CHAPTER 7

Effects of temperature and nitrogen sources on growth and nitrogen assimilation of *Curcuma alismatifolia* Gagnep.

7.1 Introduction

Nitrogen (N) plays an important role in growth and quality of *Curcuma* rhizomes and flowers (Ruamrungsri and Apavatjrut, 2003). Ohtake *et al.* (2006) also indicated that a high level of N increase flower numbers and promote continuous new rhizome formation. The form of N partially determines plant quality by affecting leaf weight, leaf area, leaf chlorophyll content, plant size and transpiration rate, (Bar-Tal *et al*., 2001). Usually plants are able to uptake N as nitrate (NO_3^-) and ammonium (NH_4^+), but some may prefer one source or another depending on plant species (Marschner, 1995). The use of N-NH_4 causes an increasing H^+ excretion, leading to a decrease in soil pH whereas the use of N-NO_3 is associated with a decrease in H^+ excretion and increasing rates of HCO_3^- or OH^-, resulting in a pH rise (Marschner and Römheld, 1996). However, when plants are supplied with N-NO_3 or N-NH_4, they may differ in many aspects related to metabolic activity and ionic composition (Kandlbinder *et al*., 1997), as a result of different physiological responses. In addition,

studies indicate that the response of plants to either NO_3^- or NH_4^+ concentration varies with species, light intensity and temperature (Edwards and Horton, 1982).

Temperature also greatly affects plant growth and development. In *Curcuma* plant, temperature affects shoot sprouting, plant growth, flowering and rhizome yields. The day and night temperatures affect plant growth and flowering of *C. alismatifolia*. Changjeraja *et al*. (2007) reported that high temperature 36/24 $^{\circ}$C (day/night) increased vegetative growth of aboveground parts and flower quality but low temperatures (24/18 $^{\circ}$C) enhanced dry weight accumulation of underground storage organs. Hongpakdee *et al*. (2010) also found that low night temperatures (30/18 $^{\circ}$C) influenced decrease in growth of *C. alismatifolia*. Temperature strongly affects nitrogen uptake, metabolism and assimilation, as well as, other physiological and biological processes. Plant growth, under lower- or higher- optimal temperatures affects the rate of N uptake, remobilization and assimilation, as well as, assimilation of nutrients by influencing assimilatory enzymes (Dubey and Pessaraki, 1994). Mengel and Kirkby (1987) indicated that uptake of N, as both NH_4^+ and NO_3^-, was temperature-dependent.

Taking into account all published informations on effects of N source on the plant growth, there is limited or no information on the interaction between temperature and N source on N assimilation of *C. alismatifolia*. Therefore, the objective of this experiment was to assess the effect of combination of temperature and N source on: (i) growth and flower quality, (ii) concentration of nitrogen, total amino-N, free amino acids, free NH_4^+ and NO_3^- in plant tissue, (iii) the activity of nitrate reductase (NR) in leaves and fibrous roots of *C. alismatifolia* plant.

7.2 Materials and Methods

The experiment was conducted at the Department of Plant Science and Natural Resources, Chiang Mai University, Thailand during November 2008- January 2009. Rhizomes of *C. alismatifolia* cv. "Chiang Mai Pink" with a diameter of 1.8-2.5 cm and 4 storage roots were planted in 5-inch pots, using sand and vermiculite mixed at a ratio of 2:1. Plants were grown in a controlled-environment growth chamber (Conther phytotron climate simulator) at 30/24 $^{\circ}$C and 30/18 $^{\circ}$C (day/night). The day length, relative humidity (% RH) and light intensity were maintained at 13 hours, 70-80% RH and 270 µmol $m^{-2}s^{-1}$PPF (photosynthetic photon flux), respectively. At six weeks after planting (6 WAP), when the shoots were sprouting, plants in each temperature treatment were treated with nutrient solutions consisting of different nitrogen sources; NO_3^- , NH_4^+, NO_3^- + NH_4^+ (1:1 ratio). The solution contained (in mg L^{-1}) 200N, 100P, 200K, 200Ca, 100Mg, 0.40Fe, 0.55Mn, 0.25Zn, 0.25Cu, 0.038Mo, and 0.22B. The initial pH of the solutions containing NO_3^- and NH_4^+ was adjusted to 5.8–6.0 by adding H_2SO_4 or NaOH. The nutrient solutions were supplied every two days at 50 ml per pot.

At flowering stage (12 WAP), plants of each treatment with four replications were collected. Plant growth and flower quality were measured. Leaves were used for measuring chlorophyll content (SPAD value) using a SPAD meter (SPAD-502, Minolta, Japan). Leaf area was measured using a leaf area meter (LI-3100, LI-COR, Lincoln, NE). NO_3^- and NH_4^+ concentration in leaves and fibrous roots were measured using a reflectometer (RQflex, Merck KgaA, Darmstadt, Germany).

The N concentration was determined by the modified indophenal method using a Kjeldahl digested solution (Ohyama *et al*., 1991). Total amino acid was determined by ninhydrin method (Appendix 9) and free amino acid was analyzed by ACQUITY UPLC^{TM} amino acid analyzer (Appendix 10). NRA was analyzed as described in 'Chapter 6'.

The data were subjected to analyses of variance (ANOVA), using generalized linear models by means of the Statistic 8 analytical software package (SXW Tallahassee, FL). In case of significant treatment effects, comparisons of means were performed using the Least Significant Difference (LSD) test at a significance level of 0.05.

7.3 Results

The effects of temperature and N-sources on the growth and flower qualities of *Curcuma* plant were shown in Tables 7.1 and 7.2. The results indicated that the low night temperature (30/18 ºC) depressed plant height and stalk length of *C. alismatifolia*. Total dry weight, chlorophyll content (SPAD value), stalk length, inflorescence length and number of pink bracts decreased when plants were grown with NH_4^+- N as the sole source of nitrogen in the nutrient solution (Table 7.1, 7.2). In addition, the low night temperature (30/18 ºC) combined with an application of NH_4^+-N significantly decreased leaf area and total dry weight.

Table 7.1 The effects of temperature and N-sources on the growth of *Curcuma* plants at flowering stage (12 WAP).

Treatment	Plant height (cm)	Chlorophyll content (SPAD Value)	Leaf area (cm^2)	Total dry weight (g)
Temperature				
30/24 ^{0}C	51.57^{a}	46.77^{a}	321.22^{a}	8.16^{a}
30/18 ^{0}C	43.05^{b}	43.56^{a}	325.43^{a}	8.15^{a}
N-source				
nitrate	50.92^{a}	50.84^{a}	365.37^{a}	8.50^{a}
ammonium	46.38^{b}	37.20^{b}	296.65^{b}	6.91^{b}
mixed	44.64^{b}	47.45^{a}	307.95^{b}	9.05^{a}
Temp. x N-source				
30/24 x nitrate	NS	NS	356.08^{ab}	7.67^{bc}
30/24 x ammonium	NS	NS	274.58^{c}	7.29^{cd}
30/24 x mixed	NS	NS	332.99^{ab}	9.53^{a}
30/18 x nitrate	NS	NS	374.67^{a}	9.34^{a}
30/18 x ammonium	NS	NS	318.73^{bc}	6.54^{d}
30/18 x mixed	NS	NS	282.90^{c}	8.58^{ab}

Means within columns followed by different letters are significantly different (**p** < 0.05**)**, NS = not significant.

Table 7.2 The effects of temperature and N-sources on flower qualities of *Curcuma* plant at flowering stage (12 WAP).

Treatment	Stalk length (cm)	Inflores-cence length (cm)	Inflores-cence width(cm)	No. green bracts	No. pink bracts
Temperature					
30/24 ^{0}C	53.38^{a}	9.47^{a}	3.75^{a}	7.88^{a}	8.67^{a}
30/18 ^{0}C	47.94^{b}	9.27^{a}	3.48^{a}	7.83^{a}	9.00^{a}
N-source					
nitrate	51.69^{a}	9.77^{a}	3.80^{b}	7.88^{a}	8.88^{ab}
ammonium	48.03^{b}	8.39^{b}	2.80^{c}	7.69^{a}	8.25^{b}
mixed	52.25^{a}	9.95^{a}	4.25^{a}	8.00^{a}	9.38^{a}
Temp. x N-source					
30/24 x nitrate	NS	NS	NS	NS	NS
30/24 x ammonium	NS	NS	NS	NS	NS
30/24 x mixed	NS	NS	NS	NS	NS
30/18 x nitrate	NS	NS	NS	NS	NS
30/18 x ammonium	NS	NS	NS	NS	NS
30/18 x mixed	NS	NS	NS	NS	NS

Means within columns followed by different letters are significantly different **(**$p < 0.05$**)**, NS = not significant.

Nitrate accumulation in leaves increased when plants were grown under low night temperature (2.34 mg g^{-1} DW) while there was no significant difference in fibrous roots. Besides, the concentration of nitrate in both fibrous roots and leaf tissues ranked in the order of nitrate>mixed-N>ammonium N sources. Ammonium concentration in fibrous roots and leaves was not significantly different under temperature treatment and the ammonium concentration in leaves was increased when observed in plant grown with NH_4^+ N-sources. There was higher NR activity in fibrous roots in plant grown under low temperature (1.69 μmoles g^{-1} FW h^{-1}) than in plant grown under high temperature (0.82 μmoles g^{-1} FW h^{-1}). Moreover, NR activity was higher in fibrous roots than in leaves under low temperature but this result was opposite in plant grown under high temperature (Table 7.3).

Total amino acid in old rhizome, old storage roots, new rhizomes and leaves were significantly different by treatments (Table 7.4). Total amino acid content in plant was higher in plants grown under low temperature than plants grown under high temperature. In addition, the decreasing of total amino acid in plant was observed in plants supplied with nitrate as N-sources (Table 7.4).

Table 7.3 The effects of temperature and N-sources on nitrate reductase activity, nitrate and ammonium concentration in fibrous roots and leaves of *Curcuma* plant at flowering stage (12 WAP).

	Nitrate (mg g^{-1} DW)		Ammonium (mg g^{-1} DW)		NRA (μmole g^{-1} FW h^{-1})	
Treatment	Fibrous roots	Leaves	Fibrous roots	Leaves	Fibrous roots	Leaves
Temperature						
30/24 ^{0}C	3.36^{a}	2.04^{b}	0.15^{a}	0.28^{a}	0.82^{b}	1.28^{a}
30/18 ^{0}C	3.28^{a}	2.34^{a}	0.12^{a}	0.29^{a}	1.69^{a}	1.12^{a}
N-source						
nitrate	5.26^{a}	3.41^{a}	0.24^{a}	0.25^{b}	1.48^{a}	1.60^{a}
ammonium	1.48^{c}	0.57^{c}	0.04^{c}	0.37^{a}	1.10^{b}	0.71^{c}
mixed	3.21^{b}	2.57^{b}	0.13^{b}	0.24^{b}	1.19^{b}	1.30^{b}
Temp. x N-source						
30/24 x nitrate	NS	3.09^{b}	0.32^{a}	NS	0.53^{d}	NS
30/24 x ammonium	NS	0.44^{e}	0.02^{c}	NS	0.94^{c}	NS
30/24 x mixed	NS	2.57^{c}	0.11^{b}	NS	1.00^{c}	NS
30/18 x nitrate	NS	3.74^{a}	0.16^{b}	NS	2.44^{a}	NS
30/18 x ammonium	NS	0.71^{d}	0.05^{c}	NS	1.26^{bc}	NS
30/18 x mixed	NS	2.57^{c}	0.14^{b}	NS	1.39^{b}	NS

Means within columns followed by different letters are significantly different **(**$p < 0.05$**)**, NS = not significant.

Table 7.4 The effects of temperature and N-sources on total amino acid of *Curcuma* plants at flowering stage (12 WAP).

Treatment	Total amino acid (mg plant^{-1})						
	Old rhizome	Old storage roots	Fibrous roots	New rhizomes	Leaves	Inflorescence	Total
Temperature							
30/24 ^{0}C	9.88^{a}	5.54^{a}	3.29^{a}	5.59^{a}	5.50^{b}	7.35^{a}	37.15^{b}
30/18 ^{0}C	7.48^{b}	6.92^{a}	3.30^{a}	6.55^{a}	8.62^{a}	8.83^{a}	41.70^{a}
N-source							
nitrate	7.90^{b}	6.10^{ab}	2.49^{b}	3.65^{c}	6.64^{b}	6.87^{b}	33.63^{b}
ammonium	10.26^{a}	5.12^{b}	3.84^{a}	8.28^{a}	9.08^{a}	7.64^{b}	44.22^{a}
mixed	7.89^{b}	7.47^{a}	3.56^{a}	6.28^{b}	5.46^{c}	9.76^{a}	40.42^{a}
Temp. x N-source							
30/24 x nitrate	8.79^{ab}	5.57^{b}	NS	2.63^{d}	4.28^{d}	NS	NS
30/24 x ammonium	10.64^{a}	5.81^{b}	NS	8.92^{a}	8.06^{bc}	NS	NS
30/24 x mixed	10.22^{a}	5.24^{b}	NS	5.23^{bc}	4.16^{d}	NS	NS
30/18 x nitrate	7.01^{bc}	6.62^{b}	NS	4.67^{cd}	9.00^{ab}	NS	NS
30/18 x ammonium	9.89^{a}	4.43^{b}	NS	7.64^{a}	10.11^{a}	NS	NS
30/18 x mixed	5.56^{c}	9.70^{a}	NS	7.33^{ab}	6.77^{c}	NS	NS

Means within columns followed by different letters are significantly different **(**$p < 0.05$**)**, NS = not significant.

At flowering stage, contents of nitrogen (mg plant^{-1}) were shown in Fig. 7.1. The highest nitrogen content was observed in leaves when plants were supplied with NO_3^- as the nitrogen source while the lowest nitrogen content was observed when plants were supplied with NH_4^+ as the nitrogen source in both temperature treatments.

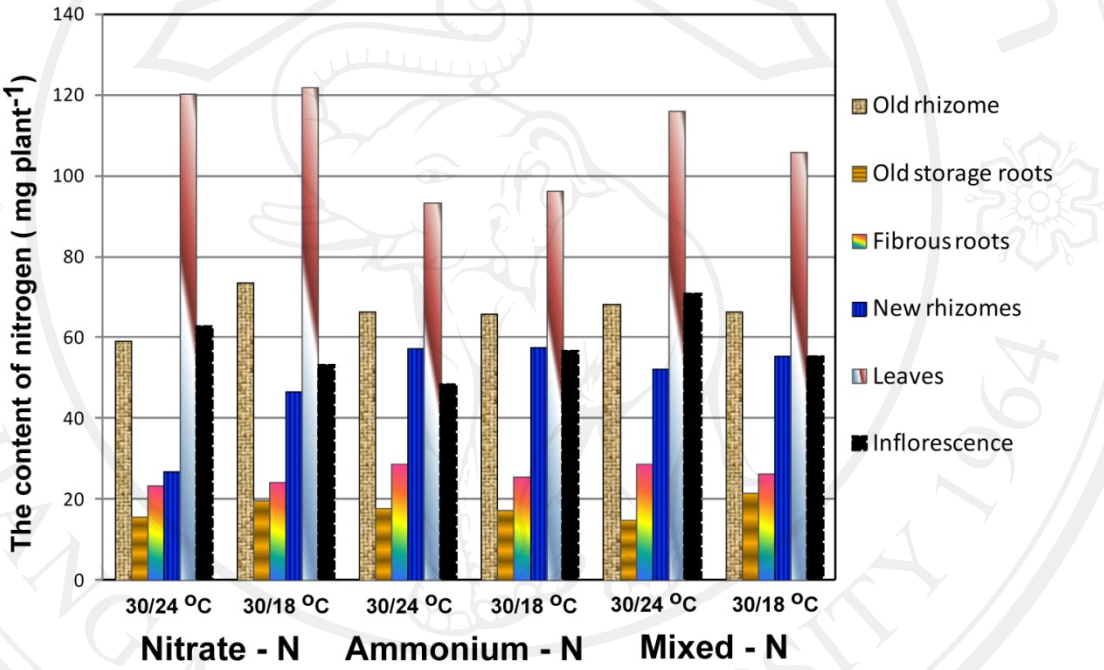

Figure 7.1 The content of nitrogen (mg plant^{-1}) in old rhizome, old storage roots, fibrous roots, new rhizomes, leaves and inflorescence of *C. alismatifolia* grown in different temperature and nitrogen sources at flowering stage (12 WAP).

Distribution of free amino acids (%) in old rhizome, old storage roots, fibrous roots, new rhizomes, leaves and inflorescence of *C. alismatifolia* grown in different temperature and N-sources were shown in Figures 7.2 and 7.3. The results showed that a large amount of Glu in old and new rhizomes of *Curcuma* plants was also detected in NO_3^- supplied treatment (Fig. 7.2a, 7.3a). The amino acids assimilated in *Curcuma* fibrous roots, were mainly gamma-aminobutyric acid (GABA), threonine (Thr) and Glu, when plants were grown in high temperature treatment (30/24 ^oC). However, N in fibrous roots was mostly incorporated into Asn and Asp when plants were grown in low temperature treatment (30/18 ^oC) (Fig. 7.2c). In this research, NH_4^+-fed plants also induced asparagines (Asn) accumulation in inflorescence (Fig. 7.3c) and such accumulation was much greater when in low temperature treatment. In addition, there was a high distribution of threonine (Thr) in both leaves and inflorescence organs when supplied with nitrate-N sources at low temperature treatment.

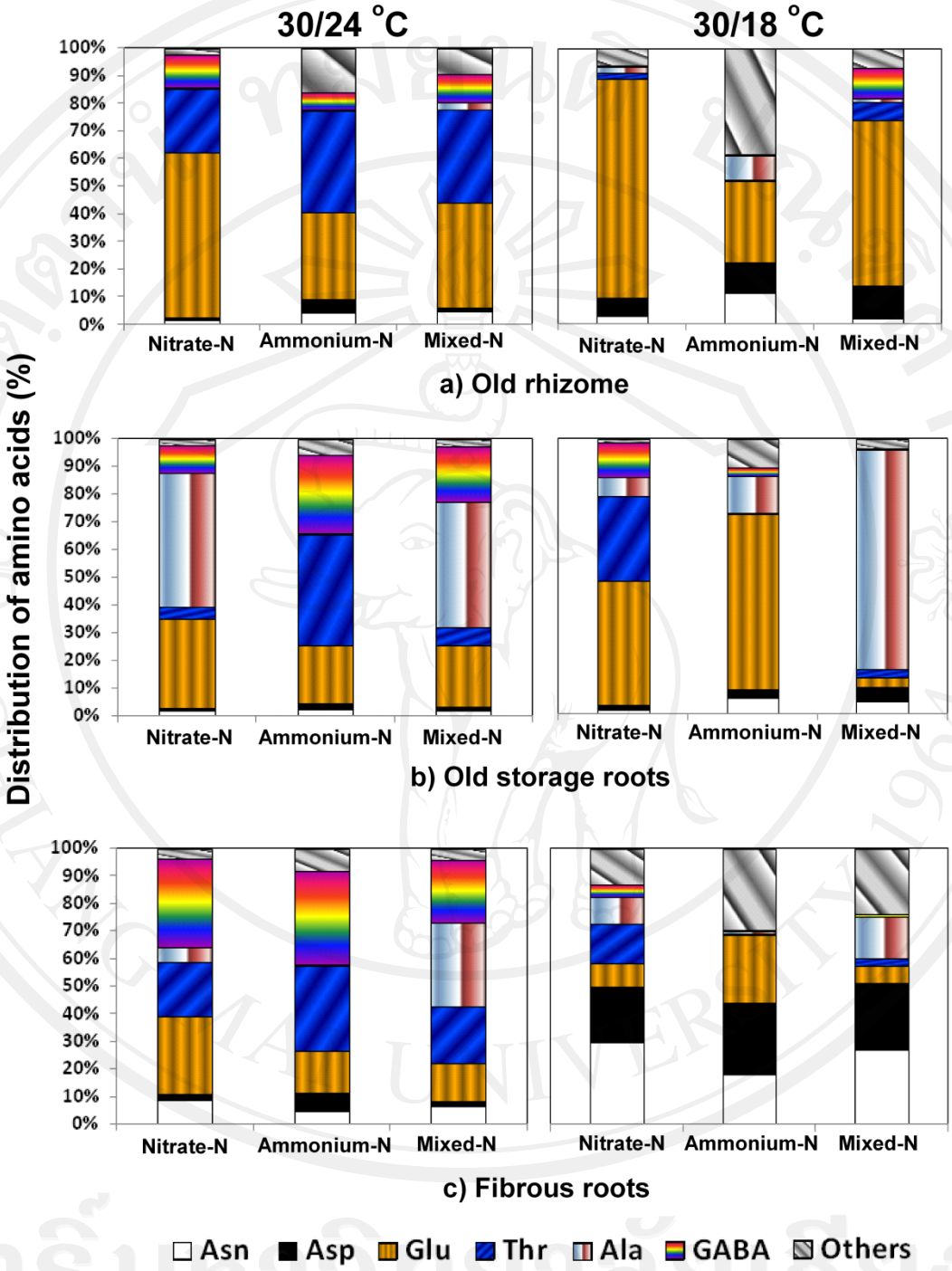

Figure 7.2 Distribution of free amino acids (%) in a) old rhizome, b) old storage roots and c) fibrous roots of *C. alismatifolia* grown in different temperatures and N-sources at flowering stage (12 WAP). Asn: asparagines, Asp: aspartic acid, Glu: glutamic acid, Thr: threonine, Ala: alanine, and GABA: gamma-aminobutyric acid.

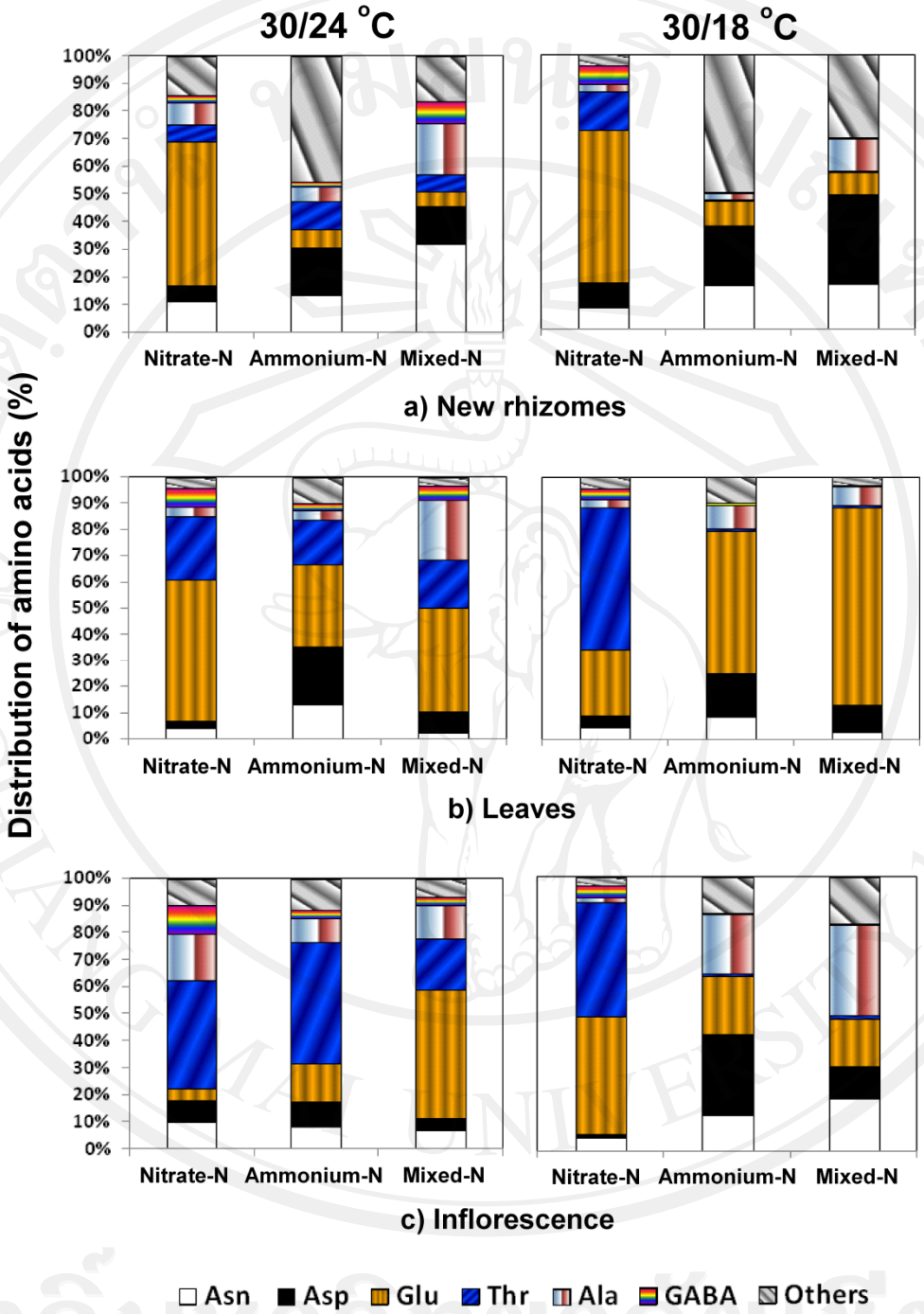

Figure 7.3 Distribution of free amino acids (%) in a) new rhizomes, b) leaves and c) inflorescence of *C. alismatifolia* grown in different temperatures and N-sources at flowering stage (12 WAP). Asn: asparagines, Asp: aspartic acid, Glu: glutamic acid, Thr: threonine, Ala: alanine, and GABA: gamma-aminobutyric acid.

7.4 Discussion

Low night temperature (30/18 ºC) decreased plant height and stalk length of *C. alismatifolia* (Tables 7.1 and 7.2), while N sources, especially NH_4^+-N, decreased chlorophyll content (SPAD value), total dry weight (Table 7.1), stalk length, inflorescence length and number of pink bracts (Table 7.2). Moreover, application of NH_4^+-N under low night temperature (30/18 ºC) significantly decreased leaf- area and total dry weight. This indicated that temperature affected the utilization of N and brought about the changes in plant growth and development. In legumes, low temperature is known to reduce nitrogen assimilation and dry matter accumulation (Gibson, 1967; Thomas and Sprent, 1984). However, *C. alismatifolia* is non-leguminous plant, the response of N assimilation to temperature therefore differed from that of leguminous plants. In this experiment, low night temperature increased NO_3^- accumulation in leaves (2.34 mg g^{-1} DW) and NR activity in roots (1.69 μmoles g^{-1} FW h^{-1}) compared with that in plant grown under high temperature (Table 7.3). On the other hand, N-sources had more influence on growth and N assimilation than temperature, since N-sources affected accumulation of NO_3^- and NH_4^+ in roots and leaves, NR activity in roots and leaves (Table 7.3) and also free amino acid concentration in all plant parts (Fig. 7.2 and 7.3).

Nitrogen content (mg $plant^{-1}$) in leaves was highest when plants were supplied with NO_3^- as the nitrogen source in both temperature treatments (Fig. 7.1). Ohyama (2010) reported that leaves played an important role in N assimilation through NO_3^- reduction and NH_4^+ assimilation to amino acid. In this experiment, the majority of nitrogen in all plant organs was in 80% ethanol insoluble fraction, as it has been

reported by Ohtake *et al*. (2006). Under 30/24 ˚C, supply with mixed-N source might be beneficial for plant growth because it increased total dry weight (9.53g) (Table 7.1).

Although most higher plants are capable of reducing NO_3^- in both roots and shoots (Marschner, 1995), NO_3^- is reduced more efficiently in leaves than in roots because of readily-available reductants, energy and carbon skeletons produced by photosynthesis (Oaks, 1994). In this experiment, it was suggested that leaves were the main site for NO_3^- assimilation in high temperature treatment. In contrast, fibrous roots were the main site for NO_3^- assimilation in low temperature treatments since NR activity was higher in fibrous roots than in leaves (Table 7.3). This result was consistent with the findings of Macduff and Trim (1986) who reported that root NRA of oilseed rape was highest when roots were grown at 3 and 7 ˚C while NRA in the petioles was highest when roots were at 11 or 17 ˚C. Other studies have actually reported increased root NR activity following low root temperature treatment (Dene-Drummond *et al*., 1980). Ding and Xi (1993) suggested 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). Ito *et al*. (2010) showed that NO_3^- was not only a substrate for nitrogen metabolism but also acted as a signal molecule for expression of the gene involved in uptake and assimilation of nitrogen (i.e., NO_3^- reduction, NH_4^+ assimilation, electron transport system, synthesis of coenzymes and carbon skeleton supply). NR is assumed to be the rate-limiting step for NO_3^- assimilation (Bussi *et al*., 1997). There is a close relationship between NR activity and NO_3^- concentration in plants (Skrdleta *et al*., 1979). In present experiment, there were positive linear relationships between the NO_3^- content and NR activity in leaves when

plants were grown under both 30/24 ˚C (R^2= 0.99) and 30/18 ˚C (R^2= 0.96) treatments, suggesting that the relationship between NR activity and NO_3^- content in the plants was dependent on the exogenous NO_3^- which might be mainly due to the cellular compartmentalization of NO_3^- (Aslam *et al*., 1976). A positive correlation between NR activity and nitrate concentration in leaf tissues implied that the activity of NR was induced by increasing NO_3^- concentration, as reported by Sivaasankar and Oaks (1996). However, NR activity decreased dramatically when plants were fed with NH_4^+ instead of NO_3^- nitrogen in eggplant, as it was dependent on plant species (Oaks, 1994). This result was probably caused by NH_4^+ which might decrease NR activity by feedback inhibition form and production of NH_4^+ assimilation (Orebamjo and Stewart, 1975).

Amino acids are used for building the proteins. Many amino acids also act as precursors of other nitrogen-containing compounds. In our experiment, a large amount of Glu in old and new rhizomes of *Curcuma* plants was also detected in NO_3^--supplied treatment (Fig. 7.2 and 7.3). The major form of inorganic nitrogen translocated to leaf cells is nitrate, which is then reduced to nitrite by NR in the cytosol. Nitrite is transported to chloroplast and reduced to ammonia by nitrite reductase. The ammonia is incorporated into an amino group of Glu by GS/GOGAT system together. The amino group of Glu is then utilized for the synthesis of a range of nitrogenous compounds (Ito *et al*., 2010). Our results showed that amino acids, assimilated in *Curcuma* fibrous roots, were mainly gamma-aminobutyric acid (GABA), threonine (Thr) and Glu, when plants were grown in high temperature treatment, indicating that high temperature could stimulate biosynthesis of GABA. The conversion of glutamate to GABA is increased under conditions that inhibit

glutamine synthesis, reduce protein synthesis or enhance protein degradation (Satya Narayan and Nair, 1990). This prompted the hypothesis that GABA is a temporary nitrogen store. However, N in *Curcuma* fibrous roots was mostly incorporated into Asn and Asp when plants were grown in low temperature treatment (Fig. 7.2). Khuankaew (2010) who reported that Asn was a major form of N accumulation in roots of *Curcuma* plants, as N was often present in very high concentrations in both xylem and phloem sap, and used to carry nitrogen away from source tissue (Lillo, 2004). Asparagine synthetase (AS) was considered as the major route for asparagines biosynthesis in plants (Lea *et al*., 1990). In an ATP-dependent reaction, AS catalyses the transfer of an amino group of glutamine to a molecule of Asp to generate a molecule of glutamine and asparagines (Coruzzi and Last, 2000). In this research, NH_4^+-fed plants also induced asparagines accumulation in inflorescence and such accumulation was much greater when in low temperature treatment. There was highly a distribution of threonine (Thr) in both leaves and inflorescence organs when supplied with nitrate-N sources in low temperature, suggesting that in low temperature, Thr was the main assimilated form of free amino acid in leaves and inflorescence when plants were supplied with nitrate-N sources.

7.5 Conclusions

Nitrogen assimilation in *Curcuma* plants was affected by both temperature and nitrogen sources. Mixed-N sources should be supplied when plants are grown under high temperatures, i.e., regular season while single form of nitrate-N or mixed-N sources could be used under low night temperature condition as off- season

production in Thailand. 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. Besides, the highest nitrogen content was observed in leaves when plants were supplied with nitrate-N sources. Assimilation of amino acids in old and new rhizome was mainly into glutamic acid (Glu) when supplied with nitrate-N sources while gamma-aminobutyric acid (GABA), threonine (Thr) and Glu, were mostly observed in fibrous roots when plants were grown in high temperature. NH_4^+-fed plants also induced asparagines (Asn) accumulation in inflorescence. Moreover, there was highly a distribution of threonine (Thr) in both leaves and inflorescence organs when supplied with nitrate-N sources in low temperature. Therefore, it was concluded that nitrogen ferilizer supply should be adjusted with respect to temperature and nitrogen sources for successful cultivation of *Curcuma*.