to a depth of 2 cm (0.8 inch) for 10 s in 1.5, 8, or 15 mmol (300, 1650, or 3000 ppm) IBA dissolved in 0.1 mol·L⁻¹ (4 g·L⁻¹ NaOH). Cuttings were stuck to a depth of 5 cm (2 inches) in the rooting substrate. Cuttings were rooted in 1 washed sand (1.0 mm maximum particle size): 1 chemically sterilized Consumo soil (clayey, mixed, isohypothermic Typic Haplohumult) (by volume), 1 perlite : 1 washed sand : 3 chemically sterilized soil (by volume), and only coarse perlite (Aero-Soil, Grefco Inc., Torrance, Calif.) that provided air-filled porosities (AFP) of 6%, 11% and 43%, respectively. The soil had 65% clay, 20% sand,

and 15% silt. Soil pH was 4.86. Air-filled porosity was determined by using Method 3 as described by Handreck and Black (1984). Water content (by mass) was 29% in 1 sand : 1 soil, 36% in 1 perlite : 1 sand : 3 soil, and 71% in perlite. Water content was determined by procedures described in Handreck and Black (1984). Media were placed into 60 × 44 × 15-cm (24 × 17 × 6-inch) propagation troughs and were maintained at 34 ± 2 °C (93 ± 4 °F) and 37 ± 3 °C (99 ± 5 °F) or unheated [29 ± 3 °C (84 ± 5 °F)]. As a result, there were nine media/temperature combinations. Media were heated with thermostatically controlled propagation mats (Progro, A.H. Hummert, Earth City, Mo.) placed at the bottom of the rooting substrate. The probe of the thermostat was inserted 5 cm into the rooting substrate. Temperature of the substrate was determined at 5 cm (2 inch) and in multiple locations with iron-constantan thermocouple probes attached to an Omega thermometer (OM-500, Omega Engineering, Stamford, Conn.). The thermostat then was set to maintain substrate temperature. A cutting from each treatment was sampled at 15, 30, and 45 d after

Table 2. Significance of F values for the effects of 1H-indole-3-butyric acid (IBA), cutting position on stock plants (position), propagation date (date), air-filled porosity of the rooting substrate (AFP), temperature of the rooting substrate, and their two way interactions on root numbers of three cultivars of *mussaenda*, following ANOVA for the quadratic model. Each cultivar was run as a separate experiment. The analysis was run on the average number of roots per cutting 30 d after propagation.

Source of variation	Cultivar		
	Ashanti Blood	Aurorae	Rosea
IBA	0.0456	0.9176	0.6771
Position (P)	0.2950	0.0551	0.3687
Date (D)	0.8350	0.1856	0.2029
AFP	0.0108	0.0969	0.2619
Temperature (T)	0.0699	0.0769	0.5956
IBA × IBA	0.2742	0.2152	0.3452
P × P	0.8076	0.1650	0.5463
D × D	0.2852	0.0256	0.4832
AFP × AFP	0.1039	0.7617	0.0265
T × T	0.0226	0.0192	0.9915
IBA × P	0.7549	0.8631	1.0000
IBA × D	1.0000	0.9725	1.0000
IBA × AFP	0.0394	1.0000	0.2561
IBA × T	0.0270	1.0000	0.7519
P × D	1.0000	0.0849	0.1162
P × AFP	1.0000	0.7434	0.6055
P × T	0.1354	0.7696	0.8405
D × AFP	0.8896	0.3472	0.8181
D × T	1.0000	0.0014	0.7312
AFP × T	0.9170	0.9725	0.2230

insertion into the rooting medium to determine the optimum time to evaluate root formation.

Cuttings were rooted within a clear plastic tunnel in an open-ended greenhouse at the University of Puerto Rico at Mayagüez (lat. 18°12' N, long. 67°8' W). Cuttings were misted with

unchlorinated tap water (pH 7.2, EC 0.407 dS·m⁻¹) for 15 s every min from 0800 to 1800 h. Fine mist and a high misting frequency were used to maintain humidity and prevent excessive temperature fluctuations within the plastic tunnel. Mean day/night air greenhouse temperatures averaged 27

± 3 °C (81 ± 5°F)/21 ± 2 °C (70 ± 4°F), respectively. Temperatures of the air within the tunnel and of the unheated medium averaged 26 ± 7 °C (79 ± 13 °F) and 29 ± 3 °C (84 ± 5°F) respectively. A data logger (LI-1000; LI-COR, Lincoln, Neb.) recorded and stored greenhouse environmental data.

The experimental design was a Box-Behnken second-order design (Box and Draper, 1986; Myers and Montgomery, 1995). The design was generated by using Design-Expert 3.0.2 Statistical Software (STATEASE, Minneapolis, Minn.). The Box-Behnken design is a three-level design for fitting second-order response surfaces (Box and Draper, 1987; Myers and Montgomery, 1995). The treatment combinations used in the design matrix are given in Table 1. The design matrix tested five factors each at three levels. Each cutting was assigned to a treatment combination outlined by the design matrix. To sample the experiment three times, three similar design matrices were used. Each cultivar was run as a separate experiment. The levels of the variables were coded 1, 0, and -1 (Table 1) because Box-Behnken designs use these three levels to analyze the data (Box and Draper, 1987; Box et al., 1978, Myers and Montgomery, 1995). Coding reduced the level of each factor to a common scale, regardless of the range of the level. Adventitious roots on each cutting were counted and their length was measured at 15 and 30 d after propagation. Rooting percentage for each cultivar was calculated at 30 d after propagation. Analysis of variance (ANOVA) and contour plots generated by Design-Expert were used to analyze data. Paired comparisons using PROC TTEST (SAS Institute, Cary, N.C.) were used to compare the number of roots and the length of roots present at 15 and 30 d after propagation.

Results and discussion

The statistical significance of the main effects and their two-way interactions are given for the number of roots produced on each cutting at 30 d (Table 2). The mean number of adventitious roots per cutting was similar at 15 and 30 d for 'Ashanti Blood' ($P \leq 0.0868$) and 'Aurorae' ($P \leq 0.3084$). Cuttings of 'Rosea' ($P \leq 0.0111$) harvested at 30 d had a larger mean number of roots per cutting

than those harvested at 15 d. For 'Ashanti Blood' ($P \leq 0.0137$), 'Aurorae' ($P \leq 0.0009$) and 'Rosea' ($P \leq 0.0032$) roots were longer at 30 d than at 15 d. Since many cuttings in all treatments senesced by 45 d, we concluded that 30 d after propagation was the best time to evaluate root numbers. For that reason, ANOVA and contour plots for the number of roots present at 30 d are given. High temperatures of the misting chamber and propagation medium may contribute to root senescence (Hartmann et al., 1996) because of increased disease and the depletion of root carbohydrate reserves (Preece, 1993).

Our study indicates that treating cuttings of 'Ashanti Blood' with IBA affected root numbers (Table 2). This agrees with reports by Bose et al. (1975) and St. Hilaire et al. (1996) that have shown IBA improves rooting of *Mussaenda*. St. Hilaire et al. (1996) showed that more root primordia were initiated in cuttings of *M. erythrophylla* 'Rosea' that were treated with IBA than cuttings not treated.

Other factors that affected root numbers in 'Ashanti Blood' were substrate AFP and substrate temperature (Table 2). For 'Ashanti Blood', a two-dimensional contour plot for the effect of IBA and rooting substrate AFP on root numbers shows that root numbers will be the highest with 15 mmol IBA and perlite (43% AFP) (Fig. 1). When the temperature of the medium was at 29 °C (84 °F), the number of roots increased with increasing concentrations of IBA (Fig. 2).

Air-filled porosity had a quadratic effect on the root numbers of 'Rosea' (Table 2). For this cultivar, root numbers were highest with perlite (data not shown). Although no ideal rooting medium exists, perlite may have a physical composition conducive to rooting this cultivar. Factors that significantly affected root numbers in *M. philippica* 'Aurorae' were date of propagation and temperature of the rooting substrate (Table 2). Seasonal variations in stock plant environment can affect adventitious rooting (Moe and Anderson, 1988), and for 'Aurorae', the largest number of roots per cutting were on cuttings taken on 28 Dec. 1993 and rooted in substrate maintained at 29 °C (Fig. 3). The contour plot trends show that approximately 100 roots per cutting are possible (Fig. 3). Our data indicates that each cut-

ting of 'Aurorae' averaged 20 roots at 30 d. In contrast, cuttings of 'Ashanti Blood' and 'Rosea' averaged 7 and 9 roots, respectively for the same period.

Averaged over all treatment combinations, root length was 4, 12, and 4 mm (0.2, 0.5, and 0.2 inch) in 'Ashanti Blood', 'Aurorae', and 'Rosea'. Strategies to increase root number and root length must be developed for 'Ashanti Blood' and 'Rosea'. For that reason, additional propagation studies have focused on techniques to improve root numbers in 'Ashanti Blood' (St. Hilaire, 1994), and 'Rosea' (St. Hilaire et al., 1996). Whether stem cuttings with increased root numbers are more likely to produce functional plants remains unknown.

Heating the substrate in which stem cuttings are rooted is a common horticultural practice (Hartmann et al., 1996). However, in 'Aurorae' the largest number of roots was produced in unheated [29 °C (84 °F)] media. Bottom heat was most beneficial for rooting 'Ashanti Blood' when IBA concentration was less than 8 mmol (Fig. 2), but bottom heat above 29 °C is dispensable when IBA levels exceed 8 mmol.

Averaged over all treatments, the rooting percentage (at 30 d) in 'Ashanti Blood', 'Aurorae', and 'Rosea' was 22%, 48%, and 39% respectively. The relatively low rooting percentages combined with the wide variation in conditions required for rooting suggest that the commercial propagation of these three cultivars of *Mussaenda* may be difficult. Other methods of clonal propagation need to be evaluated before uniform rooted cuttings of *Mussaenda* can be produced economically. On the other hand, growers willing to try the propagation of cultivars of *Mussaenda* from stem cuttings should evaluate rooting after 30 d of propagation. In addition, cuttings may be taken from any position on the stock plants and porous media, such as perlite, kept at 29 °C could be used to root stem cuttings if greater than 8 mmol of IBA are used to root cuttings.

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