Chapter

Nitrogen in Flowers

Soraya Ruamrungsri, Kanokwan Panjama, Takuji Ohyama and Chaiartid Inkham

Abstract

This chapter explores the literature and research on nitrogen in flowers. An overview of nitrogen deficiency symptoms in some flowers, i.e., *Curcuma alismatifolia* (ornamental curcuma), *Tagetes erecta* (marigold), *Zinnia violacea* (zinnia), and *Gomphrena globose* (gomphrena) were presented. Additionally, nitrogen uptake, translocation, and application in some flowers, i.e., ornamental curcuma, narcissus, orchids, and rose, were discussed in this chapter. Nitrogen affects the life cycle of flower, including vegetative and reproductive phases. Flower size, stem length, number of flowers per plant, and color were reduced by nitrogen deficiency. Therefore, the optimum level of nitrogen supply in each growth stage is important for flower crop production.

Keywords: nitrogen deficiency, nitrogen uptake, nitrogen application, nitrogen translocation, flowers

1. Introduction

Flower crops, similar to other horticultural crops, require optimum fertilizer for a good quality flower size, stem length, number of florets, stem, and petal color. Essential elements, especially nitrogen, play an important role in growth and development in each stage of the life cycle. Different genera have different nitrogen requirements. Generally, they have a nitrogen content that is enough for root emergence and shoot sprouting. However, flowers grown from seeds may require fertilizer as soon as root emergence. A lack of fertilizer supply will lead to severe nutrient deficiency. Seed germination starts with water uptake, and then food reserves, i.e., carbohydrates and storage proteins, are oxidized for the growth process [1]. Flower seedlings show deficiency symptoms when the fertilizer supply is not enough. Nitrogen is an especially important element in the life cycle of plants from seedlings to the vegetative stage and flowering until senescence. It affects flower qualities, such as size, stem length, and color. This chapter focuses on the role of nitrogen in some economic flower crops. Most information was derived from our research experiments and some are unpublished data.

2. Nitrogen deficiency symptoms in different flower species

2.1 *Curcuma alismatifolia* (ornamental curcuma)

Nitrogen deficiency (-N) affected the growth and characteristics of *C. alismatifolia* (**Table 1** and **Figure 1**). Most growth parameters, such as plant

Nutrient solution	Plant growth at flowering stage (12 weeks after planting)							
	Plant Height (cm)	No. leaves per plant	Root length (cm)	Leaf green color intensity (SPAD unit)		Leaf area (cm^2)	Total fresh weight (g)	Total dry weight (g)
				Old leaf	Young Leaf			
Complete	49.1 a	5.0 a	42.8 a	57.1 a	52.5 a	253.8 a	285.0 a	31.3 a
-N	36.0 b	4.3 a	40.6 b	34.2 b	52.8 a	191.2 b	111.4 b	14.7 b
%CV	8.7	8.8	0.9	10.8	4.9	10.6	11.0	12.4
$LSD_{0.05}$	*	NS	*	*	NS	*	*	*

**Means within the same column followed by different letters were significantly different in an LSD test; ($p \leq 0.05$). NS = not significant.*

Table 1.
Plant growth of Curcuma alismatifolia *treated with complete nutrient solution or nitrogen deficiency (-N) at the flowering stage (12 weeks after planting).*

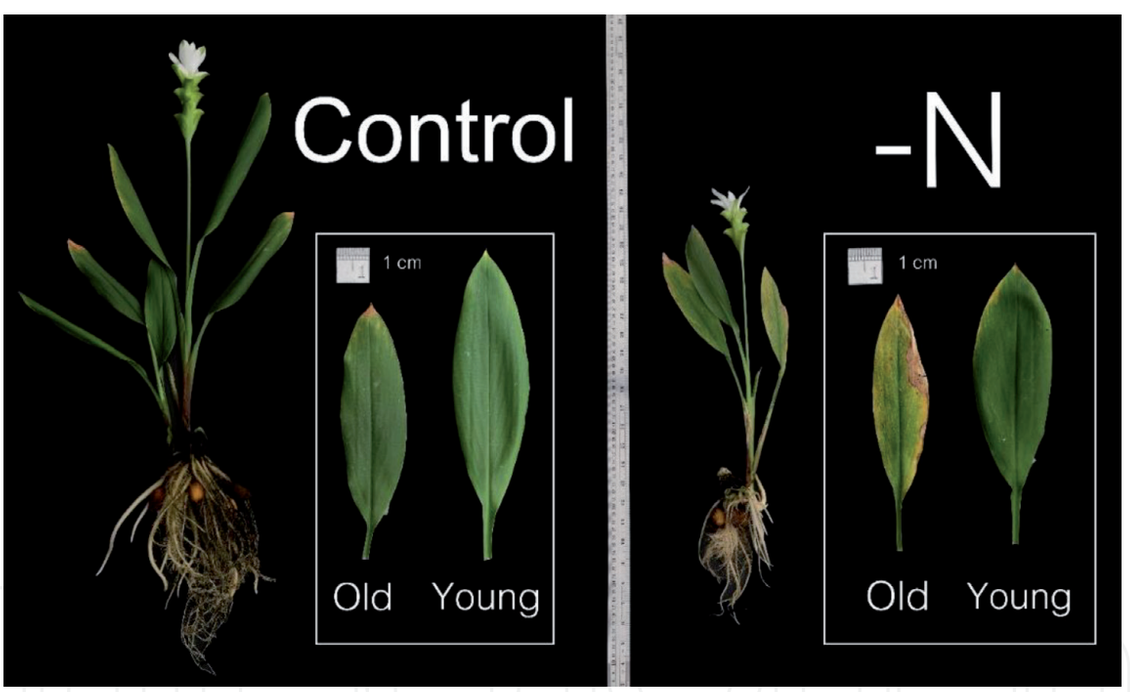

Figure 1.
Growth and flower quality of Curcuma alismatifolia *was affected by complete nutrient solution (control) and nitrogen deficiency (-N) treatment at the flowering stage (12 weeks after planting). (photo by Chaiartid Inkham).*

height, root length, leaf area, and total fresh and dry weight, were higher when plants were supplied with a complete nutrient solution, rather than -N treatment (**Table 1**). However, there was no significant difference in the number of leaves per plant between plants supplied with complete nutrient solution and -N treatment (**Table 1**). The characterization of nitrogen deficiency symptoms in *C. alismatiflolia* were evaluated at the flowering stage (12 weeks after planting). Leaves are the main plant part in which visual symptoms of the plant's response to nitrogen deficiency are usually observed. When there is a nitrogen deficit, older leaves of *C. alismatifolia* turn yellow and brown, while young leaves still appear green (**Figure 1**). The old leaves' green color intensity in -N treatment was lower than those treated with the complete nutrient solution (34.2 and 57.1 SPAD unit,

respectively). There was no significant difference among treatments in young leaves (**Table 1**). This result could explain nutrient remobilization processes in plants. Nitrogen is a macronutrient that is highly mobile in the phloem [2]; therefore, in N deficit conditions, nitrogen in old leaves of *C. alismatifolia* may be remobilized and translocate to young leaves. The remobilization of nutrients is frequently associated with foliar senescence, which makes nutrients available for younger plant organs and contributes to nutrient use efficiency [3].

Nitrogen deficiency delays flowering in *C. alismatifolia* and decreased flower quality in term of inflorescent length. However, there were no significant differences in inflorescence width, stalk length, and number of inflorescences per plant (**Table 2** and **Figure 1**). Nitrogen deficiency delayed flowering in narrow-leafed lupin [4]. The production of *C. alismatifolia,* in terms of flower quality and rhizome yield, depends on the response to N fertilizers [5]. Nitrogen-deficient plants are stunted and the quality of their flowers and rhizomes is significantly decreased. The increase of nitrogen from 0 to 50 mg L^{-1} increased the number of flowering shoots and, consequently, the number of rhizomes [6].

2.2 *Tagetes erecta* L. (Marigold)

The overall growth parameters of marigold were decreased under nitrogen deficit conditions (**Table 1** and **Figure 2**). At 8 weeks after planting, plants in the -N treatment were stunted with a plant height of only 47.5 cm, which was 42.2 cm shorter than plants in the complete nutrient solution treatment. Moreover, there was a dramatic decrease in leaf area and the total fresh weight of marigolds grown under -N treatment when compared with complete nutrient solution treatment (decreasing 82 and 90%, respectively) (**Table 3**). Leaf green color intensity of marigold was detected both in young leaves and old leaves to evaluate visual symptoms of plants grown under -N conditions. The results showed that leaf green color intensity of marigold in both young and older leaves was lower when grown under -N treatment than grown under complete nutrient solution treatment (**Table 3**). In addition, the leaves of plants under -N treatment were smaller than those under complete nutrient solution treatment. Older leaves turned yellow, red and brown, while young leaves had symptoms of chlorosis and turned light yellow (**Figure 2**). Plant height, plant spread, and the number of primary branches per plant of African marigold increased significantly with the increase in nitrogen level from 0 to 30 g m^{-2} [7]. A suitable supply of N enhanced plant growth efficiency, thus increasing plant yield and flower quality [8].

Nutrient solution	Flower quality				
	Days to flowering (day)	Inflorescence width (cm)	Inflorescence length (cm)	Stalk length (cm)	No. inflorescence per plant
Complete	70.7 b	6.9 a	11.8 a	34.3 a	1.0 a
-N	78.3 a	5.8 a	9.9 b	29.4 a	1.0 a
%CV	0.8	9.7	4.7	8.1	0
$LSD_{0.05}$	*	NS	*	NS	NS

**Means within the same column followed by different letters were significantly different in an LSD test; ($p \leq 0.05$). NS = not significant.*

Table 2.
Flower quality of Curcuma alismatifolia *treated with complete nutrient solution or nitrogen deficiency (-N) at the flowering stage (12 weeks after planting).*

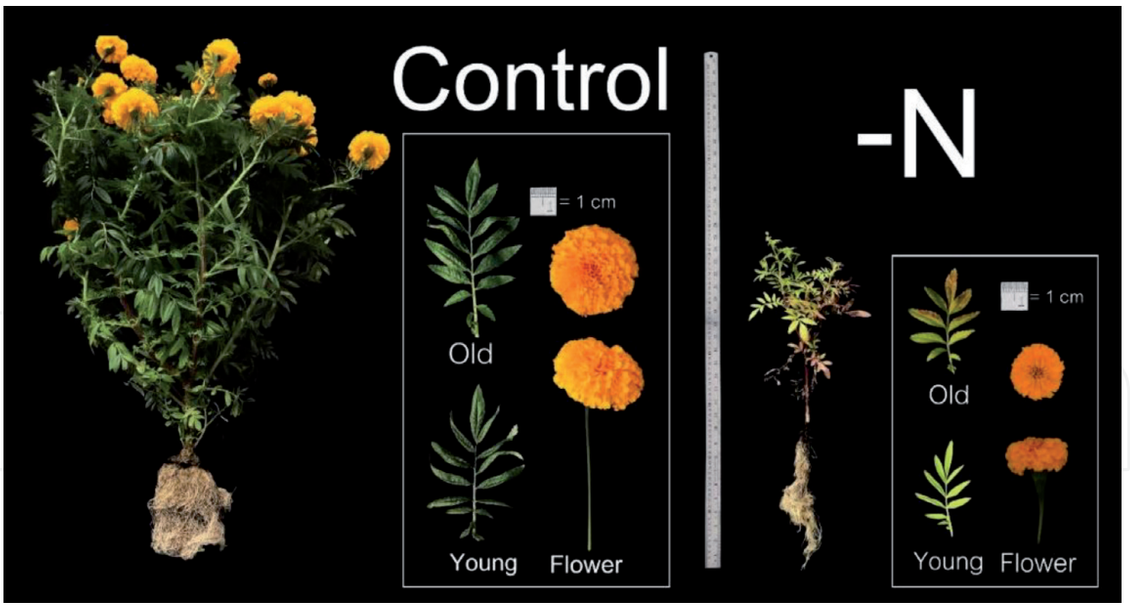

Figure 2.
Growth and flower quality of marigold was affected by complete nutrient solution (control) and nitrogen deficiency (-N) treatments at the flowering stage (8 weeks after planting). (photo by Chaiartid Inkham).

Nutrient solution	Plant growth at flowering stage (8 weeks after planting)					
	Plant Height (cm)	Root length (cm)	Leaf green color intensity (SPAD unit)		Leaf area (cm^2)	Total fresh weight (g)
			Old leaf	Young Leaf		
Complete	89.7 a	28.3 b	43.9 a	45.5 a	3,990.3 a	889.8 a
-N	47.5 b	36.5 a	23.2 b	22.2 b	706.3 b	88.7 b
%CV	8.9	5.9	14.1	19.0	40.3	2.7
$LSD_{0.05}$	*	*	*	*	*	*

**Means within the same column followed by different letters were significantly different in an LSD test; ($p \leq 0.05$).*

Table 3.
Plant growth of marigold treated with complete nutrient solution or nitrogen deficiency (-N) at the flowering stage (8 weeks after planting).

Flowering of marigold was delayed under nitrogen deficiency condition (about 12 days delay when compared with complete nutrient treatment) (**Table 4**). Furthermore, the flower quality in terms of flower width, flower length, and stalk length were also reduced in plants in the -N treatment compared to those treated with the complete nutrient solution (**Table 4**, **Figure 2**). The flower yield of marigold was highly sensitive to nitrogen deficiency, since there was a 90% decrease in the number of flowers per plant when the plants were grown under -N treatment compared with the complete nutrient solution treatment (**Table 4**). In marigold (*Calendula officinalis* L. 'TOKAJ'), nitrogen fertilization had a significant impact on the number of flower heads per plant (especially on the second-rank branches) [9].

2.3 *Zinnia violacea Cav.* (zinnia)

Nitrogen deficiency caused a decrease in plant height, number of leaves per plant, and root length of zinnia at 9 weeks after planting (**Table 5**). Additionally, yields of zinnia in terms of leaves area, total fresh weight, and total dry weight

Nutrient solution	Flower quality				
	Days to flowering (day)	Flower width (cm)	Flower length (cm)	Stalk length (cm)	No. flowers per plant
Complete	55.7 b	8.5 a	4.8 a	12.7 a	10.0 a
-N	67.7 a	4.8 b	2.0 b	4.5 b	1.0 b
%CV	0.9	18.7	15.8	27.3	25.7
$LSD_{0.05}$	*	*	*	*	*

**Means within the same column followed by different letters were significantly different in an LSD test; ($p \leq 0.05$).*

Table 4.
Flower quality of marigold treated with complete nutrient solution or nitrogen deficiency (-N) treatment at the flowering stage (8 weeks after planting).

Nutrient solution	Plant growth at flowering stage (9 weeks after planting)							
	Plant Height (cm)	No. leaves per plant	Roots length (cm)	Leaf green color intensity (SPAD unit)		Leaf area (cm^2)	Total fresh weight (g)	Total dry weight (g)
				Old leaf	Young Leaf			
Complete	25.0 a	284.7 a	50.3 a	32.8 a	30.5 a	7,950.0 a	84.4 a	11.7 a
-N	17.3 b	44.0 b	24.0 b	16.7 b	27.2 a	1,155.8 b	15.7 b	1.9 b
%CV	10.7	17.4	15.7	14.3	9.4	9.6	9.9	19.4
$LSD_{0.05}$	*	*	*	*	NS	*	*	*

**Means within the same column followed by different letters were significantly different in an LSD test; ($p \leq 0.05$). NS = not significant.*

Table 5.
Plant growth of zinnia treated with complete nutrient solution or nitrogen deficiency (-N) at the flowering stage (9 weeks after planting).

also decreased when grown plant under -N condition compared with those treated with complete nutrient solution; the percentage of reduction was 85, 81, and 84%, respectively (**Table 5**). Visual symptoms of nitrogen deficiency in zinnia were observed at the flowering stage. In -N treatment, plants were stunted with less branches (**Figure 3**). Old leaves in the -N treatment turned yellow, while young leaves remained green in both treatments (**Figure 3**). Visual symptoms observed on leaves had a similar trend with leaf green color intensity detected by a chlorophyll meter (SPAD) (**Table 5**). The results indicated that there was a significantly different leaf green color intensity in old leaves between plants treated with -N and complete nutrient solution. There was not a significant difference in young leaves (**Table 5**). Growth of *Zinnia elegans* Cv. Meteor increased with increasing nitrogen concentration from 0 to 20 g N/pot, i.e., plant growth rate (42% increase), plant height (28% increase), number of lateral shoots (56% increase), length of lateral shoots (17% increase), number of leaves (59% increase), and leaf area (40% increase) [10].

The flowering of zinnia was delayed for 15 days when plants were grown under -N treatment (**Table 6**). Nitrogen deficiency decreased flower quality in term of flower width (50% decrease), number of flower buds per plant (80% decrease),

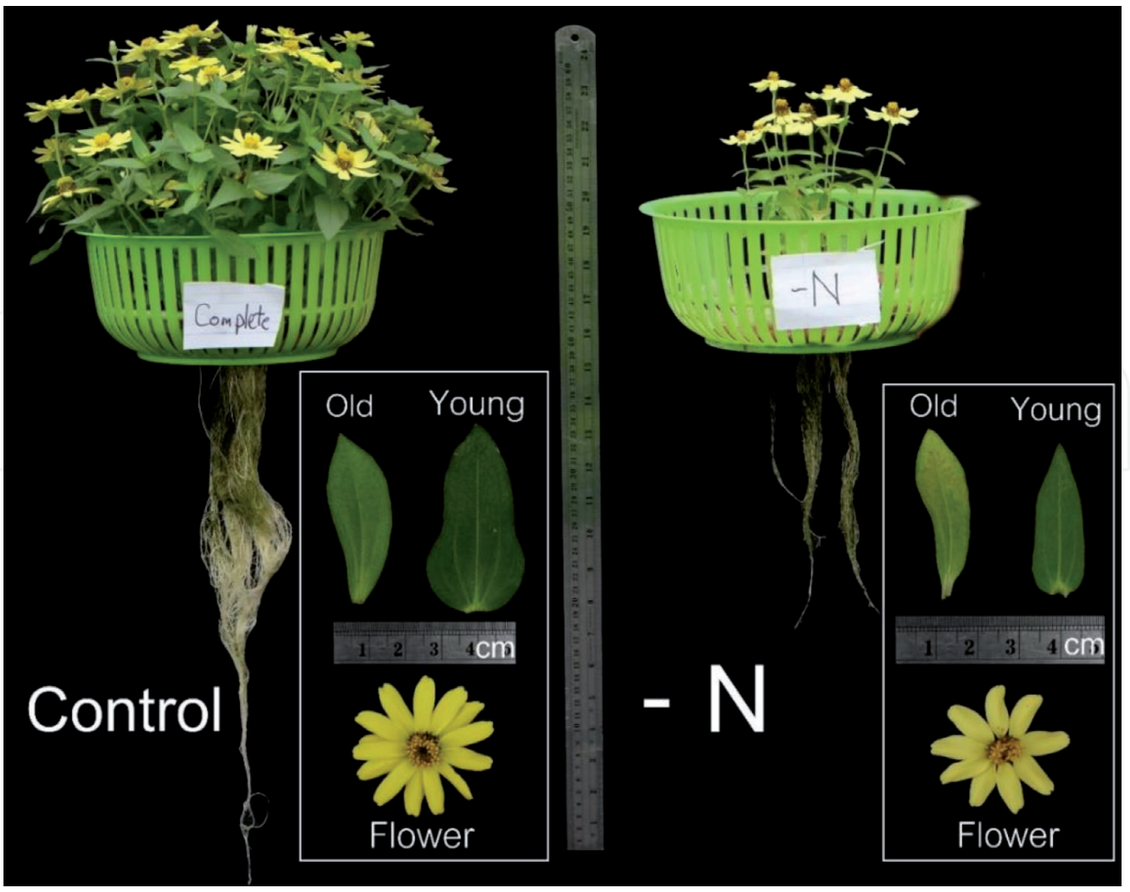

Figure 3.
Growth and flower quality of zinnia was affected by complete nutrient solution (control) and nitrogen deficiency (-N) treatment at the flowering stage (9 weeks after planting). (photo by Chaiartid Inkham).

Nutrient solution	Flower quality			
	Days to flowering (day)	Flowers width (cm)	No. flower buds per plant	No. flowers per plant
Complete	54.3 b	6.2 a	54.0 a	26.3 a
-N	69.3 a	3.1 b	10.7 b	6.7 b
%CV	3.4	9.8	11.6	18.5
$LSD_{0.05}$	*	*	*	*

**Means within the same column followed by different letters were significantly different in an LSD test; ($p \leq 0.05$).*

Table 6.
Flower quality of zinnia treated with complete nutrient solution or nitrogen deficiency (-N) at the flowering stage (9 weeks after planting).

and number of flowers per plant (74% decrease) in zinnia (**Table 6**). Visual symptoms of zinnia flowers response to -N treatment were smaller flowers and a reduced number of petals compared to those treated with complete nutrient solution (**Table 6** and **Figure 3**). In *Zinnia elegans* cv. Meteor, flower quality increased when supplied with 10 g N/pot compared with 0 g N/pot (18% increase in number of flowers per plant, 52.5% increase in flower size, and blooming stage was prolonged for 11 days) [10]. A high dose of nitrogen (20 g N/pot) negatively impacted flowering when compared with those supplied 10 g N/pot, i.e., delay emergence of first flower for 8 days, 20% decrease in the number of flowers per plant, and 17.5% decrease in flower size [10].

2.4 *Gomphrena globose* (gomphrena)

The growth of gomphrena was affected by nitrogen deficiency (**Table 7**). In the -N treatment, plants were stunted, with plant height 34.4 cm lower than those in the complete nutrient treatment (49.3 cm). Shorter root length was also observed in plants under -N treatment. Leaf area, total fresh weight, and total dry weight were dramatically decreased when plants were grown under nitrogen deficiency conditions with 80, 85, and 85% reductions, respectively (**Table 7**). Visual symptoms of nitrogen deficiency were a changed leaf color. Both old and young leaves in the -N treatment turned yellow with SPAD values lower than those under complete nutrient treatment (**Table 7** and **Figure 4**). Moreover, a decrease in the number of new branches was observed when the plant was grown under -N treatment (**Figure 4**).

Nutrient solution	Plant growth at flowering stage (13 weeks after planting)							
	Plant Height (cm)	No. leaves per plant	Root length (cm)	Leaf green color intensity (SPAD unit)		Leaf area (cm^2)	Total fresh weight (g)	Total dry weight (g)
				Old leaf	Young Leaf			
Complete	49.3 a	49.3 a	59.7 a	30.6 a	37.7 a	1,251.8 a	250.6 a	39.2 a
-N	34.4 b	21.7 b	32.6 b	22.1 b	26.6 b	254.2 b	38.0 b	6.0 b
%CV	13.2	18.0	10.0	9.67	17.8	17.9	18.0	18.0
$LSD_{0.05}$	*	*	*	*	*	*	*	*

**Means within the same column followed by different letters were significantly different in an LSD test; ($p \leq 0.05$).*

Table 7.
Plant growth of gomphrena treated with complete nutrient solution or nitrogen deficiency (-N) at the flowering stage (13 weeks after planting).

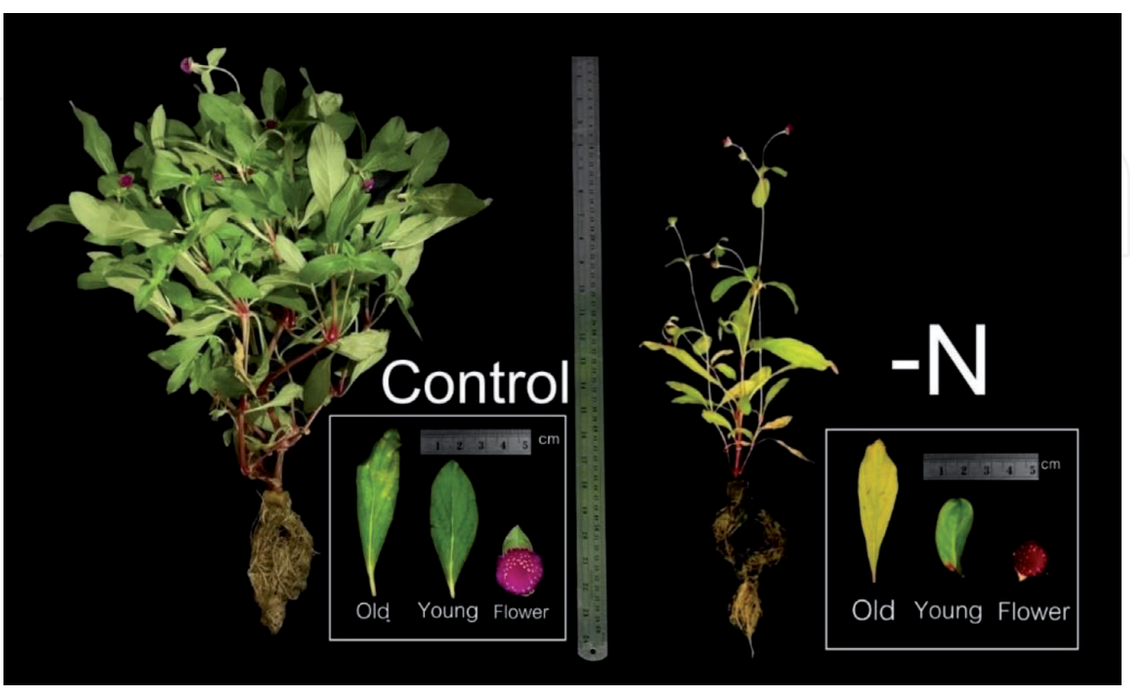

Figure 4.
Growth and flower quality of Gomphrena was affected by complete nutrient solution (control) and nitrogen deficiency (-N) treatment at the flowering stage (13 weeks after planting). (photo by Chaiartid Inkham).

Nutrient solution	Flower quality				
	Days to flowering (day)	Flower width (cm)	Flower length (cm)	Stalk length (cm)	No. flowers per plant
complete	86.7 a	2.1 a	2.6 a	14.1 a	8.7 a
-N	94.3 b	1.6 b	1.5 b	9.6 b	3.7 b
%CV	0.6	6.9	16.1	8.1	29.6
$LSD_{0.05}$	*	*	*	*	*

**Means within the same column followed by different letters were significantly different in an LSD test; ($p \leq 0.05$).*

Table 8.
Flower quality of gomphrena treated with complete nutrient solution or nitrogen deficiency (-N) treatment at the flowering stage (13 weeks after planting).

The flower quality of gomphrena was low under nitrogen deficiency conditions (**Table 8**). The flowering of gomphrena was delayed for 8 days when the plant lacked nitrogen. Additionally, flower size in terms of flower width, flower length, and stalk length were reduced under nitrogen deficit treatment. The number of flowers per plant also decreased by about 57% in the -N treatment compared with the complete nutrient treatment (**Table 8** and **Figure 4**).

3. Uptake, translocation, and nitrogen application in different flower species

Plants take up inorganic nitrogen, mostly in the form of ammonium (NH_4^+) and/or nitrate (NO_3^-). Uptake depends on the plant species and growth stage [11]. The translocation of N, including the free amino acid form, from roots to leaves could be done via the xylem. Some flowers can also utilize N via N_2-fixation by endophytic bacteria, such as *Curcuma alismatifolia* and *Vanda*. Some studies have shown the uptake, translocation, and assimilation of N-forms in flowers at different growth stages using ^{15}N-tracer feeding.

3.1 *Curcuma alismatifolia*

Curcuma alismatifolia, commonly known as the Siam tulip, is a flower bulb in the family Zingiberaceae. It is an economical flower crop in Thailand. Growers export rhizomes and cut flowers to other countries, including Japan, the Netherlands, the USA. The inflorescence of this plant is showy with pink and greenish bracts on a long peduncle (**Figure 5A**). The storage organ is the underground part of the plant and is so-called rhizome modified from the stem and attached to some storage roots (**Figure 5B**).

The rhizome is a major organ to store N, while carbohydrates are mostly stored in storage roots. The N concentration in the stubbed rhizome is 41–45 mgN gDW^{-1}, on average, while it was about 9–14 mgN gDW^{-1} in storage roots. Most of N in dormant rhizome was in PBS-insoluble form (79%), as a storage protein localized in the cytosol and cell wall, which presented as 10.6 and 12.0 kDa bands by SDS-PAGE staining. They contained five peptides and one peptide, respectively, when separated by 2D-PAGE. N in the storage roots was assimilated into different forms of free amino acids and protein compounds. The total free amino acid concentration in storage roots was higher than in the rhizome (343.8 and 109.0 μmol gDW^{-1}, respectively)

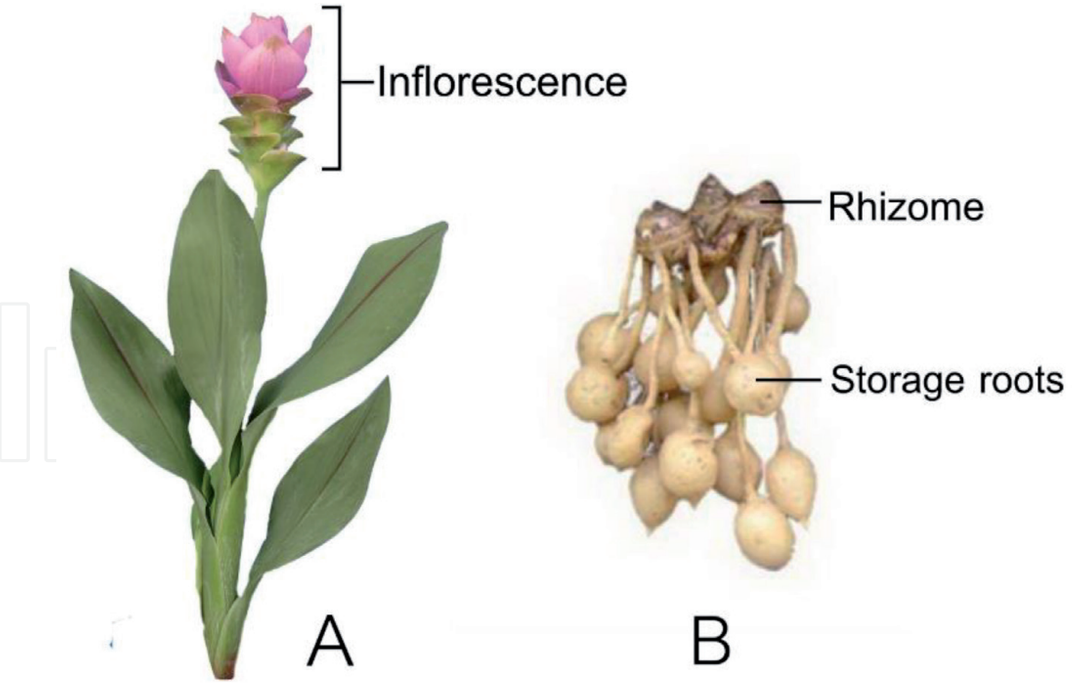

Figure 5.
Inflorescence (A) and rhizome (B) of Curcuma alismatifolia *Gagnep. (photo by Chaiartid Inkham).*

when the plants were supplied with 50 mgN L^{-1}. Most of the free amino acids in the rhizome and storage roots were arginine and glutamic acid, respectively. Proteins in the rhizome were at a higher concentration than those in storage roots with 197.4 and 46.7 mgN gDW^{-1}, respectively. A lack of N reduced the protein and free amino acid concentration in both organs. The carbon content in *Curcuma* rhizomes and storage roots was about 0.9 and 2.48 g C $plant^{-1}$, respectively, at the planting period, and it continuously decreased after planting. N and C stored in the rhizome was assimilated and utilized for root emergence and shoot sprouting [12–15].

At *Curcuma* shoot sprouting, fertilizer was generally supplied, and N was translocated via roots and leaves. Anatomical study of *Curcuma* roots and leaves showed the different sizes of cortical cells and the vascular bundle, which were larger in roots than leaves. However, the number of vascular bundles in the roots were lower than in the leaves. The abaxial leaf surface presented less barriers than the adaxial surface [16]. The N-use efficiency of fertilizer (%) via roots was 51–57%, which was higher than that via leaves (7–10%). The research by ^{15}N tracer revealed that N supply during the 1st and 2nd fully expanded leaf stage stimulated leaf growth, and N supply during the 3rd and 4th fully expanded leaf stage was translocate to utilize for flower blooming. N supply with 50 mg N L^{-1} increased the number of flowers and rhizomes compared with those at 25 mg N L^{-1} and lack of N supply. After translocation into plant organs, 81–97% of N was assimilated in an 80% ethanol insoluble fraction, mostly by proteins. N supply affected carbohydrate concentration in this plant, since nitrate reduction in roots requires carbohydrates for photosynthesis. The starch and sugar concentration in the rhizome and storage roots was high when *Curcuma* was grown under N deficiency. The translocation of carbohydrates to both storage organs was related to N deficiency conditions. From the vegetative stage until flowering, C content in leaves increased from 1.37 to 5.31 g C $plant^{-1}$. The ^{13}C exposure experiment revealed that C was accumulated in leaves during the vegetative stage and flowering, then it translocated to new storage organs (new rhizomes and storage roots) before plant dormancy [13, 15, 17, 18].

3.1.1 Effect of temperature on N uptake

Usually, N uptake and assimilation occur under normal temperature conditions. The optimum temperature for ^{15}N uptake in *Curcuma alismatifolia* was 25–35°C [19]. Day and night temperature also affected N uptake and assimilation. The nitrate concentration in leaves, nitrate reductase activity in roots, and total free amino acid content was higher in a plants grown at 30/18°C (day/night temperature) than at 30/25°C. However, a low night temperature reduced the number of shoots per plant and inflorescence quality (spike length and stalk length) in this plant [20, 21].

3.1.2 Response of curcuma to N application

Plant dry matter contains 2–4% N. The most important inorganic forms of N are ammonium (NH_4^+) and nitrate (NO_3^-), which are converted to an organic form, such as proteins, amino acid, and nucleic acids. There are three steps of N turnover in plants: 1) the conversion of inorganic N to organic N; 2) synthesis of high molecular weight N, such as protein and nucleic acids; and 3) breakdown of nitrogenous macromolecules by hydrolyzing enzymes [1]. Therefore, N supply is essential for growth, flower quality, and yield. However, the response to N was dependent on plant species, soil condition, temperature, and nutrition level. A lack of N reduced growth and development of *Curcuma*. The number of flowers and rhizome yield also decreased under 0 mg N compared with 25 and 50 mg N [13]. A field experiment was carried out with different N application rates at 3.75, 7.5, 15, 30, and 60 g N/plant, and the results demonstrated that supra-optimal N application at 60 g N/plant reduced plant height, number of shoots/plant, leaf area, and plant dry weight, but leaf N and leaf chlorophyll content increased. The research revealed that the leaf critical N for *C. alismatifolia*, calculated by the Mitscherlichs model, was 1.51% [21] The optimum fertilizer rate was different depending on the growth stage. The optimum N rates were 234, 937, and 468 kg N/hectares at the vegetative stage (45–75 days after planting), flowering stage (105 days after planting), and before rhizome harvest (135–165 days after planting), respectively (**Figure 6**) [22].

3.1.3 N_2 fixation and IAA synthesis in curcuma by endophytic bacteria

Nitrogen in the atmosphere that is fixed and converted to the organic form by microorganisms is termed N_2 fixation. In *Curcuma*, N_2 fixation by endophytic bacteria was first reported by Ruamrungsri et al. in 2009 [23]. The N-fixing rate varied depending on plant species. Eleven isolates were selected from *Curcuma alismatifolia* organs, such as the leaf, leaf base, and rhizome, and the N-fixing rate was 0.02–4.20 nmole $C_2H_4/10^6$ cells/hr. Seven isolates were derived from leaves, four isolates from the leaf base [23]. Isolates from the leaf base, i.e., ECS 202, identified using 16SrDNA, were *Sphingomonas pseudosanguinis* (99.2% similarity), ECS 203 was *Bacillus drentensis* and ECS 204 was *Bacillus methylotrophicus*. The colonization of these isolates was found in the intercellular spaces of different organs, i.e., roots, leaf base, and rhizome (**Figure 7**). Re-inoculation with these isolates into *Curcuma* plantlets derived from in vitro propagation was done by soaking roots in 10^6 cells/ml of these bacteria. Results showed that plant height, total leaf area (cm^2), and N content in roots and leaves of plants inoculated with ECS 203 was higher than the control [24].

3.2 *Narcissus*

Narcissus is a bulbs that has a storage organ that is modified from the leaf base in scales (**Figure 8**). Generally, the grower grows bulbs in autumn, and flowering

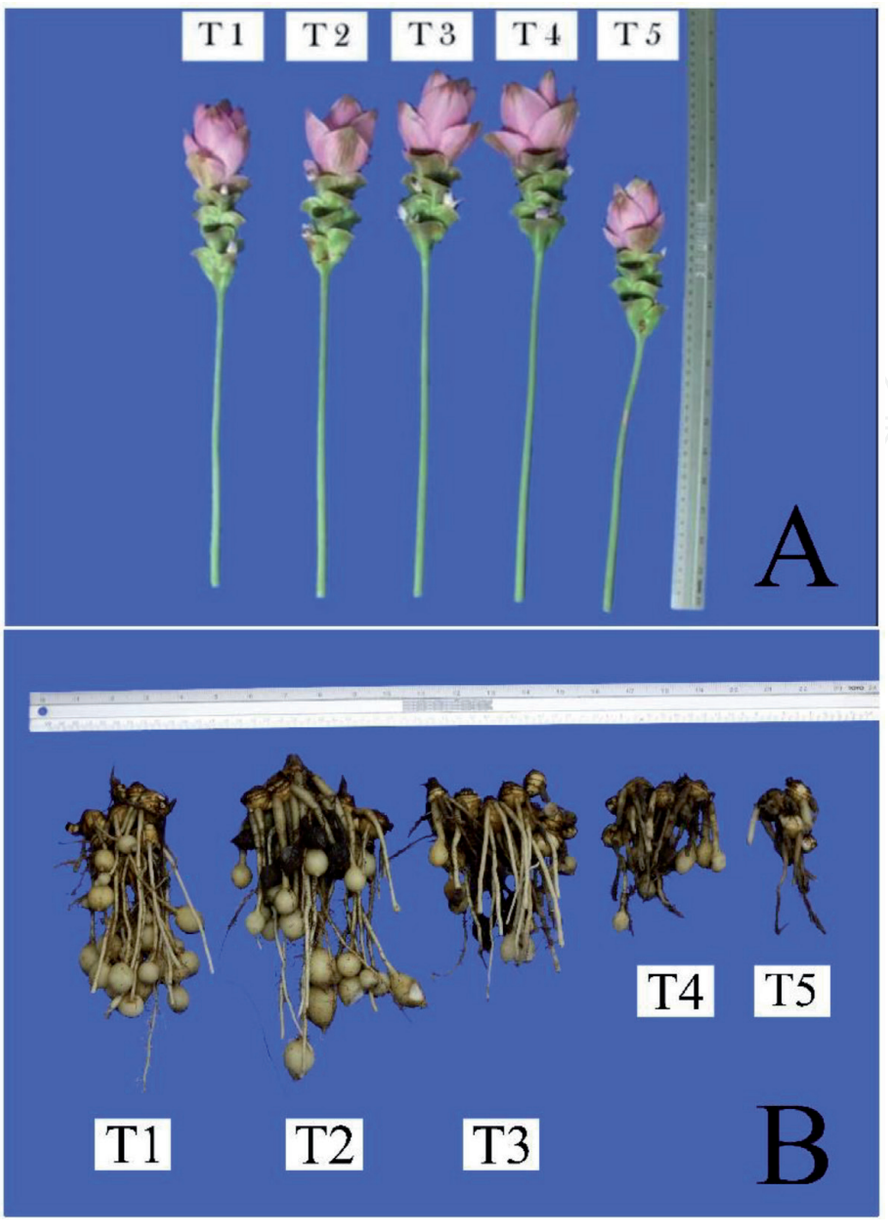

Figure 6.
Nitrogen application affected flower quality (A) and rhizome yield (B) of Curcuma alismatifolia *(T1–T5 were applied N at 234, 468, 937, 1,875, and 3,750 kg N/hectare). (Ruantip et al. [22]).*

occurs in spring. Its roots are an unbranched system that emerges under low temperatures of 9°C. A lack of nitrogen decreased shoot height, root length, chlorophyll intensity, and dry weight of the plant. N-deficient leaves were yellow and small. N concentration was about 12.47 mg gDW^{-1}, which was lower than the control plant (84.09 mgN gDW^{-1}). Sugar content in N-deficient roots was also higher than the control (with N supply), indicating that N metabolism required carbohydrates as an energy source for nitrate reduction and assimilation. Nitrogen was absorbed from fertilizer application in the winter. The N absorbed by roots was translocated to other organs after shoot emergence to promote growth and development. Therefore, N supply was required after root emergence, although the N in the mother bulb was utilized for root growth and shoot sprouting. After shoot sprouting, leaves were the sink organ to derive N from the mother bulb and fertilizer until flower senescence. The uptake of ammonium and nitrate was studied in *Narcissus* using a ^{15}N tracer. The results showed that at 2 days after fertilization with 1.0 mM of $^{15}NH_4^+$-N compared with 1.0 mM of $^{15}NO_3$-N and 0.5 mM of $^{15}NH_4^+$-N plus 0.5 mM of NO_3^--N, *Narcissus* 'Garden Giant' roots could more rapidly uptake NH_4^+-N than NO_3^--N at 2 days after

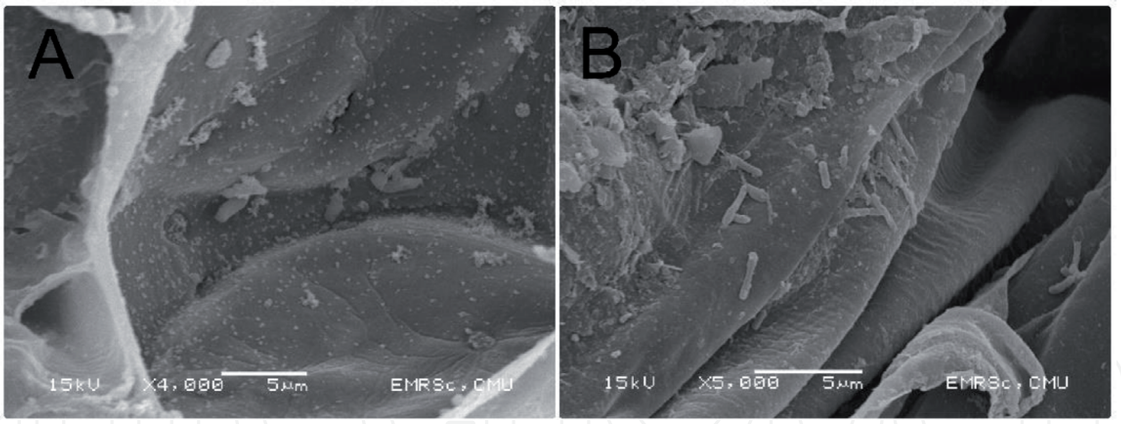

Figure 7.
Endophytic bacteria (A) isolate ECS 202 in the rhizome and (B) ECS 203 in the roots of Curcuma alismatifolia*. (photo by Soraya Ruamrungsri).*

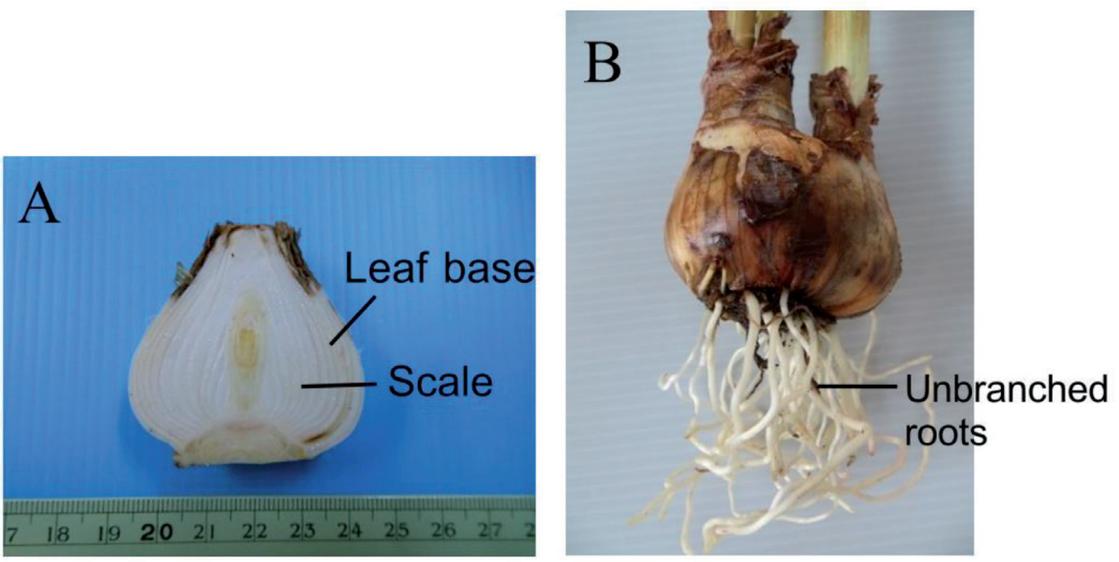

Figure 8.
Tunicated bulb of narcissus with young flower inside (A) and unbranched roots (B). (photo by Soraya Ruamrungsri).

fertilization, and the rate was equal between NH_4^+-N and NO_3^--N for 4–7 days after fertilization. Moreover, the assimilation of N into free amino acids was also different based on the N-form. At 2 days after $^{15}NH_4^+$-N fertilization, N in the roots was incorporated into free amino acids, mostly as glutamine, while asparagine and glutamine were the major assimilation forms of $^{15}NO_3^-$-N supply. [25–28].

3.3 Orchids

The growth of orchids can be presented in two ways, sympodial and monopodial habits, depending on the genus. Some orchids have enlarged bulbous organs at the base of their leaves, called a pseudobulb, with a different shape. These organs store food and mineral nutrition for plant growth and development [29].

3.3.1 Vanda

Vanda is a tropical orchid comprised of 40 species. It is an economical orchid with a high export value. The roots of *Vanda* are aerial roots, freely hanging in the

air. Growers use fertilizer applications to control flowering; however, research on plant nutrition in this plant is still rare.

The experiment on N uptake and assimilation in *Vanda* was carried out within 7 and 30 days after fertilization. The results revealed that plant uptake of $^{15}NO_3$-N occurred more rapidly than $^{15}NH_4^+$-N or their combination. However, ^{15}N use efficiency was higher in the mixed N source than the sole $^{15}NO_3$ or $^{15}NH_4^+$ form. N source affected the uptake and translocation of N to various organs, and *Vanda* prefers NO_3^--N to NH_4^+-N. N assimilation in *Vanda* was different among organs. Alanine distribution was high in leaves and roots at 7 days after feeding. Tyrosine distribution was predominant in leaves, while glutamine distribution was high in stems and roots at 30 days after fertilization [30].

3.3.2 Dendrobium

In Thailand, *Dendrobium* is an important orchid genus for export. The main area for *Dendrobium* production is in the central region of Thailand, such as Ratchaburi, Nakhon, and Pathom provinces. Fertilizer application affected the growth and development of this orchid. A combination of N sources (NO_3^- and NH_4^+) promoted the height of psuedobulbs, number of leaves and canes, spike length, and flowering percentage. The N concentration in leaves, roots, and psuedobulbs was 1.24, 0.97, and 0.61%, respectively. *Dendrobium* prefers a mixed N-source (ammonium and nitrate) to a sole N source [31].

3.3.3 Phalaenopsis

N level affected the growth of *Phalaenopsis* orchids. A concentration of 150–200 mg N L^{-1} increased the inflorescence length, stalk length, and number of flowers per stem. Supplying fertilizer with 21 N-21P_2O_5-21K_2O at 150 mg L^{-1} once a week to young plants increased the leaf area, leaf dry weight, and N concentration [32]. However, when a high N concentration (200 mg N L^{-1}) was supplied, the K concentration should also be supplied at 200 mg K L^{-1} to obtain healthy leaves and good quality flowers [33].

3.4 Rose

Rose is the highest potential cut flower in the world market. Rose quality is graded by stem length and flower size. N levels affected shoot height, shoot and flower diameter, flower fresh and dry weight, number of petals per flower, and flower yield. The N concentration in aboveground organs and roots was 19.4 and 20.9 mg N g DW^{-1} higher, respectively, when the plant was supplied with 200 mg N L^{-1}. The lack of N decreased all quality parameters and showed deficiency symptoms. The optimum N concentration for roses was 200 mg N L^{-1} $plant^{-1}$ once a week [34].

Acknowledgements

This work was partially supported by Chiang Mai University. Thanks to H.M. the King's Initiative Centre for flower and fruit propagation and the use of a greenhouse and other facilities.

Conflict of interest

The authors declare no conflict of interest.

Author details

Soraya Ruamrungsri[1,2], Kanokwan Panjama[1,2], Takuji Ohyama[3] and Chaiartid Inkham[2,4]*

1 Department of Plant and Soil Sciences, Faculty of Agriculture, Chiang Mai University, Chiang Mai, Thailand

2 Economic Flower Crop Research Cluster, Chiang Mai University, Chiang Mai, Thailand

3 Department of Agricultural Chemistry, Faculty of Applied Biosciences, Tokyo University of Agriculture, Japan

4 Science and Technology Research Institute, Chiang Mai University, Chiang Mai, Thailand

*Address all correspondence to: sunwins111@hotmail.com

References

[1] Mengel K, Kirkby and EA. Principles of plant nutrition. International Potash Institute. Bern; 1987. 687 p.

[2] White P. Long-distance transport in the xylem and phloem. In: Marschner P. editors. Marschner's Mineral Nutrition of Higher Plants. 3rd ed. Berlin: Elsevier; 2012. p. 49-70. doi: 10.1016/B978-0-12-384905-2.00003-0

[3] Avice JC, Etienne P. Leaf senescence and nitrogen remobilization efficiency in oilseed rape (*Brassica napus* L.). J. Exp. Bot. 2014; 65: 3813-3824. DOI: 10.1093/jxb/eru177

[4] Ma Q, Longnecker N, Dracup M. Nitrogen deficiency slows leaf development and delays flowering in narrow-leafed lupin. Annals of Botany. 1997; 79: 403-409. https://doi.org/10.1006/anbo.1996.0361

[5] Ruamrungsri S, Apavatjrut P. Effect of nutrient deficiency on growth and development of *Curcuma alismatifolia* Gagnep. In: Proceedings of the 3rd Symposium on the Family Zingiberaceae; 7-12 July 2003; Khon Kaen. Thailand; 2003. p. 98-104.

[6] Ohtake N, Ruamrungsri S, Ito S, Sueyoshi K, Ohyama T Apavatjrut, P. Effect of nitrogen supply on nitrogen and carbohydrate constituent accumulation in rhizomes and storage roots of *Curcuma alismatifolia* Gagnep. Soil Sci. Plant Nutr. 2006; 52: 711-716. https://doi.org/10.1111/j.1747-0765.2006.00094.x

[7] Nain S, Beniwal BS, Dalal RPS, Sheoran S. Effect of nitrogen and phosphorus application on growth, flowering and yield of African marigold (*Tagetes erecta* L.) under semi-arid conditions of Haryana. Indian Journal of Ecology. 2016; 43 (Special Issue-2): 645-649.

[8] Ahirwar MK, Ahirwar K, Shukla M. Effect of plant densities, nitrogen and phosphorus levels on growth, yield and quality of African marigold. Annals of Plant Soil Research. 2012; 14(2): 153-155.

[9] Król B. The effect of different nitrogen fertilization rates on yield and quality of marigold (*Calendula officinalis* L. 'TOKAJ') raw material. Acta Agrobotanica. 2011; 64 (3): 29-34. DOI: https://doi.org/10.5586/aa.2011.027

[10] Khan MA, Ziaf K, Ahmad I. Influence of nitrogen on growth and flowering of *Zinnia elegans* Cv. Meteor. Asian Journal of Plant Sciences. 2004; 3 (5): 571-573. DOI: 10.3923/ajps.2004.571.573

[11] Marschner P. Marschner's mineral Nutrition of Higher Plants. Academic Press, Elsevier: Amsterdam; 2012. 651 p.

[12] Anuwong C, Ohtake N, Sueyoshi K, Ruamrungsri S, Ohyama T. Characterisation of proteins in the storage organ of *Curcuma alismatifolia* Gagnep. Journal of horticultural Science & biotechnology. 2014; 89(5): 501-507. DOI: 10.1080/14620316.2014.11513112

[13] Ohtake N, Ruamrungsri S, Ito S, Sueyoshi K, Ohyama T, Apavatjrut P. Effect of nitrogen supply and carbohydrate constituent accumulation in rhizomes and storage roots of *Curcuma alismatifolia* Gagnep. Soil Science and Plant Nutrition. 2006; 52: 711-716. https://doi.org/10.1111/j.1747-0765.2006.00094.x

[14] Ruamrungsri S, Ohtake N, Sueyoshi K, Suwanthada C, Apavatjrut P, Ohyama T. Changes in nitrogenous compounds, carbohydrates and abscisic acid in *Curcuma alismatifolia* Gagnep. During dormancy. J of Horticultural Science & Biotechnology. 2001; 76 (1): 48-51. DOI: 10.1080/14620316.2001.11511325

[15] Khuankaew T, Ruamrungsri S, Ito S, Sato T, Ohtake N, Sueyoshi K,

Ohyama T. Assimilation and translocation of nitrogen and carbon in *Curcuma alismatifolia* Gagnep. Plant Biol. 2009; 12: 414-423. DOI: 10.1111/j.1438-8677.2009.00229.x

[16] Anuwong C, Ohyama T, Sueyoshi K, Ohtake N, Ruamrungsri S. Anatomical investigation of root and leaf of *Curcuma alismatifolia* Gagnep. Cv Chiang Mai Pink affecting N uptake. In: Proceedings of international graduate research conference Chiang Mai University. Chiang Mai, Thailand: 2014. p. 157-162.

[17] Marschner H. Mineral Nutrition of Higher Plants. Academic Press. Amsterdam; 1986. 674 p.

[18] Anuwong C, Ohyama T, Sueyoshi K, Ohtake N, Sato T, Ruamrungsri S. Uptake and translocation of nitrogen in Patumma (*Curcuma alismatifolia*) from foliar spray or root application. Journal of Plant Nutrition. 2017; 40(8): 1204-1212. DOI: 10.1080/01904167.2016.1264597

[19] Khuankaew T, Tanabata S, Yamamoto M, Ishikawa S, Tsutsumi K, Ohtake N, Sueyoshi K, Ohyama T, Sato T, Anuwong C, Ruamrungsri S. Temperature affects N and C assimilation and translocation in *Curcuma alismatifolia* Gagnep., The Journal of Horticultural Science and Biotechnology. 2014; 89(3): 287-292. DOI: 10.1080/14620316.2014.11513081

[20] Hongpakdee P, Ohtake N, Sueyoshi K, Ohyama T, S Ruamrungsri. Effects of low night temperature and short-day length on some phyto-hormones and nutrient status in *Curcuma alismatifolia* Gagnep. Thai journal of Agricultural Science. 2010; 43(3): 163-173.

[21] Inkham C, Sueyoshi K, Ohtake N, Ohyama T, Ruamrungsri S. Effect of temperature and nitrogen sources on growth and nitrogen assimilation of *Curcuma alismatifolia* Gagnep. Thai journal of Agricultural Science. 2011; 44(3): 145-153.

[22] Ruantip T, Ruamrungsri S. Effects of nitrogen fertilizer levels on growth, flower and rhizome qualities of *Curcuma alismatifolia* Gagnep. In: Proceeding of the 2nd CMU graduate research conference. 26 November 2010. Graduate school. Chiang Mai University; 2010. p. 4.

[23] Ruamrungsri S, Hamtisong N, Choonluchanon S. Selection of endophytic bacteria from *Curcuma alismatifolia* for N_2 fixation and IAA synthesis. In: Proceedings of the 3rd International meeting for the development of IPM in Asia and Africa, 7-9 December 2009; Bandar Lampung, Indonesia.

[24] Thepsukhon A, Choonluchanon S, Tajima S, Nomura M, Ruamrungsri S. Identification of endophytic bacteria associated with n2 fixation and indole acetic acid synthesis as growth promoters in *Curcuma alismatifolia* Gagnep. Journal of Plant Nutrition. 2013; 36(9): 1424-1438. DOI: 10.1080/01904167.2013.793712

[25] Ruamrungsri S, Ohyama T, Konno T, Ikarashi T. Deficiency of N, P, K, Ca, Mg or Fe mineral nutrients in *Narcissus* cv. 'Garden Giant'. Soil Science and Plant Nutrition. 1996; 42(4): 809-820. https://doi.org/10.1080/00380768.1996.10416628

[26] Ruamrungsri S, Ohyama T, Ikarashi T. Nutrients, free amino acid and sugar contents in *Narcissus* roots affected by N, P, K deficiency during winter. Soil Science and Plant Nutrition. 1996; 42(4): 765-771.

[27] Ruamrungsri S, Ruamrungsri S, Ikarashi T, Ohyama T. Uptake, translocation and fractionation of nitrogen in *Narcissus* organs by using ^{15}N. Acta Horticulturae. 1997; 430: 73-78. https://doi.org/10.1080/00380768.1996.10416624

[28] Ruamrungsri S, Ruamrungsri S, Ikarashi T, Ohyama T. Ammonium and nitrate assimilation in Narcissus roots. J. Hortic. Sci. Biotech. 2000; 75: 223-227. https://doi.org/10.1080/14620316.2000.11511227

[29] Hew CS, Yong JWH. The physiology of tropical orchids in relation to the industry. World Scientific: New Jersey; 1997. 370 p.

[30] Panjama K, Ohyama T, Ohtake N, Sato T, Potapohn N, Sueyoshi K, Ruamrungsri S. Identifying N sources that affect N uptake and assimilation in *Vanda* hybrid using 15N tracers. Horticulture, Environment, and Biotechnology. 2018; 59(6): 805-813. DOI: 10.1007/s13580-018-0071-6

[31] Ruamrungsri S, Khuankaew T, T Ohyama. Nitrogen sources and its uptake in *Dendrobium* orchid by ^{15}N Tracer study. Acta Horticulturae. 2014; 1025: 207-212. DOI: 10.17660/ActaHortic.2014.1025.30

[32] Ruamrungsri S, Samanit P, Pornsawatchai T, Potapohn N, Fukai S. Effect of fertilizer application on nutritional concentrations and flower quality of Phalaenopsis hybrid. Acta Horticulturae. 2007; 755: 495-498. DOI: 10.17660/ActaHortic.2007.755.68

[33] Wang YT. Phalaenopsis mineral nutrition. Acta Horticulturae. 2010; 878: 321-334. DOI: 10.17660/ActaHortic.2010.878.41

[34] Tunprasas W, Ruamrungsri S. Effect of nitrogen on cut rose quality in potted production. J of Agriculture. Chiang Mai university. 2016; 32(3): 339-345.