Calcium and nitrogen concentrations and distribution in gerbera plants as affected by nitrogen forms

Concentraciónes y distribución de calcio y nitrógeno en plantas de gerbera, afectadas por las formas de nitrógeno

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ABSTRACT

Ammonium and nitrate are the two forms of nitrogen necessary for the development, metabolism, and key processes in plants. The effects of ammonium to nitrate ratio on gerbera plants and their significance in post-harvest vase life are still poorly understood. This study aimed to investigate how different nutrient solutions affect nutrient levels in various plant organs. The experiment was conducted using a completely randomized design with three replicates at the National Research Institute of Flowers and Ornamental Plants in Mahallat City, Iran, in 2018. The factors studied were the four ratios of ammonium to nitrate: 0:100, 20:80, 40:60, and 60:40, and two gerbera varieties: Stanza and Double Dutch. The results showed that nitrogen content in different plant organs (roots, stem, and leaves) increased with higher concentrations of ammonium in the nutrient solutions. The highest nitrogen content in the roots of gerbera was observed 35 d after the first flowering stem appeared, with a concentration of 4.38 mg N g^{-1} dry weight in the 60:40 ratio of ammonium to nitrate. The lowest nitrogen content was found in the flowering stem at the time of harvest, with 2.00 mg N g^{-1} dry weight in the 0:100 ratio of ammonium to nitrate.

Key words: cut flowers, leaf nitrogen content, ratio of ammonium to nitrate.

RESUMEN

El amonio y el nitrato son las dos formas de nitrógeno necesarias para el desarrollo, el metabolismo y los procesos clave en las plantas. Los efectos de la proporción de amonio a nitrato en las plantas de gerbera y su importancia en la vida postcosecha en flores de corte aún son poco conocidos. Este estudio tuvo como objetivo investigar cómo diferentes soluciones nutritivas afectan los niveles de nutrientes en varios órganos de las plantas. El experimento se llevó a cabo utilizando un diseño completamente aleatorizado con tres repeticiones en el Instituto Nacional de Investigación de Flores y Plantas Ornamentales en la ciudad de Mahalat, Irán, en 2018. Los factores estudiados fueron las cuatro proporciónes de amonio a nitrato: 0:100, 20:80, 40:60 y 60:40, y dos variedades de flores de gerbera: Stanza y Double Dutch. Los resultados mostraron que los niveles de nitrógeno en los diferentes órganos de la planta (raíces, tallo y hojas) aumentaron con concentraciones más altas de amonio en las soluciones nutritivas. El contenido más alto de nitrógeno en las raíces de las flores de gerbera se observó 35 d después de la aparición del primer tallo floral, con una concentración de 4,38 mg N g^{-1} peso seco en la proporción de 60:40 de amonio a nitrato. El contenido más bajo de nitrógeno se encontró en el tallo floral al momento de la cosecha, con 2,00 mg N g^{-1} peso seco en la proporción de 0:100 de amonio a nitrato.

Palabras clave: flores del corte, contenido de nitrógeno en las hojas, relación de amonio a nitrato.

Introduction

In studies on crop responses to mineral nutrients, the relationships between fertilizer and water management are at the forefront. For crops to develop, grow, and produce, proper management of nitrogen fertilizer and irrigation is essential. Improving irrigation schedules and nitrogen application techniques is critical for sustainable agricultural management, with attention given to their effects on individual crops (Dai *et al*., 2019). As agriculture consumes increasingly high amounts of freshwater, the problem of scarce freshwater for irrigation worsens. High irrigation expenses, rising fertilizer costs, and environmental issues, such as pollution from the overuse of nitrogen or other chemical fertilizers in agricultural production systems, are all major concerns (Mancosu *et al*., 2015).

Received for publication: October 18, 2024. Accepted for publication: December 2, 2024.

Doi: 10.15446/agron.colomb.v42n3.117110

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Since it can precisely determine the nutrient concentrations and actual requirements for each crop, hydroponics appears to be the ideal cultivation system for both soilless crop production and the introduction of unknown plant species into intensive cultivation regimes (Varlagas *et al*., 2010). Hydroponic farming is an environmentally beneficial growing method that can be used in regions with poor soil, limited acreage, and limited water supplies. Consequently, hydroponics is promising for application in such circumstances (Marques *et al*., 2019; Pradhan *et al*., 2019). In hydroponic systems, the pH of the nutrient solution can be monitored and controlled in addition to nutrient management (Bar-Yosef *et al*., 2009; Corrêa *et al*., 2008).

Nitrogen ranks as the fourth most plentiful element in plants (Kozlowski, 1985; Hopkins, 1999). In addition to being critical for plant nutrition, nitrogen is also necessary for the synthesis of coenzymes, proteins, and nucleic acids. It is essential in photosynthesis because it is the primary constituent of the chlorophyll molecule (Barker *et al*., 2015). Nitrate (NO_3^-) and ammonium (NH_4^+) are the two main types of nitrogen that higher plants absorb (Tschoep *et al*., 2009). While plant roots can also absorb ammonium in nitrate-deficient environments, most plants prefer nitrate as a nitrogen source. According to Zhonghua *et al*. (2011) and Guo *et al*. (2012), the ideal ratio of nitrate to ammonium for plant growth and development varies depending on the genotype, growth stage, environmental factors, and total nitrogen concentration supplied. Plants exhibit diverse morphological, physiological and biochemical responses that are contingent upon the kind of nitrogen provided, species, and environmental factors (Helali *et al*., 2010; Liu *et al*., 2017; Na *et al*., 2014; Prinsi *et al*., 2020; Zhang *et al*., 2019; Zhu *et al*., 2014). Modern technology, such as hydroponic farming techniques, can be a reasonable step toward improving the efficiency of water and fertilizer usage in greenhouse crops, especially since drought and water scarcity are major global issues. The current study assessed the impact of various ammonium to nitrate ratios in the nutrient solution on the nutrient concentrations in the various organs of gerbera plants.

Materials and methods

This experiment was carried out in 2018 at the National Research Institute of Flowers and Ornamental Plants of Iran located in Mahallat City, Iran, with a geographic location of 50°30' E and 33°53' N, and an altitude of 1747 m a.s.l. The trial was conducted using a randomized complete block design with three replicates. This research was conducted under greenhouse conditions with minimum and maximum temperatures of 25-30ºC (day) and 15-20ºC (night) and relative humidity of 50 to 70%. Fans and pads, along with a shading system, were also used to cool the greenhouse.

Factors included four nutrient solutions containing 11.25 mM nitrogen at four different ammonium to nitrate ratios (0:100, 2:80, 40:60, and 60:40) in 300 L nutrient solution tanks and two varieties of gerbera: Stanza with red flowers and Double Dutch with yellow flowers. For each experimental unit, 10 pots were used, with 3 L nutrient solution and one Gerbera seedling planted in each pot. The nutrient solution used in this experiment was prepared according to the Netherlands Greenhouse Horticulture and Vegetables Research Center (de Kreij *et al*., 2003) (Tab. 1).

One molar sulfuric acid was used to adjust the pH of the nutrient solution, considering that the optimal pH for Gerbera plants is 5.5±0.1 (de Kreij *et al*., 2003). The electrical conductivity (EC) of nutrient solutions with ammonium-to-nitrate ratios of 0:100, 20:80, 40 :60, and 60:40 was 1.94, 2.28, 2.34, and 2.6 dS m^{-1}, respectively. The substrate was perlite with a particle diameter of 0.5-5 mm. After preparing the pots and substrate, gerbera seedlings (plants with 4 to 6 leaves) were planted in 3 L pots. For the first 10 d,

TABLE 1. Concentrations of mineral nutrients in nutrient solutions.

NH_4^+ : NO_3^-	Total nitrogen (mM L^{-1})	NO_3	NH_4^+	P*	K (mM L^{-1})	Ca	Mg	SO_4^{2-}
0-100	12.75	12.75	0	1.25	5.5	3	1	0.25
20-80	12.75	10.2	2.55	1.25	5.5	3	1	1.78
40-60	12.75	7.65	5.1	1.25	5.5	3	1	4.33
60-40	12.75	5.1	7.65	1.25	5.5	3	1	6.85
-	Fe (	Mn	Zn	Cu (μM L^{-1})	Mo	B	-	-
	35	5	4	0.75	0.5	30	-	-

* Phosphorus is in the form of $H_2PO_4^-$.

plants were supplied with compound fertilizer 18-18-18 (N-P_2O_5-K_2O) with a concentration of 1 g L^{-1}. Then, until the beginning of flowering (one month after planting of the seedlings), they were supplied with the nutrient solution listed in Table 1. Fertilizations were done 4 to 6 times daily, based on the greenhouse temperature. After that, all the plants received the same routine care during the growth period.

Concentrations of nitrate, calcium, ammonium, and total nitrogen in plant organs

The concentrations of nitrogen and calcium in the roots, stems, and leaves was measured at vegetative and reproductive stages. For this purpose, after drying the samples at 70°C for 24 h, the samples were crushed using an electric grinder. The Kjeldahl method was used to determine the total nitrogen concentration in plant organs (Bremner & Mulvaney, 1982). The calcium in the plant samples was measured using a UnicamSolaar atomic absorption spectrophotometer (Emami, 1996). The methods of Nelson (1983) and Cataldo *et al.* (1975) were used to measure ammonium concentration in plant organs (roots, leaves, and stems). Finally, the concentrations of ammonium and nitrate were reported in a mg g^{-1} dry weight.

Data analysis

SAS software version 9.4 was used for data analysis (ANOVA), and a Duncan test at the 5% level was applied to compare means. To understand the relationships between the studied traits, simple correlation coefficients among the traits were calculated.

Results

Concentrations of calcium and nitrogen in the roots 35 d after seedling planting

The results of the variance analysis demonstrated that, 35 d after seedling planting, the concentrations of nitrogen, nitrate, and calcium in the roots of the gerbera plants were significantly impacted by the ammonium to nitrate ratio at the 1% level. Additionally, the variety had a significant impact on calcium concentration at 5% and on nitrogen and nitrate concentrations at 1% (Tab. 2). Applying a portion of the total nitrogen in the form of ammonium (60%) caused a significant increase in the concentration of nitrogen in the roots of gerbera. The comparison of the means revealed that the ratio of ammonium to nitrate of 60:40 produced the highest amounts of calcium and nitrate at 0.77 and 2.26 mg g^{-1}, respectively. The lowest

TABLE 2. Mean squares from analysis of variance (ANOVA) for nutrient contents in roots of two gerbera cultivars grown under varying ratios of ammonium to nitrate.

SOV	df	Total nitrogen	Calcium	NO_3^-	NH_4^+
Rep	2	0.091	0.006	0.243	0.005
Cultivar	1	1.050^{**}	0.0600^{*}	2.31^{**}	0.016^{ns}
$NH_4^+ : NO_3^-$	3	0.496^{**}	0.098^{**}	2.17^{**}	0.172^{**}
Cultivar × $NH_4^+ : NO_3^-$	3	0.130^{ns}	0.020^{ns}	0.409^{ns}	0.003^{ns}
Error	14	0.043	0.007	0.133	0.0030
Cv(%)	-	6.16	14.37	21.99	10.27

ns, * and **: non-significant difference, significant difference at 5% and 1% of probability level. SOV – sources of variations.

TABLE 3. Comparison of the mean effects of ammonium to nitrate ratio and cultivar type on the concentrations of nutrients in gerbera roots at 35 d after seedling planting.

Treatment	Total nitrogen	Calcium	NO_3^-	NH_4^+
$NH_4^+ : NO_3^-$		(mg g^{-1})		
0-100	3.00c	0.77a	2.26a	0.36d
20-80	3.34b	0.66a	1.91ab	0.44c
40-60	3.43b	0.51b	1.62b	0.59b
60-40	3.70a	0.50b	0.84c	0.74a
Cultivar				
Stanza	3.58a	0.56b	1.97a	0.56a
Double Dutch	3.16b	0.66a	1.35b	0.51b

Means in each column followed by the same letters are not significantly different at the 5% probability level according to the Duncan test.

amount of calcium and nitrate was obtained from the 40:60 ammonium to nitrate ratio. The application of a nutrient solution with a ratio of 60:40 ammonium to nitrate, compared to nutrient solutions with ratios of 0:100 and 20:80 ammonium to nitrate led to a significant increase in the ammonium concentration of 105.6% and 68.2%, respectively, in the roots (Tab. 3).

Concentrations of nutrients in the roots 35 d after the appearance of the first flowering stem

The variance analysis of the data (Tab. 4) revealed that, at the 1% level, there was a statistically significant effect of the ammonium to nitrate ratio on the concentration of nitrogen, nitrate, ammonium, and calcium in the gerbera roots at 35 d after the first flowering stem appeared. Also, the effect of the gerbera cultivar on the concentrations of nitrate and calcium at the 1% level and on the concentration of nitrogen at the 5% level was significant. A comparison of mean data showed that by increasing the concentration of ammonium in the nutrient solution to 60% of the applied nitrogen, the concentrations of nitrogen and ammonium in the roots of gerbera increased at the stage of 35 d after the appearance of the first flowering stem. The concentrations of nitrogen and ammonium in the roots of gerbera increased significantly by 47.9% and 70.7%, respectively, when a nutrient solution with a ratio of 60:40 ammonium to nitrate was used instead of one with a ratio of 0:100 ammonium to nitrate (Tab. 5). Additionally, the ratio of 0:100 ammonium to nitrate produced the highest concentrations of calcium and nitrate, while the ratio of 60:40 ammonium to nitrate produced the lowest concentrations of calcium and nitrate. 35 d after the emergence of the first flowering stem, the concentration of calcium in the roots of gerbera decreased when the amount of ammonium in the nutrient solution was increased to 60% of total nitrogen (Tab. 5). The calcium concentration in gerbera roots was significantly reduced by 52.1% and 37.4%, respectively, when a nutrient solution with an ammonium to nitrate ratio of 60:40 was used instead of one with an ammonium to nitrate ratio of 0:100 and 20:80.

Concentrations of mineral nutrients in leaves 35 d after seedling planting

The ammonium to nitrate ratio had a significant effect at the 1% level on the concentrations of nitrogen, nitrate, ammonium, and calcium in gerbera leaves 35 d after seedling planting according to data variance analysis results (Tab. 6). Also, the effect of the gerbera cultivar on ammonium and nitrogen concentrations was significant at the 1% level. At 35 d after seedling planting, an increase in ammonium

TABLE 4. Mean squares from analysis of variance (ANOVA) for nutrient concentrations in the roots of gerbera at the stage of 35 d after the appearance of the first flowering stem.

SOV	df	Total nitrogen	Calcium	NO_3^-	NH_4^+
Rep	2	0.063	0.006	0.383	0.047
Cultivar	1	1.58*	1.12**	17.25**	0.036ns
NH_4^+ : NO_3^-	3	2.38**	0.66**	18.11**	0.79**
Cultivar × NH_4^+: NO_3^-	3	0.020ns	0.86ns	0.40ns	0.002ns
Error	14	0.26	0.052	1.21	0.009
Cv(%)	-	13.79	23.49	15.63	9.41

ns, * and **: non-significant difference, significant difference at 5% and 1% of probability level. SOV – sources of variations.

TABLE 5. Comparison of the mean effects of ammonium to nitrate ratio and cultivar type on the concentrations of nutrients in the roots of gerbera at 35 d after the appearance of the first flowering stem.

Treatment	Total nitrogen	Calcium	NO_3	NH_4^+
NH_4^+ : NO_3^-		(mg g^{-1})		
0-100	2.96c	1.40a	8.95a	0.89c
20-80	4.12ab	1.07b	7.95a	0.66d
40-60	3.57bc	0.75c	6.28b	1.07b
60-40	4.38a	0.67c	5.05b	1.52a
		Cultivar		
Stanza	4.02a	0.76b	7.90a	-
Double Dutch	3.50b	1.19a	6.21b	-

Means in each column followed by similar letters are not significantly different at the 5% probability level according to the Duncan test.

content in the nutrient solution to 60% of applied nitrogen resulted in an increase in nitrogen and ammonium concentration in the gerbera leaves. The concentration of nitrogen in gerbera leaves significantly increased when a nutrient solution with a ratio of 60:40 ammonium to nitrate was employed instead of other nutrient solutions. This nutrient solution, compared with the nutrient solution with a ratio of 0:100 ammonium to nitrate, caused a significant increase in nitrogen concentration in gerbera leaves by 27.9% (Tab. 7). The average data comparison demonstrated that, when compared with other ammonium to nitrate ratios, raising the concentration of ammonium in the nutrient solution to 60% of the total nitrogen resulted in a decrease in nitrate concentration in the gerbera leaves 35 d after seedling planting (Tab. 7). Application of nutrient solutions with ratios of 60:40 ammonium to nitrate contrasted in its effect to the solutions with 20:80, 40:60, and 0:100 ammonium to nitrate. The nitrate concentration in gerbera leaves significantly decreased by 55.1%, 44.2%, and 43.3%, respectively (Tab. 7). The comparison of mean data demonstrated that, 35 d after seedling planting, when ammonium in the nutrient solution was increased to 20% of the total nitrogen applied, the concentration of calcium in gerbera leaves increased significantly relative to other levels of ammonium to nitrate. Providing gerbera plants with a nutrient solution containing an ammonium-to-nitrate ratio of 20:80 caused the calcium concentration in the leaves to increase by 14.2%. The concentration of calcium in the leaves, however, significantly decreased as the amount of ammonium in the nutritional solution increased. The nitrogen (8.8%) and ammonium (12.9%) concentrations in the leaves of Stanza plants were significantly greater than those of Double Dutch plants (Tab. 7).

Concentrations of mineral nutrients in leaves 35 d after the appearance of the first flowering stem

The effect of biostimulant substances to nitrate ratio on nitrogen, nitrate, ammonium, and calcium content in gerbera leaves was significant at the 1% level according to the results of analysis of variance (Tab. 8). In comparison to other ratios of ammonium to nitrate, raising the concentration of ammonium in the nutrient solution to up to 60% of applied nitrogen resulted in a statistically significant increase in the nitrogen concentration in the leaves of gerbera. The nitrogen concentration in the leaves of gerbera increased significantly by 32.6% when they were supplied with a nutrient solution containing 60:40 ammonium to nitrate, as opposed to a nutritional solution without ammonium. 35 d after the first flowering stem appeared, the nitrate concentration in the leaves of gerbera was much lower

TABLE 6. Mean squares from analysis of variance (ANOVA) for nutrient concentrations in gerbera leaves at 35 d after transplanting.

SOV	df	Total nitrogen	Calcium	NO_3^-	NH_4^+
Rep	2	0.025	0.001	0.115	0.0022
Cultivar	1	0.055^{**}	0.113^{ns}	0.24^{ns}	0.0096^{**}
$NH_4^+ : NO_3^-$	3	0.645^{**}	0.416^{**}	1.923^{**}	0.038^{**}
Cultivar × $NH_4^+ : NO_3^-$	3	0.008^{ns}	0.005^{ns}	0.067^{ns}	0.0011^{ns}
Error	14	0.034	0.025	0.059	0.001
Cv(%)	-	5.67	13.01	12.96	11.12

ns, * and **: non-significant difference, significant difference at 5% and 1% of probability level. SOV – sources of variations.

TABLE 7. Comparison of the mean effects of ammonium to nitrate ratio and cultivar type on the concentrations of essential nutrients in gerbera leaves at 35 d after transplanting.

Treatment	Total nitrogen	Calcium	NO_3^-	NH_4^+
$NH_4^+ : NO_3^-$		(mg g^{-1})		
0-100	2.83c	1.34a	2.47a	0.39a
20-80	3.21b	1.53a	1.99b	0.25c
40-60	3.34b	1.08b	1.96b	0.30b
60-40	3.62a	0.94b	1.11c	0.21d
Cultivar				
Stanza	3.40a	-	-	0.31a
Double Dutch	3.10b	-	-	0.27b

Means in each column followed by similar letters are not significantly different at the 5% probability level according to the Duncan test.

when ammonium, up to 60% of the total nitrogen in the nutritional solution, was applied. Using nutrient solutions with an ammonium to nitrate ratio of 60:40 resulted in a significant decrease in the nitrate concentration of gerbera leaves by 34.9%, 27.3%, and 10.1%, respectively, when compared to nutrient solutions with ammonium to nitrate ratios of 0:100, 20:80, and 40:60. Compared to nutritional solutions with an ammonium to nitrate ratio of 40:60 or 60:40, the use of ammonium (20% of the total nitrogen) resulted in a considerable increase in calcium concentration of 25.4% and 52.6%, respectively. Furthermore, compared to the Stanza cultivar, 52.6% more calcium was found in the Double Dutch cultivar (Tab. 9).

Concentrations of nutrients in the stem 35 d after the appearance of the first flowering stem

The analysis of variance showed that the ammonium-to-nitrate ratio effect on nitrogen, nitrate, ammonium, and calcium concentrations was significant at the 1% level. Also, the effect of the gerbera cultivar on nitrogen and calcium concentration was significant at the 1% level (Tab. 10). The concentration of nitrogen in the stem increased significantly by 26.1% and 19.2%, and 10.1%, respectively, when nutrient solutions containing a ratio of 60:40 ammonium to nitrate were used instead of those containing ratios of 0:100, 20:80, and 40:60 ammonium to nitrate. The concentration of ammonium in the stem increased by 33.3%, 104.9%, and 15.1%, respectively. The highest amount of calcium was obtained from the ratio of 20:80 ammonium to nitrate at the rate of 0.5%. With the increase in the ammonium application, the amount of calcium in the stem of the gerbera plant decreased; this decrease was 52.9% and 67.7%, respectively, in the ratios of 40:60 and 60:40. The highest amount of nitrate was obtained from the 0:100 ammonium to nitrate nutrient solution at the rate of 2.5%, and with the increase in the amount of ammonium, the amount of nitrate decreased. The lowest amount was obtained from the ratio of 40:60 ammonium to nitrate at 1.96%. Based on the average comparison results, the Stanza cultivar had the highest concentration of stem nitrogen (2.4%), and the Double Dutch cultivar had the highest concentration of calcium (0.45%) (Tab. 11).

After 35 d of seedling planting, there was a considerable increase in the concentration of nitrogen and ammonium in the roots and leaves of gerbera in both phases when the concentration of ammonium in the nutrient solution was increased to 60% of the applied nitrogen. Moreover, 35 d after the appearance of the first flowering stem, the concentration of nitrogen and ammonium in the flowering stem

TABLE 8. Mean squares from analysis of variance (ANOVA) for nutrient concentrations in gerbera leaves at 35 d after the appearance of the first flowering stem.

SOV	df	Total nitrogen	Calcium	NO_3^-	NH_4^+
Rep	2	0.177	0.04	0.005	0.001
Cultivar	1	0.173^{ns}	2.27^{**}	0.044^{ns}	0.008^{ns}
$NH_4^+ : NO_3^-$	3	0.75^{**}	0.43^{**}	1.23^{**}	0.16^{**}
Cultivar × $NH_4^+ : NO_3^-$	3	0.01^{ns}	0.047^{ns}	0.004^{ns}	0.002^{ns}
Error	14	0.040	0.049	0.006	0.00009
Cv(%)	-	7.02	15.02	3.44	8.48

ns, * and **: non-significant difference, significant difference at 5% and 1% of probability level. SOV – sources of variations.

TABLE 9. Comparison of the mean effects of ammonium to nitrate ratio and cultivar type on concentrations of nutrients in gerbera leaves at 35 d after the appearance of the first flowering stem.

Treatment	Total nitrogen	Calcium	NO_3^-	NH_4^+
$NH_4^+ : NO_3^-$		(mg g^{-1})		
0-100	2.51c	1.64ab	2.86a	0.58a
20-80	2.70bc	1.74a	2.56b	0.41b
40-60	2.93b	1.39bc	2.07c	0.29c
60-40	3.33a	1.14c	1.86d	0.19d
Cultivar				
Stanza	-	1.17b	-	-
Double Dutch	-	1.78a	-	-

Means in each column followed by similar letters are not significantly different at the 5% probability level according to the Duncan test.

of gerbera at the stage of flower harvest was compared with the absence of ammonium in the nutrient solution (Tabs. 3, 5, 7, 9, and 11). There were positive and significant correlations between percentage of ammonium in the nutrient solution and leaf nitrogen concentration ($P<0.01$, r=0.86) as well as root ammonium concentration ($P<0.01$, r=0.97) at 35 days after seedling cultivation. Similarly, at 35 d after the emergence of the first flowering stem, significant positive correlations were observed between the percentage of ammonium in the nutrient solution and root nitrogen concentration ($P<0.05$, r=0.74), leaf nitrogen concentration ($P<0.01$, r= 0.94), leaf ammonium concentration ($P<0.05$, r=0.82), and nitrogen concentration in the flowering stem at harvest ($P<0.01$, r=0.86).

Discussion

The increase in nitrogen concentration in different organs of gerbera plants as a result of ammonium nutrition can be attributed to several reasons. Firstly, the assimilation of nitrate requires more energy compared to ammonium for its uptake and conversion (assimilation) into amino acids (Rosta, 2014). Therefore, supplying plants with nitrate alone will not result in achieving maximum growth. Assimilation of 1 mole of nitrate requires 15 moles of ATP, while one mole of ammonium requires 5 moles of ATP for assimilation (Marschner, 2012). Also, about 23% of the energy from respiration is used for nitrate assimilation in the roots, while this amount is 14% for ammonium. Therefore, ammonium nutrition saves plant energy (Marschner, 2012). In a study on how nitrogen form affects nutrient absorption by *Canna indica*, Konnerup and Brix (2010) found that the plants supplied with ammonium had higher nitrogen concentrations than the plants supplied with nitrate. According to Tabatabaei *et al.* (2008), in strawberries and rudbeckia, raising the ratio of ammonium to nitrate in a nutrient solution increased the concentration of total nitrogen in the plant organs, which is consistent with the findings of the current research. The effect of the ammonium-to-nitrate ratio of nutrient solution on the nitrate concentration of different organs of the gerbera plants varied, so that the concentration of nitrate in the root and leaves of gerbera in both stages (35 d after planting seedlings and 35 d after the appearance of the first flowering stem), as well as the concentration of nitrate in the flowering stem of gerbera in the flower harvesting stage with the application of 60% nitrogen nutrient solution to The form of ammonium, decreased significantly

TABLE 10. Mean squares from analysis of variance (ANOVA) for nutrient concentrations in gerbera stem at 35 d after the appearance of the first flowering stem.

SOV	df	Nitrogen	Calcium	NO_3^-	NH_4^+
Rep	2	6.0098	0.0082	0.082	0.0004
Cultivar	1	0.283**	0.043**	0.011ns	0.012ns
$NH_4^+ : NO_3^-$	3	0.326**	0.060**	0.564**	0.200**
Cultivar × $NH_4^+ : NO_3^-$	3	0.0070ns	0.0023ns	0.152ns	0.001ns
Error	14	0.0096	0.0043	0.056	0.005
Cv(%)	-	4.24	16.00	10.27	11.51

ns, * and **: non-significant difference, significant difference at 5% and 1% of probability level. SOV – sources of variations, df-degrees of freedom.

TABLE 11. Comparison of the mean effects of ammonium to nitrate ratio and cultivar type on the concentrations of nutrients in the stem at 35 d after the appearance of the first flowering stem.

Treatment	Total nitroge	Calcium	NO_3^-	NH_4^+
$NH_4^+ : NO_3^-$		(mg g^{-1})		
0-100	2.07d	0.46a	2.47a	0.63c
20-80	2.19c	0.52a	2.64a	0.41d
40-60	2.37b	0.34b	1.96b	0.73b
60-40	2.61a	0.31b	2.14b	0.84a
Cultivar				
Stanza	2.42a	0.37b	-	-
Double Dutch	2.20b	0.45a	-	-

Means in each column, followed by similar letters are not significantly different at the 5% probability level according to the Duncan test.

in comparison with the non-use of ammonium and the use of 20 to 80 ammonium to nitrate ratio. A negative and significant correlation between the ammonium percentage of the nutrient solution and the root nitrate concentration (r=-0.78, $P<0.01$) 35 d after seedling cultivation confirms this decrease. Similarly, at 35 d after the appearance of the first flowering stem, a negative and significant correlation was observed between the ammonium percentage of the nutrient solution and the nitrate concentration in leaves ($P<0.01$, r=-0.98) (Tab. 12). The decrease in nitrate concentration due to the increasing ratio of ammonium to nitrate in the nutrient solution can be due to decreased nitrate supply for plant absorption (Helali *et al*., 2010). Ammonium may reduce nitrate uptake by inhibiting nitrate assimilation upon entry and enhancing its efflux from the roots (Kronzucker *et al*., 1999). The type of plant cultivar was found to have an impact on the concentration of high-use and low-use nutrients in various regions of the gerbera plants. Therefore, the Stanza cultivar had much higher concentrations of most studied nutrients than the Double Dutch. Similarly, the concentrations of nitrogen and ammonium in the leaves at 35 d after the seedling planting and the concentrations of nitrate and ammonium at 35 d after the appearance of the first flowering stem was significantly higher in Stanza than in Double Dutch (Tab. 9). Additionally, during the flower harvest stage, the nitrogen concentration in Stanza stem was substantially higher than that in the Double Dutch stem (Tab. 11). Genetic differences cause the difference between plant cultivars in the concentration of nutrients, and this issue has been observed in other research as well. One of the key mineral ingredients for decorative plants and cut flower pots is calcium, which helps them to develop and last longer. The form and quantity of nitrogen included in the nutrient solution has a significant impact on its absorption. According to reports, there is a decrease in calcium absorption at high ammonium ratios in the nutrient solution (Hosseini Farahi *et al*., 2014).

Conclusions

The ammonium-to-nitrate ratio in the nutrient solution significantly influenced the concentrations of nitrogen, nitrate, ammonium, and calcium in the roots, leaves, and flower stems of gerbera. Increasing the ammonium ratio to 60% of the total nitrogen supply led to a significant rise in nitrogen and ammonium concentrations in the roots, leaves, and flower stems of gerbera at both growth stages (35 d after transplanting and 35 d after the emergence of the first flower stem). This increase may be attributed to the lower energy requirement for ammonium uptake and metabolism compared to nitrate, as nitrate requires more energy for absorption and conversion into amino acids.

On the other hand, increasing the ammonium ratio in the nutrient solution resulted in a reduction in nitrate concentrations in the roots, leaves, and flower stems of gerbera. This decrease may be due to the inhibition of nitrate uptake in the presence of ammonium, as ammonium can competitively suppress nitrate absorption. Additionally, an increase in the ammonium ratio in the nutrient solution led to a decline in calcium concentrations in the roots and leaves of gerbera, likely due to competition between ammonium and calcium ions for uptake.

Genetic differences between gerbera cultivars also affected nutrient concentrations, with the Stanza cultivar exhibiting higher concentrations of nitrogen, nitrate, and ammonium in the roots, leaves, and flower stems compared to the Double Dutch cultivar. These variations may be attributed to the specific genetic traits of each cultivar.

Overall, this study highlights that optimizing the ammonium-to-nitrate ratio in the nutrient solution can enhance nutrient uptake and improve gerbera performance. However, excessive ammonium levels may negatively impact

TABLE 12. Regression coefficients for the studied traits at different growth stages of gerbera

Plant organ		Total nitrogen	NO_3^-	NH_4^+	Calcium
Stage 35 d after seedling planting					
Root	NH_4^+	0.69	*0.78-	**0.97	*0.81-
Leaf	NH_4^+	0.86**	0.40	0.56-	**0.95-
Stage 35 d after the appearance of the first flowering stem					
Root	NH_4^+	0.74*	0.64-	0.57	0.59-
Leaf	NH_4^+	0.94**	**0.98-	*0.82	0.53-
Flower harvest stage					
Flowering stem	NH_4^+	**0.86	0.62-	0.65	0.74*-

* and **: significant differences at 5% and 1% of probability level.

calcium uptake, which is crucial for the quality and longevity of cut flowers. Therefore, maintaining an appropriate balance between ammonium and nitrate in the nutrient solution is essential for achieving optimal results in gerbera cultivation.

Acknowledgments

We thank all those who helped us implement this study. This research was not specifically funded.

Conflict of interest statement

The authors declare that there are no conflicts of interests regarding the publication of this article.

Author´s contributions

MAK and EB designed the experiments, MK carried out field and laboratory experiments, MAK and EB contributed to the data analysis, MAK, EB, and MK wrote the article. All authors approved the final version of the manuscript.

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