Research Reports

Optimal Nitrogen Concentration and Rapid Nutritional Diagnosis of Nitrogen Requirement for Container Production of Malabar Chestnut

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ADDITIONAL INDEX WORDS. *Pachira macrocarpa*, money tree, fertilization, nutrition

SUMMARY. Nitrogen (N) is a major element required for crop cultivation and an important factor affecting plant growth and development. Malabar chestnut (*Pachira macrocarpa*) is an important ornamental potted plant crop whose N requirement has been studied, and a rapid monitoring method to manage N fertilization during its commercial production is yet to be established. Malabar chestnut seedlings were fertilized weekly with 0, 4, 8, 16, or 24 mM N. After 12 weeks, 16 mM N was found to yield the greatest plant growth such as plant height, number of nodes, and total leaf area. Measurements of chlorophyll meter readings, leaf chlorophyll concentration, leaf N concentration, and leaf dry weight all indicated that the optimal level of N fertilization was 16 mM N. A chlorophyll meter can be used to monitor nondestructively whether sufficient N has been supplied to support optimal plant growth. In this study, a chlorophyll meter reading of 46.1 corresponded with a critical leaf N concentration of 2.65%, defined as the leaf N concentration when the leaf dry weight was at 90% of saturation point. Additional N supplied beyond this critical level increased foliar chlorophyll content and improved the rate of net photosynthesis. Therefore, chlorophyll meter readings, which are convenient and nondestructive can serve as a reliable reference for commercial production in monitoring N requirement for optimum growth of malabar chestnut. Weekly fertilization of malabar chestnut with 16 mM N and maintaining leaf chlorophyll meter readings between 46.1 and 58.4 are recommended.

Malabar chestnut is an essential crop and ornamental potted plant in Taiwan with a gross export value of over \$5 million

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in 2013 (Customs Administration, Republic of China, 2015). It is native to southern Mexico, Guyana, and northern Brazil, on the flooded riverbanks of the western Amazon. In its native habitat, it grows under full sunlight or partial shade and can reach 18 m in height (Robyns, 1964). In addition to being a specialty food crop, malabar chestnut has been introduced as a tropical ornamental foliage plant. Trees with large trunks can be planted individually in containers while small trees can be grown together and braided as potted foliage plants for interiorscaping. Because of its swollen stem base and flexible branches and stem, malabar chestnut is also grown as a bonsai or pseudo bonsai tree. In East Asia, the species is known as the “money tree” and is believed to afford financial fortune and to improve business (Li et al., 2009).

Nitrogen is an essential element for plant growth and influences plant quality, by affecting leaf weight, leaf area, leaf chlorophyll content, and plant size (Bar-Tal et al., 2001; Wang et al., 2012). N deficiency is commonly caused by inadequate fertilization regimes in which N supply is low to fulfill the requirements of plant growth at specific developmental stages (Locke et al., 2011). However, the application of excessive amounts of N is non-economical and contributes to environmental pollution. Soil analysis and measurements of plant N are often time-consuming, labor-intensive, and destructive to the plant. Because of the strong correlation between chlorophyll meter readings and chlorophyll content, the chlorophyll meter is increasingly used in plant cultivation for monitoring relative N content; e.g., rose [*Rosa chinensis* (Zanin and Sambo, 2006)] and apple [*Malus domestica* (Neilsen et al., 1995)]. The

Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
29.5735	fl oz	mL	0.0338
0.3048	ft	m	3.2808
2.54	inch(es)	cm	0.3937
25.4	inch(es)	mm	0.0394
6.4516	inch ²	cm ²	0.1550
1	mmho/cm	dS·m ⁻¹	1
28.3495	oz	g	0.0353
28,350	oz	mg	3.5274 × 10 ⁻⁵
0.001	ppm	mg·g ⁻¹	1000
1	ppm	mg·L ⁻¹	1
(°F–32) ÷ 1.8	°F	°C	(°C × 1.8) + 32

objectives of this study were to determine the optimal N concentration for malabar chestnut during production and to monitor the N concentration of plants by using a chlorophyll meter to establish a practical and simple N management method for commercial production of malabar chestnut.

Materials and methods

PLANT MATERIAL. For our study, fresh seeds from the supplier were sown in a naturally ventilated greenhouse in May 2013 [$\approx 300 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ photosynthetic photon flux (*PPF*), $\approx 31^\circ\text{C}$]. The substrate used was a mixture of 80% Canadian sphagnum peatmoss and 20% perlite (Growing Mix 1-P; Fafard, St. Modeste, Quebec, Canada). In June 2013, 1-month-old malabar chestnut seedlings, with a collar diameter of 6 to 7 mm and a height of 15 cm were collected and transplanted into 9-cm-diameter, 180-mL plastic containers using the same substrate. The experiment was conducted in a greenhouse under natural light conditions (day length ≈ 11 h) at the Experimental Farm, National Taiwan University. Average air temperature was 36°C during the day and 29°C at night, with a mean relative humidity of $\approx 75\%$.

FERTILIZER TREATMENTS. N treatments, consisting of five N concentrations (0, 4, 8, 16, and 24 mM) were applied weekly to the substrate of five groups of malabar chestnut seedlings. The fertilizer solutions were adapted from Hoagland and Arnon (1950) and their compositions are shown in Table 1. The electrical conductivities of the fertilizer solutions containing 0, 4, 8, 16, and 24 mM were 0.8, 1.0, 1.2, 1.6, and $2.0 \text{ dS}\cdot\text{m}^{-1}$, respectively, while the pH values were 6.37, 6.36, 6.35, 6.31, and 6.24, respectively. Each pot was fertigated with 50-mL fertilizer solution. Three days after each fertilizer application, 50-mL micronutrient solution containing $50 \mu\text{M}$ potassium chloride (KCl), $25 \mu\text{M}$ boric acid (H_3BO_3), $2 \mu\text{M}$ manganese sulfate ($\text{MnSO}_4\cdot 7\text{H}_2\text{O}$), $2 \mu\text{M}$ zinc sulfate ($\text{ZnSO}_4\cdot 5\text{H}_2\text{O}$), $0.5 \mu\text{M}$ copper sulfate ($\text{CuSO}_4\cdot 5\text{H}_2\text{O}$), $0.5 \mu\text{M}$ molybdic acid (H_2MoO_4), and $20 \mu\text{M}$ iron-ethylenediaminetetraacetic acid (Fe-EDTA) was applied to each pot.

DATA COLLECTION. Each group contained six single-plant replicates with similar heights and collar diameters. Twelve weeks after fertilizer treatments, plants were measured for plant height and the number of nodes including cotyledonal node was determined for each plant. In each plant, the largest single leaf on the third node from the top was chosen for the following measurements.

Chlorophyll meter readings, averaged from measurements at six locations on each leaf, were obtained with a chlorophyll meter (SPAD-502; Minolta, Osaka, Japan). Leaf chlorophyll *a* and chlorophyll *b* were measured spectrophotometrically in the laboratory in 80% acetone extracts following Lichtenthaler and Wellburn (1983), using a spectrophotometer (U-2800; Hitachi, Tokyo, Japan).

Photosynthesis and stomatal conductance (g_s) were measured with a portable photosynthesis system (LI-6400; LI-COR, Lincoln, NE). Measurements were carried out at 25°C with air carbon dioxide (CO_2) concentration of $380 \mu\text{mol}\cdot\text{mol}^{-1}$. Irradiance was reduced in a stepwise manner with *PPF* values ranging from 0 to $1000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Measurements were made once the leaf attained a steady net CO_2 fixation rate. Steady-state modulated chlorophyll fluorescence of single attached leaves was measured using a portable fluorimeter (Mini-PAM; Walz, Effeltrich, Germany) with a far-red source adapter. This device supplies $8 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of far-red light (700–750 nm), and together with the portable fluorimeter allowed measurements of chlorophyll fluorescence parameters, saturating pulse analysis detection and automatic calculation of fluorescence parameters of leaves, such as the fluorescence emission signal, minimum fluorescence signal (F_0), variable fluorescence signal (F_v), the maximum fluorescence signal (F_m), and the maximum quantum efficiency of photosystem II photochemistry (F_v/F_m). The F_v/F_m of chlorophyll fluorescence was estimated according to Genty et al. (1989). Measurements of F_v/F_m were made on leaves that were dark-adapted for 30 min.

Leaf area was measured with a portable leaf area meter (LI-3000, LI-COR). After the above measurements,

plants were harvested to determine leaf dry weight and N concentration. For elemental analysis of N, leaf tissue samples weighing 2 to 4 mg were packed into tin cups (8×5 mm) and analyzed with a nitrogen/carbon analyzer (Flash EA1112 Series; Thermo Fisher Scientific, Rodano, Italy). Combustion and reduction temperatures were 900 and 680°C , respectively. Atropine ($\text{C}_{17}\text{H}_{23}\text{NO}_3$; Sigma-Aldrich, St. Louis, MO) was used to standardize calibrations and system checks for the elemental analyzer.

STATISTICAL DESIGN AND ANALYSIS. The experiments were carried out in a completely randomized design, with six replications for each treatment. Data were analyzed using the statistical program Costat (version 6.29; CoHort Software, Berkeley, CA) and SigmaPlot software (version 8.0; Systat Software, San Jose, CA) was used for graphing and regression analysis. Both linear and polynomial regression models were fit to the data and significance of the models determined.

Results

After 12 weeks of treatment, the group fertilized with 16 mM N exhibited the largest increase in plant height (Fig. 1A), number of nodes (Fig. 1B), and the largest total leaf area and sampled leaf dry weight (Fig. 1D and E). Plant height, number of nodes, total leaf area, and sampled leaf dry weight showed quadratic response toward fertilizer N concentration (Fig. 1). Chlorophyll fluorescence was lower in the control plants (i.e., those given 0 mM N), but remained at normal levels in plants treated with N (Fig. 2A). Chlorophyll concentration and chlorophyll meter readings showed a similar trend, whereby they increased significantly as fertilizer N concentration increased (Fig. 2B and C). There is a significant correlation between chlorophyll meter reading and leaf chlorophyll concentration (Fig. 3). Net photosynthetic rate (P_n) did not significantly differ among treatments when the light intensity was under $400 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. At increasing light intensities (from 0 to 800 and $1000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), P_n increased and was significantly lower in plants fertilized at 0 mM (Fig. 4A). At light intensities below $100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, the 0 mM N treatment resulted in

Table 1. Concentrations of major nutrients in nitrogen (N) treatment solutions applied to malabar chestnut (adapted from Hoagland and Arnon, 1950)^a.

N treatment (mM)	Concn (mM) ^a					
	NH ₄ NO ₃	MgSO ₄ ·7H ₂ O	CaCl ₂ ·2H ₂ O	K ₂ SO ₄	Ca(H ₂ PO ₄)·2H ₂ O	CaSO ₄ ·2H ₂ O
0	0	1	2	3	1	1
4	2	1	2	3	1	1
8	4	1	2	3	1	1
16	8	1	2	3	1	1
24	12	1	2	3	1	1

^aAmmonium nitrate (NH₄NO₃); magnesium sulfate heptahydrate (MgSO₄·7H₂O); calcium chloride dihydrate (CaCl₂·2H₂O); potassium sulfate (K₂SO₄); monocalcium phosphate dihydrate [Ca(H₂PO₄)·2H₂O]; calcium sulfate dihydrate (CaSO₄·2H₂O).

Fig. 1. Effect of nutrient solution nitrogen (N) concentration on plant height (A), node number (B), collar diameter (C), total leaf area (D), and sampled leaf dry weight (E) of malabar chestnut. Bars represent SE (n = 6). **, ***Significant at $P \leq 0.01$ or $P \leq 0.001$, respectively; 1 cm = 0.3937 inch, 1 mm = 0.0394 inch, 1 cm² = 0.1550 inch², 1 g = 0.0353 oz.

Fig. 2. Effect of nutrient solution nitrogen (N) concentration on maximum quantum efficiency of photosystem II photochemistry [F_v/F_m (A)], chlorophyll meter readings (B), chlorophyll *a* + *b* (C), and leaf N concentration (D) of malabar chestnut. Bars represent SE (n = 6). **, *** Significant at $P \leq 0.01$ or $P \leq 0.001$, respectively; $1 \text{ mg} \cdot \text{g}^{-1} = 1000 \text{ ppm}$.

lower g_s in control plants (Fig. 4B). As light intensity increased from 0 to $100 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, intercellular CO_2 (C_i) level dropped significantly. C_i levels differed significantly between plants treated with N treatments and control plants (Fig. 4C).

Based on the response curve between leaf N concentration and sampled leaf dry weight, the maximum leaf dry weight response, calculated as 1.85 g, occurred when leaf N concentration was 4.25% (Fig. 5A). A critical N concentration is calculated based on 90% of the maximum dry weight (Ulrich and Hills, 1990), which is 1.66 g, and thus would be 2.65%. Therefore, when the N concentration in a leaf drops below 2.65%, N supply would be insufficient, requiring immediate replenishment to achieve appropriate growth. When the critical concentration of N was set at 2.65%, the corresponding chlorophyll meter reading was 46.1 (Fig. 5B).

Discussion

Insufficient N supply reduces plant growth and leaf area and resulted in a decrease in the rate of photosynthesis and leaf N concentration (Boussadia et al., 2010; Munoz et al., 2005). In this study, there is a definite difference in plant height, node number, and total leaf area as early as 8 weeks after treatment in 0 mM N (data not shown). Chlorophyll fluorescence represents the maximum quantum yield of PS II. In our experiment, the F_v/F_m values did not significantly differ between N treatments (4 to 24 mM), indicating that the electron transfer chain is unaffected by N concentration.

The N requirements of ornamental plants differ among species. Commercial fertilizer recommendations for zonal geranium (*Pelargonium hortorum*) range from 200 to 250 $\text{mg} \cdot \text{L}^{-1}$ N (Whipker, 1998), and lower for peace lily (*Spathiphyllum* 'Petite') or higher for dumbcane (*Dieffenbachia* 'Camille') (Campos

Fig. 3. Correlations between chlorophyll meter reading and chlorophyll *a* + *b* concentration in malabar chestnut grown with various nutrient solution nitrogen concentrations. * Significant at $P \leq 0.05$; $1 \text{ mg} \cdot \text{g}^{-1} = 1000 \text{ ppm}$.

and Reed, 1993). The optimal N level determined for malabar chestnut in our experiment (16 mM) equals $224 \text{ mg} \cdot \text{L}^{-1}$, which coincides with the recommended N level (200–300 $\text{mg} \cdot \text{L}^{-1}$) for poinsettia (*Euphorbia pulcherrima*) proposed by Ecke et al.

Fig. 4. Relationships between photosynthetic photon flux (PPF) and net photosynthetic rate [P_n (A)], stomatal conductance [g_s (B)], and intercellular carbon dioxide (CO_2) concentration [C_i (C)] of leaves in malabar chestnut. Bars represent SE ($n = 6$).

(2004). We additionally found that the largest malabar chestnut collar diameter was obtained at 8 mM N,

although both the greatest plant height and nodal number were obtained at 16 mM N. Therefore, when

Fig. 5. Relationships between leaf nitrogen (N) and sampled leaf dry weight (A) and chlorophyll meter readings (B) of malabar chestnut. ***Significant at $P \leq 0.001$; 1 g = 0.0353 oz.

maximum plant growth is the primary goal, a 16 mM N concentration is recommended. When the goal will be to increase collar diameter, a reduced N concentration is recommended for obtaining a strong and compact plant.

Chlorophyll content is a reliable index for assessing growth status and leaf N levels of plants. When leaf N is insufficient, chlorophyll meter readings will decline (Hardin et al., 2012; Mak and Yeh, 2001; Seemann et al., 1987; Wang et al., 2012). Chlorophyll meter readings reflect foliage greenness, leading to a linear relationship between readings and chlorophyll content (Schaper and Chacko, 1991). Because N is a key component of chlorophyll, a clear correlation exists between chlorophyll meter readings and leaf N levels concentration (Kantety et al., 1996).

Conclusion

Based on the results of our experiment, weekly fertilization of malabar chestnut with 16 mM N (i.e., 224 ppm N) will be recommended. Chlorophyll meter readings, which are convenient and nondestructive, can serve as a reliable reference for monitoring the N requirement for optimal growth in the commercial production of malabar chestnut. A chlorophyll meter

reading range of 46.1 to 58.4 will be recommended.

Literature cited

- Bar-Tal, A., L. Karni, J. Oserovitz, A. Hazan, M. Itach, S. Gantz, A. Avidan, I. Posalski, N. Tratkovski, and R. Rosenberg. 2001. Nitrogen nutrition of greenhouse pepper. II. Effect of nitrogen concentration and $\text{NO}_3\text{:NH}_4$ ratio on growth, transpiration, and nutrient uptake. *HortScience* 36:1252–1259.
- Boussadia, O., K. Steppe, H. Zgallai, S.B. E.I. Hadj, M. Braham, R. Lemeur, and M.C. Van Labeke. 2010. Effects of nitrogen deficiency on leaf photosynthesis, carbohydrate status and biomass production in two olive cultivars ‘Meski’ and ‘Koroneiki’. *Sci. Hort.* 123:336–342.
- Campos, R. and D.W. Reed. 1993. Determination of constant-feed liquid fertilizer rates for *Spathiphyllum* ‘Petite’ and *Dieffenbachia* ‘Camille’. *J. Environ. Hort.* 11:22–24.
- Customs Administration, Republic of China. 2015. Trade statistics search—Value of exports and imports. 20 Apr. 2015. <<https://portal.sw.nat.gov.tw/APGA/GA03E>>.
- Ecke, P., III., J.E. Faust, A. Higgins, and J. Williams. 2004. The Ecke poinsettia manual. Ball Publ., Batavia, IL.
- Genty, B., J.B. Briatais, and N.R. Baker. 1989. The relationships between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochim. Biophys. Acta* 990:87–92.
- Hardin, J.A., M.W. Smith, P.R. Weckler, and B.S. Cheary. 2012. In situ measurement of pecan leaf nitrogen concentration using a chlorophyll meter and vis-near infrared multispectral camera. *HortScience* 47:955–960.
- Hoagland, D.R. and D.I. Arnon. 1950. The water-culture method for growing plants without soil. *California Agr. Expt. Sta. Circ.* 347.
- Kantety, R.V., E.V. Santen, F.M. Woods, and C.W. Wood. 1996. Chlorophyll meter predicts nitrogen status of tall fescue. *J. Plant Nutr.* 19:881–899.
- Li, Q.S., M. Deng, J.J. Chen, and R.J. Henny. 2009. Effects of light intensity and paclobutrazol on growth and interior performance of *Pachira aquatica* Aubl. *HortScience* 44:1291–1295.
- Lichtenthaler, H.K. and A.R. Wellburn. 1983. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochem. Soc. Trans.* 11:591–592.
- Locke, J.C., J.E. Altland, and D.M. Bobak. 2011. Seedling geranium response to nitrogen deprivation and subsequent recovery in hydroponic culture. *HortScience* 46:1615–1618.
- Mak, T.Y. and D.M. Yeh. 2001. Nitrogen nutrition of *Spathiphyllum* ‘Sensation’ grown in sphagnum peat- and coir-based media with two irrigation methods. *HortScience* 36:645–649.
- Munoz, A.A., C. Celedon-Neghme, L.A. Cavieres, and M.T.K. Arroyo. 2005. Bottom-up effects of nutrient availability on flower production, pollinator visitation, and seed output in a high-Andean shrub. *Oecologia* 143:126–135.
- Neilsen, D., E.J. Hogue, L.C. Herbert, P. Parchomchuk, and G.H. Neilsen. 1995. Use of rapid techniques for estimating the N status of fertigated apple trees. *Acta Hort.* 283:211–218.
- Robyns, A. 1964. Flora of Panama. Part VI. Family 116. Bombacaceae. *Ann. Missouri Bot. Gardens* 51:37–68.
- Schaper, H. and E.K. Chacko. 1991. Relation between extractable chlorophyll and portable chlorophyll meter readings in leaves of eight tropical and subtropical fruit-tree species. *J. Plant Physiol.* 138:674–677.
- Seemann, J.R., T.D. Sharkey, J. Wang, and C.B. Osmond. 1987. Environmental effects on photosynthesis, nitrogen-use efficiency, and metabolite pools in leaves of sun and shade plants. *Plant Physiol.* 84:796–802.
- Ulrich, A. and F.J. Hills. 1990. Plant analysis as an aid in fertilizing sugarbeet, p. 429–447. In: R.L. Westerman (ed.). *Soil testing and plant analysis*. 3rd ed. Soil Sci. Soc. Amer., Madison, WI.
- Wang, Y., B.L. Dunn, D.B. Arnall, and P.S. Mao. 2012. Use of an active canopy sensor and SPAD chlorophyll meter to quantify geranium nitrogen status. *HortScience* 47:45–50.
- Whipker, B.E. 1998. Fertility management for geraniums. North Carolina State Univ. Info. Lft. 504.
- Zanin, G. and P. Sambo. 2006. Using SPAD-meter in nitrogen fertilization of *Rosa chinensis* Jacq. var. *mutabilis*. *HortScience* 41:969–970.