CHAPTER 6

Effect of ammonium: nitrate ratio on the growth and nitrogen assimilation of *Curcuma alismatifolia* Gagnep.

6.1 Introduction

Production of *C. alismatifolia* in terms of flower quality and rhizome yield depends on the response to N fertilizers (Ruamrungsri and Apavatjrut, 2003; Ohtake *et al*. 2006). Nitrogen-deficient plants are stunted and the quality of their flowers and rhizomes is significantly decreased. Ohtake *et al*. (2006) found that an increase in nitrogen from 0 to 50 mg L^{-1} increased the number of flowering shoots and consequently the number of rhizomes but depressed the growth of storage roots. Therefore, it is important to find suitable methods for increasing the number of storage roots. Providing an optimum level of nutrition is critical to ensure the maximum yield for cut-flower stems and to produce the maximum number of underground organs in *Curcuma* (Lee *et al*. 2008). Ruamrungsri *et al*. (2005) reported that 200 mg L^{-1} of nitrogen combined with 200 mg L^{-1} of potassium gave the best results in terms of plant height, number of plants/clump, diameter of new rhizomes, number of new rhizomes, length of storage roots and flower quality of *C. alismatifolia*

grown in soil-less medium. However, data on the optimum ratio of ammonium-N (NH_4^+) to nitrate-N (NO_3^-) are still lacking for this plant.

The NH_4^+: NO_3^- ratio in the root zone offers an important means of controlling the relative uptake of nutrients (Sonneveld, 2002). However, the preference for nitrate or ammonium varies according to species which is generally related to the physiological adaptations of plants to natural ecosystems (Adams and Attiwill, 1982). Although the major form of N in nutrient solutions for soil-less culture is NO_3^-, the addition of some NH_4^+ seems beneficial to plant growth (Gashaw and Mugwira, 1981; Sonneveld, 2002). Most plants, such as wheat, cucumber and bell pepper prefer nitrate as the major source of N (Heuer, 1991; Osorio *et al*. 2003). For French bean, an increase in the ratio of NH_4^+ to NO_3^- in the root zone impairs growth and reduces yield (Guo *et al*. 2002). However, while toxicity is observed in many plant species when NH_4^+ is provided alone, it can be alleviated by co-provision of nitrate (Britto and Kronzucker, 2002). For species such as cotoneaster (*Cotoneaster dammeri* Schneid., a woody ornamental) and rudbeckia (*Rudbeckia fulgida* Ait., a herbaceous perennial), NH_4^+ -N or a combination of NH_4^+ -N and NO_3^- –N yield greater growth than NO_3^- -N alone (Kraus and Warren, 2002). Modification of the NH_4^+: NO_3^- ratio in the nutrient solution affects the relative uptake of anions and cations (Sonneveld, 2002). Wang and Below (1992) 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. Similarly in potatoes, mixed form of N increased total and tuber dry weights, plant size, leaf area and specific leaf area as compared to either NH_4^+ or NO_3^- alone and the enhanced growth was greatest at 8% to 20% NH_4^+-N (Cao and Tibbitts, 1993).

NH_4^+: NO_3^- ratio may influence nitrate reductase activity (NRA). NRA is a measure of capacity for plants to reduce NO_3^- into usable forms for assimilation and the location of NO_3^- reduction has a critical influence on plant energy costs. Reduction of NO_3^- in the roots is associated with the increase of NO_3^- (Zogg *et al*, 1996). Conversely, assimilation of NO_3^- in leaves occurs at less energy cost to plants because reduction in leaves is powered mostly from direct products of photosynthesis (Schrader, 1984). Ding and Xi (1993) suggested that most NO_3^- was transported to leaves for assimilation but in some cases, NO_3^- was primarily reduced in the roots (Adams and Attiwill, 1982; Rothstein *et al*., 1996; Toselli *et al*., 1999). Mixed N source may optimize NRA and NO_3^- is known to induce NRA (Hageman, 1980). On the other hand, NH_4^+ may decrease NRA because of feedback inhibition form and product of NH_4^+ assimilation (Orebamjo and Stewart, 1975). By contrast, other studies contend that NH_4^+ inhibition rarely occurs and NH_4^+ is often required for optimal NRA (Mohanty and Fletcher, 1976; Beevers and Hageman, 1980).

The form and concentration of nitrogen influence the production of cytokinins (Sattelmacher and Marschner, 1978; Darrall and Wareing, 1981; Mercier and Kerbauy, 1991; Santokh *et al*., 1992; Smiciklas and Below, 1992; Wagner and Beck, 1993). Cytokinins are thought to be required for the initiation of cell division in tiller buds (Skoog and Armstrong, 1970). As they are mainly synthesized in the root apical meristem and transported to the shoot, a close relationship may exist between root growth and the overall cytokinin content of the plant (Skene, 1975; Carmi and Staden, 1983).

The effects of mixed applications of NH_4^+: NO_3^- on growth, yield and cytokinin productions in *C. alismatifolia* Gagnep. are largely unknown. In this study, we assess the impact of the NH_4^+: NO_3^- ratio on growth, NR activity, cytokinin levels, free amino acid and yield of *Curcuma* plant.

6.2 Materials and methods

6.2.1 Plant materials and experimental conditions

Rhizomes of *C. alismatifolia* cv. "Chiang Mai Pink" with a diameter of 1.8-2.5 cm and 4 storage roots were grown in 5-inch pots, using sand and vermiculite mixed at a ratio of 2:1, in a plastic greenhouse. Average temperatures were 30/24 °C (day/night). At six weeks after planting (6 WAP) when the first leaf had expanded, plants were treated with nutrient solutions consisting of different proportions of NO_3^- and NH_4^+. All solutions contained (in mg L^{-1}) 200N, 100P, 200K, 200Ca, 100Mg, 0.40Fe, 0.55Mn, 0.25Zn, 0.25Cu, 0.038Mo, and 0.22B (Table 6.1). Changes to the NH_4^+: NO_3^- ratio by varying the concentration of at least one or more nutrient were inevitable due to the charge balance constraint (Schrevens and Cornell, 1993; Savvas *et al*., 2003). The initial pH of the solution 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/pot.

Table 6.1 The concentration of N (200 mg L^{-1}) and other nutrients in the solution.

$NH_4^+ : NO_3^-$									
100:0		**75:250**		**50:500**		**25:75**		**0:100**	
NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-
200	0	150	50	100	100	50	150	0	200
Nutrient concentrations (mg L^{-1})									
P	K	Ca	Mg	Zn	B	Cu	Fe	Mn	Mo
100	200	200	100	0.25	0.22	0.25	0.4	0.55	0.038

6.2.2 Sampling

At the flowering stage (12 weeks after planting: WAP), plants of each treatment with four replications were randomly collected. Plant growth in terms of plant height, number of shoots per cluster and flower quality were measured. Leaf area was measured using a leaf- area meter (LI-3100, LI-COR, Lincoln, NE). The sampled plants were separated into the old rhizomes, old storage roots, fibrous roots, new rhizomes, leaves and inflorescence, and hot air oven dried at 60 °C for 7 days to determine dry weight. At the harvest stage, rhizome quality and yield in terms of the number of new rhizomes, rhizome diameter, the number of new storage roots, the diameter and length of storage roots and the total fresh weight of rhizomes per clump were measured.

6.2.3 Chemical analysis

Nitrogen analysis

Total nitrogen was determined by a modified indophenol method using a Kjeldahl-digested solution (Ohyama *et al.*, 1991) (Appendix 6).

Nitrate and ammonium analysis

Nitrate and ammonium concentration in leaves and fibrous roots were measured using a reflectometer (RQflex, Merck KgaA, Darmstadt, Germany) (Appendix 7).

NR activity analysis

Nitrate reductase activity was assessed using methodology in Truax *et al*. (1994) with the following modifications. Leaves and fibrous roots were rinsed and then dried of surface water before sampling to minimize contamination and ensure that weights were not skewed by water on the samples. The samples were cut into small pieces and mixed thoroughly. A 0.2 g of sample were placed in a test tube containing 3 ml incubating solution (100 mM phosphate buffer [pH 7.5], 40 mM KNO_3, and 1.2% 1-propanol) and placed in the dark for 1 hour at room temperature. The enzymatic reaction was stopped by removing the plant tissue. A 1 mL aliquot was taken from the tube with a pipette, mixed with 1 mL naphthyl ethylenediaminehydrochloride (0.02%) and 1 mL sulfanilamide (1% in 3M HCl), then measured at absorbance 540 nm after 30 min. NRA values were expressed as μmoles $NO_2^- g^{-1}$ fresh weight h^{-1} (Appendix 8).

Free amino acid

Free amino acid was analyzed by ACQUITY UPLC^{TM} amino acid analyzer (Appendix 10).

Cytokinin analysis (t-ZR)

At the flowering stage, plants were separated into leaves and fibrous roots, washed with water and immediately frozen in liquid nitrogen. They were then freeze-dried, weighed and ground into a fine powder and stored at -20 °C until the analysis. The extraction was modified following Potchanasin *et al*. (2009), 100 mg DW of sample powder was extracted with 50 mL of 80% cold methanol overnight at 4 °C. The extract was filtered through a G-4 glass-inter-filter (max. pore size, 10-16 μm). The filtrate was evaporated at 40 °C and the residues re-dissolved in 0.01M ammonium acetate (pH 7.5) and frozen at -20 °C overnight. After thawing, the extracts were centrifuged at 22,000 rpm for 25 min at 4°C. The supernatant was passed through a pre-conditioned column filled with PVP followed by Sep–Pak. The cytokinin (CK) was eluted directly from the removed Sep-Pak with 30% methanol in 0.01 M acetic acid, evaporated overnight, re-dissolved in absolute methanol and refrigerated at 4 °C until assayed. The measurements of CK (*t*-ZR) were carried out by using a Phytodetek CK (*t*-ZR) Test Kit for competitive ELISA, monoclonal antibody (Agdia, Inc. Elkhart, IN) (Appendix 11).

6.2.4 Statistical analysis

The experiment was a randomized complete block design with four replications. Analyses of variance (ANOVAS) were carried out using the Statistic 8 analytical software package (SXW Tallahassee, FL). In the case of significant treatment effects, a comparison of means was performed using LSD at a significance level of 0.05.

6.3 Results

Plant height at flowering stage was significantly shorter when NH_4^+ (100%) was used as the sole source of nitrogen in the nutrient solution. The number of shoots per clump was not affected (data not shown) but the number of leaves per plant was significantly greater with 100% NO_3^- than the other treatments (Fig. 6.1).

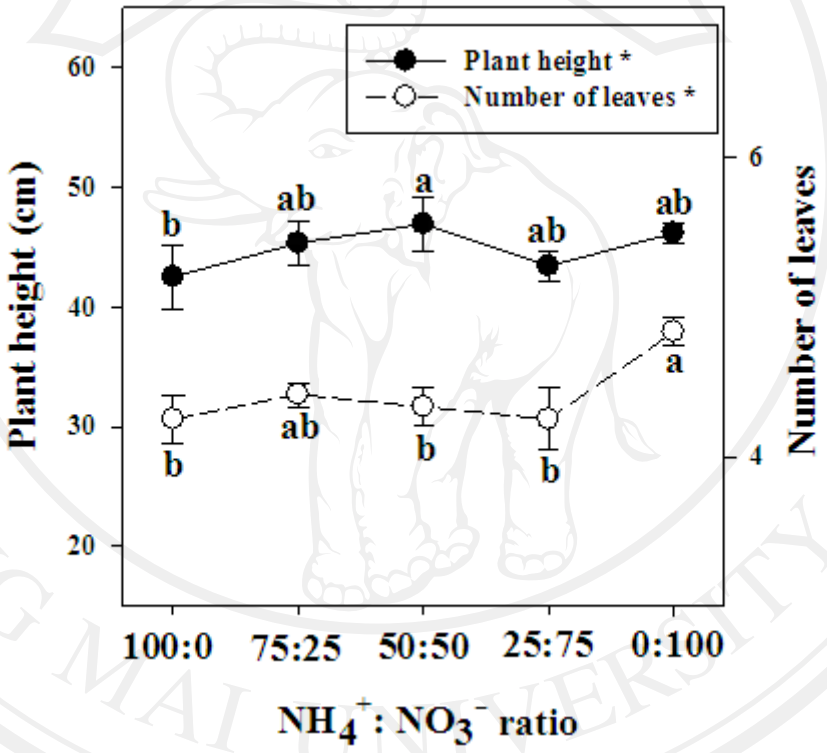

Figure 6.1 Effect of the NH_4^+: NO_3^- ratio on the growth of *C. alismatifolia*. at flowering stage (12 WAP). The vertical bars represent the SE of the mean.*Significantly different among treatments at $p < 0.05$; NS= not significantly different.

There was a trend toward higher total plant dry weights as the concentration of NO_3^- in the nutrient solution increased (Fig. 6.2). The plants grown with 100% NH_4^+ showed the lowest dry weights of leaves, inflorescence and total plant weight (Fig. 6.2) but there were no significant effects of the NH_4^+: NO_3^- ratio on the dry weight of old rhizomes, old storage roots, fibrous roots or new rhizomes (Fig. 6.2).

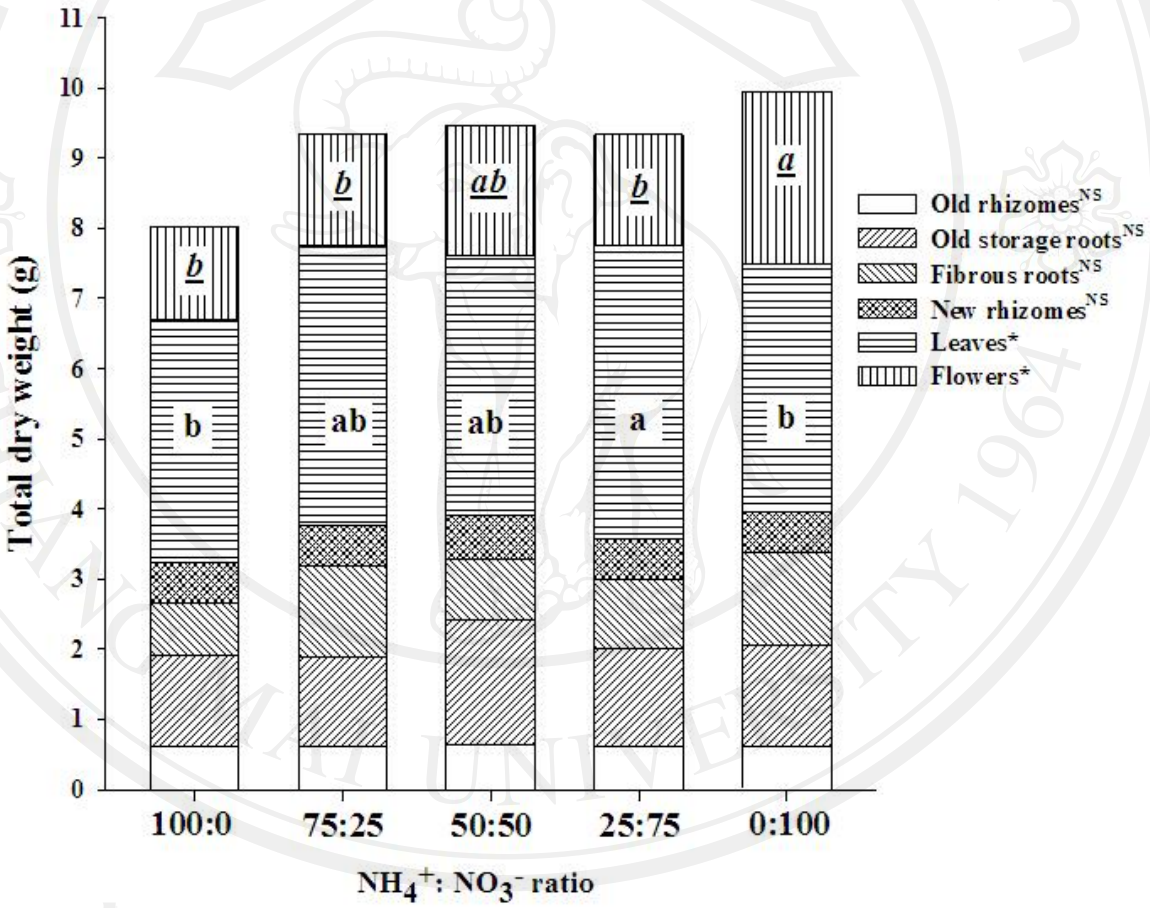

Figure 6.2 Effect of the NH_4^+: NO_3^- ratio on the dry weight of plant parts of *C. alismatifolia*. at flowering stage (12 WAP). *Significantly different among treatments at $p < 0.05$; NS= not significantly different.

Leaf area was greatest in the plants grown under the highest concentration of NO_3^- (0:100) and not significantly different at 100:0, 75:25, 50:50 and 25:70 (Fig. 6.3). An increase of NO_3^- from 0% to 50% also increased the SPAD value which then progressively decreased at 50% to 100% NO_3^- (data not shown).

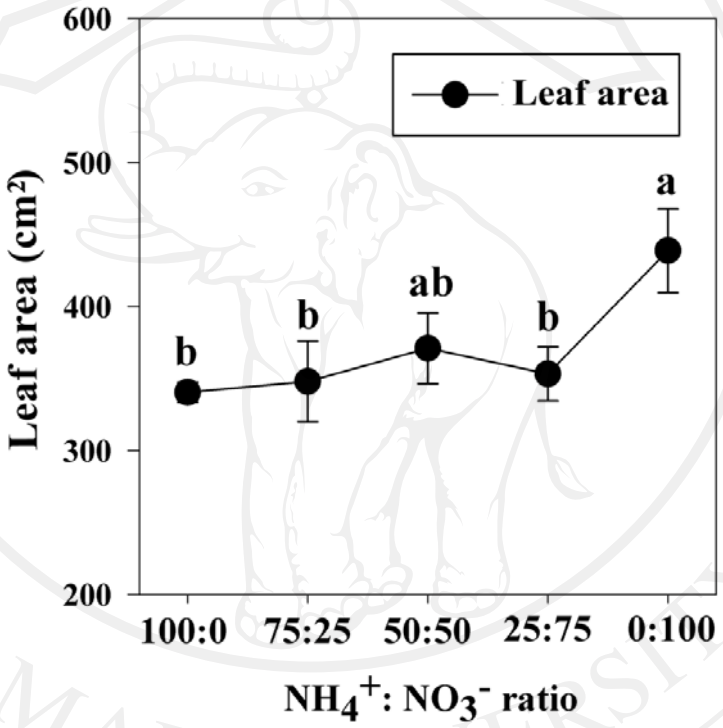

Figure 6.3 Effect of the NH_4^+: NO_3^- ratio on the leaf area of *C. alismatifolia*. at flowering stage (12 WAP). The vertical bars represent the SE of the mean.*Significantly different among treatments at $p < 0.05$; NS= not significantly different.

Stalk lengths were shorter in the plants grown with 100% NH_4^+ (46.8 cm) or 100% NO_3^- (51.4 cm) than in those grown with mixed nitrogen sources (Fig. 6.4, 6.5). Flower spike length was greatest (17.5 cm) in the plants grown with 100% NO_3^- (Fig. 6.4). The numbers of green and pink bracts were not affected by the treatment (data not shown).

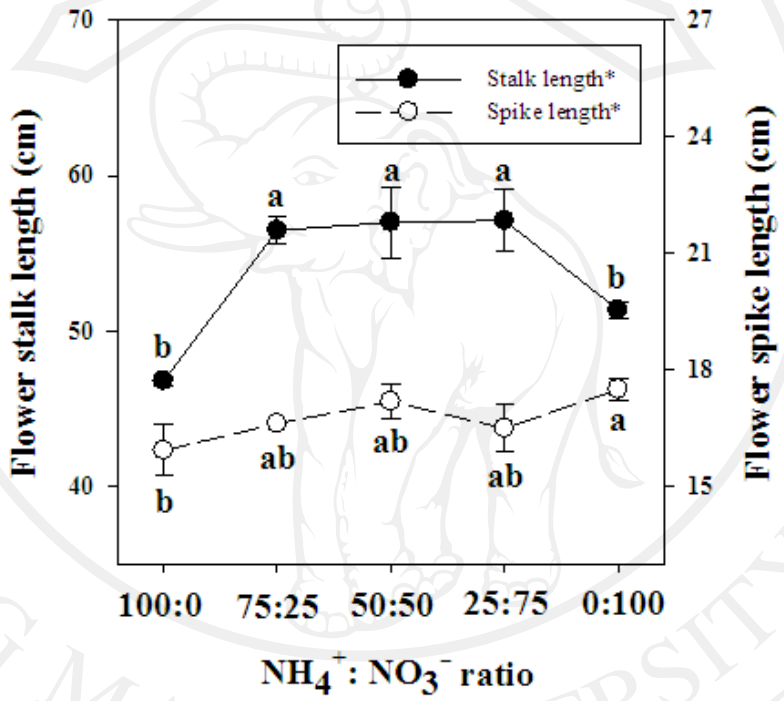

Figure 6.4 Effect of the NH_4^+: NO_3^- ratio on the stalk length and spike length of *C. alismatifolia*. at flowering stage (12 WAP). The vertical bars represent the SE of the mean.*Significantly different among treatments at $p < 0.05$; NS= not significantly different.

Figure 6.5 Effect of NH_4^+: NO_3^- ratio on the inflorescence quality of *C. alismatifolia*. at flowering stage (12 WAP).

There was significantly more NRA in the leaf compared to the fibrous roots and in fibrous roots was not significantly different among treatments. In leaf, there was a trend toward higher NRA with increasing NO_3^- ratio in the nutrient solution and the highest NRA in leaf was observed in 100% NO_3^- treatment (Fig. 6.6).

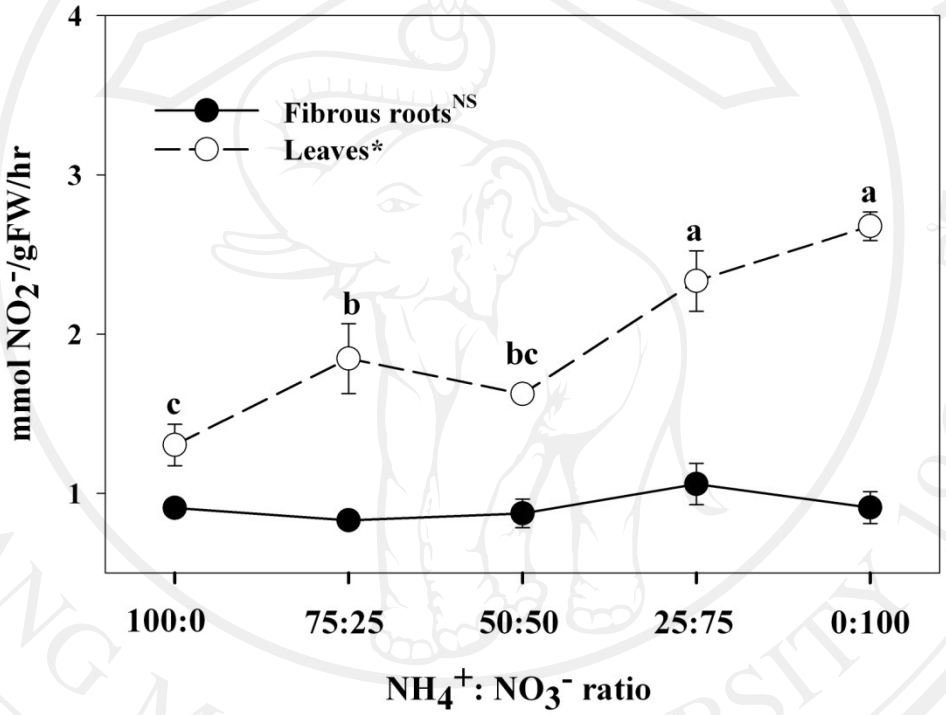

Figure 6.6 Effect of NH_4^+: NO_3^- ratio on nitrate reductase acticity of *C. alismatifolia*. at flowering stage (12 WAP). The vertical bars represent the SE of the mean.*Significantly different among treatments at $p < 0.05$; NS= not significantly different.

The different ratios of nitrate had a significant effect on the nitrate content of the leaves and fibrous roots. The nitrate content was always higher in the leaves. However, nitrate concentrations in leaves increased linearly as the percentage of NO_3^- increased from 0% to 50%, and in fibrous roots at 0% to 75 % NO_3^- (Fig. 6.7A).

The ammonium concentration in fibrous roots was relatively constant ranging from 137.4 to 208.2 mg/kgDW. In leaves, however, it was constantly low, i.e., at 27.8 to 47.9 mg/kgDW, and not affected by the NH_4^+: NO_3^- ratio (Fig. 6.7B).

The highest and lowest endogenous cytokinin levels in fibrous roots were obtained when plants were supplied with 100% NH_4^+ and 100% NO_3^-, respectively. In contrast, cytokinin levels in leaves were highest in the plants grown with 100% NO_3^-. In fibrous roots, increasing the proportion of NO_3^- in the nutrient solution from 0% to 50% significantly decreased cytokinin levels from 111.5 to 81.9 ng/gDW. On the other hand, cytokinin levels in leaves significantly increased from 81.7 ng/gDW to 136.2 ng/gDW when the percentage of NO_3^- increased from 25% to 100% (Fig. 6.7C).

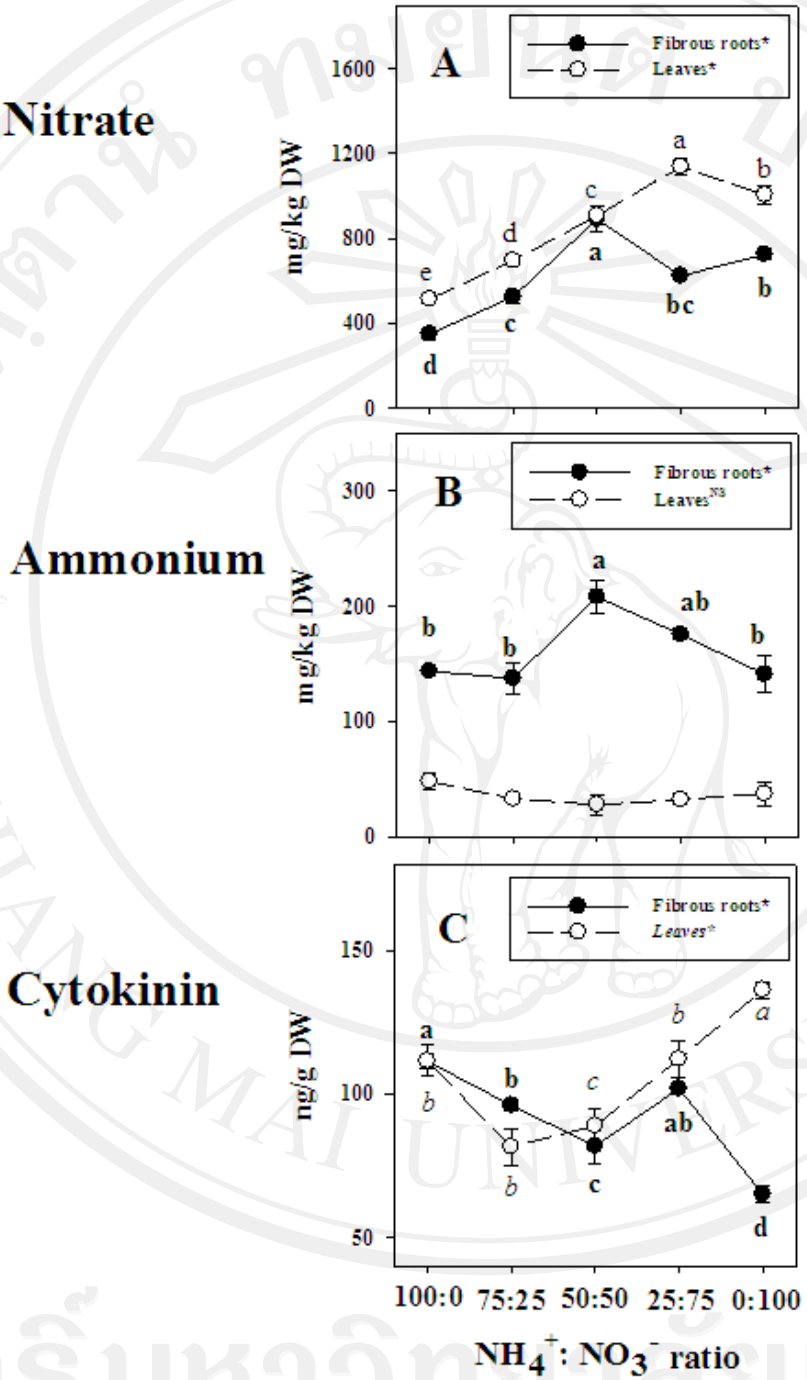

Figure 6.7 Effect of NH_4^+: NO_3^- ratio on nitrate (A), ammonium (B) and cytokinin (C) concentrations in fibrous roots and leaves of *C. alismatifolia*. at flowering stage (12 WAP). The vertical bars represent the SE of the mean.*Significantly different among treatments at $p < 0.05$; NS= not significantly different.

Distributions of free amino acids (%) in old rhizome, old storage roots, fibrous roots, new rhizomes, leaves and inflorescence of *C. alismatifolia* grown in different NH_4^+: NO_3^- ratios were shown in Fig. 6.8. In curcuma old rhizome, glutamic acid (Glu) was recognized as a major form of the accumulation N, particularly in plant supplied with 25-100 % NO_3^- treatments while glutamic acid (Glu), alanine (Ala) and gamma-aminobutyric acid (GABA) were recognized as a major form of the accumulation N in 100% NH_4^+ (Fig. 6.8a). In old storage roots, aspartic acid (Asp) and glutamic acid (Glu) were major form of accumulation N in all treatments, and alanine (Ala) and gamma-aminobutyric acid (GABA) were added in 100% NH_4^+ treatments (Fig. 6.8b). In fibrous roots, leaves and inflorescence, aspartic acid (Asp), glutamic acid (Glu) and threonine (Thr) were major form of accumulation N in all treatments and alanine (Ala), gamma-aminobutyric acid (GABA) were added in 100% NH_4^+ treatments (Fig. 6.8c, 6.8e, 6.8f). In new rhizomes, asparagines (Asn), aspartic acid (Asp) and glutamic acid (Glu) were major form of accumulation N in all treatments, which threonine (Thr), alanine (Ala) and gamma-aminobutyric acid (GABA) were added in 100% NH_4^+ treatment (Fig. 6.8d).

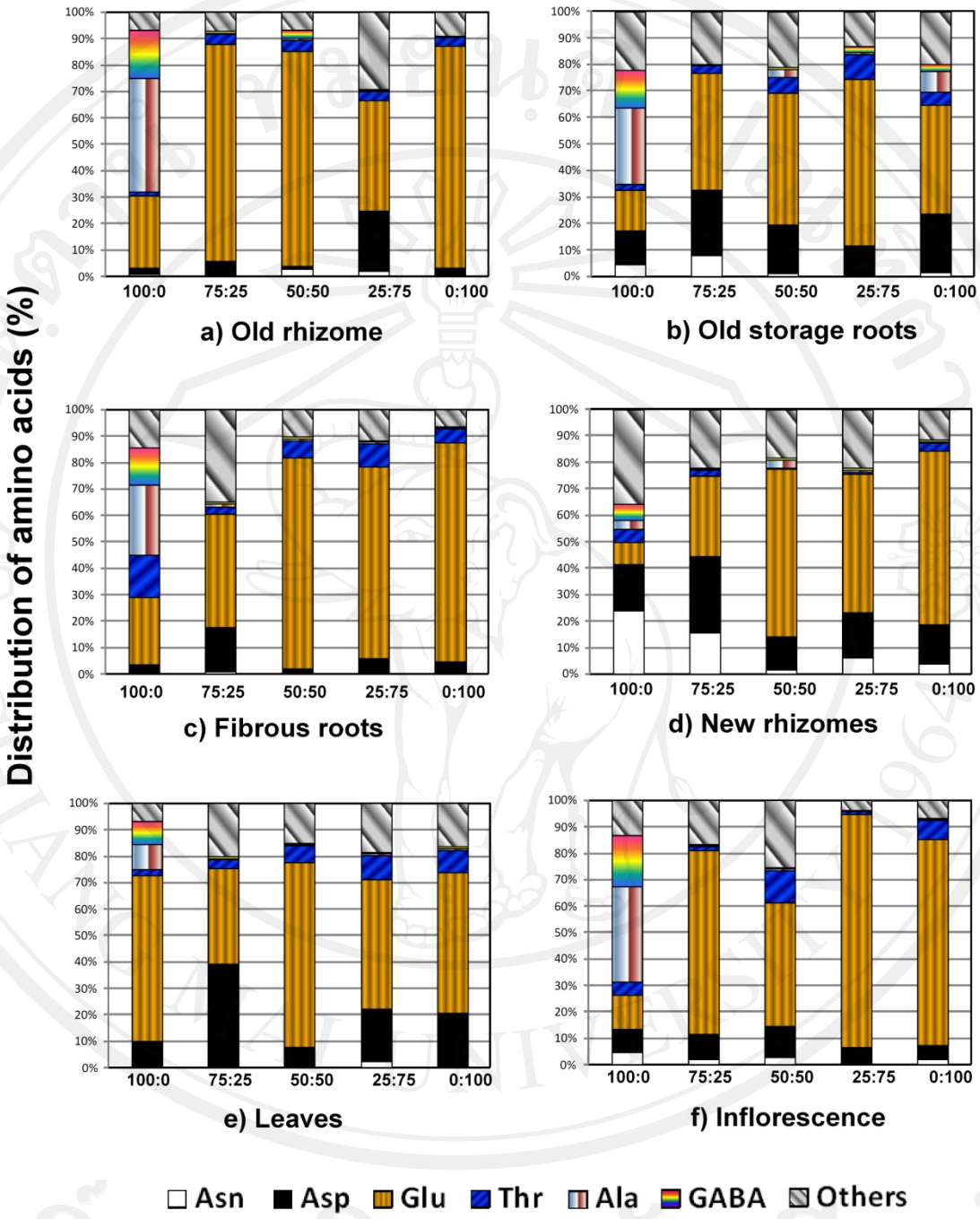

Figure 6.8 Effect of NH_4^+: NO_3^- ratio on distribution of free amino acids (%) in a) old rhizome, b) old storage roots, c) fibrous roots, d) new rhizomes, e) leaves and f) inflorescence of *C. alismatifolia*. at flowering stage (12 WAP). Asn: asparagines, Asp: aspartic acid, Glu: glutamic acid, Thr: threonine, Ala: alanine, and GABA: gamma-aminobutyric acid.

Rhizome quality, in terms of the number of storage roots and total rhizome fresh weight per clump, was highest after the $50NH_4^+$: $50NO_3^-$ treatment (Fig. 6.9, 6.10). The number of new storage roots and the total rhizome fresh weight per clump were increased by 45% and 33%, respectively, when the plants were grown with $50NH_4^+$: $50NO_3^-$ compared to when they were grown with 100% NH_4^+.

Figure 6.9 Effect of NH_4^+: NO_3^- ratio on rhizome quality at harvest stage.

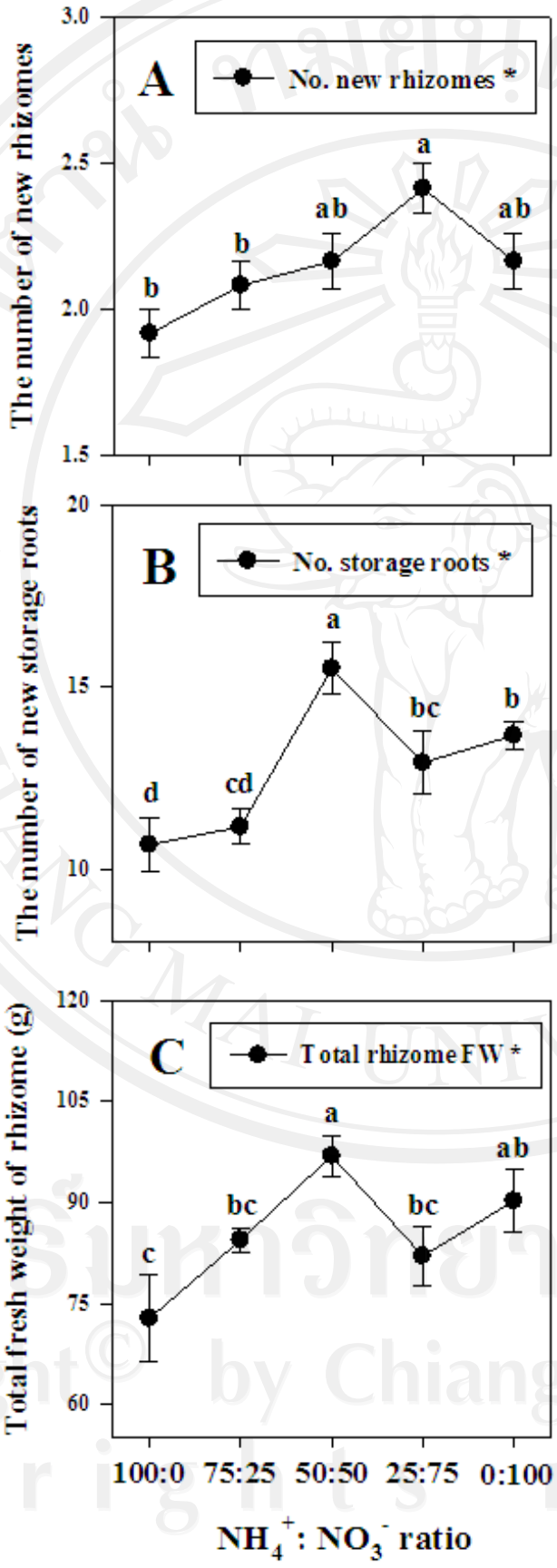

Figure 6.10 Effect of NH_4^+: NO_3^- ratio on the quality of *C. alismatifolia*. rhizomes. The vertical bars represent the SE of the mean.*Significantly different among treatments at $p< 0.05$; NS= not significantly different.

6.4 Discussion

The preference source of nitrogen varies with plant species (Haynes, 1986). In many cases, mixture of NH_4^+ and NO_3^- is preferred, but in some cases, one form is favored over another (Hageman, 1980; Haynes, 1986). When *C. alismatifolia* was grown in a solution containing 100 % NH_4^+, the number of leaves per plant (≈ 12%), inflorescence dry weight (≈ 47%), total dry weight (≈ 15%) and leaf area (≈ 25%) decreased significantly compared to values for plants grown in 100% NO_3^-. These results might indicate that NO_3^- is favored by *C. alismatifolia*, as it is by tomato (Claussen, 2002) and strawberry (Tabatabaei *et al*., 2006). NH_4^+ at high concentrations can be toxic, causing a deficiency of nutrients, acidification of the root zone, alterations in the osmotic balance, changes in phytohormone levels and impairment of N metabolism (Gerendas *et al*., 1997; Lorenzo *et al*., 2000). The acidification of the root zone did not occur in this experiment since the pH of the solution was maintained at 5.8- 6.0. However, NH_4^+: NO_3^- at 25:75, 50:50 and 75:25 caused an increase in both leaf dry weight and stalk length. This result was similar to that observed in *Tetragonia tetragonioides* treated with both NO_3^- and NH_4^+ (Ahmed and Johnson, 2000). In spinach, enhanced growth was also observed when NO_3^- and NH_4^+ (25:75, 50:50) were supplied compared with just NO_3^- or NH_4^+ alone (Zhang *et al*., 2005). The appropriate combination of these two forms appears to be beneficial to plant growth (Gashaw and Mugwira, 1981; Ikeda and Osawa, 1983; Salsac *et al*., 1987). Several explanations have been offered for the nitrate-ammonium synergism. One possibility is that the amount of energy consumed is higher for the assimilation of nitrate than that of ammonium. In fact, the energy requirement for the assimilation of

NO_3^- is 20–21 ATPmol^{-1}, compared to only 5 ATPmol^{-1} for the assimilation of NH_4^+ (Traore and Maranville, 1999).

The concentration of nitrate in fibrous roots and leaves generally increased with the proportion of NO_3^- . However, the ammonium concentration was relatively constant in fibrous roots and fairly low in leaves regardless of the ratio of NH_4^+ to NO_3^-. These results indicate that *C. alismatifolia* prefers nitrate even when both NH_4^+ and NO_3^- are supplied and the absorbed ammonium is retained in fibrous roots.

The growth indices of aboveground parts, such as the length of the stalk and flower spike and leaf area, were also altered by changing the ratio of NO_3^- to NH_4^+ in the nutrient solution. It is possible that the production of cytokinins is affected by varying the proportions of NO_3^- and NH_4^+ (Smiciklas and Below. 1992; Chen *et al*., 1998). In our studies, the leaf area of *Curcuma* plants decreased as the percentage of NH_4^+ in the solution increased. This result is consistent with the findings of Raab and Terry (1994) who reported that the effect of NH_4^+ could be attributed mainly to a reduction in leaf area. The reduced leaf area may be due to reduced cell numbers (MacAdam *et al*., 1989) and/or smaller cell sizes (Taylor *et al*., 1993; Palmer *et al*., 1996; Snir and Neumann, 1997). The negative effect of NH_4^+ on leaf area is explained in part by decreased root-to-shoot translocation of cytokinins (Fig. 6.6, Walch *et al*., 2000).

Although most higher plants are capable of reducing NO_3^- in both roots and shoots (Marschner, 1995), nitrate is reduced more efficiently in leaves than in roots because of readily available reductants, energy and carbon skeletons produced by photosynthesis, which is dependent on plant species (Solomonson and Barber, 1990;

Oaks, 1994). In *C. alismatifolia*, the leaves were the main site for nitrate assimilation since NRA was found higher in leaves than roots. Nitrate taken up by a plant is reduced or stored in the vacuoles or transported in the xylem transpiration stream to the leaf for reduction, and most is stored in the vacuole until released for reduction in cytosol (Cardenas-Navarro *et al*., 1999). The NR is assumed to be the rate-limiting step for nitrate assimilation (Caba *et al*., 1995; Bussi *et al*., 1997), there is a close relationship between NRA and nitrate concentration in plants (Skrdleta *et al*., 1979). In present experiment, the regression analysis indicated positive linear relationship between the NO_3^- content in leaves and the proportion of NO_3^- -N in the nutrient solution (r=0.8842). Moreover, there were positive linear relationships between the NO_3^- content and NRA in leaves (r = 0.6896), suggesting that the relationship between NRA and nitrate content in plant was dependent on the exogenous nitrate, which might be mainly due to the cellular compartmentation of nitrate (Ferrari *et al*., 1973; Aslam *et al*., 1976) and it was insufficient to evaluate the nitrate content in plant only by NRA. However, the relationship between NRA and nitrate concentration has been still uncertain. Some studies indicated that the higher NRA was, the more nitrate might be reduced, so there was negative relationship between NRA and nitrate concentration (Hu *et al*., 1992). Most studies show that, with NR being a substrate-induced enzyme, the higher substrate-nitrate concentration was in plant, the higher NRA might be, so there was positive correlation between them (Skrdleta *et al*., 1979; Reddy and Menary, 1990).

The present results showed that the concentration of cytokinins in fibrous roots decreased as the amount of NO_3^- increased. Similarly in maize, plants grown with mixed N had higher concentrations of cytokinins in roots than plants grown with

predominantly NO_3^- (Smicilas and Below, 1992). The mixed-N or 100% NH_4^+ may favor greater cytokinin production in plant roots (Wang and Below, 1996). However, there were higher cytokinin concentrations in leaves of plants grown with 100% NO_3^- than 100% NH_4^+ (Fig. 6.6C). Similarly, the application of cytokinins to one side of wheat *(Triticum aestivum* L.) plants grown in a 'split' root system resulted in increased N retention by the shoot under NO_3^--N limiting conditions (Richard *et al*., 1982). On examining different N-form regimes, Ei-D *et al*. (1979) found that NO_3^--N stimulated plant growth and increased the endogenous zeatin concentration in exudate from sunflower *(Helianthus annuus* L.) roots, compared with NH_4^+-N. In contrast, NH_4^+-N did not inhibit the growth of young apple *(Pyrus malus)* rootstocks, with higher zeatin concentrations found in xylem exudates than when NO_3^--N was supplied (Buban *et al*., 1978). Thus, our results suggested that the increase in nitrate in nutrient enhanced not only the production of cytokinins in roots, but also the root-shoot translocation of cytokinins.

At flowering stage, distributions of free amino acids in plant organs were mostly incorporated into asparagines (Asn), aspartic acid (Asp), glutamic acid (Glu), and threonine (Thr) in all NH_4^+: NO_3^- treatments. 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. Following the assimilation of ammonia into glutamine and glutamic acid, these two amino acids act as important nitrogen donors in many cellular reactions, including the biosynthesis of aspartic acid and asparagine (Givan, 1980; Lea, 1993). In addition, our results also suggested that alanine (Ala) and gamma-aminobutyric acid (GABA) were mainly distributed in plant organs, especially in 100% NH_4^+ treatments (Fig. 6.8). GABA

syntheses play an integral role in ammonium metabolism and represent a homeostatic mechanism important for plant stress tolerance (Aurisano *et al*., 1995). An accumulation of GABA was also characteristic responses to environmental stress (Roberts *et al.,* 1992). Roberts *et al*. (1992) reported that increases in Ala and Glu had also been observed following the onset of hypoxia and in the case of hypoxic maize root tips and the accumulation of Ala, Glu, and GABA was enhanced by pretreatment with ammonium. The accumulation of these amino acids was consistent with cytoplasmic pH, being regulated by changes in primary metabolism because the syntheses of Ala, arising from the decarboxylation of malate as a result of malic enzyme activation by cytoplasmic acidosis, the conversion of Gln to Glu, and the decarboxylation of Glu to GABA were all proton-consuming reactions (Roberts *et al*., 1992).

Numbers of green and pink bracts were not affected by the NH_4^+: NO_3^- ratio, however, flower quality in terms of stalk length and spike length were significantly increased by $50NH_4^+$: $50NO_3^-$ (Fig. 6.5). The increased rhizome yield in terms of fresh weight of rhizome per clump with the $50NH_4^+$: $50NO_3^-$ treatment was the result of increases in the numbers of new rhizomes and the numbers of new storage roots per clump. The lower rhizome yield of plants supplied with NH_4^+ compared with NO_3^- has been shown to be due to various factors. The optimal nutritional balance in cultivation depends on the specific response desired (photochemical composition or dry-weight production) of a plant species.

6.5 Conclusion

In this experiment, it is may conclude that the increase in nitrate in nutrient enhanced both the production of cytokinins in roots and the root-shoot translocation of cytokinins. Moreover, the leaves were the main site for nitrate assimilation since NRA was found higher in leaves than roots. Free amino acids in plant organs were mostly assimilated into asparagines (Asn), aspartic acid (Asp), gutamic acid (Glu) and threonine (Thr). Morover, alanine (Ala) and gamma-aminobutyric acid (GABA) were mostly found in 100% NH_4^+ treatments. The results of the present experiment indicated that *C. alismatifolia* prefers a combination of NH_4^+ and NO_3^- -N rather than a sole source of N. A combination of these two forms of N in an appropriate ratio ($50NH_4^+$: $50NO_3^-$) appears to be beneficial to growth, inflorescence quality and rhizome production of this plant.