



Effects of different $\text{NO}_3^-:\text{NH}_4^+$ ratios on the photosynthesis and ultrastructure of lettuce seedlings

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Abstract

Nitrate nitrogen (NO_3^-) and ammonium N (NH_4^+) are the two main forms of nitrogen (N). The effects of the $\text{NO}_3^-:\text{NH}_4^+$ ratio (0:100, 25:75, 50:50, 75:25, and 100:0) in the nutrient solution on growth, pigment content, photosynthetic parameters, chlorophyll (Chl) precursor content, and chloroplast mitochondrial ultrastructure of lettuce leaves were evaluated to determine the appropriate N ratio for the growth of lettuce. The results showed that a ratio of nitrate to ammonium of 75:25 compared with the single N form resulted in significantly higher fresh weights in the shoots and the lower parts of the lettuce. The growth rate, as indicated by the CO_2 response curve, was increased because of the carboxylation efficiency and dark respiration rate (Rday) of the lettuce seedlings, and the difference between the light compensation point and CO_2 compensation point was reduced under single nitrate N treatment. The contents of δ -aminolevulinic acid (ALA), porphobilinogen, uroporphyrinogen III, protoporphyrin IX, and Mg-protoporphyrin IX in seedlings at 75:25 nitrate:ammonium were higher than those in seedlings under the other treatments. The contents of Chl a and b were also the highest when the ratio of nitrate ammonium was 75:25. The chloroplast structure of lettuce leaves was intact, the cell membrane was completely closed to the cell wall, and the organelles were closely arranged on the cell membrane. The chloroplast was spindle-shaped and full of vesicles. The whole mitochondria were rich, the base particles were rich, the base granules were clear and rich, and the mitochondria exhibited the overall best state with a complete structure, rich sputum, and dense inclusions. The above results show that the nutrient solution with a ratio of nitrate to ammonium of 75:25 was more conducive to Chl synthesis and the promotion of photosynthesis and root growth in lettuce seedlings than the other treatments. The single nitrate N nutrient solution reduced Chl synthesis and photosynthesis in lettuce seedlings, while the single ammonium N nutrient solution affected the growth of lettuce seedlings, resulting in lower growth of lettuce shoots and roots. Therefore, the nutrient solution ratio of nitrate to ammonium of 75:25 is the most suitable for the lettuce seedling growth.

Keywords Chlorophyll (Chl) · Chl precursor · Lettuce · Nitrogen (N) ratio · Photosynthetic parameters

1 Introduction

Lettuce (*Lactuca sativa* L.) belongs to the genus *Heliconia* and is an annual or biennial herb that is also called leaf lettuce because the leaves are its edible parts (Ferrerres et al. 1997). Soilless culture includes substrate cultivation and hydroponic culture, both of which are free of the constraints of traditional land-based agricultural production and have the characteristics of saving water, fertilizer, and labour; producing high yields; and reducing pollution (Wahome et al. 2011).

In the past few decades, with the increase in global food production, the use of nitrogen (N) fertilizer has gradually increased, and excessive application of N fertilizer and soil N contents have a certain impact on crops (Singh and

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Sekhon 1990). Different N application rates have different effects on plants. Appropriate N application can promote plant growth, crop yield, and quality (Cai et al. 2012); an inappropriate N application rate to plant crops not only does not have a growth-promoting effect but also has an inhibitory effect to a certain degree, and excessive N use can also cause environmental pollution (Rios-Gonzalez et al. 2002). N sources are mainly divided into nitrate N ($\text{NO}_3\text{-N}$) and ammonium N ($\text{NH}_4\text{-N}$) (Mifflin and Habash 2002). Crops differ in metabolic processes such as absorption, storage, transportation, and assimilation (Sun et al. 2016), which affect the physiological and biochemical metabolism of plants. Plants have different ways of absorbing N in its two forms. The absorption of $\text{NO}_3\text{-N}$ is an active process (Glass et al. 1992), while the absorption of $\text{NH}_4\text{-N}$ is divided into the reverse transport of NH_4^+ and H^+ and the absorption of NH_4^+ protons in the form of NH_3 (Mengel and Kirkby 1982). The two forms of N have different effects on plants, but studies have found that nutrient solutions with mixtures of the two N forms are superior to single-form nutrient solutions (Roosta and Schjoerring 2008).

Photosynthesis is a biological process in which plants (as well as photosynthetic bacteria) convert light energy into chemical energy that can be used in life processes to synthesize and effectively accumulate organic matter. Enhancing photosynthesis is an effective means to improve vegetable yield and quality (Müller et al. 2001). N affects chloroplasts, photosynthesis, photorespiration, dark respiration, and enzyme synthesis and can directly or indirectly affect photosynthesis (Guo et al. 2007a). Chlorophyll (Chl) is a tetrapyrrole macrocyclic molecule containing Mg^{2+} , phytol chains, and characteristic closed loops (Tanaka and Tanaka 2007). In plants, Chl is the most abundant tetrapyrrole and acts as a photosynthetic pigment to harvest light energy and transfer the absorbed energy to the reaction centre where charge separation occurs (Fromme et al. 2003). N greatly influences Chl accumulation and is an essential element for plant growth. Lack of N causes leaves to shrink, which in turn affects Chl (Huang et al. 2004).

Chloroplasts are structures that absorb light energy in cells and are used for photosynthesis. Mitochondria are structures that make energy in cells and are the main site of cellular respiration. The ultrastructure of chloroplasts and mitochondria can reflect the growth of plants (Talla et al. 2011). In a suitable growth environment, the cell membrane completely adheres to the cell wall, and the organelles are closely arranged on it. The chloroplast should be spindle-shaped, full of vesicles, rich in granules, and clear (Wang et al. 2016). The mitochondria are structurally intact, rich in internal organs, and dense in inclusions (Lage-Pinto et al. 2008).

Lettuce is one of the most widely used vegetables in hydroponic culture worldwide, necessitating the study of the

appropriate N ratio in the nutrient solution under graduated hydroponic culture (Kimura and Rodriguez-Amaya 2003). Previous studies on the growth of crops with different ratios of N forms have been reported for gerbera (Savvas 2001), tomato (Claussen 2002), and rice (Britto and Kronzucker 2004). However, there are few reports on the changes in Chl precursor content, photosynthesis, and the cell ultrastructure in lettuce under different N ratios. In this paper, a hydroponic method was used to study the growth, pigment content, light response curve, CO_2 response curve, photosynthetic parameters, Chl precursor content, and chloroplast and mitochondria characteristics of seedling leaves under different N forms. The influence of the microstructure in determining the appropriate N ratio for the growth of lettuce was studied as a reference for high-yield, high-quality, and N-efficient synergy. In addition, determining the appropriate N ratio for lettuce growth provides theoretical and technical information for the corresponding physiological mechanism of N use.

2 Materials and methods

2.1 Plant materials and treatments

The experiment was conducted under hydroponic conditions. The seeds of ‘No. 1 Beisansheng’ lettuce (*L. sativa* L.) (provided by the Beijing University of Agriculture and the Beijing Leaf Vegetable Innovation Team) were placed in Petri dishes for germination. After 24 h of germination, the germinated seeds were placed in a sponge block, which was placed in a light incubator. The light cycle was 14 h/10 h (day/night), and the relative humidity was controlled at 75%. The light intensity was $800 \mu\text{mol m}^{-2} \text{s}^{-1}$. When the seedlings had three leaves and one heart (2 weeks), strong seedlings of similar size were transplanted into the 10-L hydroponic device, and the water was slowed for 3 days. Hoagland nutrient solution was applied (see Table 1 for recipe details).

The five treatments were defined as follows: T1, $\text{NO}_3\text{-N}:\text{NH}_4\text{-N}$ ratio of 0:100; T2, $\text{NO}_3\text{-N}:\text{NH}_4\text{-N}$ ratio of 25:75; T3, $\text{NO}_3\text{-N}:\text{NH}_4\text{-N}$ ratio of 50:50; T4, $\text{NO}_3\text{-N}:\text{NH}_4\text{-N}$ ratio of 75:25; and T5, $\text{NO}_3\text{-N}:\text{NH}_4\text{-N}$ ratio of 100:0.

For each of the 16 strains, samples were taken every 2 days from the start of the treatment, that is, samples were taken at 0, 2, 4, 6, and 8 days, and morphological index measurements were performed on the samples taken on the 8th day.

2.2 Measurements of plant physiological characteristics

Samples were taken on the 8th day of the test. Each of the samples comprised five plants of the same size with

Table 1 Hydroponic nutrient solution N distribution ratio

Nutrient composition (mmol L ⁻¹)	NO ₃ -N:NH ₄ -N				
	0:100	25:75	50:50	75:25	100:0
NaNO ₃	0	1.25	2.5	3.75	5
NH ₄ Cl	5	3.75	2.5	1.25	0
K ₂ SO ₄	1	1	1	1	1
MgSO ₄	0.36	0.36	0.36	0.36	0.36
CaCl ₂ 2H ₂ O	0.33	0.33	0.33	0.33	0.33
NaH ₂ PO ₄	0.25	0.25	0.25	0.25	0.25
CuSO ₄ 5H ₂ O	0.016	0.016	0.016	0.016	0.016
ZnSO ₄ 7H ₂ O	0.015	0.015	0.015	0.015	0.015
MnCl 4H ₂ O	0.01	0.01	0.01	0.01	0.01
FeCl ₃	0.008	0.008	0.008	0.008	0.008
C ₂ H ₂ N ₄	0.007	0.007	0.007	0.007	0.007
Na ₂ MoO ₄ 2H ₂ O	0.005	0.005	0.005	0.005	0.005
H ₃ BO ₃	0.002	0.002	0.002	0.002	0.002

moderate growth. The roots were washed with deionized water and wiped clean, and the aboveground and belowground parts were sampled separately. The fresh weight of the belowground part of the lettuce sample was measured, and the root length was measured. Subsequently, the test sample was placed in an electrothermal constant temperature drying oven (DHG-9245A, YiHeng, Shanghai, China) at 105 °C for 30 min and dried at 75 °C until reaching constant weight, and the dry weight of the sample was measured. The water content, root-to-shoot ratio, and dry and fresh weight of the lettuce were calculated.

2.3 Photosynthetic index

The photosynthetic index of the lettuce was measured by a portable photosynthetic apparatus (CIRAS-3, PP Systems, Massachusetts, Amesbury, USA) on the 8th day of treatment from 8:00 to 11:30 AM. The leaf chamber temperature was set at 25 ± 1 °C, the relative humidity was set at 75–80%, and the effective photosynthetic photon flux density (PPFD) was controlled by an instrument and was set to 2000, 1800, 1600, 1400, 1200, 1000, 800, 600, 400, 200, 150, 100, 50, 0 μmol m⁻² s⁻¹. The net photosynthetic rate (Pn) of the sample was measured under each light intensity, and the PPFD was taken as the abscissa and Pn as the vertical coordinate in the light response curve to obtain the apparent quantum efficiency (AQY), light compensation point (LCP), and dark respiration rate (Rday) (Lu et al. 2012).

Using a closed gas path, the CO₂ concentration was set to 1800, 1600, 1400, 1200, 1000, 800, 600, 400, 300, 200, 150, and 100 at 1000 μmol m⁻² s⁻¹ light intensity. A Pn of 50 μmol m⁻² s⁻¹ was measured, and the Pn-Ci curve was prepared with the intercellular CO₂ concentration (Ci) as the horizontal axis, and the carboxylation efficiency (CE)

and CO₂ compensation point were obtained. Five seedlings were randomly selected for each treatment, and the leaves used for detection were the third true leaf from the first true leaf of each plant. When the instrument value became stable, it was recorded and selected at 800 μmol m⁻² s⁻¹. The data described in the following sections were recorded under this light intensity, and the Pn, stomatal conductance (Gs), Ci, and transpiration rate (Tr) were measured and averaged (Farquhar and Sharkey 1982).

2.4 Pigment index

The leaves of the seedlings were sampled 8 days after the treatments began. Ethanol (95%) and acetone were mixed at a ratio of 1:2 to ensure complete Chl extraction in the samples. The samples were completely immersed in the mixture and protected from light overnight. After the extraction, the mixture to be tested was measured at absorbances of 645, 663, and 470 nm, and the contents of Chl *a* and *b*, total Chl, and carotenoid were calculated by the formula (Qin et al. 2017).

2.5 Chl precursor substance content index

The Chl precursor content was determined by measuring 0.5 g of 0-, 2-, 4-, 6-, and 8-days-old seedlings. The δ-aminolevulinic acid (ALA) content was determined according to the method of Mauzerall and Granick (1956). Four milliliters of 4% trichloroacetic acid extract was added to 0.4 g of the leaves, thoroughly ground, and centrifuged at 12,000 rpm for 10 min. Took the extract, added 1 mL of 1 mol L⁻¹ sodium acetate and acetylacetone, boil water for 10 min, then cool to room temperature, then centrifuge at 12,000 rpm to take the supernatant, took 2 mL of supernatant and added 2 mL of Ehrlich-Hg reagent to the developer, in the dark. Fifteen minutes after the reaction, the absorbance was measured at 553 nm.

The porphobilinogen (PBG) content was determined according to the method of Bogorad (1962). Weighed the leaves 0.2 g, ground the liquid N, add 4 mL extraction buffer (0.6 M Tris, 0.1 M EDTA, pH = 8.2), and then homogenized them, centrifuged at 12,000 rpm for 10 min, and took 2 mL of supernatant to added 2 mL of Ehrlich-Hg, was reacted for 15 min in the dark, the absorbance was measured at 553 nm.

Urinary protoporphyrin III (Uro III) content was determined by the method of Bogorad (1962). Weighed the leaves 0.2 g, added 4 mL (0.067 M, pH = 6.8) phosphate buffer extract, centrifuge at 12,000 rpm for 10 min, take 3 mL supernatant, added 180 μl Na₂S₂O₃ solution (1%), violently shook, illuminate for 20 min. Added glacial acetic acid to pH 3.5, extract with ether 3 times, and measure the absorbance of water at 405.5 nm after combining.

Proto IX and Mg-protoporphyrin IX (Mg-proto IX) contents were determined according to Hodgins and Van Huys-tee (1986).

For Ehrlich-Hg, 1 g of *p*-dimethylaminobenzaldehyde was dissolved in 30 mL of glacial acetic acid, 8 mL of 70% perchloric acid was added and diluted to 50 mL with glacial acetic acid, and 0.2 g of HgCl₂ was added. This can be stored in a refrigerator at 4 °C for a long time.

Acetate buffer (pH 3.5): Took ammonium acetate 25 g, added 25 mL of water to dissolve, added 38 mL of 7 mol L⁻¹ hydrochloric acid solution, accurately adjust the pH value with 2 mol L⁻¹ hydrochloric acid solution or 5 mol L⁻¹ ammonia solution To 3.5, diluted to 100 mL with water.

2.6 Chloroplast and mitochondrial ultrastructure

On the 8th day, seedling leaves (3rd leaf from the top) from the different treatment groups were randomly selected for electron microscopy. The leaves were washed with phosphate-buffered saline (PBS) and cut with blades on both sides of the main veins of the leaves. A 1 × 1-mm tissue block was immediately placed in a 3% glutaraldehyde fixative (ZB-S1040, Zhongxingbairui, Beijing, China), pumped to the blade with a manual air pump, and fixed at room temperature for 2 to 4 h. After that, keep the cut blade tissue block in a 4° C refrigerator for use. Samples were rinsed eight times with 0.1 M PBS with a pH of 7.2 for 10 min; fixed with citrate for 1 h; rinsed with PBS three to four times, each time for 10 min; dehydrated with an ethanol gradient at concentrations of 30%, 50%, 70%, 80%, 90%, 95%, and 100% three times, for 10 min each time; and held at 4 °C. The transmission electron microscope sample was rinsed with 100% acetone three times, each time for 10 min; the resin-embedding agent was embedded, the resin block was trimmed with a trimming machine after polymerization, and the ultrathin section was cut with an ultrathin slicer (EM UC6 type slicer, Leica, Wetzlar, Germany). After double staining with uranyl acetate (Zhongxingbairui, Beijing, China) and lead citrate (Zhongxingbairui, Beijing, China),

the subcellular ultrastructure of cells and chloroplasts was observed by transmission electron microscopy (H-7650, Hitachi, Tokyo, Japan).

2.7 Statistical analysis

The experimental design was a completely randomized block comprising 16 replicates, and each seedling constituted one replicate. The data were subjected to an analysis of variance and an 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 ($p < 0.05$).

3 Results

3.1 Effects on lettuce morphological characteristics

With a gradual increase in NO₃-N in the nutrient solution, the total fresh weight of lettuce biomass was the smallest in treatment T2, and the fresh weight in the T4 treatment was 9.34 g, which was significantly higher than that in the other treatments (Table 2). The fresh weight of the shoots reached the lowest and highest values, 4.96 g and 8.67 g, in the T2 and T4 treatments, respectively. The fresh weight of the aboveground parts treated with T4 and T5 was significantly higher than that of the other three treatments. The trend of fresh weight for the belowground parts was the same as that for the aboveground parts, which was the highest for T4 at 0.67 g and the lowest for T2 and T3 at 0.36 g. As the nitrate-to-ammonium ratio increased, the fresh weights of the lettuce overall, the shoots, and the belowground parts of the lettuce first decreased and then increased. Lettuce exhibited the best growth when the ratio of nitrate to ammonium (N:A) was 75:25. The heavy upper and lower parts had the highest fresh weights.

The total dry weight of lettuce biomass showed that the value reached a peak of 1.85 g in the T4 treatment, which

Table 2 The growth of lettuce under different NO₃⁻:NH₄⁺ ratios

NO ₃ ⁻ :NH ₄ ⁺ ratios	Shoot fresh weight (g)	Root fresh weight (g)	Fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)	Dry weight (g)	Root-to-shoot ratio (%)	Root length (cm)	Water content (%)
0:100 ^z	5.89b ^y	0.41b	6.30b	1.22ab	0.09a	1.31b	0.09a	10.76ab	79.60a
25:75	4.96b	0.36b	5.32c	1.13b	0.08ab	1.21c	0.07ab	9.16b	77.69a
50:50	5.97b	0.36b	6.33b	1.18ab	0.07ab	1.25c	0.06bc	11.16ab	80.58a
75:25	8.67a	0.67a	9.34a	1.77a	0.08ab	1.85a	0.04c	12.74a	79.22a
100:0	8.11a	0.41b	8.52a	1.31ab	0.05b	1.36b	0.04c	9.68ab	83.60a

^z0:100: NO₃-N:NH₄-N ratio of 0:100; 25:75: NO₃-N:NH₄-N ratio of 25:75; 50:50: NO₃-N:NH₄-N ratio of 50:50; NO₃-N:NH₄-N ratio of 75:25; 100:0: NO₃-N:NH₄-N ratio of 100:0

^yMean separation within columns by Tukey's honestly significant difference test at $p < 0.05$. The values are the means of five replications

was significantly higher than that of the other treatments. The trends of the total dry weight and the shoot dry weight changes were the same, and the lowest and highest values were reached in the T2 and T4 treatments with values of 1.13 and 1.77 g, respectively. The dry weight of the T4 treatment was significantly higher than that of the T2 treatment. The dry weight of the belowground parts showed that the lowest value in the T5 treatment was 0.07 g and the highest in the T1 treatment was 0.09 g, which was 0.01 g, 0.02 g, 0.01 g, and 0.04 g higher than that in the T2–T5 treatments, respectively. There was no significant difference between treatments with T2–T5. T1 had significantly higher values than the other treatments. As the nitrate-to-ammonium ratio increased, the total dry weight and aboveground and belowground dry weights of lettuce showed a decreasing-increasing trend. When the N:A was 75:25, the total dry weight and shoot dry weight were the highest.

As the N:A ($\text{NO}_3\text{-N:NH}_4\text{-N}$) increased, the root-to-shoot ratio of lettuce plants decreased gradually, reaching a minimum of 0.04% with the T4 and T5 treatments. There was no significant difference among the T3, T4, and T5 treatments, and the highest T1 treatment value was 0.09%. When T2 and T5 were applied, the water content reached the lowest and highest values, which were 77.69% and 83.60%, respectively, but the difference among the five treatments was not significant.

3.2 Effects on photosynthetic pigments of lettuce

The Chl *a*, Chl *b*, and Chl *a* + *b* were the highest in the T4 treatment (Table 3). In the T1–T5 treatments, the content of Chl *a* reached a high peak during T2 and T4 treatments, and the content of Chl *a* under T2 and T4 was significantly higher than that of the other three treatments. The content of Chl *b* under T1, T2, and T3 was significantly higher than that under the other two treatments. The content of Chl *a* + *b* reached a high peak under T2 and T4, significantly higher than the Chl *a* + *b* contents of the other three treatments. Similarly, the carotenoid content also showed a rising-decreasing-rising-decreasing trend, reaching a high peak

during T2 and T4 that was significantly higher than those of the other three treatments.

3.3 Effects of Pn-PPFD and Pn-Ci response curves on leaves of lettuce seedlings

When PPFD ranged from 0 to 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$, the Pn of each treatment increased and the growth in T4 and T2 was the largest. When the light intensity increased to 600 $\mu\text{mol m}^{-2} \text{s}^{-1}$, the Pn value of the T2 treatment was stable. When the light intensity reached 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$, the Pnmax was 9.15 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1}$, while that of T4 was 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and the Pn value was stable. When the light intensity was 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$, the Pnmax was 9.29 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1}$; other treatments also increased light intensity to 600 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and the change in Pn values was stable. The Pnmax values of T1, T3, and T5 were 7.38 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1}$, 7.02 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1}$, and 6.05 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1}$, respectively, and the growth rate of these three treatment groups was T3 > T1 > T5. The highest photosynthetic activity was observed in the T4 treatment, and the light intensity of the T2 treatment Pn was 0.14 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1}$, lower than that of the T4 treatment, and that of the T4 treatment was stable (Fig. 1a).

When the Ci was between 0 and 2000 $\mu\text{mol mol}^{-1}$, all of the treated Pn gradually increased with increasing Ci, and the maximum increase was observed for T4. When the Ci reached 1000 $\mu\text{mol mol}^{-1}$, the Pn tended to change slightly. The Pnmax was 10 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1}$ (Fig. 1b). The other treatments also tended to be stable after the Ci reached 600 $\mu\text{mol mol}^{-1}$, and the change trend was similar. The Pnmax values of the treatments were 8.7 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1}$, 7.6 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1}$, 7.5 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1}$, and 6 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1}$, respectively. The Pnmax of T5 was the lowest, and the Pn value was lower than that of the other treatments. The Pn value of T1 increased with increasing Ci, and the Pn values of the other treatments also increased slowly but not as clearly as that of T1 (Fig. 1b). Several main photosynthetic parameters in Table 4 were obtained by simulating and calculating the parameters of the Pn-PPFD

Table 3 Photosynthetic pigment contents in lettuce leaves under different $\text{NO}_3\text{-N:NH}_4\text{-N}$ ratios

$\text{NO}_3\text{-N:NH}_4\text{-N}$ ratios	Chl <i>a</i> (mg g ⁻¹)	Chl <i>b</i> (mg g ⁻¹)	Chl <i>a</i> + <i>b</i> (mg g ⁻¹)	Carotenoids (mg g ⁻¹)
0:100 ^z	0.35b ^y	0.24ab	0.59b	0.13b
25:75	0.39a	0.25a	0.65a	0.15a
50:50	0.33b	0.21c	0.54c	0.12c
75:25	0.41a	0.26a	0.68a	0.15a
100:0	0.34b	0.22bc	0.55bc	0.13bc

^z0:100: $\text{NO}_3\text{-N:NH}_4\text{-N}$ ratio of 0:100; 25:75: $\text{NO}_3\text{-N:NH}_4\text{-N}$ ratio of 25:75; 50:50: $\text{NO}_3\text{-N:NH}_4\text{-N}$ ratio of 50:50; 75:25: $\text{NO}_3\text{-N:NH}_4\text{-N}$ ratio of 75:25; 100:0: $\text{NO}_3\text{-N:NH}_4\text{-N}$ ratio of 100:0

^yMean separation within columns by Tukey's honestly significant difference test at $p < 0.05$. The values are the means of five replications

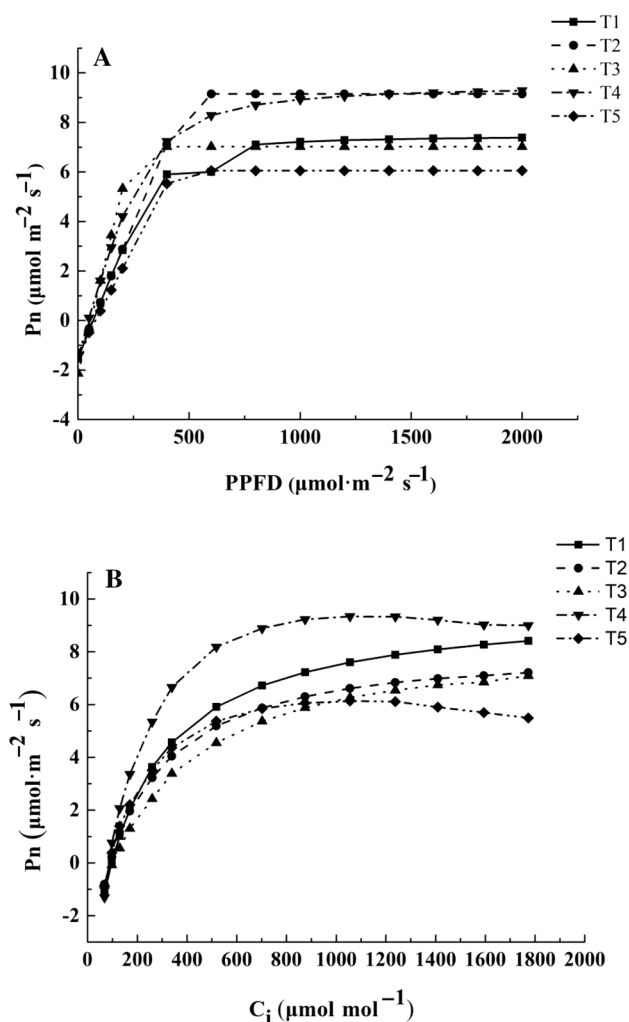


Fig. 1 Pn-PPFD and Pn-Ci response curve parameters for lettuce leaves under different NO_3^- - NH_4^+ ratios. **a** Pn-PPFD response curve parameters on lettuce leaves under different NO_3^- - NH_4^+ ratios; **b** Pn-Ci response curve parameters on lettuce leaves under different NO_3^- - NH_4^+ ratios. T1: NO_3^- -N: NH_4^- -N ratio of 0:100; T2: NO_3^- -N: NH_4^- -N ratio of 25:75; T3: NO_3^- -N: NH_4^- -N ratio of 50:50; T4: NO_3^- -N: NH_4^- -N ratio of 75:25; T5: NO_3^- -N: NH_4^- -N ratio of 100:0

Table 4 Pn-PPFD response curve parameters for lettuce leaves under different NO_3^- - NH_4^+ ratios

NO_3^- - NH_4^+ ratios	AQY (mol mol^{-1})	Rday ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	CE ($\text{mol m}^{-2} \text{s}^{-1}$)	LCP ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	CCP ($\mu\text{mol m}^{-2} \text{s}^{-1}$)
0:100 ^z	0.023bc ^y	1.55b	0.092b	65.79b	97.52b
25:75	0.021c	1.40b	0.074b	54.94b	88.70b
50:50	0.025b	0.77d	0.077b	49.90c	109.09a
75:25	0.035a	2.13a	0.262a	58.96b	82.07c
100:0	0.017d	1.22c	0.095b	78.99a	81.05c

^z0:100: NO_3^- -N: NH_4^- -N ratio of 0:100; 25:75: NO_3^- -N: NH_4^- -N ratio of 25:75; 50:50: NO_3^- -N: NH_4^- -N ratio of 50:50; 75:25: NO_3^- -N: NH_4^- -N ratio of 75:25; 100:0: NO_3^- -N: NH_4^- -N ratio of 100:0

^yMean separation within columns by Tukey's honestly significant difference test at $p < 0.05$. The values are the means of five replications

AQY apparent quantum yield, Rday dark respiration rate, CE carboxylation efficiency, LCP light compensation point, CCP CO_2 compensation point

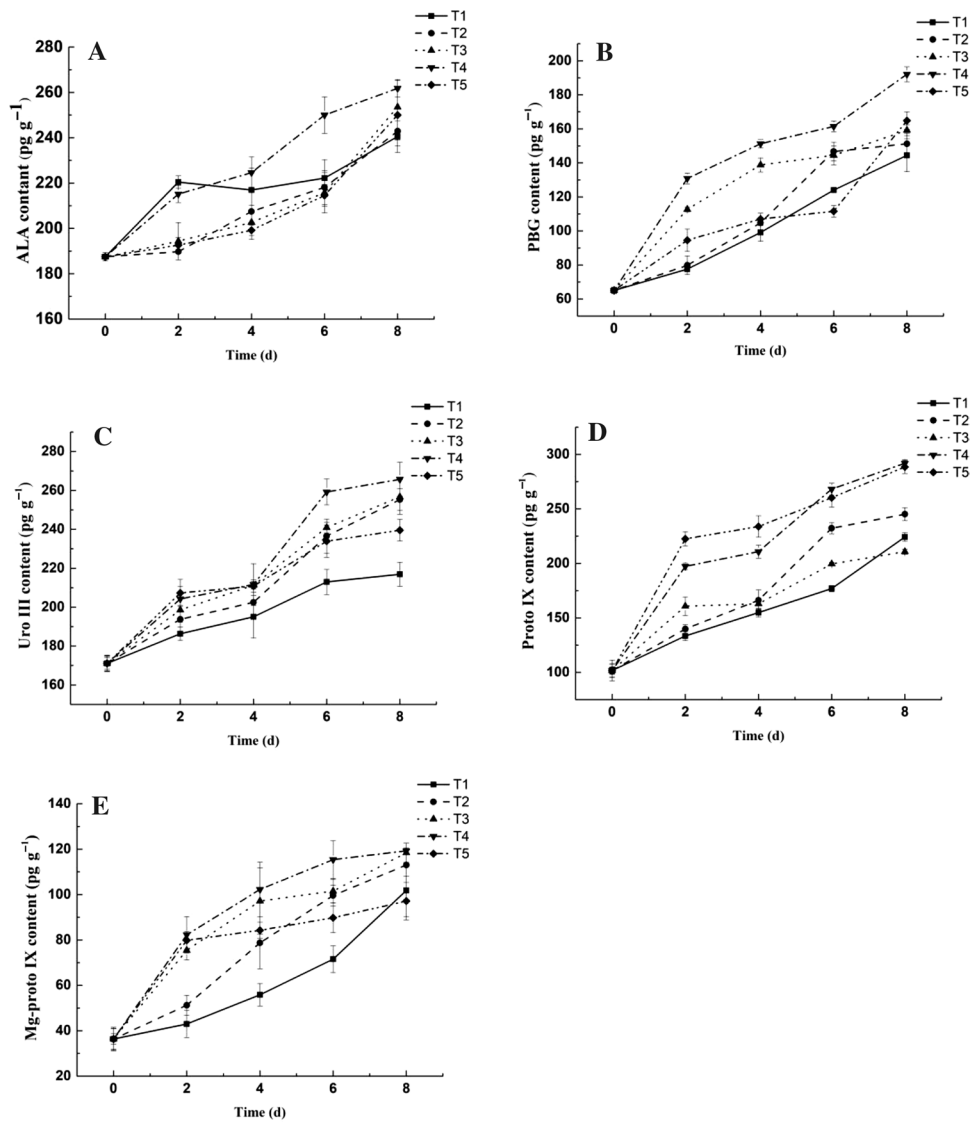
and Pn-Ci response curves for each treatment. It can be seen from Table 4 that the AQY and CE of T4 were the highest; the AQY was 106% higher than that of the lowest, with T5, and was significantly different from those of the other treatments, and the T4 CE was different from that of the other treatments. Significantly, the LCP of T4 was 185%, 254%, 240%, and 176% higher than those of the other treatments, and the lowest LCP was observed for T3, which was significantly different than that of the other treatments. The CO_2 compensation point was the lowest with T5 but was not significantly different from that under T4. The highest Rday was significantly different for the T4 treatment, with the lowest being observed under the T3 treatment. From the five indicators in Table 4 it can be seen that with the N:A of 75:25 (T4), the AQY and CE are higher than with other treatments, and both the CO_2 compensation point and the LCP are lower.

3.4 Effects on Chl precursor content

Throughout the process, the ALA content gradually increased over time. The ALA content of the T4 treatment was higher than that of the other treatments. ALA is a substrate for the synthesis of the next PBG, and the increase in the substrate concentration of ALA promotes the synthesis of the next step. The similar differences in ALA contents and growth trends of T2, T3, and T5 were not obvious, but the ALA content increased sharply on the second day of T1 treatment and then increased more gradually.

The PBG content trend for all treatments was the same as the ALA content trend. It can be seen from Fig. 2 that the PBG content of T4 was always higher than that of the other treatments and gradually increased with time; there was no sudden increase or decrease. The Chl gradually accumulated with the growth of the seedlings and was significantly different among treatments on the 8th day. The PBG content of T1 was lower than that of the other treatments.

Fig. 2 The content of chloroplasts in lettuce leaves under different NO_3^- - NH_4^+ ratios. T1: NO_3^- -N: NH_4^+ -N ratio of 0:100; T2: NO_3^- -N: NH_4^+ -N ratio of 25:75; T3: NO_3^- -N: NH_4^+ -N ratio of 50:50; T4: NO_3^- -N: NH_4^+ -N ratio of 75:25; T5: NO_3^- -N: NH_4^+ -N ratio of 100:0. The bars represent the SEs of the means ($n=3$). **a** ALA content; **b** PBG content; **c** Uro III content; **d** Proto IX content; **e** Mg-Proto IX content



As shown in Fig. 2, the trend of Uro III content in all treatments showed a stepwise increase in the 8-days treatment, at which the Uro III content (day 8) was the highest for T4. The Uro III content was high, and the trends observed were the same as those for ALA and PBG. The Uro III content of T1 increased but was the same as the PBG content and was lower than that of the other treatments.

It can be seen from Fig. 2 that the trend of Proto IX content is similar to that of Uro III, especially for T2 and T4, which also have a staged growth trend, which indicates that the content of Proto IX is affected to some extent by Uro III. The T4 treatment had the highest Proto IX content (day 8), but it was not significantly different from that of T5, with the lowest being observed for T3. The previous precursor content was consistently high with T3 (Fig. 2), but the lower Proto IX content was probably due to the decrease in Proto IX content due to more synthesis of Mg-Proto IX.

It can be seen from Fig. 2 that Mg-Proto IX gradually increased with time, and the difference between T3 and T4 was not significant, but both were significantly different from T1 and T2. Although the content of magnesium ions can also affect the synthesis of Mg-Proto IX, the trend of Mg-Proto IX and the trend of Proto IX were similar, as shown in Fig. 2, indicating the main factors affecting the content of Mg-Proto IX here. Proto IX showed the highest content under T4, while the lower contents under T1 and T2 with single N forms indicated that the mixed nutrient solution containing more nitrate N promoted the Chl synthesis of lettuce seedlings, and the single N form could not promote the synthesis of Chl in lettuce seedlings and inhibited its synthesis to some extent.

3.5 Effects on the ultrastructure of leaves

3.5.1 Chloroplast ultrastructure

Under different N ratios, the chloroplasts in the leaves of each lettuce plant had different degrees of influence. Under the T1 treatment, the chloroplasts were enlarged and deformed, and large vacuoles appeared, and the chloroplast membranes degraded and appeared broken. The structure of the thylakoids was disordered. In the chloroplast, the

matrix was relatively sparse near the granule layer; the granule layer was disorderly, loose, and unclear; and there were also different degrees of degradation, small vacuoles, many eosinophilic granules, a loose connection between the chloroplasts, and an unsatisfactory overall growth state (Fig. 3a, b). The chloroplasts under T2 treatment had deformation and swelling, but the chloroplast membrane was clear and had no degradation and blurring, and the structure was firm. In the chloroplast, the matrix is denser than the T1 treatment and is rich in no significant large vacuoles. The granules were

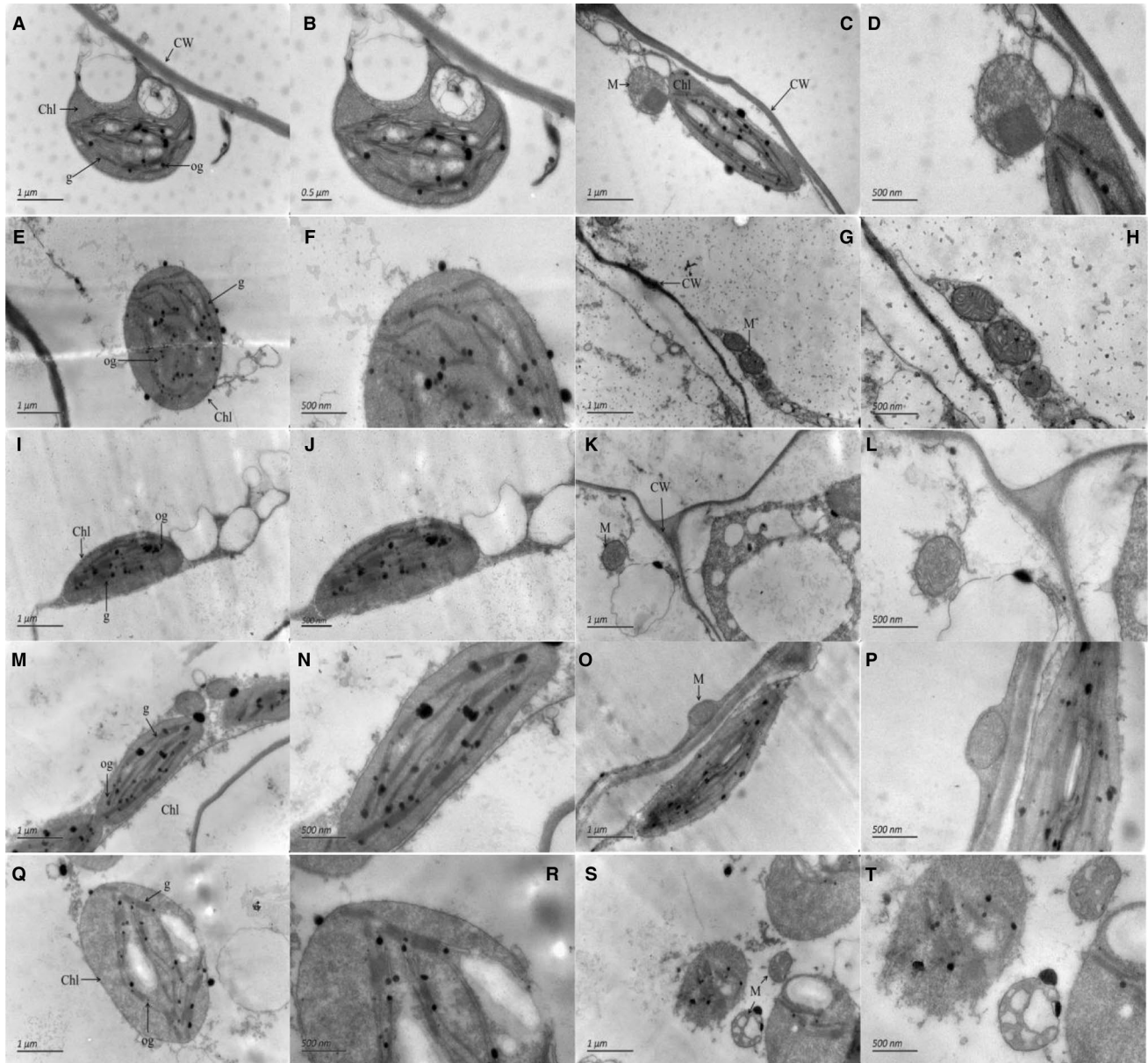


Fig. 3 Effects of different N ratios on leaf mesophyll cell chloroplast and mitochondrial ultrastructure. **a–d** T1: $\text{NO}_3\text{-N}:\text{NH}_4\text{-N}$ ratio of 0:100; **e–h** T2: $\text{NO}_3\text{-N}:\text{NH}_4\text{-N}$ ratio of 25:75; **i–l** T3: $\text{NO}_3\text{-N}:\text{NH}_4\text{-N}$ ratio of 50:50; **m–p** T4: $\text{NO}_3\text{-N}:\text{NH}_4\text{-N}$ ratio of 75:25; **q–t** T5:

$\text{NO}_3\text{-N}:\text{NH}_4\text{-N}$ ratio of 100:0. *Chl* Chloroplast; *CW* cell wall; *M* mitochondria; *Og* osmium particles; *g* grana layer; magnification: **a, e, i, m, q, c, g, k, o, s** $\times 30,000$; **b, d, f, h, j, l, n, p, r, t** $\times 60,000$

clear, and there were many layers. However, the arrangement was disorderly, and there were many eosinophilic particles. The chloroplasts were loosely connected, and the growth state was better than that observed with T1 but was still affected to a certain extent (Fig. 3e, f). Under T3 treatment, the chloroplast had a slight deformation, the chloroplast membrane was intact and clear, and the overall form was fusiform and oblong. The internal matrix of the chloroplast was dense, and the thylakoid was parallel to the long axis of the chloroplast. The layers were arranged neatly but were somewhat unclear, and the chloroplasts were loosely connected and had an intact growth state (Fig. 3i, j). Under the treatment T4, the chloroplasts showed no fusiform deformations, and the membrane structure was intact and clear. The inner chloroplast was densely packed, the thylakoid was parallel to the long axis of the chloroplast, the granule layer was clear, there were many neatly arranged lamellae, the highest number of granules among all treatments, and there were relatively few eosinophils in the interior. The granules and chloroplasts were closely attached to the edge of the cell membrane and had the best growth state (Fig. 3m, n). The T5 chloroplasts were deformed and expanded, the membrane was broken, and large vacuoles appeared. The internal matrix of the chloroplast was sparse, and the granules were arranged neatly. However, due to the occurrence of vacuoles, some of the granules were squeezed and deformed, and the granules were clear but broken. The chloroplasts were loosely connected and grew stronger than those of T1 but were also unsatisfactory in terms of growth (Fig. 3q, r).

According to the above, the chloroplast ultrastructure of lettuce under different N ratios has different effects on the growth state. The best treatment was T4 and the other treatments showed the following order from best to worst: T3 > T2 > T5 > T1. Except for the close relationship between the chloroplasts under T4, the chloroplasts were loosely distributed and had different degrees of deformation. Under the treatments T1 and T5, the chloroplasts of the mesophyll cells of lettuce seedlings were destroyed, indicating a single N form. The nutrient solution had a certain inhibitory effect on the growth of lettuce seedlings, and the mixed N ratio had a certain growth-promoting effect. The optimal chloroplasts were observed with T4 (N:A of 75:25), and its growth state was the best.

3.6 Mitochondrial ultrastructure

The mitochondria of lettuce seedlings varied under different treatments. Under T1 treatment, the mitochondrial outer membrane was clear and partially damaged, and internal degradation was rare and clotting occurred (Fig. 3c, d). The structure of the mitochondrial bilayer membrane treated by T2–T4 was complete and clear. Under T2 treatment, internal sputum was rare, and the inclusions were reduced with

vacuoles (Fig. 3g, h). The T3 mitochondria were clear and intact, and the inclusions were dense, but the double layer of the membrane was distorted (Fig. 3k, l). The mitochondria under T4 treatment were rich in number, and the contents were dense and in intact condition (Fig. 3p). Under the T5 treatment, the mitochondrial bilayer membrane was broken, and the internal contents were largely lost. A large number of vacuoles appeared in the intrinsic sputum (Fig. 3s, t). The chloroplast ultrastructure of lettuce under different N ratios has different effects on the growth state. The best treatment was T4 and the other treatments showed the following order from best to worst: T3 > T2 > T5 > T1. Except for the close relationship between the chloroplasts under T4, the chloroplasts were loosely distributed and had different degrees of deformation. Under the T1 and T5 treatments, the chloroplasts of the mesophyll cells of lettuce seedlings were destroyed, indicating a single N form. The nutrient solution had a certain inhibitory effect on the growth of lettuce seedlings, and the mixed N ratio had a certain growth promoting effect. The optimal chloroplasts were observed with T4 (N:A of 75:25), and its growth state was the best.

In conclusion, the mitochondrial ultrastructure of lettuce under different N ratios was damaged by the T1 and T5 treatments. The other treatments resulted in an intact mitochondrial structure, and the best growth state was observed with T4 treatment. The bilayer membrane was intact and had intact biological activity. The other treatments showed the following order from best to worst: T3 > T2 > T1 > T5; especially noteworthy are T1 and T5. The mitochondria under these treatments showed basic loss of biological activity and function, indicating that the ratio of the enzymes under T1 and T5 caused stress. The most suitable ratio was T4.

3.7 Mesophyll cell ultrastructure

Cleavage of the mesophyll cells occurred under the treatments of T1, T2, and T5 (Fig. 4a, b, e). Plasma membrane fragmentation occurred under T1 treatment; the intercellular space was large, and the whole cell structure was destroyed and distorted. T2 treatment resulted in the lower mesophyll cells showing deformation, and the destruction of the plasma membrane caused the cell structure to be destroyed. Although T5 resulted in plasmolysis, the cytoplasmic membrane remained intact and still maintained its intact structure and function. The separation of the plant cell wall was not apparent under T3 and T4, and the cell arrangement was tight and structurally complete. The cell state under T4 was the best; the cells were tightly arranged, and the chloroplast and mitochondria cell wall distribution showed intact biological activity and function (Fig. 4e, f).

In conclusion, T1, T2, and T5 resulted in the separation of the sarcoplasmic cells of lettuce seedlings, which led to the destruction of the cell membrane structure and the reduction

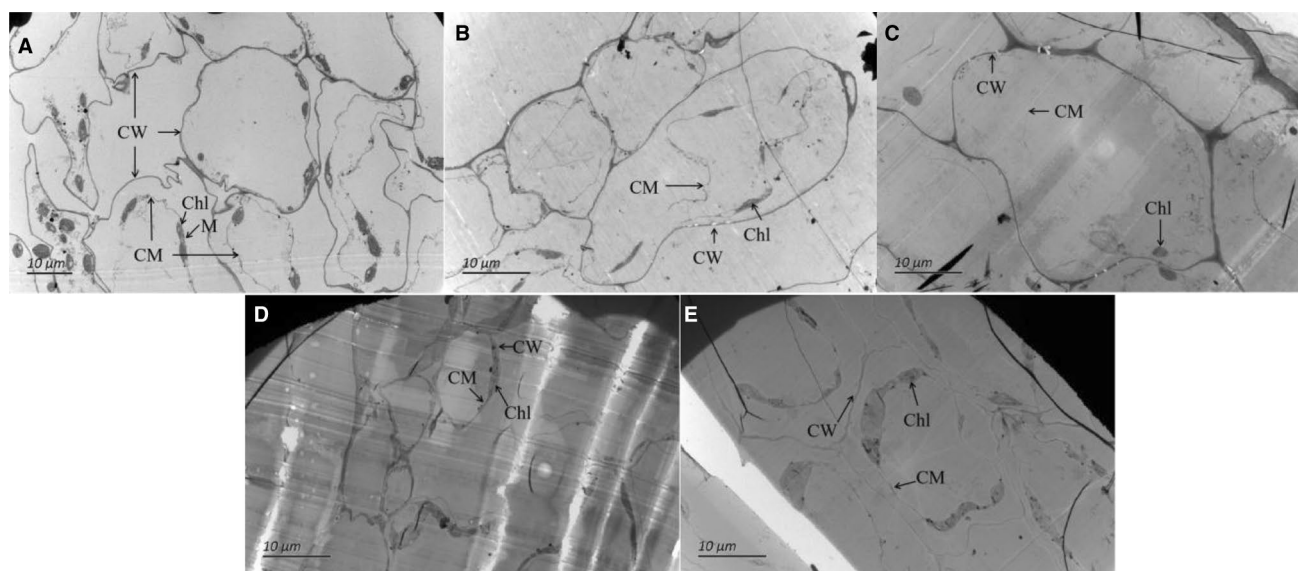


Fig. 4 Effects of different N ratios on the ultrastructure of the mesophyll cells of lettuce leaves. **a** T1: $\text{NO}_3\text{-N}:\text{NH}_4\text{-N}$ ratio of 0:100; **b** T2: $\text{NO}_3\text{-N}:\text{NH}_4\text{-N}$ ratio of 25:75; **c** T3: $\text{NO}_3\text{-N}:\text{NH}_4\text{-N}$ ratio of

50:50; **d** T4: $\text{NO}_3\text{-N}:\text{NH}_4\text{-N}$ ratio of 75:25; **e** T5: $\text{NO}_3\text{-N}:\text{NH}_4\text{-N}$ ratio of 100:0. *Chl* Chloroplast; *CW* cell wall; *M* mitochondria; *CM* cell membrane; magnification: T1–T5 $\times 2000$

of normal photosynthetic capacity. The normalization of mesophyll cells was promoted in lettuce seedlings under T3 treatment. The optimal biological activity was observed for plant cells treated with T4 (75:25 N:A).

4 Discussion

4.1 Effects of different N forms on morphological indexes of lettuce seedlings

Different N ratios have different effects on different crop plants, probably because the way the plants absorb N in these two forms is different (Mengel and Kirkby 1982; Glass et al. 1992), and single N forms have a certain inhibitory effect on plant growth. Ammonium N alone will poison the plant and cause it to wilt, and the rhizosphere will be negatively affected. Nitrate N alone will cause excessive accumulation in the plant. In this experiment, under the treatment of total ammonium N, that is, T1, the aboveground and belowground dry weights were not as high as those with other treatments, and the sole nitrate N treatment, T5, resulted in better quality than T4. Thickening and length of the root system were also the most notable under the T4 treatment. Moreover, as the nitrate N increased, the amount of material in the lettuce seedlings also increased.

Many studies have shown that the growth of different crops and plants, such as Chinese cabbage (Song et al. 2011), corn (Xu et al. 2002), and sweet pepper (Wang et al. 2003), is promoted with a mixture of nutrients in the growth

solution, with each crop having different suitable proportions of mixed nutrients in the solution. In this study, five treatments ($\text{NO}_3\text{-N}:\text{NH}_4\text{-N}$ ratio of 0:100, 25:75, 50:50, 75:25, and 100:0) were applied, and the root and aboveground biomasses of lettuce seedlings were the greatest in terms of water volume under treatment T4. The fresh weight of the seedlings under T4 was 1.75 times that of the lowest value, which occurred with T2, and the fresh weight of the belowground parts was 1.86 times higher than that of the lowest value, also the result of treatment T2. The roots were densely grown with T4, significantly more so than with other treatments. When the N:A was 75:25, an increase in the biomass of lettuce seedlings was observed.

4.2 Effects of different N forms on photosynthesis parameters of lettuce seedlings

Photosynthesis is affected by the plant itself and the environment. Any adverse environment will affect it, and different plants and environments have different mechanisms of influence in this process (Jie et al. 2001; Zhang et al. 2003; Zhu et al. 2010). The light response curves and CO_2 response curves of the plants are affected by many important light parameters, such as the AQY, CE, Rday, LCP, and CO_2 compensation point, which can reflect different environments and the effects of plants themselves on photosynthesis. Therefore, there is much attention given to these factors (Anselmi et al. 2004). In the case of consistent illumination in this experiment, the maximum Pn increased with the increase in nitrate N and peaked with T3 and T4 (the difference between

the two was not significant), indicating that in the same light environment under the mixed nitrate:ammonium nutrient solution, lettuce plants can better absorb N and prefer the nitrate N to the ammonium N. In addition, the maximum net photosynthetic value of the lettuce seedlings under both single N forms are at a lower level, suggesting that a single N form reduces plant uptake and conversion by reducing the supply of photosynthetic energy or that a single N form in the nutrient solution creates a mild stress environment for lettuce seedlings, resulting in the overall decline in the photosynthetic capacity of lettuce seedlings.

In this experiment, the light response curve and CO₂ response curve of the lettuce seedlings treated with the five treatments were determined and plotted. From the five indicators in Table 4, the optimal N:A was 75:25 (T4). The AQY and CE of the following treatments were higher than those of the other treatments, and the CO₂ compensation point and the LCP were lower. Although the Rday was higher, lettuce seedlings under T4 treatment had significantly enhanced CO₂ assimilation. The CE increases the AQY, thereby enhancing the Pn of lettuce seedlings. The Pnmax of T5 was the lowest, and the Pn value was lower than that of the other treatments, indicating that the photosynthesis under the single N form of nitrate N was weaker than that under the other treatments. The results of this experiment are similar to previous studies. Previous studies have shown that both single N form and mixed N can increase the photosynthesis rate of rice, but the effect of mixed N fertilizer is better (Guo et al. 2007b). Warren's study showed that nitrate ammonium had no significant effect on the assimilation of wheat CO₂ (Warren et al. 2000), but the optimal N:A in this experiment was 75:25, which greatly improved the CE and reduced CO₂. The compensation point indicates that the ratio of CO₂ utilization of lettuce seedlings is increased when the N:A is 75:25, and the assimilation of CO₂ is further enhanced and photosynthesis is better promoted.

4.3 Effects of different N forms on Chl precursors in lettuce seedlings

Chl is a basic indicator for measuring the growth and development of lettuce plants, and it regulates and utilizes light energy. Carotenoids play an important role in the photosynthetic process of lettuce growth and can promote the absorption of light energy by chloroplasts and protect them from glare and high temperature, occupying a prominent position in the photoreaction and light transmission pathways in photosynthesis. N is an essential element affecting the growth of plants. N is also a necessary element for Chl synthesis. An increase in N increases the synthesis of Chl and enhances the synthesis of organic matter in photosynthesis (Huang et al. 2004). Bondada and Syvertsen (2003) showed that N is involved in Chl development, synthesis, and photosynthetic

enzyme activity, which in turn regulate photosynthesis. The content of Chl and carotenoids is positively correlated with the photosynthetic rate, and their contents directly affect the photosynthetic rate (Maynard et al. 1976). Studies have also shown that an appropriate N ratio is more conducive to the synthesis of Chl, such as those considering Chinese cabbage (Song et al. 2011), cucumber (Kotsiras et al. 2005), corn (Xu et al. 2002), and sweet pepper (Wang et al. 2003), so the appropriate N ratio affects photosynthesis by affecting the content of Chl. In this test, with T2 and T4, the content of Chl a and b was higher than that with the other treatments, indicating that a certain proportion of mixed nutrient solution was better than a single N nutrient solution. However, the 50:50 N:A treatment resulted in a Chl content that was not as high as that under other treatments, which may be because the simultaneous absorption of two forms of N at the same concentration caused the conversion of nitrate N to ammonium N in plants. Schelbert et al. (2009) showed that Chl b can absorb more light than Chl a and has a certain protective effect; higher Chl b content ensures that the Chl content is maintained at a high level (Hortensteiner 2009), consistent with the results of this test.

N is a synthetic factor in amino acids, and glutamate, as a raw material for Chl synthesis, affects the synthesis of Chl. The synthesis of Chl includes two major reactions from L-glutamyl-tRNA to Proto IX and Proto IX to Chl, and ALA, which is a universal precursor for all tetrapyrrole compounds and a key step in Chl synthesis, is composed of glutamate and is synthesized by an enzymatic reaction (Tanaka et al. 2011). The steps of Chl synthesis include the following: glutamyl-tRNA → ALA → PBG → urinary porphyrinogen → decompressed protoporphyrin III → Proto IX → Mg-proto IX → protophyll Chl, and changes in any of these steps will affect the synthesis of Chl (Lu et al. 2010). Sun et al. (2010) found that the reduction in Chl in spinach under seawater stress is due to the inhibition of its synthesis. Chen et al. (2012) found that the decrease in Chl content in spinach under salt stress was due to the inhibition of the PBG → uroporphyrinogen III transformation. The results in this experiment are similar to those of previous studies (Sun et al. 2010; Chen et al. 2012). The seedling ALA, PBG, uroporphyrinogen III, Proto IX, and Mg-proto IX contents under T4 were higher than those under the other treatments, and ALA was used as a tetrapyrrole. The first universal precursor content has a great influence on the subsequent Chl synthesis. The formation of ALA is the rate-limiting step of Chl synthesis. An increase in ALA content is beneficial to Chl synthesis. Therefore, the ALA content under T4 was higher than that under the other treatments. T4 promoted the synthesis of ALA, which provided more substrate for better synthesis of Chl in the next step. Two molecules of ALA condense to form PBG, which is an intermediate product between glutamate and Proto IX. The PBG content can

reflect the conversion efficiency of ALA to PBG and can also reflect the final product to some extent (Tanaka et al. 2011). The steady decrease in ALA content and increase in PBG content indicates the normal synthesis of Chl in lettuce seedlings. The high PBG content also lays a good foundation for the subsequent Uro III synthesis, so T4 better promoted the production of Proto IX and subsequent Chl synthesis. Uro-porphyrinogen III (Uro III) is involved in the next step of PBG conversion, which is obtained by condensation of four molecules of PBG, and its content is affected to some extent by PBG (Eckhardt et al. 2004). Uro III and PBG are also intermediates in the synthesis of Proto IX, and their contents are higher under T4 than under other treatments, which proves that T4 better promoted Chl synthesis. Proto IX is the last common precursor of haem and Chl. At Proto IX, two branches will appear, one branch will add ferrous ions to form haem, and the other branch will add magnesium ions to form Chl (Stenbaek and Jensen 2010). Proto IX is the last common precursor (after the haemoglobin and Chl branches), and its content is related to Chl synthesis (Santos 2004). Proto IX is involved in the next step to synthesize Mg-Proto IX and its contents provide a suitable indication that T4 promotes the synthesis of Chl. Mg-Proto IX (Mg-Proto IX) is the first product of the Chl branch, which is formed by inserting Mg^{2+} into Proto IX for Chl biosynthesis, which is key to Chl synthesis. The amount of Mg-Proto IX can be well correlated with the level of Chl synthesis (Stenbaek and Jensen 2010).

4.4 Effects of different N forms on the cellular ultrastructure of lettuce seedlings

The shape and state of the chloroplast can reflect the photosynthesis of plants and whether they are stressed so that the growth of plants can be assessed. Many studies have shown that the chloroplasts will have corresponding changes when subjected to stress. Wang et al. (2016) found that drought stress results in destruction of the membrane structure and granule layer of chloroplasts in tomato (*Solanum lycopersicum*); in this experiment, the single N form nutrient solution produced greater damage to the chloroplasts of lettuce leaves, and the chloroplasts showed a corrupted membrane structure. The granule layer was blurred and reduced, probably because the single-form N caused a slight stress environment for the lettuce seedlings, resulting in chloroplast damage, and this experiment proves that the single-form N environment reduces the Chl synthesis of lettuce seedlings. The combination of the two causes the Chl content to decrease and the growth to decrease. With the N:A of 75:25, the chloroplast structure of the lettuce seedlings was complete, the cell membrane was completely closed to the cell wall, the organelles were closely arranged on the cell membrane, and the chloroplasts were spindle-shaped.

In addition, the thylakoid capsule was complete and rich in basal granules, the basal granules were clear and rich, and Chl synthesis was higher than with other treatments, allowing the lettuce seedlings to grow well. Zhang et al. (2010) found that eosinophils are an important indicator of the degree of lipid peroxidation in chloroplasts. In this experiment, the single N form increased the eosinophilic granules in the chloroplast, which was consistent with the performance of the plants under stress (Shao et al. 2008). We suggest that the single N form nutrient solution causes slight stress in lettuce seedlings.

The integrity of the mesophyll cell ultrastructure determines the photosynthetic capacity, and chloroplasts and mitochondria are the most important and most sensitive organelles in photosynthesis (Xu et al. 2009). In a stressful environment, the mitochondria will also be damaged to a certain extent. Our results show mitochondrial damage under pressure, similar to the results of previous studies by Ali et al. (2013), Garces et al. (2017), and Salem-Fnayou et al. (2016). This test is similar to previous studies. The mitochondrial state was the best under T4. The mitochondrial structure was complete, the inner sputum was rich, and the inclusions were dense, which provides suitable conditions for photosynthesis. The cleavage of the mesophyll cells in this experiment may have been caused by the mild stress environment, and the cell vacuole breakage may have led to the increase in osmotic pressure and damage to the plasma membrane. The overall cellular ultrastructure was consistent with that reported in previous studies on hyacinth (Lage-Pinto et al. 2008), tea (Das et al. 2015), and barley (Chen et al. 2011).

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