CHAPTER 8

General discussion

The element nitrogen (N) is an essential nutrient for all organisms, and as a critical component of proteins and nucleic acids, N is fundamental to the structures and biochemical processes that defines life. Thus, it is not surprising that this nutrient and its cycle have been studied more extensively than any other nutrient element. In the production of *Curcuma alismatifolia*, nitrogen fertilizer is an important factor since growth and yields respond to the amount of nitrogen fertilizer supplied (Ohtake *et al*., 2006). Therefore, the understanding of the nitrogen requirements for *Curcuma* makes it easier to meet their supplement needs.

To achieve the objective of the studies, the experiments were divided into 2 parts; the first part, Experiments 1, 2 and 3, were concerned about an evaluation of N requirement by using leaf analysis and N critical value and the second part, in Experiments 4 and 5, were concerned about growth and nitrogen assimilation in plant which was affected by ammonium and nitrate ratio, N-source and temperature.

The results for N requirement evaluation were obtained by three steps as follows. The first step was done in 4 farmer's fields with having different fertilizer practices (Chapter 3). The results revealed that highest and lowest nitrogen supply rate (15 and 1.95 g N/plant, respectively) was not suitable for *Curcuma* production while nitrogen rate of 4.1 – 6.9 g N/plant was required to enhance rhizome yields. Korsaeth *et al*., (2002) reported that normally it was obvious that farmers would rather choose the cheapest and most convenient fertilizer option which often lost a significant proportion of applied fertilizer N due to a lack of precise predictions of N availability for plants as a function of agricultural practice and the weather conditions. If the synchronization of fertilizer application with plant N demand is not achieved, this would therefore result in additional economical losses. Thus, a major challenge is how to maximize the benefits of fertilizer N application by increasing yield production while minimizing its unwanted consequences. This reason brings us to the second step to approach N requirement evaluation in *Curcuma* plant.

In the second step, we tried to establish N critical value of *Curcuma* plant in each growing stage which was based on a mathematically-established, predictable and functional relationship between yield and leaf N concentration. The nitrogen supply rates were limited to assess nitrogen status in plant and the N supply rates were varied as: 3.75 and 7.5 g N/plant (represented as suboptimal range), 15 g N/plant (represented as optimal range according to GAP recommended), and 30 and 60 g N/plant (represented as supra-optimal range) to get the deficient, adequate and toxic zone of growth. The results showed that there were differences in N fertilizer demand in each growth stage. The recommended N supply rates 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. However, the recommended N supply rate to promote rhizome yields was 7.5 g N/plant since there was the highest rhizome fresh weight derived from plants supplied with 7.5 g N/plant or 0.47 t/ha at the harvest stage (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. Nitrogen concentration in tissue is an importance criterion for diagnosis of nutritional status in crop plant. In present results, the concentration of nitrogen in various tissues ranked from the highest to the lowest N supply rate. In addition, there was a downward trend in most of tissue nitrogen concentrations 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*., 1981). In this experiment, the leaf N critical level for *C. alismatifolia* in each growth stage (45, 75, 105 and 135 DAP), calculated using the Mitscherlich model and expressed as a percentage of leaf dry weight, was 1.51, 1.75, 1.51 and 1.80 % respectively. N critical levels as reported here are not dependable but can serve as a guide in the interpretation of suitably-evaluated analytical results. Moreover, 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. 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. However, N fertilizer at 3.75 g N/plant seemed to be in the marginal range between deficient and adequate ranges (Chapter 4). Therefore, further experiments should be performed in the deficient range to fulfill the standard curve.

The next step to confirm the results from the previous experiment was to compare the N supply rate (7.5 g N/plant) to farmer's field practice. The results showed that supplying with 7.5 g N/plant could enhance yields of *Curcuma* plant when compared with nitrogen application by farmer's method (1.95 g N/plant). N critical value on Chapter 4 was used for evaluation of N requirement on Chapter 5. At flowering stage, the results of leaf N concentration obtained from nitrogen application based on farmer's method was 1.01% which was lower than critical N value obtained in Chapter 4 (1.51%). It might be concluded that if farmers needed to increase their yield, they had to increase N supply rate. However, leaf N concentration obtained from our supplied rate was 1.23% which was still lower than critical N value obtained in Chapter 4 that might be mainly due to environmental effects. Westerveld *et al.* (2003) reported that several environmental factors may affect the results of plant analysis such as soil type, soil moisture content, fertilizer source, and climate. Further studies may try to test this fertilizer nitrogen rate by varying from field-to-field and from year-to-year to find out the variation in both crop nitrogen demand and nitrogen supply. The relationship between yield and N fertilizer need may be more appropriate for long-term, large-scale analyses to increase the database for making regional recommendations.

The results on N assimilation in *Curcuma* plant were divided into two experiments. The first experiment studied the effect of ammonium and nitrate ratio (Chapter 6) and the second one studied the effects of N-source and temperature (Chapter 7) on growth and N assimilation of *Curcuma* plants. In general, NO_3^- and NH_4^+ are the major sources of inorganic N taken up by the roots of crop plants. The assimilation of NH_4^+ or NO_3^- begins with its uptake from the soil solution by epidermal and cortical cells of the root. The results in our experiment indicated that a combination of mixed-N sources in an appropriate ratio ($50NH_4^+$: $50NO_3^-$) appeared to be beneficial to the growth, inflorescence quality and rhizome production of this plant in regular reason while completely 100% ammonium in nutrient solution depressed plant growth and yield. Similarly in Wang and Below (1992) who demonstrated that growth and yield were enhanced when wheat plants were provided with mixtures of NO_3^- and NH_4^+, compared with either form alone. Moreover, our results showed that single form of nitrate-N or mixed-N sources could be used under low night temperature condition as off-season production. N assimilations in our experiment were mostly explained in terms of nitrate and ammonium content, nitrate reductase activity (NRA) and distribution of free amino acid in plant organs-. The results of nitrate content in fibrous roots were generally increased with the proportion of NO_3^- while the ammonium concentration was relatively constant in fibrous roots. These results indicate that *Curcuma* plant prefers nitrate even when both NH_4^+ and NO_3^- are supplied and the absorbed ammonium is retained in fibrous roots.

Leaves were the main site for nitrate assimilation since NRA was higher found in leaves than roots when plants were grown in regular season. Oaks, (1994) reported that NO_3^- is reduced more efficiently in leaves than in roots because of readily-available reductants, energy and carbon skeletons produced by photosynthesis. However, in low night temperature/off-season, fibrous roots were the main site for nitrate assimilation since NRA was higher in fibrous roots than in leaves. Other studies have actually reported increased root NR activity following low root temperature treatment (Dene-Drummond *et al*., 1980). Ding and Xi (1993), suggesting that most NO_3^- was transported to the leaves for assimilation but in some cases, NO_3^- was primarily reduced in the roots (Toselli *et al*., 1999).

Distributions of free amino acids in plant organs were mostly incorporated into asparagines (Asn), aspartic acid (Asp), gutamic acid (Glu) and threonine (Thr). Lam *et al*. (1996) reported that inorganic nitrogen was assimilated into the amino acids, glutamine (Gln), Glu, Asn and Asp, which serve as important nitrogen carriers in plants. In addition, our results also indicated that alanine (Ala) and gamma-aminobutyric acid (GABA) were mainly distributed in plant organs especially in ammonium N-source treatments. Aurisano *et al*. (1995) reported that GABA syntheses play an integral role in ammonium metabolism and represent a homeostatic mechanism important for plant stress tolerance. An accumulation of GABA was also characteristic responses to environmental stress (Roberts *et al.*, 1992). Large amount of Glu in old and new rhizomes of *Curcuma* plants was also detected in NO_3^--supplied treatment. Ito *et al*. (2010) reported that the amino group of Glu was then utilized for the synthesis of a range of nitrogenous compounds. N in fibrous roots was mostly incorporated into Asn and Asp when plants were grown in low temperature treatment. Khuankaew (2010) reported that Asn was a major form of N accumulation in roots of *Curcuma* plants, as N is often present in very high concentrations in both xylem and phloem sap, and used to carry nitrogen away from source tissue (Lillo, 2004). NH_4^+-fed plants also induced Asn accumulation in inflorescence and such accumulation was much greater under low temperature.

Results from this study can lead to the conclusions as follow:

1. Fertilizer management in commercial *Curcuma* farm is varied widely from one individual to another and the nitrogen supply was related to nitrogen concentration in plant parts at different growth stages. Therefore, soil analysis data should be used for fertilizer application management.

2. Results from leaf analysis can be used to evaluate fertility programs, to predict the levels of nitrogen required by plants at critical growth stages. N critical level in this experiment was 1.51, 1.75, 1.51, and 1.80% at 45, 75, 105, and 135 days after plant, respectively.

3. In this experiment, critical nitrogen values were 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.

4. Optimal nitrogen fertilization that is essential for achieving commercial rhizome production and results in maximum economic return in these studies was N 7.5 g N/plant which this rate can improve rhizome yield of *Curcuma* in commercial-field production.

5. Nitrate in nutrient enhanced both the production of cytokinins in roots and the root-shoot translocation of cytokinins. Moreover, the leaf parts were the main site for nitrate assimilation since NRA was higher found in leaves than roots.

6. Nitrate accumulation in leaves was increased when plants were grown under low night temperature. Fibrous roots were the main site for NR activity under

low temperature while the leaves were the main site for NR activity under high temperature.

7. Free amino acids in plant organs were mostly assimilated into asparagines (Asn), aspartic acid (Asp), gutamic acid (Glu) and threonine (Thr). In addition, alanine (Ala) and gamma-aminobutyric acid (GABA) were mostly found when supplied N with ammonium- N sources.

8. When supplied with nitrate-N sources, assimilation of amino acids in old and new rhizome was mainly into glutamic acid (Glu). In addition, gamma-aminobutyric acid (GABA), threonine (Thr) and Glu were mostly observed in fibrous roots when plants were grown in high temperature.

9. NH_4^+-fed plants also induced asparagines (Asn) accumulation in inflorescence and there was highly a distribution of threonine (Thr) in both leaves and inflorescence organs when supplied with nitrate-N sources in low temperature.

10. A combination of two forms of N in an appropriate ratio ($50NH_4^+$: $50NO_3^-$) appears to be beneficial to the growth, inflorescence quality, and rhizome production of this plant in regular season while single form of nitrate-N or mixed-N sources could be used under low night temperature condition as off-season production.

11. Nitrogen assimilation in *Curcuma* plants was affected by both temperature and nitrogen sources. Thus, nitrogen ferilizer supply should be adjusted with respect to temperature and nitrogen sources for successful cultivation of *Curcuma*.