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Effect of ammonium/nitrate ratio on pak choi (*Brassica chinensis* L.) photosynthetic capacity and biomass accumulation under low light intensity and water deficit

H.Q. SHANG and G.M. SHEN^{+}

Key Laboratory of Plant Biology, Department of Life Sciences, Heze University, Heze 274015, Shandong, China

Abstract

We conducted a hydroponic experiment in order to study effects of the ammonium/nitrate ratio (0:15, 5:10, 7.5:7.5, and 10:5) on photosynthetic characteristics and biomass accumulation in *Brassica chinensis* under low light intensity and water stress. Results showed that net photosynthetic rate, transpiration rate, intrinsic water-use efficiency, stomatal conductance, intercellular CO_2 concentration, effective quantum yield of PSII photochemistry, electron transport rate, and nonphotochemical quenching were lower in the treatment (low light intensity and water deficit) than those in the control, whereas stomatal limitation increased. Minimum fluorescence, maximal quantum yield of PSII photochemistry, and photochemical quenching were largely unchanged. Pigment contents first increased and then decreased as the ammonium/nitrate ratios were altered, with significant differences between treatment and control observed at all ratios except for 10:5. Biomass first increased slightly and then decreased both in treated and control plants. Results suggest that economic losses caused by extreme conditions can be minimized by a proper adjustment of the ammonium/nitrate ratio.

Additional key words: chlorophyll fluorescence; electron transport rate; nitrate reductase; NH_4^+, NO_3^-; stomatal conductance; water-use efficiency.

Introduction

Water, light conditions, and nitrogen concentrations are the main factors influencing vegetable crop production. During winter and spring seasons, light has a great influence on vegetable formation, with most vegetable crops requiring strong or intermediate light exposure (Shen 2016). Weak light hinders vegetable growth (Hou *et al.* 2002), while water stress, which impedes photosynthesis, is the main reason for declining vegetable crop production in arid and semiarid areas (Deng *et al.* 2006, El Bassam 2010, Brittain Jr. 2016). Nitrogen nutrition is an important regulator of crop growth and photosynthetic productivity. Excess nitrogen supplementation causes agricultural, ecological, and environmental pollution and prevents improvement of vegetable crop yield and quality (Oerke *et al.* 1994, Gill *et al.* 2011, Hirel *et al.* 2011, Xu and Zhou 2005, 2006).

Extensive research has been carried out on physiological mechanisms related to the use of fertilizer to regulate water utilization (Angus and van Herwaarden 2001, Deng *et al.* 2006, Cramer *et al.* 2009, Yan *et al.* 2008, Waraich *et al.* 2011). When soil is used as a culture medium for vegetable growth, ammonium nitrogen (NH_4^+-N) fertilizer performs better than nitrate nitrogen (NO_3^--N) (Lastra *et al.* 2009, Yu *et al.* 2015). Under hydroponic conditions, in contrast, a NO_3^--N source is better, however, supplementation with a large concentration of nitrate-based fertilizer can readily lead to the accumulation of NO_3^--N, which is unfavorable for effective plant nitrogen uptake (Gigon and Rorison 1972, Yan *et al.* 2008). Studies have shown that the addition of specific amounts of NH_4^+-N to nutrient solution can increase crop biomass and chlorophyll (Chl) contents (Ruiz-Martinez *et al.* 2012, Wang *et al.* 2009). In pak choi (*Brassica chinensis* L.), low light intensities decrease the

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$^{+}$Corresponding author; phone: +86 5305525804, e-mail: gmshen@tzc.edu.cn

Abbreviations: Car – carotene; Chl – chlorophyll; C_i – intercellular CO_2 concentration; E – transpiration rate; ETR – electron transport rate; F_m – maximum fluorescence; F_0 – minimum fluorescence; F_s – fluorescence at stable state; F_v – variable fluorescence; F_v/F_m – maximal quantum yield of PSII ; g_s – stomatal conductance; L_s – stomatal limitation; NPQ – nonphotochemical quenching; NR – nitrate reductase; PEG – polyethylene glycol; P_N – net photosynthetic rate; q_P – photosynthetic quenching; WUE – intrinsic water-use efficiency (= P_N/g_s); Φ_{PSII} – effective quantum yield of PSII photochemistry; Ψ_w – water potential.

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photosynthetic rate and reduce leaf area, which leads to a decline in biomass and inhibition of growth (Zhang *et al.* 2007). Light, nitrogen, and their cross-regulation affect leaf pigment contents, the ratios of various pigments, leaf fluorescence characteristics, and energy conversion. A study using the nitrogen-tracer technique to investigate the effect of NH_4^+-N and NO_3^--N nutrition on nitrate accumulation in vegetables *in vivo* revealed that increasing the ratio of NH_4^+-N reduced nitrate accumulation in vegetables (Ai *et al.* 2002). Under shade conditions, the relative proportions of NH_4^+-N and NO_3^--N have been found to affect crop yields and quality as well as nitrogen metabolism (Leleu *et al.* 2000, Vyas 2004, Kuang *et al.* 2014).

The aim of this study was to find the optimal ratio of NH_4^+-N to NO_3^--N in a nutrient solution to relieve water and light stresses on vegetable crop production and reduce NO_3^--N contents in vegetables. To accomplish this goal, pak choi was cultivated under similar nitrogen contents using different proportions of NH_4^+-N and NO_3^--N in the nutrient solution. The effects of these different proportions on pak choi leaf photosynthetic characteristics, fluorescence parameters, and biomass accumulation were measured under various water- and light-stress conditions. Our findings should contribute to a better understanding of reasonable water and fertilizer management practices for vegetable production.

Materials and methods

Plant material and culture conditions: Experiments were carried out during the 2016 cabbage-growing season (late March to early May) in the greenhouse of the Zijingang Campus Experimental Station, Zhejiang University, Hangzhou, China (30°46′–30°50′N, 119°46′–119°48′E). Seeds of pak choi (*Brassica chinensis* L.), cultivar Hangzhou Youdonger, were germinated under the same conditions as described in Shen (2016). On 12 April, 18-d-old seedlings were transplanted into plastic pots containing 7 L of Hoagland-Arnon nutrient solution for further experiments.

Imposition of stress: Stress experiments were performed using four NH_4^+-N/NO_3^--N ratios, namely, 0:15, 5:10, 7.5:7.5, and 10:5, with the total nitrogen concentration maintained at 15 mmol L^{-1} and with 7 μmol(dicyanamide) L^{-1} added as a nitrification inhibitor. Stress conditions consisted of exposure to 32% of full sunlight (established by placing a layer of shade cloth 1 m above plants) and 25 g (PEG 6,000) L^{-1} ($\Psi_w = -0.1671675$ MPa, Michel and Kaufmann 1973). Full sunlight exposure and no PEG 6,000 ($\Psi_w = 0$ MPa) were used as the control. Concentrations of different ions in the Hoagland-Arnon nutrient solution were adjusted to maintain constant ion contents. Concentrations of essential nutrients in all treatments were 7 mmol(K) L^{-1}, 10 mmol(Ca) L^{-1}, 2 mmol(Mg) L^{-1}, 2 mmol(P) L^{-1}, 0.02 mmol(Fe) L^{-1}, 46 μmol(B) L^{-1}, 9.2 μmol(Mn) L^{-1}, 0.32 μmol(Cu) L^{-1}, 0.76 μmol(Zn) L^{-1}, and 0.5 μmol(Mo) L^{-1}. The solution was continuously aerated during the day time with an aerobic pump. The pH of the nutrient solution was checked daily and adjusted as needed with 0.5 mol(HCl or NaOH) L^{-1} to maintain a value of approximately 6.5. The nutrient solution was replaced weekly. Each treatment consisted of five replicates.

Sampling and biomass determination: Pak choi plants were harvested 30 d after transplanting. The plants were removed from the nutrient solution, washed with distilled water, and surface-blotted with absorbent paper. The plants were cut at the base to separate shoots from roots. The shoots were weighed and the values recorded as fresh mass (FM).

Nitrate content and nitrate reductase (NR) activity: Nitrate content was measured according to GB/T-5009.33-2010 (Determination of nitrite and nitrate in food of China). Nitrate reductase (NR, E.C.1.6.6.1) activity was measured *in vitro* as described by Leleu *et al.* (2000) with modifications as follows. Accurately weighed 0.5 g portions of fresh specimens were immersed in liquid nitrogen and ground to a powder over an ice bath. After addition of 4 mL of extraction buffer (containing 0.1211 g cysteine and 0.0372 g EDTA per 100 mL of 0.025 mol L^{-1} phosphate buffer, pH 8.7), the homogenate was ground and then centrifuged for 15 min (2,200 × *g* at 4°C). The resulting supernatant was used as the enzyme extract.

A mixture of 0.4 mL of the enzyme extract, 1.2 mL of phosphate buffer (containing 0.2 mM KNO_3, pH 7.5), and 0.4 mL of 0.2% NADH solution was incubated in a water bath for 30 min at 25°C. (A similar mixture with 0.4 mL of phosphate buffer substituted for the NADH served as the control.) After incubation, 1 mL of 1% sulfanilamide was added to terminate immediately the reaction, which was followed by the addition of 1 mL of 0.02% naphthyl ethylene amine solution. After staining for 15 min and centrifugation for 15 min (2,200 × *g* at 4°C), the O.D. of the solution was measured at 540 nm (*UV2600/2700, Shimadzu*, Japan). The activity of NR was expressed per gram of protein extracted from fresh mass (FM) of plant materials in nmol min^{-1} g^{-1}(protein) or (FM).

Photosynthetic rate, pigment contents and Chl fluorescence: On 12 May, after 30-d treatment, plant photosynthetic rate, pigment contents, and Chl fluorescence parameters were measured. Photosynthetic rate was determined according to the method of Maroco *et al.* (2002). Briefly, photosynthesis was measured from 8:00 to 11:30 h using a *Li-6400* portable photosynthetic apparatus (*Li-Cor 6400*, Lincoln, NE, USA) equipped with a red and blue LED light source under the following conditions: a leaf temperature of 23 ± 1°C, relative humidity of 40 ± 2%, CO_2 concentration of 400 ± 5 μmol mol^{-1}, and leaf indoor light intensity of 800 ± 1 μmol(photon) m^{-2} s^{-1}. Three to six fully expanded upper leaves per plant were used to determine net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i). Stomatal limitation (L_s) was calculated as $(1 – C_i/C_a) \times 100\%$, where C_a was the air CO_2 concentration. Water-use efficiency (WUE) was calculated as $WUE = P_N/E$.

Pigments in plant samples were determined by micro-column high performance liquid chromatography (HPLC) (De Las Rivas *et al.* 1989), with 90% acetone. Chl *a* was determined at 649.5 nm, Chl *b* at 664.5 nm, and Car at 452.1 nm. Chl fluorescence determination was carried out according to the method of Schreiber *et al.* (1986) in a *Li-Cor 6400-40* fluorescence leaf chamber (*Li-Cor 6400-40,* Lincoln, NE, USA) from 8:00 to 11:30 h. Fluorescence parameters of pak choi leaves were measured at the light-adapted state using three to six fully expanded upper leaves. From 19:00 to 21:00 h, dark fluorescence parameters were determined after acclimating leaves to darkness for 30 min. Measured parameters included basic fluorescence (F_0), maximum fluorescence yield (F_m), variable fluorescence (F_v), maximum PSII intrinsic light energy conversion efficiency (F_v/F_m), potential activity (F_v/F_0), and coefficient of photochemical quenching (q_P). The following parameters were then calculated based on the measured data: leaf photosynthetic electron transport quantum efficiency, namely, $\Phi_{PSII} = (F_m' - F_s)/F_m'$, relative electron transfer rate (ETR) = $\Phi_{PSII} \times PAR \times 0.84 \times 0.5$, and the nonphotochemical quenching (NPQ) coefficient = $(F_m - F_m')/F_m'$ (Živčák *et al.* 2014).

Statistical analysis: Statistical analyses were performed in *SPSS 16.0*. Results from analysis of variance (*ANOVA*) and post-*ANOVA Duncan*'s test were considered significant at the 0.05 probability level. All of the measurements were performed five times, and the means and calculated standard deviations (SD) are reported.

Results

Biomass: Statistically significant differences in biomass were observed between control and treatments at all NH_4^+-N/NO_3^--N ratios except for 10:5 (Fig. 1). Biomass of treated plants under the four nitrogen ratios was reduced by 46.8, 55.3, 54.5, and 58.7% compared with the control, which indicates that low light density and water deficit had profound effects on biomass accumulation in the studied plants. When the NH_4^+-N/NO_3^--N ratio was 5:10, plant biomass was relatively higher than that under the other nitrogen conditions, with control and treated plant biomass values of 53.1 ± 3.13 and 23.7 ± 1.43 g(FM) per plant, respectively. Plants exposed to 10:5 NH_4^+-N/ NO_3^--N had the lowest biomass accumulation, with control and treated-plant biomass recorded as 36.6 ± 3.14 and 15.1 ± 3.04 g(FM) per plant, respectively. The biomass reduction in the control group was 30.1% [theoretical maximum biomass = 56.23 g(FM) per plant, Table 1], while that in the treatment group was 36.3% [theoretical maximum = 25.23 g(FM) per plant, Table 1], implying that the combination of low light, water deficit, and high ammonium concentration was not conducive to the accumulation of biomass.

Nitrate content and NR activity: Water and light stress had a strong influence on NR activity in leaves, which suggests that the addition of appropriate amounts of ammonia is an effective measure to reduce nitrate uptake. In both treated and control plants, the lowest nitrate content and the highest NR activity was observed at a NH_4^+-N/NO_3^--N ratio of 5:10 (Fig. 2).

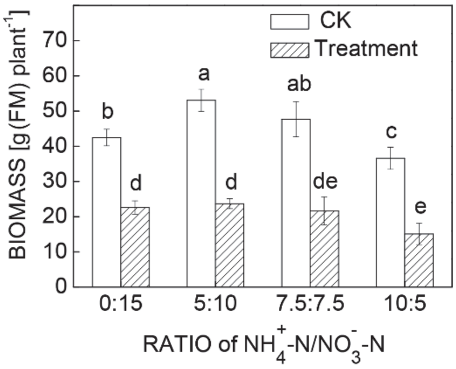

Fig. 1. Effects of NH_4^+-N/NO_3^--N ratio on the biomass in leaves of pak choi under low light intensity and water stress. CK – full sunlight with no PEG 6,000; the treatment at 32% full sunlight with 25 g(PEG 6,000) L^{-1}. *Lowercase letters* mean statistically significant differences in 0.05 level ($P<0.05$), the same in the following illustrations. $n = 5$.

Table 1. Effects of NH_4^+-N/NO_3^--N ratio on the biomass of pak choi (*Brassica chinensis*). ^{a} – the maximum fresh mass of every single plant in range of NH_4^+ 0–10 mM, NO_3^- 5–15 mM.* – statistically significant differences at 0.05 level.

Treatment	Ratio of NH_4^+-N/ NO_3^--N	Regression equation	Coefficient of correlation	Maximum biomass [g(FM) per plant] ^{a}
32% full sunlight with 25 g(PEG 6,000) L^{-1}	NH_4^+-N	$y = 10^{(1.6447-0.0185 x)}$	$r = -0.7556^*$	25.23
full sunlight without PEG 6,000	NH_4^+-N	$y = 62.374 + 5.247 x – 0.871 x^2$	$R^2 = 0.9997^*$	56.23

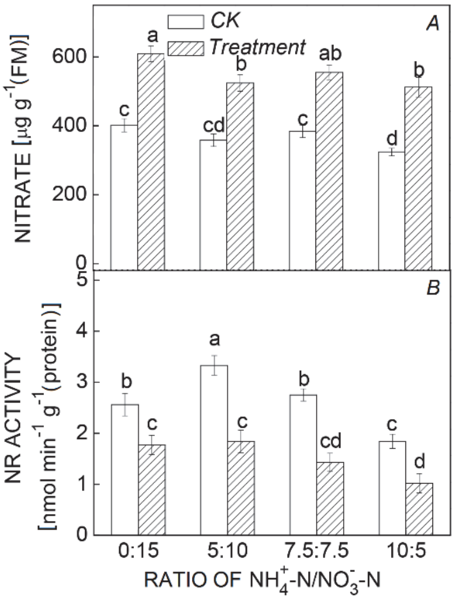

Fig. 2. Nitrate content and NR activity of pak choi leaves with different NH_4^+-N/NO_3^--N proportion. CK – full sunlight with no PEG 6,000; treatment: 32% full sunlight with 25 g(PEG 6,000) L^{-1}. *Lowercase letters* mean statistically significant differences in 0.05 level ($P<0.05$), the same in the following illustrations. $n = 5$.

Pigment contents: At all NH_4^+-N/NO_3^-N ratios, Chl *a*, Chl *b*, total Chl, and carotenoid (Car) contents exhibited great variations (Fig. 3*A–D*). Overall, nitrate-N increased pigment contents: as nitrate-N decreased and ammonium-N increased, the pigment contents decreased. Under stress conditions at a NH_4^+-N/NO_3^--N ratio of 10:5, however, pigment contents increased suddenly. This change might be due to a sharp decline in biomass, which caused pigment contents to be increased to boost photosynthesis to compensate for the biomass loss caused by low light intensity. Under stress conditions, the content of Chl *a* relative to total Chl increased (Fig. 3*E*). When nitrate-N decreased and ammonium-N increased, the ratio of total Chl/Car increased (Fig. 3*F*). No statistically significant differences between biomass and Chl were detected (Table 2).

Photosynthetic capacity: At all ammonium/nitrate-N ratios, P_N, *E*, WUE, g_s, and C_i in the treated group [32% full sunlight with 25 g(PEG 6,000) L^{-1}] were lower than those of the control group (full sunlight with no PEG 6,000), whereas L_s was higher (Fig. 4). As nitrate-N decreased and ammonium-N increased, P_N, *E*, and C_i in the control group declined (Fig. 4*A*,*D*,*E*). *E* first decreased slightly, then rose and subsequently declined (Fig. 4*B*),

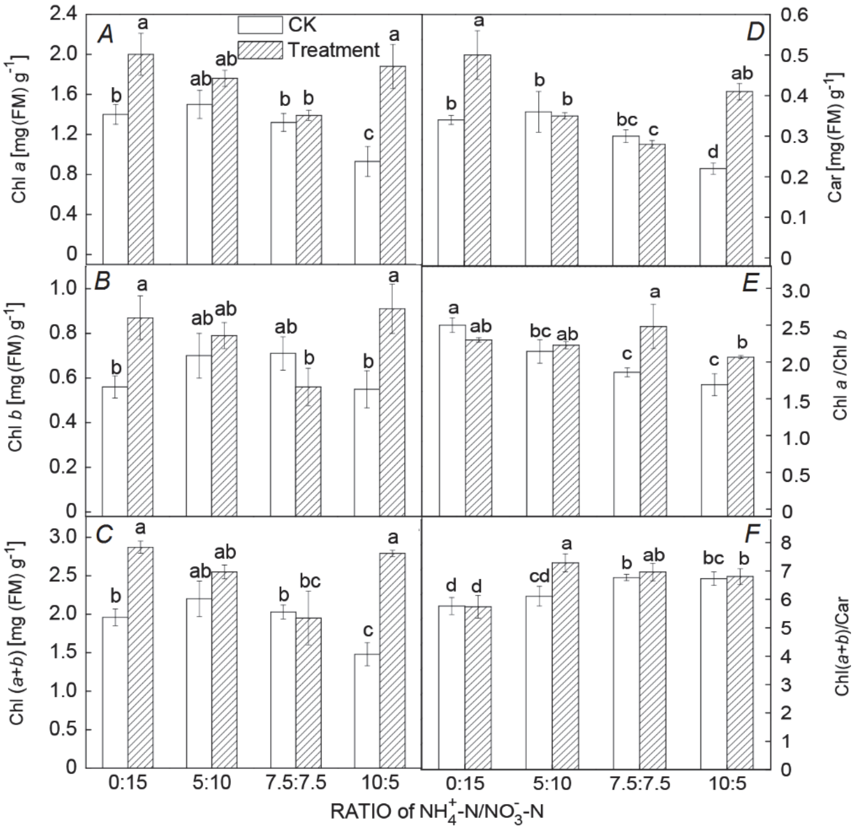

Fig. 3. Effects of NH_4^+-N/NO_3^--N ratio on chlorophyll (Chl) *a*, Chl *b*, Chl (*a*+*b*), Car content, and Chl *a*/*b*, Chl (*a*+*b*)/Car proportion of *Brassica chinensis* leaves under low light intensity and water stress. CK – full sunlight with no PEG 6,000; treatment: 32% full sunlight with 25 g(PEG 6,000) L^{-1}. *Lowercase letters* mean statistically significant differences in 0.05 level ($P<0.05$), the same in the following illustrations. $n = 5$.

Table 2. Correlation between the biomass and chlorophyll fluorescence parameters, Chl (*a*+*b*) content, Φ_{PSII}, NPQ, and P_N of *Brassica chinensis*. *r* – correlation coefficient; Sig. – significant level;*,** – significant at 0.05 and 0.01 level, respectively.

Variable	Parameter	Chl (*a*+*b*)	Φ_{PSII}	NPQ	P_N
Biomass [g(FM) per plant]	*r*	-0.071	0.762*	-0.714*	0.833**
	Sig.	0.867	0.028	0.046	0.01

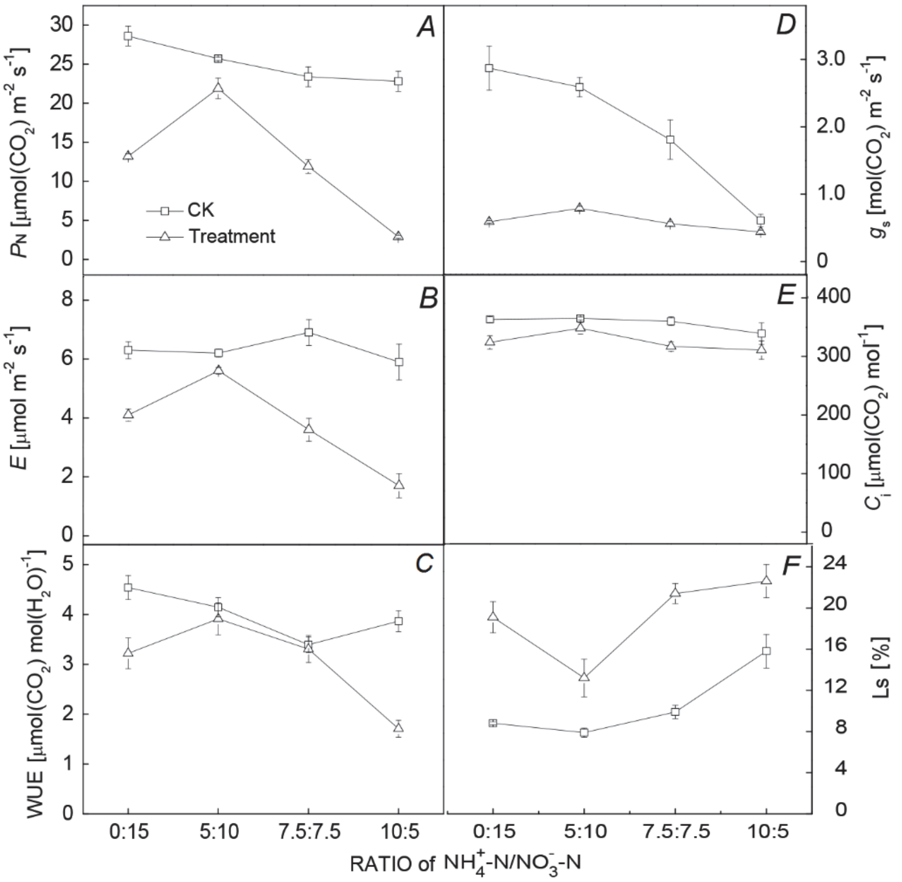

Fig. 4. Effects of NH_4^+-N/NO_3^--N ratio on net photosynthetic rate (P_N), transpiration rate (*E*), intrinsic water-use efficiency (WUE), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), and stomatal limitation (L_s) of *Brassica chinensis* leaves under low light intensity and water stress. CK – full sunlight with no PEG 6,000; treatment: 32% full sunlight with 25 g(PEG 6,000)·L^{-1}. *Lowercase letters* mean statistically significant differences in 0.05 level (P<0.05), the same in the following illustrations. *n* = 5.

while WUE and L_s showed the upward trend after initial drop. WUE reached the lowest value at a NH_4^+-N/NO_3^--N ratio of 7.5:7.5 (Fig. 4*C*), and the minimum stomatal limitation was observed at a ratio of 5:10 (Fig. 4*F*). In the treated group, changes were regular, with a transition point occurring at a NH_4^+-N/NO_3^--N ratio of 5:10, except that L_s (Fig. 4*F*), P_N, *E*, WUE, g_s, and C_i declined after an initial rise (Fig. 4*A*–*E*). P_N was the most important indicator of biomass, with an extremely significant correlation observed between both parameters in this experiment (Table 2).

Chl fluorescence parameters: Φ_{PSII}, ETR, and NPQ in the treated groups were lower than those of the control group at all ammonium/nitrate-N ratios (Fig. 5*C*,*D*,*F*), whereas F_o, F_v/F_m, and q_P were largely unchanged (Fig. 5*A*,*B*,*E*). All Chl fluorescence parameters remained relatively stable for each ammonium/nitrate-N ratio in both control and treated groups. The relationships of biomass to Φ_{PSII} and NPQ were statistically significant (Table 2).

Discussion

The NH_4^+-N/NO_3^--N ratio had a statistically significant effect on the biomass of pak choi (Table 1), with a substantial increase in the relative amount of ammonium-N causing a sharp decrease in biomass (Fig. 1).

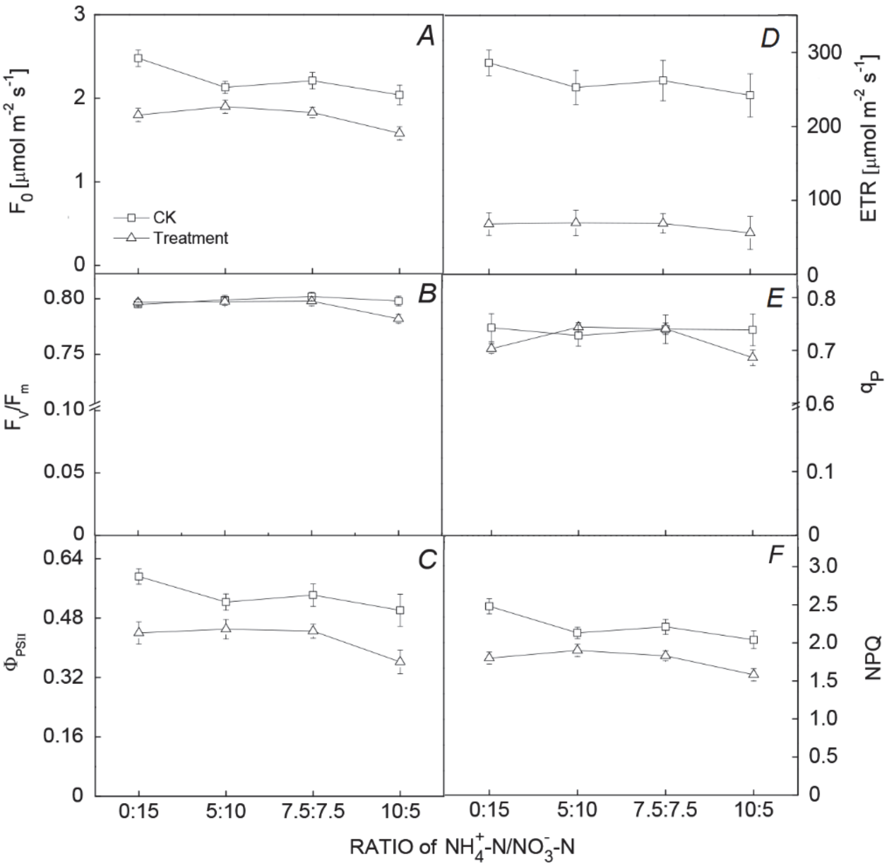

Fig. 5. Effects of NH_4^+-N/NO_3^--N ratio on minimum fluorescence (F_0), maximal quantum yield of PSII (F_v/F_m), effective quantum yield of PSII photochemistry (Φ_{PSII}), electron transport rate (ETR), photosynthetic quenching (q_P), and nonphotochemical quenching (NPQ) of *Brassica chinensis* under low light intensity and water stress. CK – full sunlight with no PEG 6,000; treatment: 32% full sunlight with 25 g(PEG 6,000) L^{-1}. *Lowercase letters* mean statistically significant differences in 0.05 level ($P<0.05$), the same in the following illustrations. $n = 5$.

Since ammonium inhibits NR activity, most studies have found that the relationship between leaf photosynthetic rate and nitrogen supply follows a quadratic curve (Gill *et al*. 2011). When nitrogen supply or leaf nitrogen content reaches a certain value, the photosynthetic rate no longer increases (Zeng *et al*. 2000). In this study, the relationship between pak choi biomass and nitrogen supply under full sunlight without water stress followed a quadratic curve, whereas an exponential relationship was observed under 32% full sunlight with 25 g(PEG 6,000) L^{-1} (Table 1).

Since water deficit and light stress have a strong influence on pak choi leaf NR activity (Shen 2016), the addition of appropriate concentrations of ammonium in order to reduce nitrate uptake is an effective measure to reduce leaf nitrate content (Fig. 2). On the basis of high NR activity, the optimal NH_4^+-N/NO_3^--N ratio in this study was 5:10 (Fig. 2*B*). More nitrogen assimilation thus occurred at this ratio, and plants accumulated relatively higher biomass (Fig. 1) and relatively lower nitrate (Fig. 2*A*). This proportion of ammonium- *vs.* nitrate-N is therefore valuable as a reference point for agricultural production. Similar findings have been reported by Luo *et al*. (2005).

Under different experimental treatments, no significant correlation was found between the Chl content and biomass (Table 2), but an obvious correlation was observed between Φ_{PSII} and pak choi biomass. NPQ and pak choi biomass were obviously negatively correlated, and the correlation between pak choi biomass and P_N reached an extremely significant level (Table 2). These results demonstrated that a relationship exists between biomass accumulation and solar energy utilization in plants, with biomass and P_N the most closely related. No correlation was found between the Chl content and yield, which rules out the use of the Chl content as an indicator of crop photosynthetic capability or biomass accumulation. For comprehensive assessment, all the above factors must be taken into account.

Studies have shown that a decline in P_N under drought stress is the result of both stomatal and nonstomatal limitations. Under mild stress, the decline is mainly due to stomatal limitation, whereas nonstomatal limitation is a more important contributor under severe stress (Guan *et al*. 1995). In this study, P_N was positively correlated with C_i

(Fig. 4*A*,*E*), L_s was negatively correlated with both g_s and C_i (Fig. 4*D*–*F*), and P_N was significantly negatively correlated with L_s (Fig. 4*A*,*F*). These results indicated that the main reason for a decline in P_N was the stomatal limitation. Chl fluorescence parameters can reflect the series of critical adjustment processes in photosynthetic organs (Wu *et al*. 1997). In this study, ETR was positively correlated with Φ_{PSII} (Fig. 5*C*,*D*), and q_P was positively correlated with NPQ (Fig. 5*E*,*F*). An improvement in q_P due to increased concentrations of ammonium-N can increase the proportion of open PSII reaction centers, thus improving PSII electron transfer ability, while NPQ reflects the radiation energy dissipation capacity of the PSII reaction center. The level of q_P is maintained by adjustment of NPQ, thus ensuring consistency of electron transfer and CO_2 fixation rates. This phenomenon explains why increasing NH_4^+-N initially improved q_P, with further increases causing q_P to decline.

Water influences plant photosynthetic capacity mainly through indirect effects on factors limiting photosynthesis. Plant leaves under well-watered conditions have stronger photosynthetic capacity, with water deficit causing photosynthetic rates to decrease obviously. Various hypotheses have been advanced to explain why water deficit attenuates photosynthesis. Some researchers have proposed that water deficit decreases photosynthetic rate primarily by reducing stomatal conductance (*i.e.*, increasing stomatal resistance), thus affecting CO_2 transport into leaves (Guan *et al*. 1995, Fukutoku 1996) such that blades hydrolyze more starch, accumulate sugars, and experience slowed photosynthetic product output (Saglio and Pradet 1980, Guan *et al*. 1995). In the present investigation, the positive correlation of E with P_N (Fig. 4*A*,*B*) and E with g_s (Fig. 4*B*,*D*) might cause WUE to increase under water stress. Under our experimental conditions of reduced light intensity and high nitrate and ammonium, however, WUE was relatively low (Fig. 4*C*). This observation suggests that even under low light conditions, proper adjustment of ammonium-N and nitrate-N can improve WUE.

Nitrogen, one of the most important nutritional elements required by plants, is also the most common factor limiting plant growth. Plants using the C_3 pathway, the main plant photosynthetic physiological metabolic process on the Earth, have a leaf nitrogen content of 75%. In C_3 plants, this leaf nitrogen is used in chloroplast physiological processes, mostly photosynthesis, consequently, nitrogen often becomes the limiting factor of plant growth (Luo *et al*. 2005). Fluctuations in plant nitrogen nutrition directly affect photosynthetic rate and growth and development, and, ultimately, biomass and light energy utilization. Studies have shown that application of ammonium- and nitrate-N has a positive effect on most crops. Mixed application of nitrate- and ammonium-N fertilizers is able to reduce vegetable NO_3^- content and increase vitamin C content, with the optimum ratio depending on crop growth period (Huang *et al*. 2011). Under the treatment conditions of our study, P_N, E, Φ_{PSII}, and ETR first increased and then decreased, with the maximum values occurring at the lowest content of NH_4^+-N (5 mM). Chl *a*, Chl *b*, and Chl (*a*+*b*) contents initially decreased and then increased (Fig. 3*A*–*C*). At a NH_4^+-N/NO_3^--N ratio of 10:5, pigment contents increased suddenly, possibly because of a sharp decline in biomass. The resulting increase in pigment contents enhanced photosynthetic processes to compensate for the loss of biomass due to low light intensity.

Conclusion: The results of our study imply that the relative proportions of ammonium- and nitrate-nitrogen affect biomass accumulation in water-deficient and low light-intensity environments as well as under nonstressed conditions and all have a positive correlation to biomass. At the same time, we observed no correlation between biomass and chlorophyll contents. Biomass accumulation positively correlated with Φ_{PSII} and P_N, but negatively correlated with NPQ.

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