CHAPTER 4

Establishment of critical nitrogen concentrations in *Curcuma alismatifolia* Gagnep.

4.1 Introduction

Among mineral nutrients, nitrogen generally produces the greatest growth response in plants. Nutrient management is the practice of using nutrients wisely for optimum economic benefit while minimizing impact on the environment. The extent of N emissions can often be reduced substantially by appropriate fertilizer application regimes (Janzen *et al*., 2003). 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, there is no detailed information available concerning relationships between tissue nutrient concentrations and yields of *Curcuma*. It is essential to develop tools and strategies for *Curcuma* growers that assist them to apply "the right N fertilizer rate at the right time and place". The final step is to convince growers to use means of maximizing income through adequate rhizome yields while respecting environmental constraints leading to significantly higher N fertilizer use efficiency.

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 the 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 better match needs and supply, as a consequence, they can improve N fertilizer efficiency (Vos and Mackerron, 2000; Alva, 2004). Such an approach also allows to better cover 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 would require 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 correction in the same growing season. Information on N status can be obtained either from the crop side or from the soil side. A common definition of the critical level in the literature is the tissue nutrient concentration corresponding to 90% maximum yield when only that nutrient limits growth (Ulrich, 1952). The concept of N critical 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 system 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 might 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 topdressing. This approach has not yet been used for determining critical levels in *Curcuma* plants.

4.2 Materials and methods

This experiment was based on field experiments conducted at training Units, H.M. The King's Initiative Centre for Flower and Fruit Propagation, Yang Kharm village, Chiang Mai, Thailand. The soil was a grey sandy loam and the chemical analysis of the soil before planting could be seen in Table 4.1. The experiment was set up in a randomized complete block design with five N supplying treatments and three blocks treatment. The treatments were 3.75, 7.5, 15, 30 and 60 g N/plant (designated respectively: N1, N2, N3, N4 and N5 in the text) applied as urea by splitting into 12 equal dressings for six months. 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.

Table 4.1. Chemical analysis of soil from an experimental area at Chiang Mai.

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

[a] Whiley (1974)

Rhizomes of *C. alismatifolia* 'Chiang Mai Pink' with a diameter of 1.8 -2.5 cm and 4 storage roots were planted, 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 plant density was approximately 62,500 plants/ha. 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 at five growth stages, i.e., 1) the 1st fully-expanded leaf (45 days after planting: DAP) 2) the 3rd – 4th fully-expanded leaves (75 DAP) 3) flowering stage (105 DAP) 4) pre-resting stage (135 DAP) and 5) harvest stage (165 DAP). 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 were counted. Chlorophyll content (SPAD value) was 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). Samples were put in paper bags and dried in an oven at 60 ºC until dry mass become stable. Fresh and dry weights of various plant parts were recorded. The N concentration was determined by a modified indophenol method using a Kjeldahl digested solution (Ohyama *et al*. 1991).

For the determination of critical tissue N levels, the relationship between yield and the N concentrations in the first fully expanded leaf from the bottom of 1st order shoots as investigated. Total plant dry weight was represented as a yield at stage 1-2 (45 – 75 DAP) and total rhizome dry weight was represented as a yield at stage 3-4 (105 – 135 DAP). A curve was visually fitted to the data using an equation for the Mitscherlich fertilizer yield function (Ware *et al*. 1982), which results in the following model:

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

where y represents the net dry weight yield at a N concentration in the first leaf 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 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 significant level of 0.05. A linear regression analysis was performed with SigmaPlot version 11 (Systat Software Inc., San Jose, CA).

4.3 Results

4.3.1 The 1st fully-expanded leaf stage (45 DAP)

As shown in Table 4.2, after 45 DAP the lowest plant height (25.0 cm) was found in plant supplied with the N2 treatment (7.5 g N/plant). The largest leaf area (103.8 cm^2) was observed for the N1 treatment (3.75 g N/plant) and not significantly different from the N3 (15 g N/plant) (88.6 cm^2) and N4 (30 g N/plant) (98.7 cm^2) treatments.

Table 4.2 Effect of N supply on plant height and leaf area at the 1st fully-expanded leaf stage (45 DAP).

N supply (g/plant)	Plant height* (cm)	Leaf area* (cm^2)
3.75 (N1)	27.9a	103.8a
7.5 (N2)	25.0b	81.2bc
15 (N3)	28.4a	88.6abc
30 (N4)	28.1a	98.7ab
60 (N5)	27.8a	78.7c
$LSD_{0.05}$	2.18	19.25

(*)Within each column, the same letters indicate no significant difference between treatments ($p < 0.05$).

The effect of N supply on dry weight was significant for aboveground parts, underground parts and whole plant (Table 4.3). The highest dry weight for aboveground parts was 2.69 g, derived from the N5 treatment, and the lowest was 2.20 g from the N2 treatment. The N2-N5 treatments gave higher underground dry weights than the N1 treatment. The highest and lowest total plant dry weights were obtained when plants received the N5 treatment (4.32 g) and N2 treatment (3.84 g), respectively.

Table 4.3 Effect of N supply on aboveground, underground, and whole plant dry weight at the 1st fully-expanded leaf stage (45 DAP).

N supply (g/plant)	Dry weight (g)		
	Aboveground parts*	Underground parts*	Whole plant*
3.75 (N1)	2.54ab	1.42b	3.95bc
7.5 (N2)	2.20c	1.64a	3.84c
15 (N3)	2.38bc	1.64a	4.02abc
30 (N4)	2.55ab	1.64a	4.20ab
60 (N5)	2.69a	1.64a	4.32a
$LSD_{0.05}$	0.29	0.18	0.33

(*)Within each column, the same letters indicate no significant difference between treatments ($p < 0.05$).

The leaf N concentrations at the first fully-expanded leaf stage were relatively constant when supplied at N2-N5 treatments but decreased with the amount of N supply at N1 treatment. Leaf chlorophyll content had a significant difference by N supply treatments. The higher of SPAD units were obtained when plants received the N1, N2 and N4 treatments, compared with those of the N3 and N5 treatments (Table 4.4).

Table 4.4 Effect of N supply on N and chlorophyll level in the first leaf from bottom of plant at the 1st fully-expanded leaf stage (45 DAP).

N supply (g/plant)	Leaf N* (%)	Leaf chlorophyll* (SPAD units)
3.75 (N1)	1.48b	46.9a
7.5 (N2)	2.02a	47.0a
15 (N3)	2.18a	41.0b
30 (N4)	2.25a	47.3a
60 (N5)	2.45a	40.9b
LSD 0.05	0.46	3.93

(*)Within each column, the same letters indicate no significant difference between treatments ($p < 0.05$).

There was a strong positive correlation between N supply and N concentrations in underground parts, aboveground part and the first leaf from the bottom of 1st order shoots (Fig. 4.1C, 4.1D and 4.1E, respectively). Higher concentrations of N were observed in the first leaf from the bottom of 1st order shoots than in other tissues. Moreover, N concentrations in underground part, aboveground part and the first leaf from bottom increased when high concentration of N was supplied.

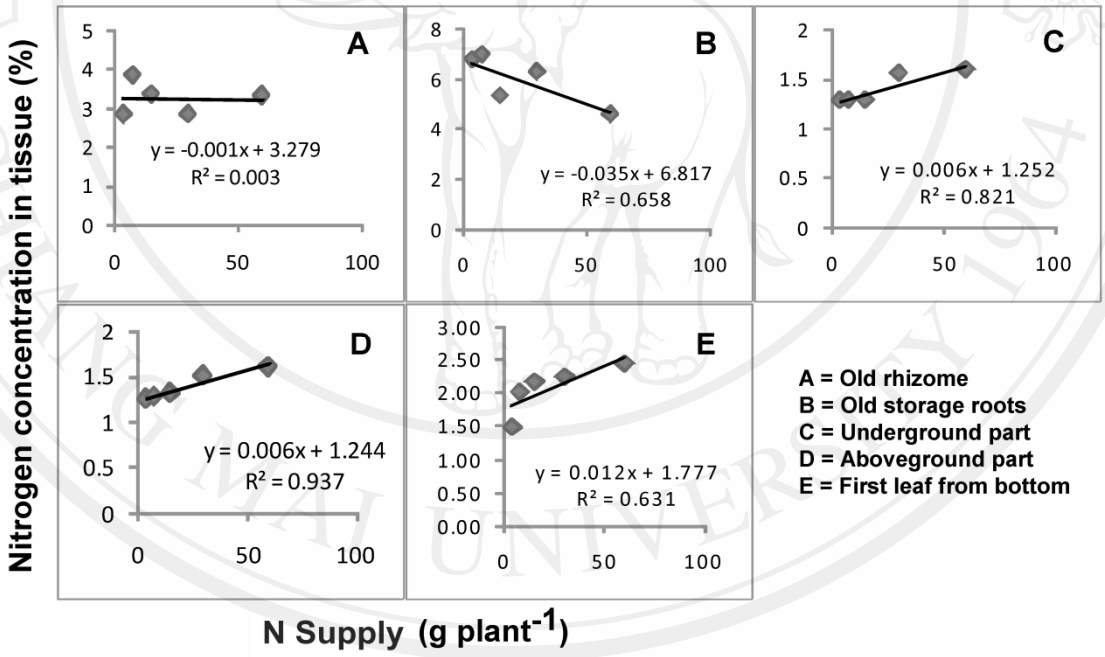

Figure 4.1 Effect of nitrogen supply on nitrogen concentration in selected plant parts at the 1st fully-expanded leaf stage (45 DAP).

The leaf N concentrations at the first fully-expanded leaf (45 DAP) were between 1.04% to 2.95% (Fig. 4.2). The fit of the Mitscherlich function to these data was $y = 4.56(1 - 238.42e^{-5.15x})$. The critical N level corresponding to 90% maximum yield was 1.51% N (Fig. 4.2).

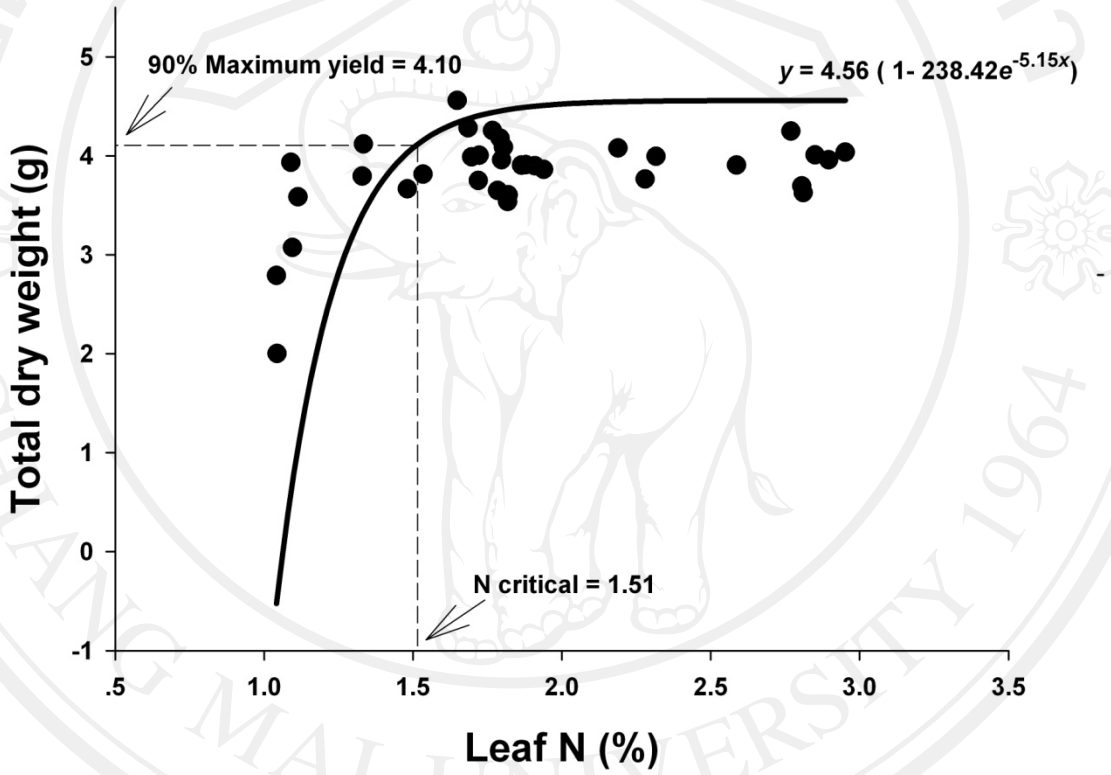

Figure 4.2 The relationship between the leaf N concentrations of *C. alismatifolia* 'Chiang Mai Pink' and total dry weight per plant at 45 DAP. The solid line corresponds to the Mitscherlich equation fit to the data. Dotted lines indicate the leaf N concentration corresponding to 90% of the maximum yield.

4.3.2 The 3rd- 4th fully-expanded leaves stage (75 DAP)

After 75 DAP, the least plant height (28.7 cm) and lowest number of new shoots per cluster (1.42) were found in plants that received the N5 treatment. The largest leaf area (320.9 cm^2) was observed for the N3 treatment and not significantly different from the N2 (302.9 cm^2) and N4 (316.7 cm^2) treatments (Table 4.5).

Table 4.5 Effect of N supply on plant height, number of new shoots per cluster, and leaves area at the 3rd- 4th fully-expanded leaves stage (75 DAP).

N supply (g/plant)	Plant height* (cm)	No. of new shoots per cluster*	Leaf area* (cm^2)
3.75 (N1)	32.2a	3.0a	285.1bc
7.5 (N2)	31.8a	3.0a	302.9abc
15 (N3)	31.8a	3.0a	320.9a
30 (N4)	30.9a	2.0b	316.7ab
60 (N5)	28.7b	1.4b	281.2c
$LSD_{0.05}$	2.00	0.69	37.94

(*)Within each column, the same letters indicate no significant difference between treatments ($p < 0.05$).

The effect of N supply on dry weight was significant for aboveground parts, underground parts and whole plant (Table 4.6). The highest dry weight of aboveground parts was 9.72 g, for the N3 treatment and the lowest was 5.68 g, for the N5 treatment. The N1-N3 treatments gave higher underground dry weights than the N4-N5 treatments. The highest and lowest of whole dry weights were obtained when plants received the N3 (11.8 g) and N5 treatments (6.51 g), respectively.

Table 4.6 Effect of N supply on aboveground, underground and whole plant dry weight at the 3rd- 4th fully-expanded leaves stage (75 DAP).

N supply (g/plant)	Dry weight (g)		
	Aboveground parts*	Underground parts*	Whole plant*
3.75 (N1)	6.79cd	1.83a	8.62b
7.5 (N2)	7.72bc	1.80a	9.51b
15 (N3)	9.72a	2.12a	11.8a
30 (N4)	8.45b	1.32b	9.78b
60 (N5)	5.68c	0.83c	6.51c
$LSD_{0.05}$	1.25	0.39	1.39

(*)Within each column, the same letters indicate no significant difference between treatments ($p < 0.05$).

The N concentration of the first fully-expanded leaf was higher when supplied N as the N3- N5 treatments than the N1-N2 treatments. The trend showed that plants which received higher level of N tended to accumulate more N concentration in tissue than those receiving less N. Leaf chlorophyll content was significantly higher in the N2, N3 and N4 treatments than the N1 and N5 treatments (Table 4.7).

Table 4.7 Effect of N supply on N and chlorophyll level in the first leaf from bottom of plant at the 3rd- 4th fully-expanded leaves stages (75 DAP).

N supply (g/plant)	Leaf N* (%)	Leaf chlorophyll* (SPAD units)
3.75 (N1)	1.63b	52.0b
7.5 (N2)	1.74b	59.4a
15 (N3)	1.95ab	64.3a
30 (N4)	2.04ab	59.2a
60 (N5)	2.20a	51.6b
$LSD_{0.05}$	0.41	6.19

(*) Within each column, same letters indicate no significant difference between treatments ($p < 0.05$).

Nitrogen concentrations in various tissues increased when increasing N supply, except in old storage roots organ and the 3rd leaf from bottom of 1st order shoot. There was a strong positive correlation between N concentrations and N supply in underground parts (Fig. 4.3C), the first leaf from the bottom of 1st-order shoots

(Fig. 4.3E), whole 2nd-order shoots (Fig. 4.3I), and whole 3rd order shoots (Fig. 4.3J) with R^2 = 0.99, 0.85, 0.90, 0.92, respectively.

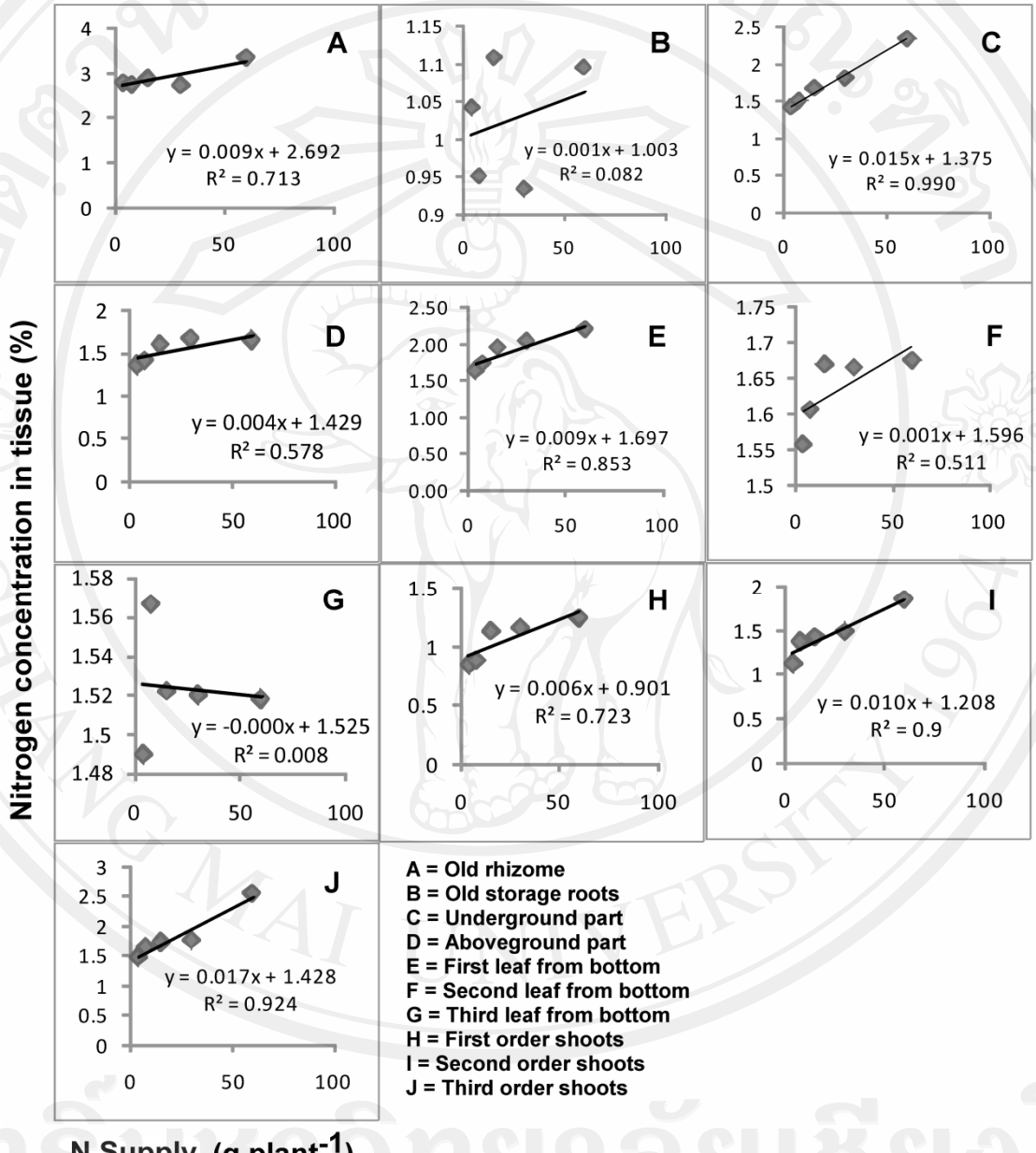

Figure 4.3 Effect of nitrogen supply on nitrogen concentration in selected plant parts of *C. alismatifolia* 'Chiang Mai Pink' at 75 DAP.

The relationship between the leaf N concentrations of *C. alismatifolia* and total dry weight was shown in Figure 4.4. The fit of the Mitscherlich function to these data was y = 13.26 (1- 70.07 $e^{-3.75x}$). The leaf critical N level corresponding to 90% maximum yield was 1.75% N.

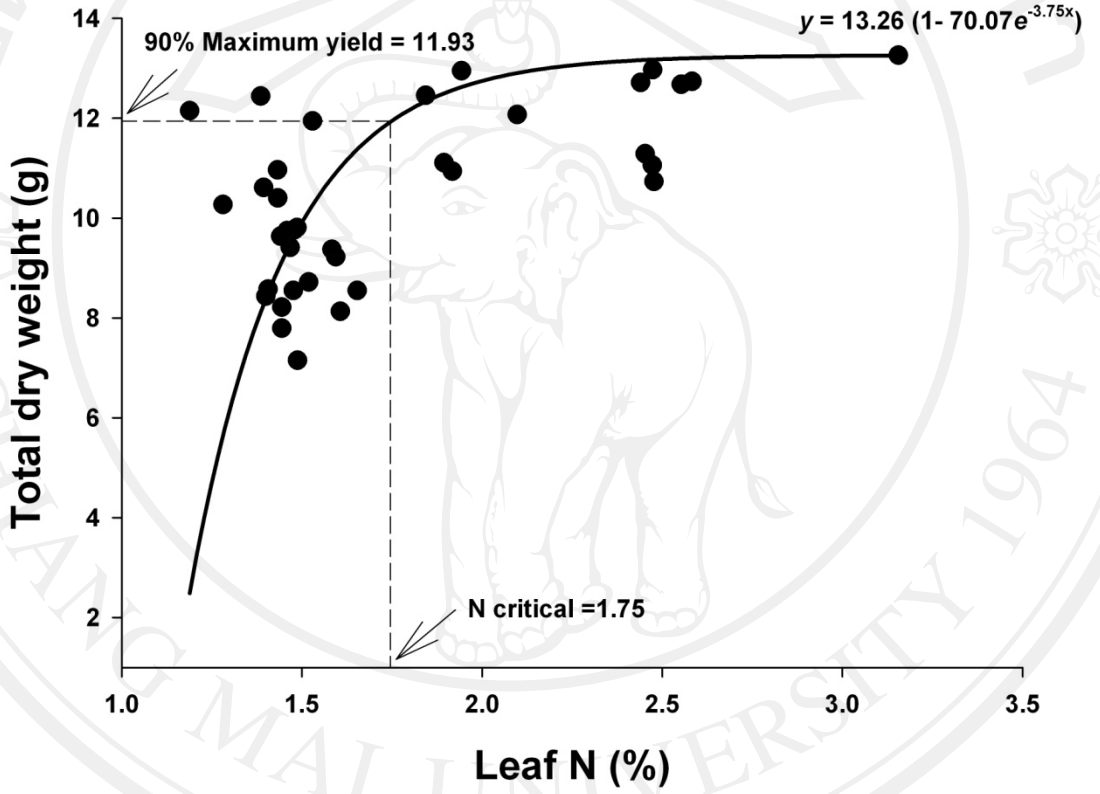

Figure 4.4 The relationship between the leaf N concentrations of *C. alismatifolia* 'Chiang Mai Pink' and total dry weight per plant at 75 DAP. The solid line corresponds to the Mitscherlich equation fit to the data. Dotted lines indicate the leaf N concentration corresponding to 90% of the maximum yield.

4.3.3 At flowering stage (105 DAP)

As shown in Table 4.8, after 105 days of planting and supplied with different N rates, the least plant height (28.7 cm) and lowest number of new shoots per cluster (3.5) were found in plants that received the N5 treatment. The largest leaf areas were observed for the N2 treatment and not significantly different from the N3 treatment.

Table 4.8 Effect of N supply on plant height, number of new shoots per cluster and leaf area at flowering stage (105 DAP).

N supply (g/plant)	Plant height* (cm)	No. of new shoots per cluster*	Leaf area* (cm^2)
3.75 (N1)	32.2a	5.8a	915.0bc
7.5 (N2)	31.8a	5.7a	1129.6a
15 (N3)	31.8a	5.8a	1011.8ab
30 (N4)	30.9a	4.3a	779.5c
60 (N5)	28.7b	3.5b	275.2d
$LSD_{0.05}$	2.00	0.91	326.40

(*) Within each column, the same letters indicate no significant difference between treatments ($p < 0.05$).

The effect of N supply on dry weight was significant for aboveground parts, underground parts and whole plant (Table 4.9). The highest dry weight for aboveground parts was 22.9 g for the N2 treatment, and the lowest was 7.4 g for the N5 treatment. The N1-N3 treatments gave higher underground dry weights than the N4-N5 treatments. The lowest total plant dry weights were obtained when plants received the N5 treatment.

Table 4.9 Effect of N supply on aboveground, underground and whole plant dry weight at flowering stage (105 DAP).

N supply (g/plant)	Dry weight (g)		
	Aboveground parts*	Underground parts*	Whole plant*
3.75 (N1)	20.0b	4.8a	24.7a
7.5 (N2)	22.9a	4.6a	27.5a
15 (N3)	21.6ab	4.3a	25.9a
30 (N4)	15.9c	2.6b	18.5b
60 (N5)	7.4d	1.2c	8.5c
$LSD_{0.05}$	4.37	0.91	4.42

(*)Within each column, the same letters indicate no significant difference between treatments ($p < 0.05$).

There was no significant difference in leaf N concentrations among N treatments with the exception of the 60 g N/plant (Table 4.10). The N concentration of the first fully-expanded leaf was relatively constant when N was supplied at amounts of less than 30 g N/plant, but increased when the amount of N supply was at 60 g N/plant. Leaf chlorophyll content decreased when the N supply decreased.

Table 4.10 Effect of N supply on N and chlorophyll level in the first leaf from bottom of plant at flowering stage (105 DAP).

N supply (g/plant)	Leaf N* (%)	Leaf chlorophyll* (SPAD units)
3.75 (N1)	1.37b	43.15d
7.5 (N2)	1.38b	50.89c
15 (N3)	1.44b	55.70bc
30 (N4)	1.61ab	60.95ab
60 (N5)	1.92a	63.56a
$LSD_{0.05}$	0.44	5.50

(*) Within each column, the same letters indicate no significant difference between treatments ($p < 0.05$).

Differences of N concentrations were found among N treatments and individual plant parts. Most N concentrations in the various tissues were increased when increasing N was supplied. There was a strong positive correlation between N concentrations and N supply in underground parts (Fig. 4.5C), the first leaf from the

bottom of 1st-order shoots (Fig. 4.5E), whole 2nd-order shoots (Fig. 4.5I), and whole 3rd-order shoots (Fig. 4.5J) with the R^2= 0.99, 0.97, 0.99 and 0.98, respectively.

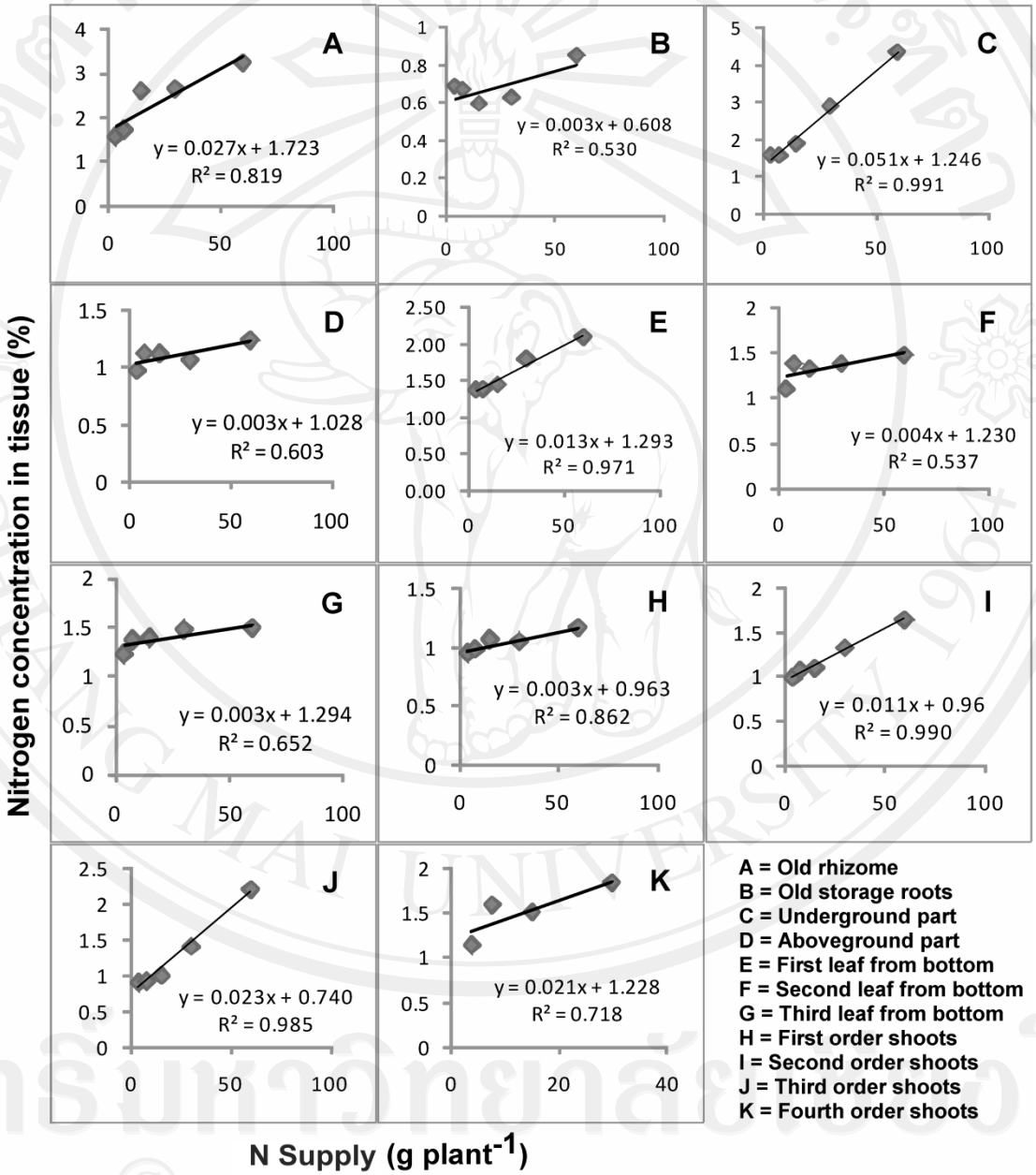

Figure 4.5 Effect of nitrogen supply on nitrogen concentration in selected plant parts of *C. alismatifolia* 'Chiang Mai Pink' at flowering stage (105 DAP).

At this stage, the total rhizome dry weight was represented as yield of this plant because plant started to form a new rhizome. The total rhizome dry weight increased sharply with increasing N concentrations in the first fully-expanded leaf at the values of 0.91% to 1.48% (Fig. 4.6). Above this range, rhizome dry weight gain did not increase appreciably in relation to leaf N. The fit of the Mitscherlich function to these data was $y = 6.14 (1\text{-}7.88\ e^{-2.88x})$. The leaf critical N level corresponding to a 90% maximum yield was 1.51% N in the first fully-expanded leaf

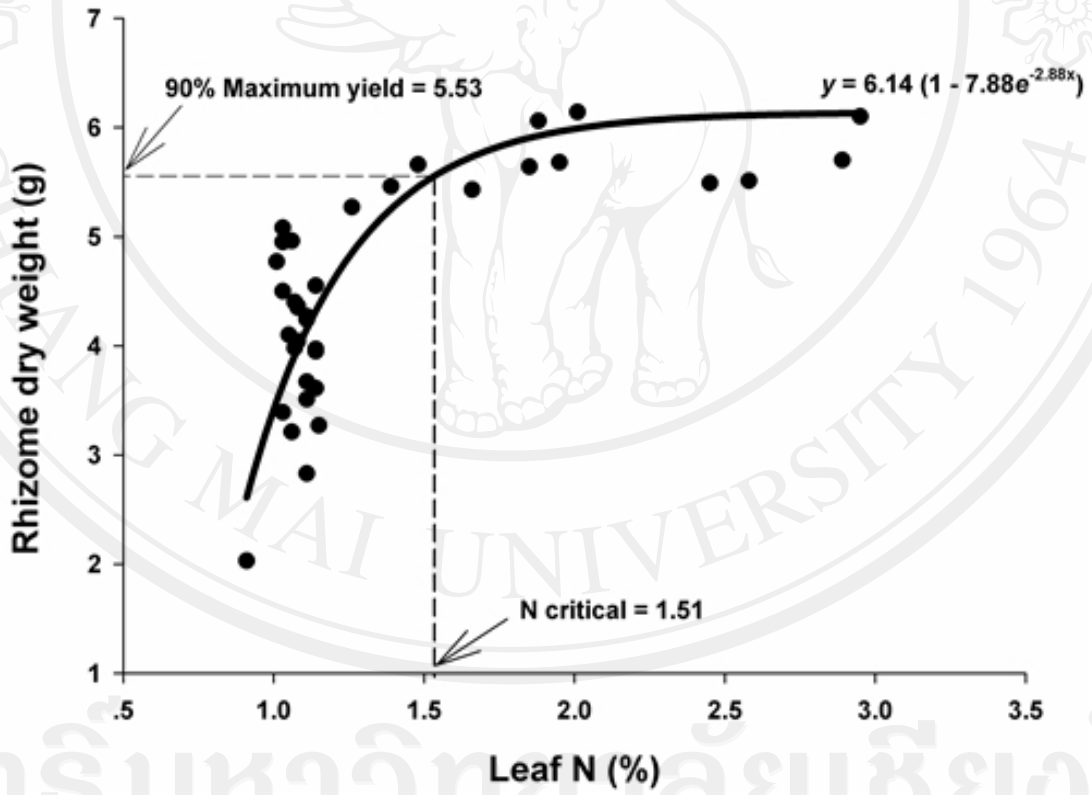

Figure 4.6 The relationship between the leaf N concentrations of *C. alismatifolia* 'Chiang Mai Pink' and rhizome dry weight per plant at flowering stage (105 DAP). The solid line corresponds to the Mitscherlich equation fit to the data. Dotted lines indicate the leaf N concentration corresponding to 90% of the maximum yield.

4.3.4 At pre- resting stage (135 DAP)

The least plant height (28.7 cm) and lowest number of new shoots per cluster (3.6) were found in plants that received the N5 treatment (Table 4.11). The smallest leaf area was also observed for the N5 treatment.

Table 4.11 Effect of N supply on plant height, number of new shoots per cluster and leaf area at pre-resting stage (135 DAP).

N supply (g/plant)	Plant height* (cm)	No. of new shoots per cluster*	Leaf area* (cm^2)
3.75 (N1)	32.2a	9.6a	1123.3a
7.5 (N2)	31.8a	8.9a	778.6a
15 (N3)	31.8a	8.6a	802.4a
30 (N4)	30.9a	6.6b	882.3a
60 (N5)	28.7b	3.6c	263.9b
$LSD_{0.05}$	2.00	1.79	487.79

(*) Within each column, the same letters indicate no significant difference between treatments ($p < 0.05$).

The effect of N supply on dry weight was significant for aboveground parts, underground parts and whole plant (Table 4.12). The highest dry weight for aboveground parts was 14.4 g, for the N1 treatment which was not significantly different from the N2, N3 and N4 treatments, and the lowest was 4.3 g, for the N5 treatment. The N1-N3 treatments gave higher underground dry weights than the N4-

N5 treatments. The lowest total plant dry weights were obtained when plants received the N5 treatment.

Table 4.12 Effect of N supply on aboveground, underground and whole plant dry weight at pre-resting stage (135 DAP).

N supply (g/plant)	Dry weight (g)		
	Aboveground parts*	Underground parts*	Whole plant*
3.75 (N1)	14.4a	8.0a	22.4a
7.5 (N2)	12.6a	7.9a	19.7ab
15 (N3)	11.9a	7.1a	19.0ab
30 (N4)	10.8a	3.7b	16.3b
60 (N5)	4.3b	1.9c	6.2c
$LSD_{0.05}$	5.02	1.58	5.42

(*) Within each column, the same letters indicate no significant difference between treatments ($p < 0.05$).

N concentrations in the various tissues were increased when increasing N was supplied except the 8th-order shoots ($R^2 = 0.12$). Most of selected plant parts showed a strong positive correlation ($R^2 = 0.74$ -0.99) between N concentrations and N supply in the most of selected plant parts. (Fig. 4.7).

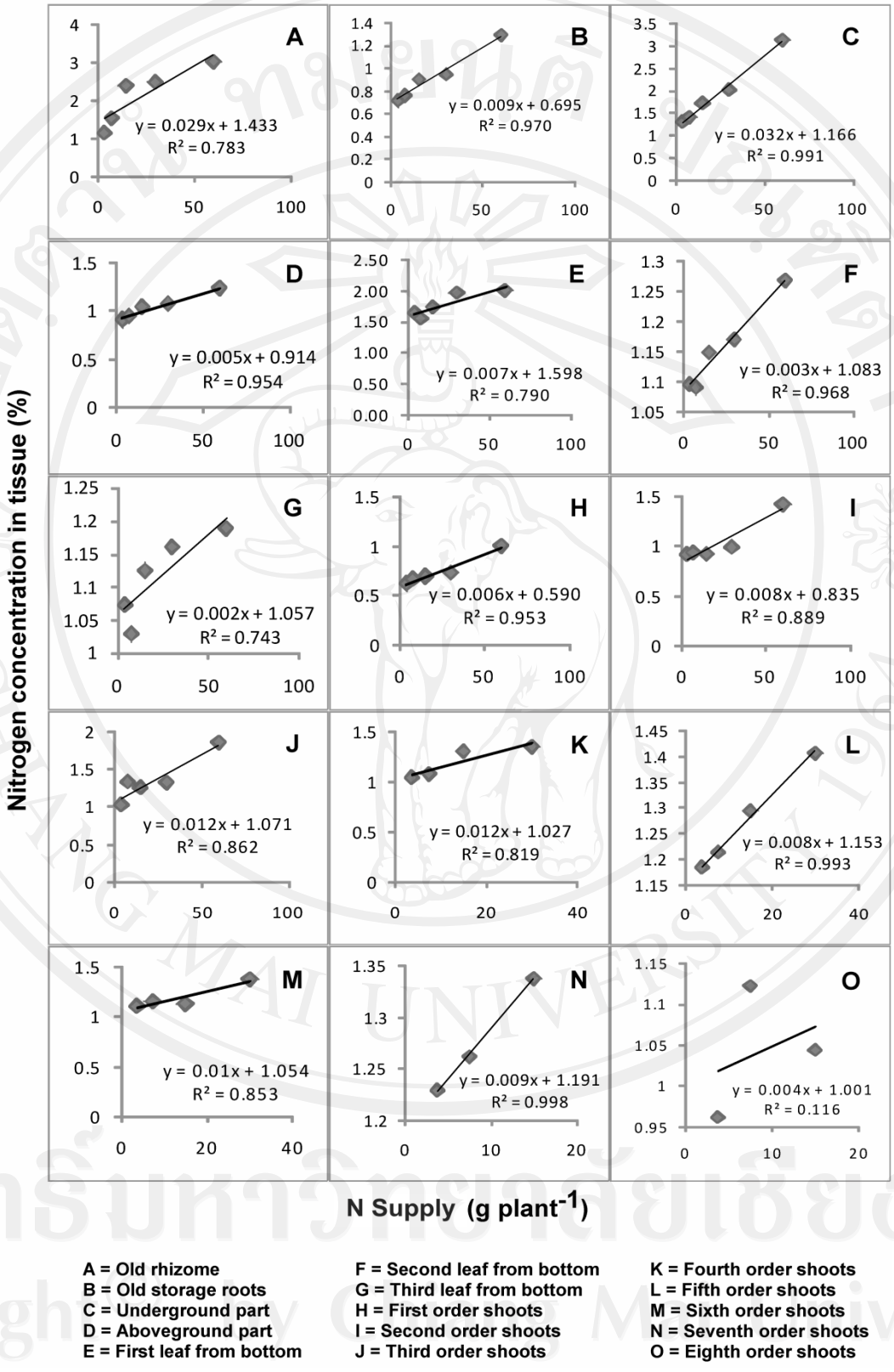

Figure 4.7 Effect of nitrogen supply on nitrogen concentration in selected plant parts of *C. alismatifolia* 'Chiang Mai Pink' at pre-resting stage (135 DAP).

The relationship between leaf N concentrations of *C. alismatifolia* and rhizome dry weight was shown in Fig. 4.8. The fit of the Mitscherlich function to these data was $y = 10.8 (1- 8.14\ e^{-2.44x})$. The leaf critical N level corresponding to 90% maximum yield was 1.80% N.

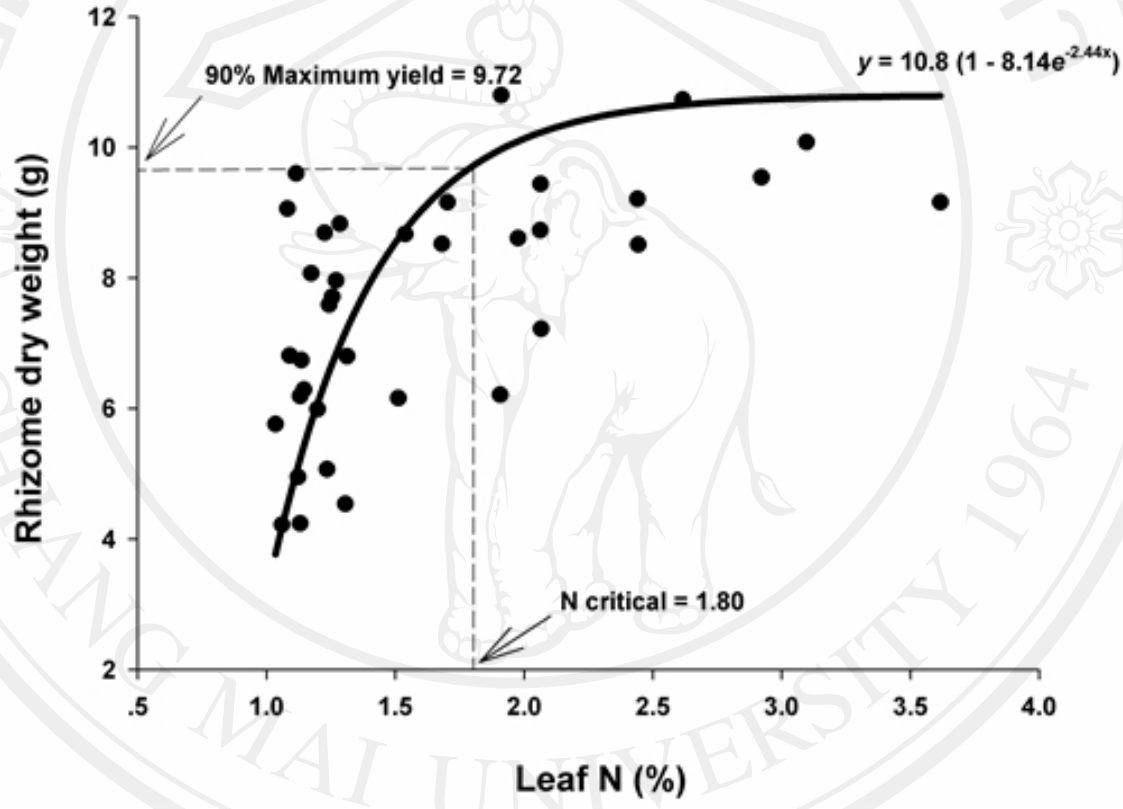

Figure 4.8 The relationship between leaf N concentrations of *C. alismatifolia* 'Chiang Mai Pink' and rhizome dry weight per plant at pre-resting stage (135 DAP). The solid line corresponds to the Mitscherlich equation fit to the data. Dotted lines indicate the leaf N concentration corresponding to 90% of the maximum yield.

4.3.5 At harvest stage (165 DAP)

At harvest stage, rhizome quality in terms of the number of storage roots and total rhizome fresh and dry weight per cluster was highest when plants received the N2 treatment while the highest number of new rhizome per cluster (8.0) was observed when plants received the N1 treatment (Table 4.13).

Table 4.13 Effect of N supply on rhizome quality of *C. alismatifolia* 'Chiang Mai Pink' at harvest stage (165 DAP).

N supply (g/plant)	No. of new rhizome per cluster*	No. of storage roots per rhizome*	Total rhizome* FW (g)	Total rhizome* DW (g)
3.75 (N1)	8.0a	1.9b	95.0b	13.1b
7.5 (N2)	6.1b	3.0a	124.6a	16.4a
15 (N3)	6.7ab	1.0c	60.1c	8.6c
30 (N4)	6.2b	0.5cd	44.0c	7.1c
60 (N5)	3.9c	0.0d	23.0d	3.5d
$LSD_{0.05}$	1.63	0.54	18.48	3.42

(*)Within each column, the same letters indicate no significant difference between treatments ($p < 0.05$).

Differences in N concentrations were found among the N treatments and individual plant parts. Most N concentrations in various tissues were increased when increasing N was supplied. There was a strong positive correlation between N concentrations and N supply in underground parts (Fig. 4.9C) with $R^2 = 0.93$.

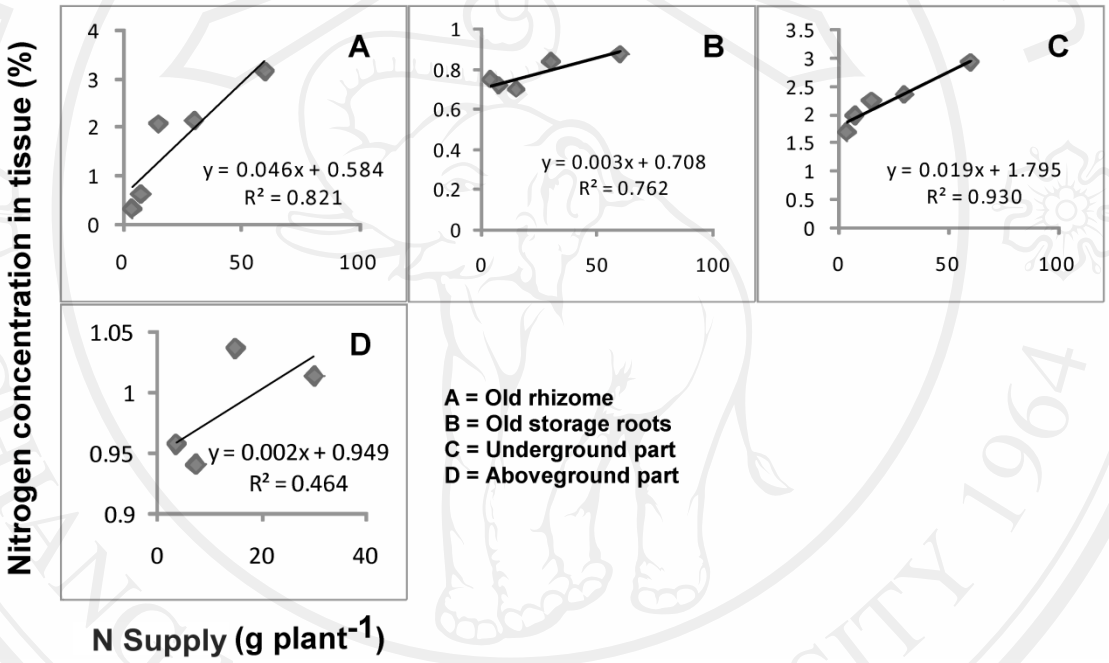

Figure 4.9 Effect of nitrogen supply on nitrogen concentration in selected plant parts of *C. alismatifolia* 'Chiang Mai Pink' at 165 DAP.

4.4 Discussion

The different rates of N supply in N1, N2, N3, N4 and N5 treatments were 3.75, 7.5, 15, 30 and 60 g N/plant, respectively and could be calculated in t/ha as 0.23, 0.47, 0.94, 1.88, 3.75, respectively (62,500 plants/ha). Dry weights of *C. alismatifolia* 'Chiang Mai Pink' for each of five treatments at different growth stages were lower for plant receiving the N5 treatment than the other treatments (Appendix 4). The N5 treatment reduced growth, in terms of leaf area and leaf chlorophyll, at 45 DAP but not for the other parameters. On the other hand, it reduced the values of growth parameters thereafter at 75, 105 and 135 DAP, suggesting that the response of plant to fertilizer application was different along growing stage. The highest N application in the N5 treatment seemed to be 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). Lang and Pannkuk (1998) found that low dry weight was often attributable to frequent fertilizer applications. When N was supplied in excess, it is necessary to quantify the rate of N uptake for maximal growth. In plants grown with high fertilization rates, part of the N uptake was not immediately assimilated. This extra N was in the NO_3^- form for early stages and in the reduced N form after the beginning of elongation in wheat (Justes *et al*., 1994). The excess of N could be stored in different chemical forms, comprising simple inorganic and complex organic compounds in plant tissues (Brégard *et al*., 2000).

At the 1st fully-expanded leaf stage (45 DAP), the total plant dry weight which represented as yield on this stage was increased with higher N supply rate (Table 4.3), suggesting this stage was the period of rapid growth of plant that might demand

higher N fertilizer to promote their growth and development. Similarly, Thomas *et al*. (1999) reported that nitrogen demand was high during vegetative growth of potato plant (the three to four weeks after seeding) and the uptake was rapid. Thus, in our experiment, high nitrogen supply rate as N3- N5 treatments (15 – 60 g N/plant) seemed to be beneficial to increase total plant dry weight at the 1st fully-expanded stage (45 DAP) . However, at the 3rd – 4th fully-expanded leaves stage (75 DAP), supply of high nitrogen rate as N4-N5 or low nitrogen rate as N1-N2 treatments decreased total plant dry weight when compared with N3 treatment (Table 4.6). This indicated that supplying with N3 treatment was a suitable rate to enhance total plant dry weights at 75 DAP. Moreover, the highest total plant dry weight at flowering (105 DAP) and pre-resting stage (135 DAP) were obtained when supply with N1-N3 treatments (3.75 - 15 g N/plant) (Table 4.9 and 4.12), suggesting that there was high N demand of *Curcuma* plant in vegetative growth stage through pre-flowering stage and then decreased from flowering through pre-resting stage. Thus, the recommended rate for N supply in each growth stage which was based on total plant dry weight were 15 g N/plant at 45 and 75 DAP and 3.75 g N/plant at 105 and 135 DAP. At harvest stage, the highest rhizome fresh weight derived from plants supplied with the N2 treatment (7.5 g N/plant or 0.47 t/ha) (Table 4.13). This amount was in the range of 0.34 – 0.83 t/ha as recommended by Lee (1975) to ginger industry for the highest commercial yields of fresh rhizomes, suggesting that supplying with the N2 treatment could be suitable for rhizome production in *Curcuma* plants.

Nitrogen concentration in tissue is an important criterion for diagnosis of nutritional status in crop plant. In present results, there was a highly significant difference of nitrogen concentration in plant tissue depending on nitrogen supply,

individual plant parts and times of sampling (Appendix 5). Of the most sampling times, the concentration of nitrogen in various tissues ranked N5>N4>N3>N2>N1. In addition, there was a downward trend in most of tissue nitrogen concentration as the growth stage progressed, despite the fact that an attempt had been made to match the size and frequency of nitrogen fertilizer additions to crop requirements for nitrogen (Lee *et al*., 1981b). However, in underground organ, a peak in nitrogen concentration was observed at 105 days after planting (flowering stage) (Fig. 4.11).

In our experiment, the response of yields to nitrogen supply shown here largely followed the law of diminishing returns, with supply of an increment of extra nitrogen at an already-high rate of N application giving a smaller increment of extra crop yield than supply of increment of nitrogen at low rate of application, and very high rate of N application giving no further yield increases. This was the standard response of crop yield to supply of nitrogen and formed the basis of the Mitscherlich curves used in recommendation for rate of N fertilizer application.

Critical levels determined with various plant parts and fertilizer systems differed 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 N critical level since there was a strong correlation between N concentrations in the first fully-expanded leaf and the amount of N applied and it could be sampled over a longer period than those of shoots of higher orders. Lee (1975) studied on nitrogen in ginger by using data on nitrogen concentration in the third leaf from the top of shoots of various orders and the frequency of occurrence of each shoot order at selected times throughout the growing season and he suggested that restriction of leaf sampling to

shoots of particular order shoot might be unnecessary. Jones (1972) considered that mature leaves just below the growing tip should be sampled, preferably at the flowering stage. Leaves were also a major site of carbohydrate and mineral storage. Consequently, the mineral status of the leaves not only influenced the efficacy of photosynthesis but also reflected the nutrient status of the plant. Although other organs within the plant might act in a similar manner, the leaf was the most readily-available source of tissue for analysis (Mooney, 1992).

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 N supply (Griffin *et al*., 1999). Since it is necessary for an external source of N to be applied during production for maximum growth to be obtained, this approach is often justified (Barnett and Ormrod, 1985). However, there are limitations to this approach. It is indirect, relying on the assumptions that the N remains present and available to the plant in all cases, that there are no physical or chemical interactions in the soil that alter the effective rates of addition, that all of the N fertilizer is used by the plant, and that plant N demand does not exceed soil availability at any time during the crop period (Tanji and Stevenson, 1982).

In this experiment, growth curves relating to yields with leaf N concentrations were 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. [2] (Table 4.14). The leaf N critical level for *C. alismatifolia* in each growth stage (45, 75, 105 and 135 DAP) calculated, using the Mitcherlich model and expressed as a percentage of leaf dry weight, was 1.51, 1.75, 1.51 and 1.80%, respectively. 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 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 with maximum growth was in the range of 1.89 to 2.70 %, which is similar to 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).

Table 4.14 Estimated parameter and nitrogen critical level for the Mitscherlich model used to characterize the relationship between leaf N concentration and yield of *C. alismatifolia* 'Chiang Mai Pink'.

Days after planting (DAP)	Parameter			N Critical level (%)
	α	β	γ	
45	5.15	4.56	238.42	1.51
75	3.75	13.26	70.07	1.75
105	2.88	6.14	7.88	1.51
135	2.44	10.8	8.14	1.80

The equation is $\mathbf{y = \beta (1 - \gamma e^{-\alpha x})}$.

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. The experimental point giving rise to these effects usually must be deleted from the data set before attempting to fit the model (Ware *et al*., 1982). The same way in our experiment, the data from N4 and N5 treatments were deleted before fitting with the model since those treatments decreased plant growth and yields.

4.5 Conclusion

The use of plant analyses to evaluate nutrient status can be useful and sound practice. Results from plant tissue analysis also can be used to evaluate fertility programs, to predict the levels of essential elements required by plants at critical growth stages. In our experiment, a field - growing system with known N concentrations allowed for analysis of plant N status and determination of the first fully-expanded leaf. N critical level was 1.51, 1.75, 1.51 and 1.80% at 45, 75, 105 and 135 DAP, respectively. Critical plant composition values, as reported here, are not dependable but can serve as a guide in the interpretation of suitably evaluated analytical results. 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 apply suitable amounts of N fertilizer, resulting in maximum yield and lower production costs.