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Critical Nitrogen Level Determination in *Curcuma alismatifolia* Gagnep.

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Summary

We determined the critical nitrogen (N) level in field-grown *Curcuma alismatifolia* Gagnep. by applying N at a rate of 3.75, 7.5, 15, 30, and 60 g N plant^{-1} as urea. Plant growth and N critical levels were determined 105 d after planting (flowering stage). To establish critical N levels in plant tissue, the relationship between rhizome yield and the N concentrations in the first fully expanded leaf from the bottom of 1st order shoots as investigated. The rate of N application had a significant effect on growth, plant dry weight, leaf N concentrations, and leaf chlorophyll content. Leaf N concentrations were similar for all treatments up to 30 g N plant^{-1}, but increased when N was applied at 60 g N plant^{-1}. Rhizome yields increased with increasing leaf N up to about 1–1.5 % N, but were relatively constant at a higher leaf N. The critical leaf N level response to 90 % of the maximum yield was 1.51 %. Most N concentrations in the various tissues increased with increasing N supply. The method for determining critical N levels provides an accurate description of the relationship between leaf N and rhizome yield and may be used by growers to predict the N requirement of *Curcuma* plants.

Key words. critical nitrogen level – *Curcuma alismatifolia* – leaf analysis – nitrogen status

Introduction

The potential impact of agricultural pollution particularly that of N, has become a major concern during the last few decades. The extent of N emissions can often be reduced substantially by appropriate fertilizer application regimes (Janzen et al. 2003). *Curcuma alismatifolia* Gagnep. is an ornamental plant from Thailand, exports of which total more than four million rhizomes per year on the world market. Nitrogen fertilizers used in *C. alismatifolia* production are important since growth, flower quality, and rhizome yields respond to the amount of N applied, and a deficiency of N can substantially reduce yields (Ohtake et al. 2006). However, no detailed information is available concerning the relationship between tissue nutrient concentrations and yields of *Curcuma*.

With regard to the application of the total recommended amount of N fertilizer at planting, it is generally agreed that the establishment of a provisional field-specific N recommendation for *Curcuma* at planting time can never be accurate. It is impossible to accurately predict total crop N requirements and soil mineral N supply during the growing season (Vos and Mackerron 2000). Such variables are influenced by several predictable or unpredictable factors, such as weather conditions, chemical and physical soil properties, type and evolution of organic matter previously accumulated in the soil, cultural practices, maturity of the variety, and crop duration. N fertilization strategies that combine splitting of field specific N fertilizer recommendations with the assessment of crop N requirements during the growing season can help to better match needs with supply; as a consequence N fertilizer efficiency can be improved (Vos and Mackerron 2000; Alva 2004). Such an approach also allows better cover age of large variations in optimal N for *Curcuma* crops among growing seasons (related to weather), within season (related to region, soils, and crop management), among fields of the same farm (related to crop rotation and cropping practices), and within specific fields (related to variable N supply and other variable soil characteristics).

Adjusting the N input to an economically and ecologically compatible level requires reliable information on *Curcuma*. An ideal indicator of crop N status should be able to detect deficiencies and excesses of N supply and provide a fast diagnosis to allow for corrections in the same growing season. Information on N status can be obtained either from the crop side or the soil side. A common definition of the critical level in the literature is the tissue nutrient concentration corresponding to 90 % of

the maximum yield when only that nutrient limits growth (Ulrich 1952). The concept of critical N has been successfully applied to various crops. In a study of fish geranium (*Pelargonium hortorum* Bailey), the critical level was defined as the percentage dry weight of a mineral element in leaves at the onset of visual deficiency symptoms (Kofranek and Lunt 1969). Evans et al. (2008) defined the critical level of *Euonymus fortunei* 'Colorata' grown in non-leached systems by using the equation for the Mitscherlich fertilizer yield function. Since plant species and cultivars within species have been found to vary in response to nutrient levels in the soil, appreciable errors may occur result if critical concentrations determined for one cultivar were used for another. Therefore, the objective of this study was to determine the critical leaf N level in field-grown *C. alismatifolia* Gagnep. based on a mathematically established, predictable, and functional relationship between yield and nutrient concentration. This insight can be integrated in the development of new fertilizer strategies to decide on the need for supplementary fertilizer N application as a top dressing. This approach has not yet been used for determining critical levels in *Curcuma* plants.

Materials and Methods

This experiment was based on field experiments conducted in northern Thailand as a part of H.M. The King's Initiative Centre for Flower and Fruit Propagation. The soil was a grey sandy loam and the chemical analysis of the soil before planting can be seen in Table 1. The experiment was set up in a randomized complete block design with five N treatments and three blocks. The treatment was 3.75, 7.5, 15, 30, and 60 g N plant^{-1} (designated respectively: N1, N2–N5 in the text) applied as urea. Other essential elements were supplied equally for plants in each treatment group as = 15 g P_2O_5, 20 g K_2O, 0.25 g $MgSO_4$, and 0.54 g $CaSO_4$ per plant.

Rhizomes of *C. alismatifolia* 'Chiang Mai Pink' with a diameter of 1.8–2.5 cm and 4 storage roots were planted by hand to ensure uniform spacing, the planting depth being about 10 cm and the distance between plants along the row being 30 cm. Two-row beds were used, the distance between rows being approximately 30 cm. The planting rate represents a density of approximately 62,500 plants ha^{-1}. Crop water requirements were completely satisfied by sprinkler irrigation and pests and diseases were completely controlled by chemical treatments.

Crop development was followed by sampling 105 d after planting (flowering stage). On the day of sampling, the plants were separated into rhizomes and shoots arising from various new rhizomes. The numbers of shoots and new rhizomes of each cluster (group of new shoots which come from one mature rhizome) were counted. Chlorophyll content (SPAD value) as measured using a SPAD meter (SPAD-502, Minolta, Japan) and leaf area was measured using a leaf area meter (LI-3100, LI-COR, Lincoln, NE). Fresh and dry weights of various plant parts were obtained, with samples being dried in paper bags in an oven at 60 °C until dry mass stable. The N concentration was determined by a modified indophenol method using a Kjeldahl digested solution (Ohyama et al. 1991).

Table 1. Chemical analysis of soil from an experimental area at Chiang Mai, Northern Thailand.

Element	Value obtained	Interpretation
Total Nitrogen	0.06 %	Low
Available Phosphorus	161 mg kg^{-1}	Probably adequate
Exchangeable Potassium	68 mg kg^{-1}	Low
Exchangeable Calcium	965 mg kg^{-1}	Adequate
Exchangeable Magnesium	44 mg kg^{-1}	Low
pH (1: 2 soil: water)	6.5	pH 6.5 recommended[a]

[a] Whiley (1974)

For the determination of critical tissue N levels, rhizome dry weight was used as a yield parameter related to the N concentration of the leaves. A curve was visually fit to the data using an equation for the Mitscherlichs fertilizer yield function (Ware et al. 1982), which results in the following model:

$$y = \beta(1-\gamma\, e^{-\alpha x}) \quad [1]$$

where y represents the net dry weight yield at a tissue N concentration x, β is the asymptotic maximum yield, and α is the constant of proportionality. The term γ is the y-intercept when the N concentration is zero, such that for $x = 0$, $\gamma = (\beta - y_0)/\beta$. Observed data were used to estimate the α, β, and γ parameters. To find the tissue nutrient concentration associated with a 90 % maximum yield let

$$y/\beta = 1-\gamma\, e^{-\alpha x} \quad [2]$$

where $y/\beta = 0.9$ the solution of Eq. [2] for the critical nutrient level(x), yields:

$$x = -\ln (0.1/\gamma)/\alpha \quad [3]$$

Data for the leaf area, leaf chlorophyll content, shoot growth, and biomass were subjected to an analyses of variance (ANOVA) using generalized linear models by means of the Statistic 8 analytical software package (SXW Tallahassee, FL). The significance of the treatment effects presented as ns, not significant; *$P < 0.05$. In the case of significant treatment effects, the comparison of means was performed by LSD at a significance level of 0.05. A linear regression analysis was performed with SigmaPlot version 11 (Systat Software Inc., San Jose, CA).

Results

As shown in Table 2, after 105 d of planting and application with different N rates, the lowest plant height (28.7 cm) and lowest number of new shoots per cluster (3.5) were found in plants that received the N5 treatment (60 g N plant^{-1}). The highest leaf areas were observed with the N2 treatment (7.5 g N plant^{-1}) and the N3 treat-

Table 2. Effect of applied N on plant height, number of new shoots per cluster, and leaf area of *C. alismatifolia* 'Chiang Mai Pink' at 105 d after planting.

Treatment	Applied N (g plant^{-1})	Plant height (cm)	No. of new shoots per cluster	Leaf area (cm^2)
N1	3.75	32.2 a	5.8 a	915.0 bc
N2	7.50	31.8 a	5.7 a	1129.6 a
N3	15.00	31.8 a	5.8 a	1011.8 ab
N4	30.00	30.9 a	4.3 a	779.5 c
N5	60.00	28.7 b	3.5 b	275.2 d
Significance		*	*	*

(*) Significant effect at P < 0.05. Within each column, the same letters indicate no significant difference between treatments (P < 0.05).

Table 3. Effect of applied N on *C. alismatifolia* 'Chiang Mai Pink' aboveground and belowground dry weight, and whole plant dry weight at 105 d after planting.

Treatment	Applied N (g plant^{-1})	Dry weight (g)		
		Aboveground parts	Belowground parts	Whole plant
N1	3.75	20.0 b	4.8 a	24.7 a
N2	7.50	22.9 a	4.6 a	27.5 a
N3	15.00	21.6 ab	4.3 a	25.9 a
N4	30.00	15.9 c	2.6 b	18.5 b
N5	60.00	7.4 d	1.2 c	8.5 c
Significance		*	*	*

(*) Significant effect at P < 0.05. Within each column, the same letters indicate no significant difference between treatments (P < 0.05).

ment (15 g N plant^{-1}). The effect of applied N on dry weight was significant for the aboveground parts, belowground parts, and the whole plant (Table 3). The highest dry weight for aboveground parts was 22.9 g with the N2 treatment, and the lowest was 7.4 g with the N5 treatment. The belowground dry weight was divided by a factor 2 between N1 and N5 treatment. The N1–N3 treatment gave higher belowground dry weights than the N4–N5 treatments, and, as aboveground, belowground dry weight was divided by two between N4 and N5. The lowest total plant dry weights were obtained when plants received N5 treatment.

There was no significant difference in leaf N concentrations among N treatments with the exception of the 60 g N plant^{-1} (Table 4). The N concentration of the first fully expanded leaf was relatively constant at applied N amounts of less than 30 g N plant^{-1}, but increased when the amount of applied N was at 60 g N plant^{-1}. Leaf chlorophyll content decreased when the applied N decreased.

Differences in N concentrations were found among N treatments and individual plant parts (Fig. 1). Most N concentrations in various tissues increased when increasing N was applied from N1 to N5. There was a strong positive correlation between N concentrations and applied N in the belowground parts (Fig. 1C), the first leaf from the bottom of 1st order shoots (Fig. 1E), whole 2nd order shoots (Fig. 1I), and whole 3rd order shoots (Fig. 1J).

Table 4. Effect of applied N on N and chlorophyll levels in the first leaf from the bottom of *C. alismatifolia* 'Chiang Mai Pink' at 105 d after planting.

Treatment	Applied N (g plant^{-1})	Leaf N (% DW)	Leaf chlorophyll (SPAD units)
N1	3.75	1.37 b	43.2 d
N2	7.50	1.38 b	50.9 c
N3	15.00	1.44 b	55.7 bc
N4	30.00	1.61 ab	61.0 ab
N5	60.00	1.92 a	63.6 a
Significance		*	*

(*) Significant effect at P < 0.05. Within each column, the same letters indicate no significant difference between treatments (P < 0.05).

Higher concentrations of N were observed in belowground parts and old rhizome parts than in other tissues.

The total rhizome dry weight increased sharply with increasing N concentrations in the first fully expanded leaf at leaf N values of 0.91 % to 1.48 % (Fig. 2). Above

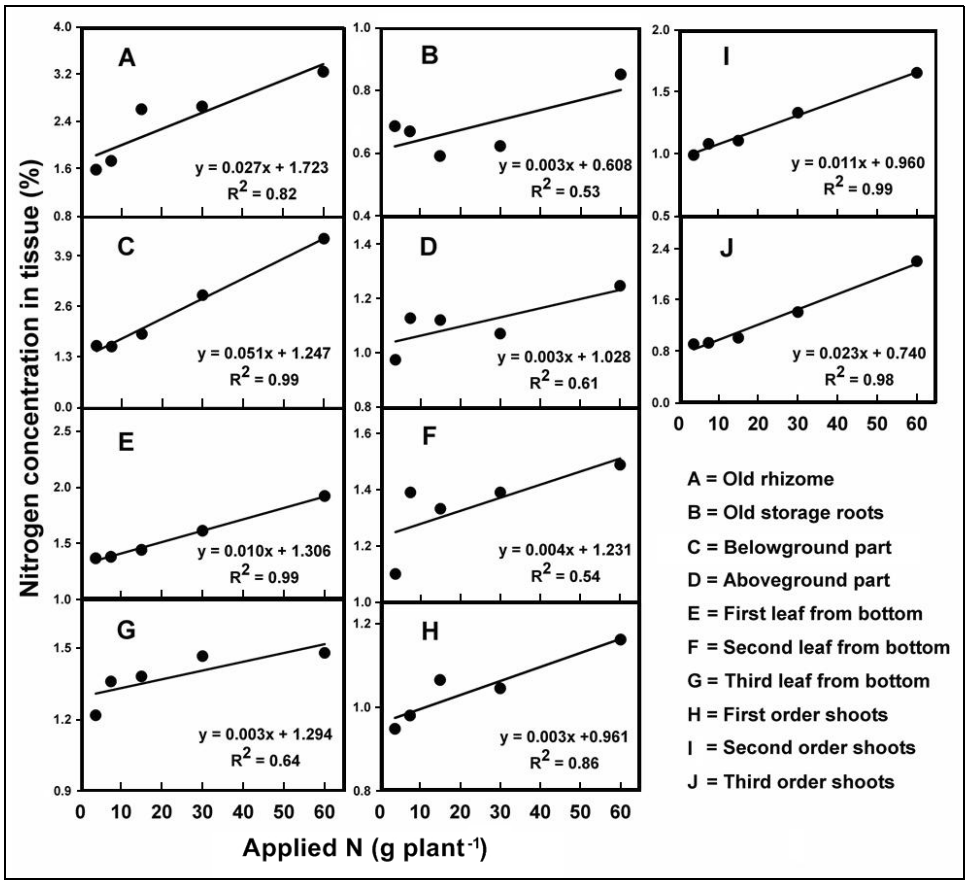


Fig. 1. Effect of nitrogen supply on nitrogen concentration in selected plant parts of *C. alismatifolia* 'Chiang Mai Pink' at 105 d after planting.

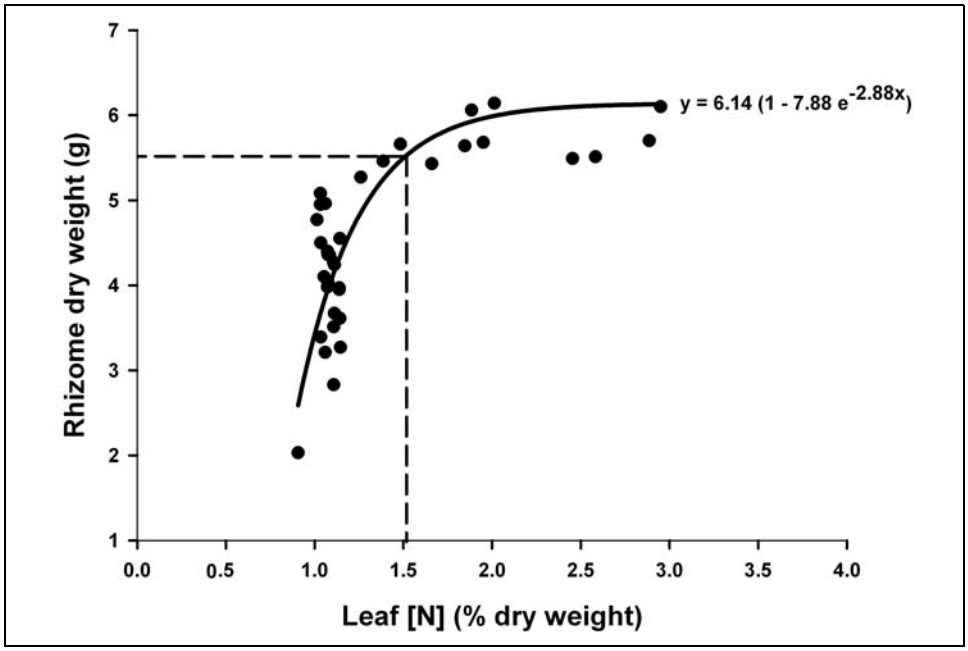


Fig. 2. The relationship between leaf N concentrations of *C. alismatifolia* 'Chiang Mai Pink' and rhizome dry weight per plant at 105 d after planting. The solid line corresponds to the Mitscherlich equation fit to the data. Dotted lines indicate the leaf N concentration corresponding to 90 % of the maximum yield.

that concentration, rhizome dry weight gain did not increase appreciably in relation to leaf N. The fit of the Mitscherlichs function to these data is $y = 6.14\ (1-7.88\ e^{-2.88x})$. The leaf critical N level corresponding to a 90 % maximum yield was 1.51 % N in the first fully expanded leaf (Table 5).

Discussion

Several plant phenotype characteristic decreased when *C. alismatifolia* 'Chiang Mai Pink' received the N5 fertilizer treatment, 10 % for plant height, 39 % for number of shoots per cluster, 76 % for leaf area, and 69 % for plant

Table 5. Estimated parameter and nitrogen critical level for the Mitscherlich model used to characterize the relationship between leaf N concentration and rhizome dry weight of *C. alismatifolia* 'Chiang Mai Pink' at 105 d after planting.

Parameter	Values
α	2.88
β	6.14
γ	7.88
Nitrogen Critical level	
Model	1.51

The equation is: $y = 6.14\ (1 - 7.88\ e^{-2.88\ x})$.

dry weight compared to values for plants that received the N2 fertilizer treatment. This shows that the highest N application rate was supra-optimal for growth of *Curcuma*. A similar response was previously reported for celery, lettuce, broccoli, and pepper (Tremblay et al. 1987; Tremblay and Senécal 1988). In plants grown with high fertilization rates, part of the N uptake is not immediately assimilated. The excess of N can be stored in different chemical forms, comprising simple inorganic and complex organic compounds in plant tissues (Brégard et al. 2000). In this experiment, extremely high levels of N fertilizer were experimentally supplied to derive the data of leaf N concentration in a toxic zone. However, N fertilizer at 3.75 g N plant^{-1} seemed to be in the marginal range between deficient and adequate ranges because it significantly decreased only the total leaves area, aboveground parts dry weight, and leaf chlorophyll (Table 2–4). Therefore, further experiments should be performed in the deficient range to fulfill the standard curve in Fig. 2.

Critical levels determined with various plant parts and fertilizer systems often differ greatly (Westerveld et al. 2003). In this experiment, the first fully expanded leaf from the bottom of the 1st order shoot was used to establish the critical N level since there was a strong correlation between N concentrations in the first fully expanded leaf and the amount of N applied (Fig. 1E). Leaves are also a major site of carbohydrate and mineral storage (Embleton et al. 1973). Consequently, the mineral status of the leaves not only influences the efficacy of photosynthesis, but also reflects the nutrient status of the plant. Although other organs within the plant may act in a similar manner, the leaf is the most readily available source of tissue for analysis. Jones (1972) considered that mature leaves just below the growing tip should be sampled, preferably at the flowering stage. Previous reports showed that leaf N dominantly translocates to the new rhizome from the flowering stage, and continuous supply of N fertilizer from a flowering to pre-harvest rhizome is very important in promoting growth and development of *C. alismatifolia* (Ruamrungsri et al. 2006; Khuankaew et al. 2010). Therefore, we used the flowering stage (105 d after planting) to adjust fertilizer application in order to promote rhizome yield.

In this experiment, growth curves relating to the rhizome yield with leaf N concentrations were preliminary described in mathematical terms based upon a hypothesis underlying the growth process. The estimates of parameters were obtained by solving Eq. [1], and the estimates of critical N level were obtained by solving Eq. [3] (Table 5). The leaf critical N level for *C. alismatifolia*, calculated using the Mitscherlichs model and expressed as a percentage of leaf dry weight, was 1.51 %. Recommended optimum leaf tissue N concentration ranges have been published for some related species based on numerous fields sampling in nurseries. The optimum leaf N concentration range reported for the container-grown spindle tree (*Euonymus japonicus* L.) is 1.05 % to 2.32 %, and that for container- and field-grown winged euonymus (*Euonymus alatus* Sieb.) is 2.37 to 2.62 % (Mills and Jones 1996). In our experiment, the N concentration of the first fully expanded *C. alismatifolia* 'Chiang Mai Pink' leaf that corresponded to maximum yields was 2.01 % (Fig. 2), in the range of 1.8 to 3.5 % leaf N reported for maximum growth of some other ornamental crops (Gilliam and Wright 1977; Stratton et al. 2001; Cabrera 2003). Expression of dry weight as a function of the first fully expanded leaf N concentration is not reported commonly in the literature for *Curcuma* plants. However, this relationship has been expressed for several agronomic crops (Ulrich 1949). The most common discussion of critical N levels centers on maximum net dry weight accumulation as a function of applied N (Griffin et al. 1999).

According to Johnson and Ulrich (1959), a sensitive method for critical level determination will also result in a sharp transition from the deficiency to the adequacy zone on the critical level curve, a result consistent with the observations of this experiment. This sharp transition provides a confined region of the graph corresponding to 90 % maximum growth, and an increased probability of determining the actual critical level. In addition, careful attention must be given when applying the growth model to a nutrient calibration curve which exhibits toxicity or the Piper-Steenbjerg effect; a negative relationship between growth and internal nutrient concentration (Wikstrom 1994). The experimental point giving rise to these effects must usually be deleted from the data set before attempting to fit the model (Ware et al. 1982). In our experiment, the data from the N4 and N5 treatments were deleted before fitting with the model since those treatments decreased plant growth and rhizome yields. At low leaf N concentrations in *Curcuma*, they were relatively constant over a wide range of dry weight rhizome yields (Fig. 2). Above the critical level, on the other hand, there is great variation in leaf N concentrations and little variation in yield. This sharp change in the relationship allows for a reasonably precise measure of N sufficiency in the plant.

In conclusion, a field – growing system with known N concentrations allowed for analysis of plant N status and determination of the first fully expanded leaf critical N level of 1.51 % in *C. alismatifolia* 'Chiang Mai Pink' at 105 d after planting. Knowledge of critical levels from leaf analysis must be integrated with soil analysis for N fertilizer recommendations developed in a particular growing stage or site. The efficiency of this approach will provide growers with a rapid, accurate method for assessing crop N status which may enable growers to supply the optimal amounts of N fertilizer, resulting in lower production costs and high yields.

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