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

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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 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)].

Units

| 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         | 3.208   |
| 2.54          | inch(es)                           | cm        | 0.0937  |
| 6.4516        | inch²                              | cm²       | 0.1550  |
| 1             | mmho/cm                            | dS·m⁻¹    | 1       |
| 28.3495       | oz                                 | g         | 0.0353  |
| 28,350        | ppm                                | mg·g⁻¹    | 1000    |
| 0.001         | ppm                                | mg·L⁻¹    | 1       |
| (°F–32) + 1.8 | °F                                 | °C        | (°C × 1.8) + 32 |

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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 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 ≈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 dS·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 μM potassium chloride (KCl), 25 μM boric acid (H₃BO₃), 2 μM manganese sulfate (MnSO₄·7H₂O), 2 μM zinc sulfate (ZnSO₄·5H₂O), 0.5 μM copper sulfate (CuSO₄·5H₂O), 0.5 μM molybdc acid (H₂MoO₄), and 20 μM 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 (1985), using a spectrophotometer (U-2800; Hitachi, Tokyo, Japan).

Photosynthesis and stomatal conductance (gs) were measured with a portable photosynthesis system (LI-6400; LI-COR, Lincoln, NE). Measurements were carried out at 25 °C with air carbon dioxide (CO₂) concentration of 380 μmol·mol⁻¹. Irradiance was reduced in a stepwise manner with PPFD values ranging from 0 to 1000 μmol·m⁻²·s⁻¹. Measurements were made once the leaf attained a steady net CO₂ 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 μmol·m⁻²·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 (F₀), variable fluorescence signal (Fᵥ), maximum fluorescence signal (Fₘ), and the maximum quantum efficiency of photosystem II photochemistry (Fₖ/Fₘ). The Fᵥ/Fₘ of chlorophyll fluorescence was estimated according to Genty et al. (1989). Measurements of Fᵥ/Fₘ 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 (C₁₇H₂₃NO₅; 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 (Pn) did not significantly differ among treatments when the light intensity was under 400 μmol·m⁻²·s⁻¹. At increasing light intensities (from 0 to 800 and 1000 μmol·m⁻²·s⁻¹), Pn increased and was significantly lower in plants fertilized at 0 mM (Fig. 4A). At light intensities below 100 μmol·m⁻²·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).  

| N treatment (mM) | NH₄NO₃ | MgSO₄·7H₂O | CaCl₂·2H₂O | K₂SO₄ | Ca(H₂PO₄)·2H₂O | CaSO₄·2H₂O |
|------------------|--------|------------|------------|--------|----------------|------------|
| 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          |

*Ammonium 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 ≤ 0.01 or P ≤ 0.001, respectively; 1 cm = 0.3937 inch, 1 mm = 0.0394 inch, 1 cm² = 0.1550 inch², 1 g = 0.0353 oz.
lower $g_c$ in control plants (Fig. 4B). As light intensity increased from 0 to 100 μmol·m$^{-2}$·s$^{-1}$, intercellular CO$_2$ (Ci) level dropped significantly. Ci 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 (Bouassadia 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 mg·L$^{-1}$ N (Whipker, 1998), and lower for peace lily (Spathiphyllum ‘Petite’) or higher for dumbcane (Dieffenbachia ‘Camille’) (Campos and Reed, 1993). The optimal N level determined for malabar chestnut in our experiment (16 mM) equals 224 mg·L$^{-1}$, which coincides with the recommended N level (200–300 mg·L$^{-1}$) for poinsettia (Euphorbia pulcherrima) proposed by Ecke et al.
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 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.

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