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EFFECTS OF VARYING NO_3^-:NH_4^+ RATIOS ON LETTUCE (*LACTUCA SATIVA* L.) NITROGEN METABOLISM

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Abstract

The two main forms of nitrogen (N) in plants are Nitrate (NO_3^-) and ammonium (NH_4^+). The effects of different nutrient solution NO_3^-:NH_4^+ ratios (0:100, 25:75, 50:50, 75:25, and 100:0) on the biomass, photosynthetic parameters, activity of key N metabolism enzymes, mineral contents and quality of hydroponically grown lettuce were evaluated. The results showed that total dry biomass, the total fresh biomass and root length were the maximum at the 75:25 NO_3^-:NH_4^+ ratio and that the water content was the highest by a significant margin at 25:75. The leaf intercellular CO_2 concentration (Ci) , net photosynthesis rate (Pn),and stomatal conductance (Gs) of lettuce were the highest at 75:25, and the transpiration rate (Tr) was the maximum at 50:50. The Ca and Fe contents in lettuce were the highest at 100:0, and the N and Mg contents were the highest at 75:25. The activities of key enzymes related to N metabolism, nitrite reductase (NiR), nitrate reductase (NR), glutamine synthetase (GS), asparagine synthetase (AS), glutamate synthase (GOGAT), and glutamate dehydrogenase (GDH), were the greatest at 75:25, and the nutritional quality of lettuce in terms of the soluble sugar, vitamin C (Vc) and soluble protein contents was the highest at 75:25. The results showed that when the ratio of NO_3^-:NH_4^+ was 75:25, the most conducive to lettuce growth.

Key words: Enzyme activity, *Lactuca sativa*, Nitrogen ratio, Nutritional quality, Photosynthesis.

Introduction

As an essential element for plant growth, nitrogen (N) is participated in a variety of physiological and biochemical processes for plant production (Belyaev *et al*., 2020). It affects the yield, quality and growth of plants and crops and plays an important role in the synthesis of various organic compounds, such as proteins, chlorophyll, vitamins, nucleic acids and enzymes (Zhao *et al*., 2020). Reasonable use of nitrogen can improve plant quality and yield, whereas inappropriate N applications can reduce plant yields and even cause ecological problems (Das *et al*., 2020). The main N forms absorbed by plants are nitrate (NO_3^-) and ammonium (NH_4^+). The two forms of N have significant effects on plant absorption, transport and assimilation pathways, as well as on plant normal growth and development and plant physiology (Domiciano *et al*., 2020). There are many reports stating that the presence of only NO_3^- or NH_4^+ as a N source is not conducive to plant growth; the metabolism and nutritional quality of the plants are greatest under an appropriate NO_3^-:NH_4^+ ratio (Yu *et al*., 2020). For example, replacing a portion of NO_3^- in nutrient solutions with NH_4^+ is beneficial to the growth of poinsettia (Zou *et al*., 2020). Furthermore, cabbage (Hu *et al*., 2017), Gerbera (Khalaj *et al*., 2017), rose (Farahi *et al*., 2019) and ryegrass (Mia *et al*., 2019) grew best under an appropriate NH_4^+ and NO_3^- mixture. Leaves of strawberry plants presented the greatest dry weight at ratios of 75:25 or 50:50 (Tabatabaei *et al*., 2008), and the dry weight of tomato (Claussen, 2002) was significantly increased by 10.3% at 75:25. Thus, many reports have shown that applications of various proportions of NH_4^+ and NO_3^- can promote plant growth.

Lettuce (*Lactuca sativa* L.), a member of the Chrysanthemum family, contains vitamin C (Vc), sugars, Ca, Fe, P, crude fibre and other nutrients and has high nutritional value (Medina-Lozano *et al*., 2020) However, there have been few reports on lettuce, despite this species being widely cultivated in recent decades. As such, the effects of different N forms on the growth and N metabolism of hydroponic lettuce were investigated, the effects of NO_3^- and NH_4^+ on plant growth while considering data from other studies. The effects of different NO_3^-:NH_4^+ ratios on the growth, photosynthetic parameters, N metabolism, activity of key enzymes, contents of mineral elements and quality of lettuce were studied. This study aims to determine the suitable nitrogen form ratio for lettuce growth and to provide corresponding theoretical guidance and a technical reference for high yields, high quality and high N-use efficiency.

Materials and Methods

Materials and treatments: Lettuce variety Beisansheng No.1. was used for the experiment. The test was conducted at the Key Laboratory of Vegetables of the Beijing University of Agriculture from March to December 2020. Lettuce seeds were sown on a sponge that was placed in a hydroponic device. The relative humidity in the greenhouse was maintained at 60%~80%, and the light power was approximately set about 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$. When the seedlings started producing three leaves, robust seedlings displaying the same growth trend were selected and moved to a 20 L hydroponic system. Hoagland as the nutrient solution was chosen for the hydroponic system (Table 1). The seedlings were grown in modified Hoagland and Arnon nutrient solution.

The experiment involved five different N form ratios, T1 to T5, with respective NO_3^-:NH_4^+ ratios of 0:100, 25:75, 50:50, 75:25, and 100:0.

Table 1. Distribution of nutrient elements in the nutrient solutions.

Nutrient component ($mmol·L^{-1}$)	$NO_3^-:NH_4^+$				
	0:100	**25:50**	**50:50**	**75:25**	**100:0**
$NaNO_3$	0	1.25	2.5	3.75	5
NH_4Cl	5	3.75	2.5	1.25	0
K_2SO_4	1	1	1	1	1
$MgSO_4$	0.36	0.36	0.36	0.36	0.36
$CaCl_2·2H_2O$	0.33	0.33	0.33	0.33	0.33
NaH_2PO_4	0.25	0.25	0.25	0.25	0.25
$CuSO_4·5H_2O$	0.016	0.016	0.016	0.016	0.016
$ZnSO_4·7H_2O$	0.015	0.015	0.015	0.015	0.015
$MnCl_2·4H_2O$	0.01	0.01	0.01	0.01	0.01
$EDTA-FeNa_2·3H_2O$	0.008	0.008	0.008	0.008	0.008
$C_2H_4N_4$	0.007	0.007	0.007	0.007	0.007
$NaMoO_4·2H_2O$	0.005	0.005	0.005	0.005	0.005
H_3BO_3	0.002	0.002	0.002	0.002	0.002

Twenty-two plants were treated, (replicated three times). When the lettuce seedlings developed 5 leaves, samples were collected, and various indicators were measured. To prevent nitrification during the tests, $C_2H_4N_4$ was added to the nutrient solution, and the initial pH of the nutrient solution was set to 6.5±0.2 with phosphoric acid (H_3PO_4). The lettuce seedlings were grown in the nutrient solution until the 8th day, after which 5 processed lettuce seedlings were sampled, washed with deionized water and wiped clean.

Measurements of plant physiological characteristics: The fresh biomass of shoots and roots of lettuce seedlings was measured with an electronic balance (precision of 0.0001), and the length of the roots was measured by ruler. The experiment lettuce sample was subsequently put into an electric constant temperature air drying oven (DHG-9245 model, YiHeng, Shanghai, China) and the temperature is adjusted to 75°C until the mass was constant, after which the dry biomass was measured with a balance. The lettuce leaves water content and the root-shoot ratio were calculated.

Pigment index: At a ratio of 95% ethanol and the acetone were mixed, after which chlorophyll extraction was performed by immersing 0.05 g of leaf tissue into the mixture. The absorbance was measured at wavelengths of 645 nm, 663 nm and 470 nm, after which the contents of chlorophyll a (Chla) and b (Chl b) and the total content of carotenoids were determined according to the formula (Saunkaew *et al*., 2011).

Photosynthetic parameters: Determination of the photosynthesis index of lettuce plants by CIRAS-3 portable photosynthesis meter, and the measurements occurred from 8:00-11:30 a.m. on the eighth day of treatment. The leaf chamber temperature was adjusted to 25±1°C, and the relative humidity was set to 75%-80%, and the light power was also adjusted to 800 $\mu mol·m^{-2}·s^{-1}$. Five leaves were randomly selected per treatment. When the photosynthesis apparatus was relatively stable, the net photosynthesis rate (Pn), stomatal conductance (Gs), intercellular CO_2 concentration (Ci), and transpiration rate (Tr) of the lettuce leaves were measured (Zhu *et al*., 2020).

Mineral element content: Dried lettuce samples (0.5g) were subjected to potassium nitrate (KNO_3) oxidative spectrophotometry to directly measure the NO_3^-, NH_4^+ and total N contents in the plants. The mineral element content was determined according to method of Singh *et al*., (2010).

Activities of key enzymes: The nitrite reductase (NiR) and nitrate reductase (NR) activities were analysed *in vivo* (Abbasifar *et al*., 2020), glutamine synthetase (GS) and asparagine synthetase (AS) enzyme activities were determined according to Wang *et al*., (2008), and the activities of glutamate synthase (GOGAT) and glutamate dehydrogenase (GDH) were determined according to Ye (Ahanger *et al*., 2017).

Nutritional quality: The content of soluble sugar was determined via anthrone colourimetry (Yang *et al*., 2015), the content of vitamin C was determined by 2,6-dichlorophenolin-dophenol colorimetry (Yang *et al*., 2015) and the soluble protein content was determined by Coomassie brilliant blue G-250 staining (Lin *et al*., 2019).

Statistical analysis

The experimental design was a completely randomized block design comprising sixteen replicates, and each seedling constituted one replicate. The data were subjected to analysis of variance and analysis of significance using SPSS 16.0 (SPSS Inc. Chicago, IL, USA). Different letters were used to represent significant differences as determined by Tukey's honestly significant difference test ($p<0.05$).

Table 2. Effects of different nitrogen ratios on the growth of lettuce.

Treatment	Plant biomass (g)	Shoot fresh biomass (g)	Root fresh biomass (g)	Root:shoot (%)	Plant dry biomass (g)	Shoot dry biomass (g)	Root dry biomass (g)	Root length (cm)	Water content (%)
T1	10.21a	8.91a	1.3a	0.15b	0.49ab	0.42a	0.07a	9.75b	95.28a
T2	10.12a	8.66a	1.46a	0.17ab	0.46ab	0.40ab	0.06a	10.02b	95.38a
T3	6.3b	5.41b	0.89b	0.17ab	0.32b	0.28c	0.04b	13.14b	94.3b
T4	10.62a	9.12a	1.50a	0.16a	0.6a	0.52a	0.08a	19.3a	95.08ab
T5	6.75b	5.97b	0.78b	0.13b	0.34b	0.30bc	0.04b	12.32b	94.9ab

*Note: The values followed by different letters in the same column indicate significant differences at the 5% level.
T1-T5: NO_3^-:NH_4^+ ratios of 0:100, 25:75, 50:50, 75:25, and 100:0, respectively.

Table 3. Effects of different nitrogen ratios on photosynthetic pigments of lettuce.

Treatment	Chlorophyll a ($mg \cdot g^{-1}$)	Chlorophyll b ($mg \cdot g^{-1}$)	Total chlorophyll ($mg \cdot g^{-1}$)	Carotenoids ($mg \cdot g^{-1}$)
T1	0.55bc	0.69b	1.24ab	0.21ab
T2	0.60ab	0.60b	1.2b	0.23ab
T3	0.56b	0.39c	0.95c	0.24ab
T4	0.62a	0.84a	1.46a	0.27a
T5	0.34c	0.38c	0.72bc	0.14c

*Note: The values followed by different letters in the same column indicate significant differences at the 5% level.
T1-T5: NO_3^-:NH_4^+ ratios of 0:100, 25:75, 50:50, 75:25, and 100:0, respectively.

Results

Effects on lettuce morphological characteristics: Table 2 shows that total dry biomass and entirel fresh biomass were the greatest under T4 (75:25). The results exhibited the following trend: T4>T1>T2>T5>T3. In addition, the total fresh biomass under T1 and T2 were significantly different from those under T3 and T5. The length of the roots under T1 to T5 were first went up, then it went down, reaching a maximum of 19.3 cm under T4, and the root length under T1 was the lowest (9.75 cm). The water content was the greatest under T2, and the difference between T4 and T2 was not significant.

Effects on photosynthetic pigments in lettuce: The results can be simplified appropriately (Table 3). For example: "Under T4, the content of chlorophyll a was the highest, and the increase in chlorophyll a was more apparent under T4 than under the other treatments. The content of chlorophyll b reached 0.85 mg·g-1 under T4; it was significantly different from that under the other treatments, 1.21, 1.40, 2.15, and 2.22 times the chlorophyll b contents under the other treatments. The total chlorophyll content under the T4 treatment was the greatest; it was 0.186, 0.245, 0.476, and 0.622 mg·g-1 greater than that under the other four treatments".

Effects on photosynthetic parameters of lettuce: As shown in Table 4, the Pn of lettuce was greatest under T4 at 5.42 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The Pn of lettuce under treatments T1 to T5 was as follows: T4>T2>T5>T1>T2. Moreover, the T4 treatment resulted in the greatest Ci, which was significantly different from other treatments. Gs was the greatest under T4 and was significantly different from that under the other treatments. T3 presented the greatest Tr (7.83 $mmol \cdot m^{-2} \cdot s^{-1}$), which was 0.86 $mmol \cdot m^{-2} \cdot s^{-1}$ greater than that under the T4 but was less than that under the others. The Tr exhibited the following trend: T3>T4>T2>T5>T1. In summary, under the T4 treatment, the Pn, Ci, and Gs of lettuce were maximum, and the Tr was highest under the T3 treatment.

Effects on mineral element contents in lettuce: As shown in Table 5, the N content in the lettuce leaves was highest under T4, T5 was the lowest, which was significantly different to the other treatments. The content of N reached 342.87 $mg \cdot kg^{-1}$ under T5, which was 105.3, 88.54, 74.5, and 31.8 times greater than that under the other treatments. With respect to the Fe content, the trend among the five treatments was as follows: T5>T4>T1>T2>T3. With respect to P, the trend among the five treatments was as follows: T2>T3>T1>T5>T4. In terms of Mg, the content were greatest under T4, and the difference between T4 and T3 was not significant, while the other treatments were significantly different from T4. The K content was the largest underT5 treatment, followed by T4 treatment.

Effects on N metabolism and key related enzymes in lettuce: The maximum content of NO_3^--N in the lettuce was observed under T5; the difference between T5 and the other treatments was significant (Table 6). The behaviour of the root was similar to that of the shoot. The NH_4^+ content in the lettuce shoots under T5 was 0.37, 0.34, 0.29, and 0.25 $mg \cdot g^{-1}$ less than that under the other treatments; the NH_4^+ content in roots under T5 was 0.40, 0.39, 0.36, and 0.35 $mg \cdot g^{-1}$ less than that under the other treatments. In summary, when the NO_3^-:NH_4^+ ratio was 100:0, the content of NO_3^--N was the greatest, and the content of NH_4^+-N was the greatest when the NO_3^-:NH_4^+ ratio was 0:100.

Table 4. Effects of different nitrogen ratios on the photosynthetic parameters of lettuce.

Treatment	Net photosynthesis rate ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Intercellular CO_2 concentration ($\mu mol \cdot mol^{-1}$)	Stomatal conductance ($mmol \cdot m^{-2} \cdot s^{-1}$)	Transpiration rate ($mmol \cdot m^{-2} \cdot s^{-1}$)
T1	4.23b	425.50b	201.33b	3.76c
T2	4.90ab	432.17ab	440.33b	6.18ab
T3	2.62c	444.00ab	675.33ab	7.83a
T4	5.42a	459.67a	1070.50a	6.97ab
T5	4.25b	417.00b	345.67b	5.66b

*Note: The values followed by different letters in the same column indicate significant differences at the 5% level.
T1-T5: NO_3^-:NH_4^+ ratios of 0:100, 25:75, 50:50, 75:25, and 100:0, respectively.

Table 5. Effects of different nitrogen ratios on the mineral elements in lettuce.

Treatment	N ($mg \cdot g^{-1}$)	P ($mg \cdot g^{-1}$)	K ($mg \cdot kg^{-1}$)	Ca ($mg \cdot kg^{-1}$)	Mg ($mg \cdot kg^{-1}$)	Fe ($mg \cdot kg^{-1}$)
T1	44.23a	0.76a	480.90c	237.57c	102.18b	3.53ab
T2	44.22a	0.81a	558.47c	254.33bc	116.10ab	2.74b
T3	44.19a	0.79a	640.10c	268.37bc	135.17a	1.72c
T4	45.13a	0.55b	882.03b	311.07b	137.63a	3.56ab
T5	30.64b	0.67a	1074.47a	342.87a	105.88ab	7.10a

*Note: The values followed by different letters in the same column indicate significant differences at the 5% level.
T1-T5: NO_3^-:NH_4^+ ratios of 0:100, 25:75, 50:50, 75:25, and 100:0, respectively.

Table 6. Effects of different nitrogen ratios on NO_3^- and NH_4^+ contents in lettuce.

Treatment	Ammonium ($mg \cdot g^{-1}$)			Nitrate ($mg \cdot g^{-1}$)		
	Shoot	Root	Plant	Shoot	Root	Plant
T1	0.46a	0.47a	0.92a	0.01b	0.02c	0.02c
T2	0.43a	0.46a	0.87a	0.05b	0.03bc	0.09b
T3	0.38a	0.43a	0.77a	0.06ab	0.05b	0.12b
T4	0.34a	0.41a	0.70a	0.08ab	0.06b	0.15b
T5	0.09b	0.07b	0.18b	0.11a	0.10a	0.22a

*Note: The values followed by different letters in the same column indicate significant differences at the 5% level.
T1-T5: NO_3^-:NH_4^+ ratios of 0:100, 25:75, 50:50, 75:25, and 100:0, respectively.

As shown in Fig. 1, the NR activity was the greatest under T4, which was significantly different from that under the other treatments. The NiR activity was the same as that of NR and was greatest under T4, which was significantly different from other treatments. GOGAT activity was the greatest under T4; the levels were significantly different from those under the other treatments. The GOGAT activity was significantly lower under T5 than under the other treatments. The activity of GS was greatest under T4 and differed significantly to the other treatments. Under T1 to T5, the activity of AS and GDH increased first but then decreased, peaking under T4. In summary, the activities of key enzymes involved in lettuce N metabolism were greatest under T4.

Effects on the nutritional quality of lettuce: In Fig. 2, the soluble sugar content of lettuce leaves was greatest under T4 and significantly differed from that under T1, T2 and T5, with levels 33.00%, 32.69% 24.69%, and 27.65% greater than those under the other four treatments. The soluble protein content in the lettuce leaves significantly differed between the T1, T2 and T4 treatments. The soluble protein was the highest under T4, with levels 6.84, 5.66, 2.11, and 2.53 $mg \cdot g^{-1}$ greater than those under the other treatments. Whereas soluble protein content was the lowest under T1. NH_4^+ treatment alone was not conducive to the accumulation of several soluble proteins in the lettuce plants. The Vc content was the greatest under the T4 treatment and significantly differed from that under the T1 treatment. The Vc contents under T1-T5 were 6.03, 7.35, 7.36, 8.62 and 8.16 $mg \cdot g^{-1}$, respectively. In summary, the best nutritional quality of lettuce occurred under T4.

Discussion

An appropriate NO_3^-:NH_4^+ ratio can promote the growth of plants and crops. For example, Gerbera cut flowers (Khalaj *et al*., 2017) presented the best growth and quality at NO_3^-:NH_4^+ ratio of 80:20. On the basis of the present experiments, it was determined that a NO_3^-:NH_4^+ ratio of 75:25 resulted in significant positive effects on the relative fresh biomass, root: shoot ratio, water content, root length, and leaf area of lettuce. These results were similar to the results of previous studies (Fujii *et al*., 2019), in which the yield and quality of plants were greatly improved at a suitable NO_3^-:NH_4^+ ratio.

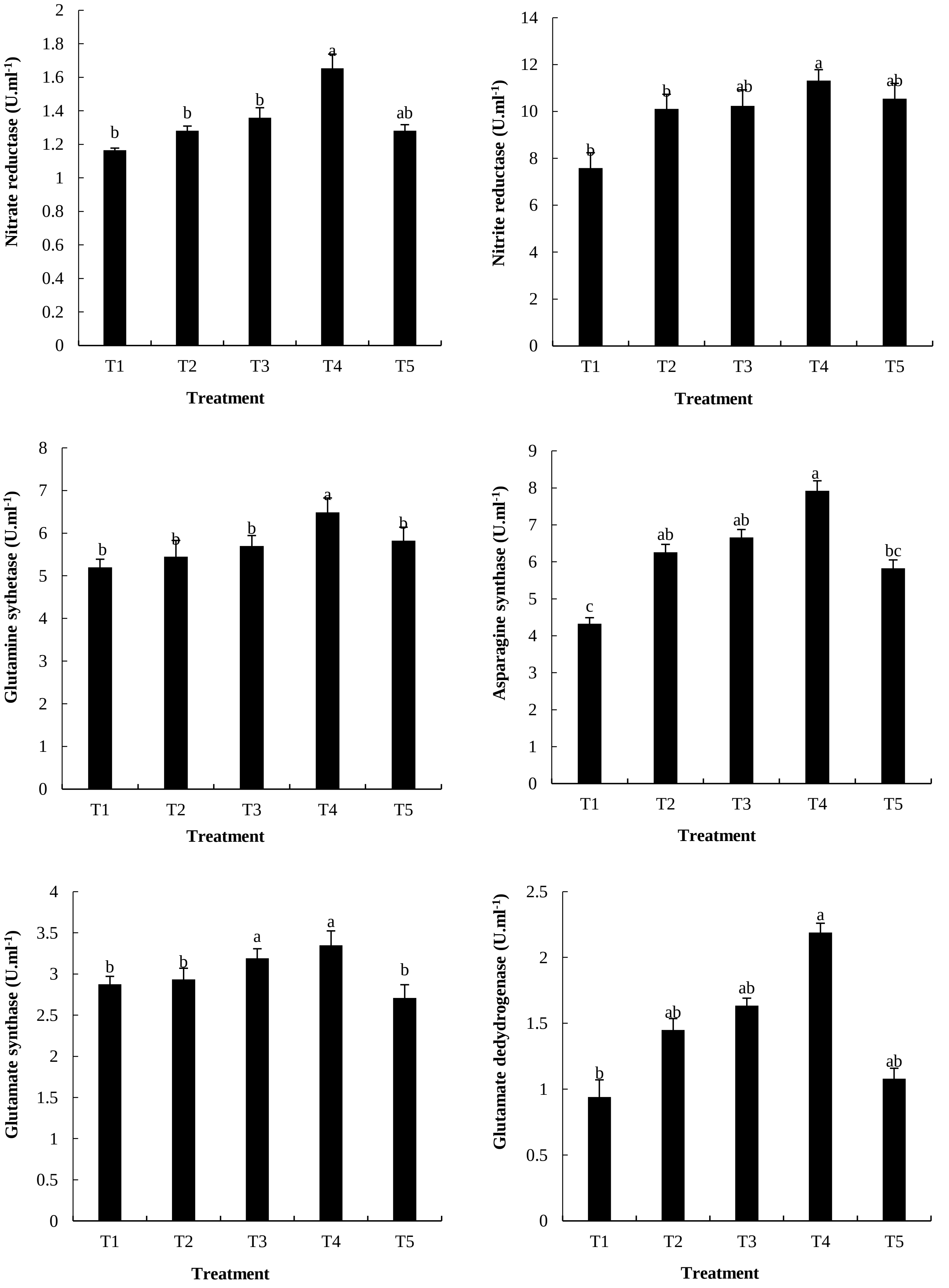

Fig. 1. Effects of different nitrogen ratios on the key enzymes involved in N metabolism in lettuce
*On the eighth day, seedlings displaying consistent growth were selected for measurements. T1-T5: NO_3^-:NH_4^+ ratios of 0:100, 25:75, 50:50, 75:25, and 100:0, respectively. The values are the means of 6 replications.

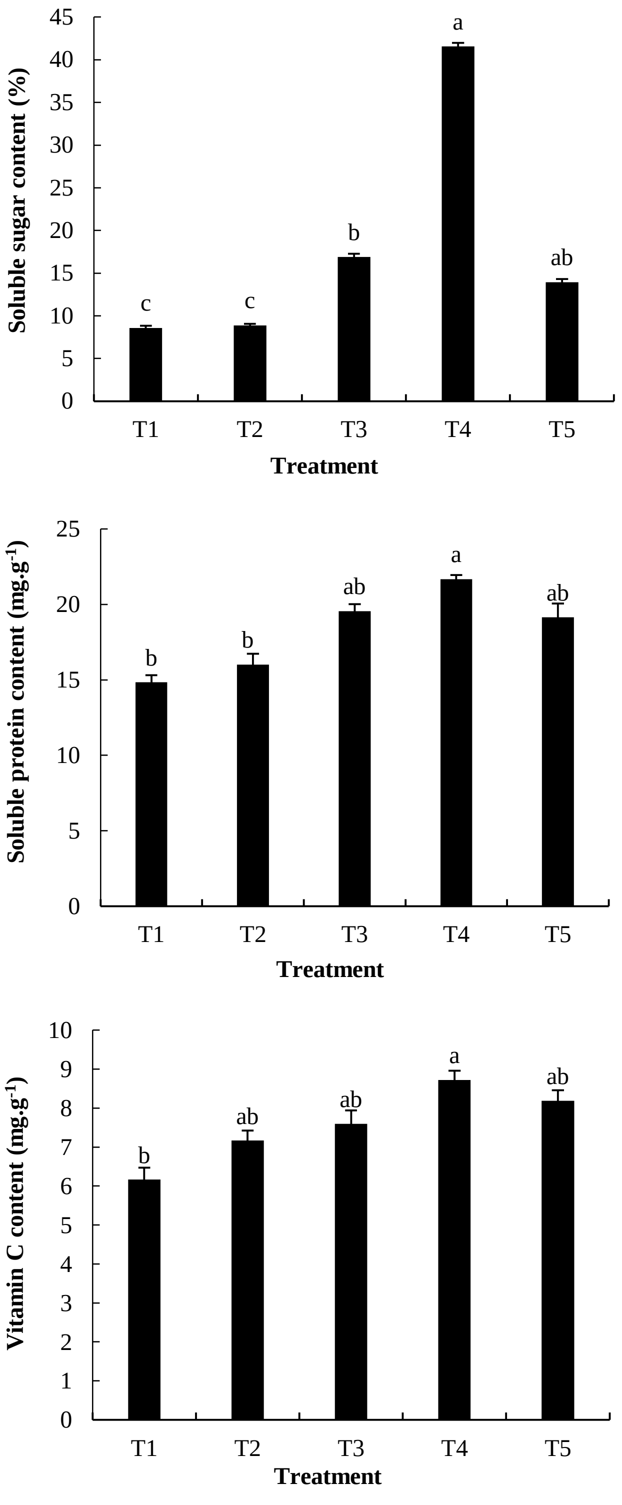

Fig. 2. Effects of different nitrogen ratios on the nutritional quality of lettuce

*On the eighth day, seedlings displaying consistent growth were selected for measurements. T1-T5: NO_3^-:NH_4^+ ratios of 0:100, 25:75, 50:50, 75:25, and 100:0, respectively. The values are the means of 6 replications.

Jampeetong & Brix (2009a) reported that when NO_3^--N was supplied alone, the amount of photosynthetic pigments in the leaves decreased. In the present study, changes in Chlorophyll A and Chlorophyll B and carotenoid contents were observed such as increasing levels followed by decreasing levels. When the NO_3^-:NH_4^+ ratio was 75:25, the contents of lettuce Chlorophyll A and Chlorophyll B increased, while under the treatment of NH_4^+ alone or NO_3^- alone, the synthesis of lettuce Chlorophyll A and Chlorophyll B was inhibited. The effect of sole N source application was not conducive to the accumulation of Chlorophyll A and Chlorophyll B (Jampeetong & Brix, 2009b). A NO_3^-:NH_4^+ ratio of 75:25 was most beneficial for pigment accumulation and positively affects photosynthesis.

An appropriate NO_3^-:NH_4^+ ratio helps to improve light energy conversion efficiency and can enhance plant photosynthesis. The results of this study showed that when the NO_3^-:NH_4^+ ratio in the nutrient solution was 75:25 (T4), the Gs, Ci and Pn of lettuce were greatest and significantly differed from those under the other treatments. The Tr was maximum when the NO_3^-:NH_4^+ ratio was 50:50, and the difference was small compared with the Tr under T4. The results were similar to those of Zhu *et al*., (2020). The photosynthesis of lettuce was optimal when the NO_3^-:NH_4^+ ratio was 75:25.

Zhang *et al*., (2009) showed that an increasing N content resulted in the increase in the contents and accumulation of Mg, Mn and Fe in soybean vegetative tissue first but then decreased, while in the grain, Ca decreased sharply under low N contents but then gradually increased. Chen *et al*., (2010) reported that under a relatively high proportion of NH_4^+-N (75%) in the nutrient solution, the contents of total N and P in soybean seeds increased significantly, while the contents of mineral nutrients such as Ca, K and Mg decreased significantly. In contrast, under appropriate NO_3^-:NH_4^+ ratios (75:25 and 50:50), mineral nutrients such as Ca, K, and Mg were absorbed and accumulated in the soybean seeds. This indicates that N has a significant effect on the absorption of mineral elements by plants. In the present study, the P content gradually increased under T1 to T2, but the difference between T1 and T2 was not significant. When the NO_3^-:NH_4^+ ratio was 75:25 (T4), the content of N was the greatest recorded, but when the NO_3^-:NH_4^+ ratio was 100:0, the N content decreased significantly, indicating that the accumulation of N was inhibited when the N source was provided by NO_3^--N only. These findings were similar to those of Daryanto *et al*., (2019). Plants consume energy when absorbing NO_3^--N, which is not conducive to the accumulation of N. NH_4^+-N can be directly absorbed by plants. Adding a small amount of NH_4^+-N to the nutrient solution was beneficial to the absorption of N by plants. Because the uptake of NO_3^- occurs simultaneously with the uptake of Ca^{2+} or K^+, the content of Ca^{2+} and K^+ in leaves increases with an increasing proportion of NO_3^--N. Roosta and Schjoerring (2007) reported that 5.0 and 10.0 mmol·L^{-1} NH_4^+ significantly reduced the contents of Ca^{2+} and Mg^{2+} but significantly increased the P content in cucumber (*Cucumis sativus* L. cv Styx). As in the study, the trends of Ca^{2+} and K^+ in lettuce was positively correlated with the proportion of NO_3^--N. Under the total NO_3^- treatment, the contents of Ca^{2+} and K^+ peaked. The content of P was high when the proportion of NH_4^+-N was relatively high (25:75). The Mg content was greatest when the NO_3^-:NH_4^+ ratio was 75:25, and the sole NH_4^+ treatment was not conducive to the accumulation of Mg. Different N forms strongly influence the absorption of trace elements by plant roots, especially Fe. In the present study, when the NO_3^-:NH_4^+ ratio was 50:50, the content of Fe was the lowest recorded, and the contents of total NO_3^- and total NH_4^+ greatly differed. Under the sole NO_3^- treatment, the Fe content was the greatest recorded, and the difference between the sole NH_4^+ treatment (T1) and the treatment in which the NO_3^-:NH_4^+ ratio was 75:25 (T4) was small.

The results of this study showed that as the ratio of ammonium nitrogen in the nutrient solution increased, the content of N, P and other elements in the lettuce also increased significantly, while the content of K, Ca, and Mg decreased, especially when the ratio of ammonium nitrate was 25:75, the content of N and P in the lettuce were higher than that of other treatments, while the content of K, Ca, and Mg was obviously insufficient. Obviously, this uncoordinated nutritional status will have an adverse effect on the growth and development of grains. When the ratio of nitrate：ammonium was 75:25, the contents of K^+, ca_2+, and Mg_2^+ in the lettuce increased significantly, indicating that a proper ratio of ammonium nitrate was beneficial to promote the absorption of cations, thereby improving the nutritional status and quality of vegetable.

Soluble sugars and soluble proteins are the most important plant nutrient components, reflecting the efficiency and metabolism of C and N assimilation. Our results of the present study were similar to a study of rape by Qin *et al*., (2017) who reported that the soluble protein and soluble sugar contents were the greatest when the NO_3^-:NH_4^+ ratio was 1 and the lowest with greater or lower NO_3^-:NH_4^+ ratios. In the present study, the soluble protein content in the lettuce plants was the highest when the NO_3^-:NH_4^+ ratio was 75:25, indicating that the C and N assimilation efficiency reached their maximum levels under this treatment. Moreover, Vc is an important indicator for measuring the edible standards of leafy vegetables, and its level is closely related to the human nutrition. In a study of spinach, as the proportion of NO_3^--N increased, the Vc content decreased, and an appropriate increase in the NO_3^-:NH_4^+ ratios (Sui *et al*., 2018) was beneficial to the increase in Vc. In the present study, the Vc content increased gradually with the increase in the proportion of NO_3^--N. The Vc content in the lettuce peaked under T4; however, under sole NO_3^- treatment, the Vc content began to decrease, and the nutrient quality of the lettuce began to decline.

Nitrate nitrogen needs to be converted into ammonium nitrogen through the action of NR and NIR before it can be absorbed by plants. Under the catalysis of As and GS or GOGAT and GDH, the ammonium absorbed by plants was converted into amino acids through the glutamine-glutamate cycle pathway or glutamate dehydrogenase pathway, and then participated in other metabolic activities of plants(Wang *et al*., 2019) .There are many studies on N metabolism and the activities of related enzymes in plants and crops. NR and NiR act as key rate-limiting enzymes involved in the assimilation of NO_3^- (Wang *et al*., 2019). In the study of kidney bean, when the ratio of ammonium nitrate was 75:25, the activity of NR and NIR was higher Relevant experiments had shown that the activities of NR and NiR in plants were positively correlated with the content of NO_3^--N (Jia *et al*., 2019). In the present experiment, when the NO_3^-:NH_4^+ ratio was 75:25, the activities of NiR and NR were relatively high. Under the sole NH_4^+ treatment, however, the activities of NiR and NR were inhibited, similar to the findings of the above research, and the activities of NiR and NR were significantly increased when the proportion of NO_3^--N was relatively high. The level of GS activity is directly related to the level of N metabolism and transport. Furthermore, Yang *et al*., (2010) demonstrated that NO_3^--N was more conducive to GS and AS activity at an appropriate NO_3^-:NH_4^+ ratio in rice (Bybordi *et al*., 2012). An appropriate N ratio can increase the GOGAT activity in the leaves. A NO_3^-:NH_4^+ ratio of 50:50 or 25:75 was shown to improve the activity of GOGAT in grape plants. Moreover, GDH plays an vital role in the N metabolism of plants. GDH is present in mitochondria, and in the grape study, a NO_3^-:NH_4^+ ratio of 0:100 (sole NH_4^+ treatment) resulted in extremely high GDH activity. The results of this experiment were similar to other studies, the activity of lettuce GS、AS、GOGAT、GDH increased with a decrease in the proportion of NH_4^+-N, and the activity of GS was greatest under T4.

Conclusion

When the NO_3^-:NH_4^+ ratio was 75:25, the biomass, Pn, Gs and metabolic enzyme activity of lettuce was increased significantly. Under this treatment, the chlorophyll content in the lettuce leaves was the greatest, and photosynthesis was significantly greater than the other treatments. When the ratio of NO_3^-:NH_4^+ was 75:25 in the nutrient solution, the accumulation of N was increased. A suitable NO_3^-:NH_4^+ ratio in nutrient solutions is 75:25, as this ratio is most conducive to the growth of lettuce among those tested.

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