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EFFECTS OF AMMONIUM AND NITRATE RATIOS ON PLANT GROWTH, NITRATE CONCENTRATION AND NUTRIENT UPTAKE IN FLOWERING CHINESE CABBAGE

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Abstract

In order to clarify the effect of different nitrogen forms on growth, nitrate concentration and nutrient uptake of flowering Chinese cabbage, hydroponic experiment was conducted under four ammonium and nitrate ratios (0 : 100, 25 : 75, 50 : 50, 75 : 25) with three cultivars. The results indicated that, compared to complete nitrate treatment (CK) in nutrient solution, plant biomass of flowering Chinese cabbage was promoted in low ammonium enhancement (25%) (T1) treatment, while it was decreased in medium (50%) (T2) and high (75%) (T3) ammonium enhancement treatments. With the increase of ammonium ratio, nitrate concentration in leaf and flower stalk of flowering Chinese cabbage for 3 cultivars reduced gradually. Compared to CK, the accumulations of plant total nitrogen, phosphorus and potassium were increased significantly in T1 treatment, while it was decreased significantly in T3 treatment. Plant biomass and nutrient uptake were enhanced and nitrate concentration in product organs was reduced by 25% ammonium enhancement in nutrient solution, so it was appropriate in hydroponics for flowering Chinese cabbage.

Introduction

Nitrogen (N) nutrition has an important role in plant growth, yield and quality. Plants have developed efficient mechanisms to sense the varying levels of N forms and uptake them (Reddy and Ulaganathan 2015). Ammonium and nitrate ions are the two main forms of N uptake by plant in natural conditions. Uptake of both the forms is strictly under genetic control by plants (Mitra 2015). Ammonium seems to be a more significant N source, because less energy is required to uptake by plants (Guo *et al*. 2007).

However, too much ammonium accumulation in plant will affect many important physiological reactions, such as rhizosphere environment acidification, nutritional imbalance, restrict of photosynthesis and so on (Britto and Kronzucker 2002, Chen *et al*. 2013). Thus for most plants, the nitrate is relatively safe and act as the main N source (Marschner 1995). Plants can assimilate much more nitrate and store them in the vacuole. Generally, vegetable crops, especially leafy vegetables, tend to absorb and accumulate nitrate. Sole nitrate application could lead to high nitrate accumulation in vegetable products, which results in potential threat to human health (Demsar and Osvald 2003).

Previous studies indicated that appropriate proportion of ammonium added in the single nitrate N nutrient solution had much advantages, not only had high biomass (Wang *et al*. 2009, Wang and Shen 2011), but also had good nutritional quality (Song *et al*. 2012a), and it could reduce nitrate concentration significantly (Demsar and Osvald 2003). Therefore, the study for suitable ratio of ammonium and nitrate in nutrient solution is an important way to obtain high yield and good quality of vegetables.

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The flowering Chinese cabbage (*Brassica campestris* L. ssp. *chinensis* var. *utilis* Tsen et Lee) is one of the famous and special vegetables in south China, also it has the largest grown area and yield in local area (Cai *et al*. 2010). Flower stalk is the edible organ of flowering Chinese cabbage, which is crisp and full of nutrition. It was easy to accumulate nitrate, and the accumulation had close relationships with different genotypes (Song *et al*. 2012b).

Three different cultivars of flowering Chinese cabbage (early, middle and late maturity) were used in the experiment. The aims of the experiment were to identify: (1) the effect of different ratios of ammonium and nitrate on plant growth, nitrate accumulation and the uptake of N, P, K; (2) the effect of N forms on 3 different cultivars of flowering Chinese cabbage.

Materials and Methods

The experiment was carried out in the plastic greenhouse of South China Agricultural University, from November 2010 to January 2011. Three cultivars of flowering Chinese cabbage were used in the experiment, in which cultivars "Lvbao 70", "Youlv 80" and "Chixin NO.2" were early, middle and late maturity, respectively. Plug seedlings were started with the medium of perlite, and seedlings with 3 true leaves were transplanted in the nutrient solution. Eleven seedlings were transplanted in one plastic hydroponic box, which was filled with 15 litre nutrient solution. Each plastic box was noted as a repeat, and each cultivar had 4 treatments and 3 repeats, with randomized block arrangement.

Half dose of Hoagland formula was used as basic nutrient solution, with the total N 7.5 mM, total P 0.5 mM, total K 3.0 mM, total Ca 2.5 mM and total Mg 1.4 mM. The treatments were 4 different ammonium and nitrate ratios (NH_4^+-N : NO_3^--N = 0:100, 25:75, 50:50 and 75:25), with the same amount of total N (Table 1).

Nutrient solution in each treatment was replaced weekly and ventilated through pump every 15 minutes per hour. pH value of the solution was adjusted to around 6.2 every day.

Table 1. Nutrition formula of different ammonium and nitrate ratios (mM/l).

NH_4^+:NO_3^-	Nutrient source						
	KNO_3	$Ca(NO_3)_2 \cdot 4H_2O$	KH_2PO_4	$MgSO_4 \cdot 7H_2O$	$(NH_4)_2SO_4$	K_2SO_4	$CaCl_2$
0 : 100 (CK)	2.5	2.5	0.5	1.4	-	-	-
25 : 75 (T1)	0.625	2.5	0.5	1.4	0.9375	0.9375	-
50 : 50 (T2)	-	1.875	0.5	1.4	1.875	1.25	0.625
75 : 25 (T3)	-	0.9375	0.5	1.4	2.8125	1.25	1.5625

Plant materials were taken randomly when they reached marketable maturity. Plant organs (over the 4th leaf node) were divided into leaf and flower stalk, in which nitrate concentrations were quantified as described by Patterson *et al*. (2010).

Three plants were put together as a sample for biomass measurement. The plant was divided into above-ground and root part respectively, and was weighed for the dry weight after drying at 70ºC to constant weight.

Approximately 0.5 g of dried plant sample (milled into powder) was digested using sulfuric acid and hydrogen peroxide (Lowther 1980). Kjeldahl N concentration in the digestion solution was determined with an auto-analyzer (Kjeltec 2300 Analyzer Unit, Foss Tecator, Sweden). Total P concentration in the digestion solution was determined by a modified molybdenum blue procedure

(Murphy and Riley 1962) at 660 nm using a spectrophotometer (UV-1800, Shimadzu, Japan). Total K concentration in the digestion solution was determined by atomic absorption spectrophotometer (AA-6800, Shimadzn, Japan).

Statistical analysis of the data was performed with DMRT at $p < 0.05$ level, using the SPSS software package (Version 16.0).

Results and Discussion

For whole plant dry weight of flowering Chinese cabbage, including above ground part and root, the late-maturity cultivar (Chixin NO.2) was the largest, followed by the mid-maturity cultivar (Youlv 80), and the early-maturing cultivar (Lvbao 70) was the least (Fig. 1). The plant biomass was positively correlated with their maturity period.

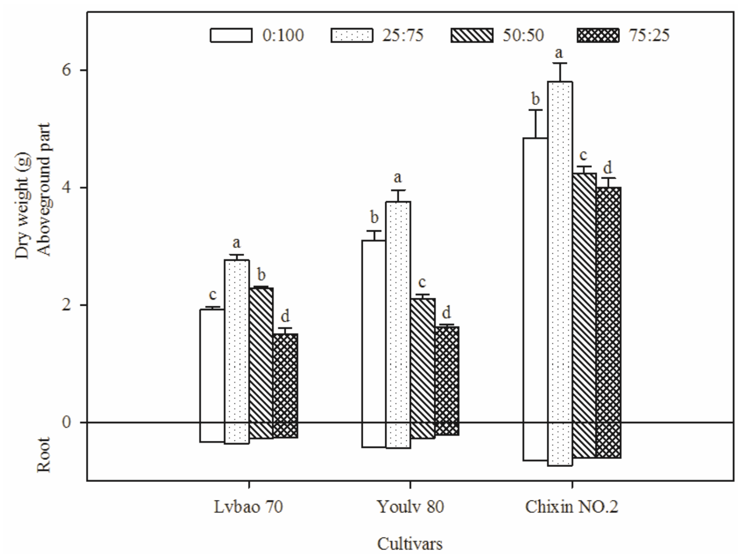

Fig. 1. Biomass of flowering Chinese cabbage in different ammonium and nitrate ratios. The bars in the figure indicate means ± the standard errors (n = 3) for the whole plant. Different letters in each cultivar indicate significant difference at $p < 0.05$.

Different ratios of ammonium and nitrate significantly affect the dry weight of flowering Chinese cabbage. Dry weight was the highest in T1 among the 4 treatments, and it was significantly higher than the other three treatments ($p < 0.05$). With the increasing of ammonium in nutrient solution, dry weight of flowering Chinese cabbage was decreased. The dry weight in T3 treatment was significantly lower than that of CK. This indicated that, too much ammonium (75%) would inhibit plant growth of flowering Chinese cabbage.

Nitrate concentration in flower stalks was much higher than that of leaves for three cultivars of flowering Chinese cabbage (Fig. 2). Different ratios of ammonium and nitrate in nutrient solution significantly affected nitrate content in both organs. Nitrate concentration was the highest in CK, with the increasing of ammonium, it decreased gradually, and it reached the lowest level for T3 treatment. The difference of nitrate concentration among the four treatments was significant. This trend was the same between two organs for all three cultivars.

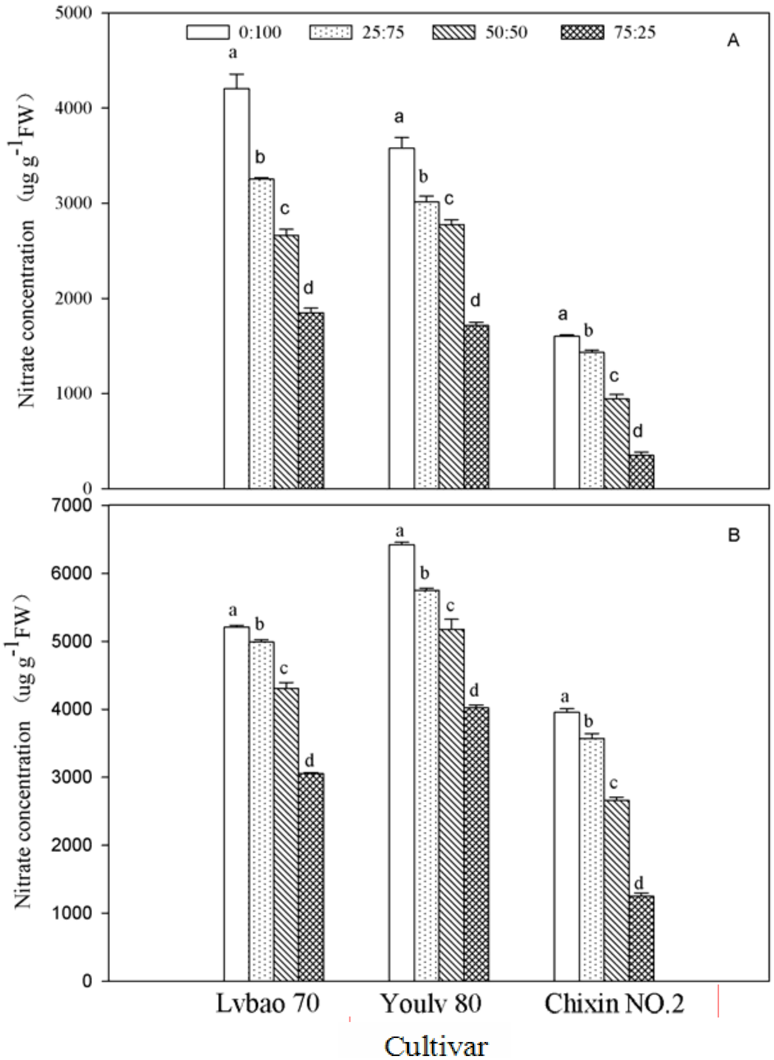

Fig. 2. Nitrate concentration in product organs of flowering Chinese cabbage in different ammonium and nitrate ratios. A: leaf, B: flower stalk. The bars in the figure indicate means ± the standard errors (n = 3). Different letters in each cultivar indicate significant difference at $p < 0.05$.

Compared to CK, the nitrate concentration of T3 treatment in leaves of "Lvbao 70","Youlv 80" and "Chixin NO.2" cultivars was decreased by 56.0, 52.1 and 78.0%, respectively, and it was decreased by 41.4, 37.3 and 68.4% in flower stalk, respectively.

Different ratios of ammonium and nitrate treatment significantly influenced plant N, P and K concentrations and accumulations of flowering Chinese cabbage (Fig. 3).

With the increase of ammonium proportion in nutrient solution (0 ~ 75%), total N concentration of the whole plant for 3 cultivars increased firstly, T2 treatment reaching the highest, and then decreased for T3 treatment.

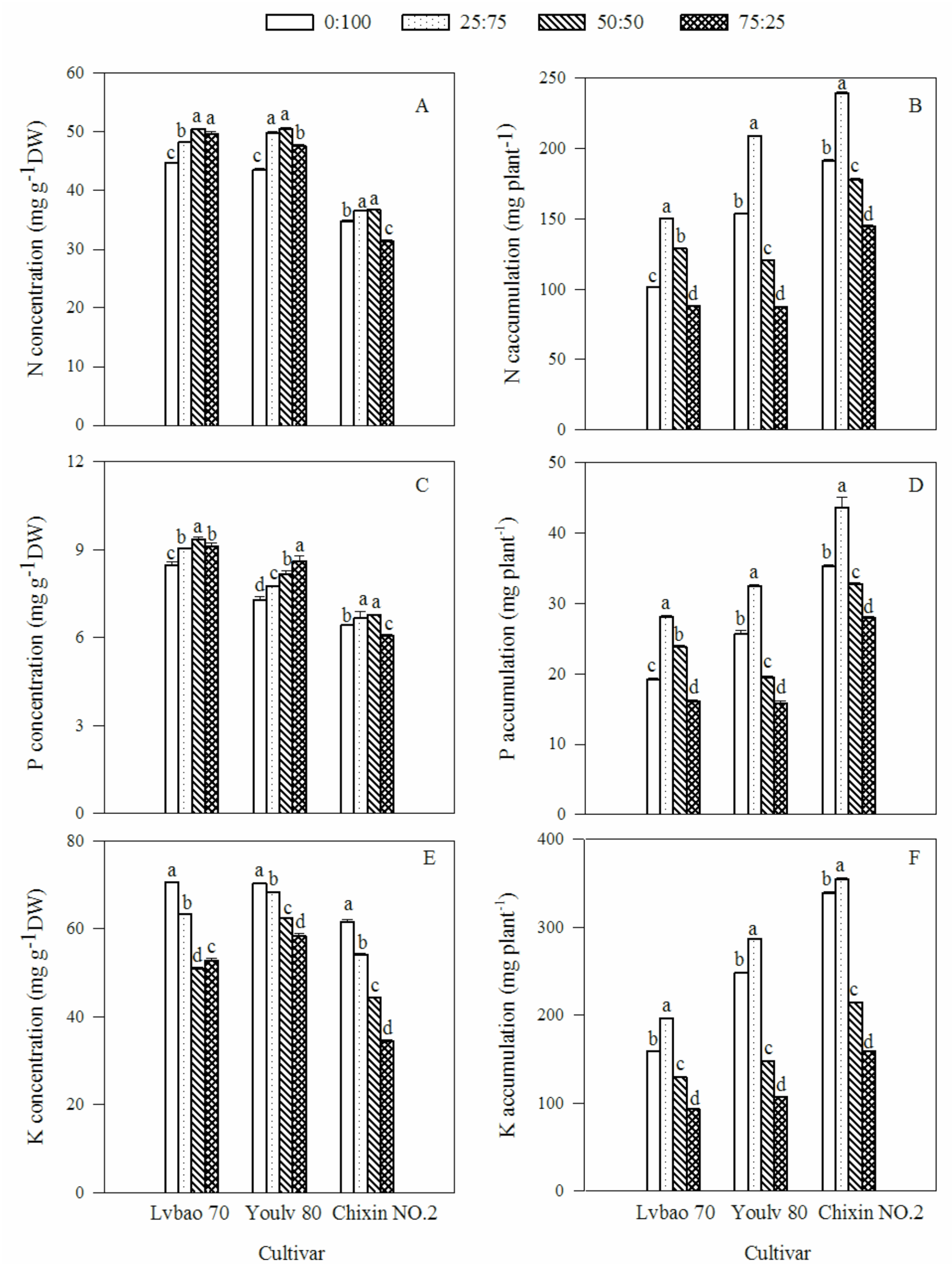

Fig. 3. Total N, P and K concentrations and accumulations of flowering Chinese cabbage in different ammonium and nitrate ratios. A: N concentration; B: N accumulation; C: P concentration; D: P accumulation; E: K concentration; F: K accumulation. The bars in the figure indicate means ± the standard errors (n = 3) for the whole plant. Different letters in each cultivar indicate significant difference at $p < 0.05$.

The tendency of plant N accumulation was similar with that of N concentration. N accumulation was the highest in T1 treatment and the lowest in T3 treatment, and the difference among four treatments was significant ($p < 0.05$). Compared to CK, the N accumulation of T1 treatment was increased by 48, 36 and 25%, respectively of "Lvbao 70","Youlv 80" and "Chixin NO. 2" cultivars, and it was decreased by 13, 43 and 24% of T3 treatment, respectively of the former three cultivars.

With the increase of ammonium proportion, total P concentration increased in the beginning and then decreased in "Lvbao 70" and "Chixin NO.2" cultivars, with T2 treatment the highest level, and then decreased for T3 treatment. However, in "Youlv 80" cultivar, total P concentration increased gradually with the increasing proportion of ammonium, with it reached the highest level for T3 treatment.

P accumulation in the whole plant among the four treatments had the same tendency as that of N accumulation. P accumulation was the highest in T1 treatment and lowest in T3 treatment, and the difference among four treatments was significant ($p < 0.05$). Compared to CK, the P accumulation of T1 treatment was increased by 47, 27 and 24%, respectively of "Lvbao 70","Youlv 80" and "Chixin NO.2" cultivars, and it was decreased by 16, 38 and 21% of T3 treatment, respectively of the former three cultivars.

With the increase of ammonium proportion, total K concentration decreased gradually for the three cultivars, and the difference among 4 treatments was significant ($p < 0.05$).

With the increase of ammonium proportion, K accumulation increased in the beginning, and then decreased, showing the tendency T1 > CK > T2 > T3, also the difference among four treatments was significant ($p < 0.05$). Compared to CK, the K accumulation of T1 treatment was increased by 23, 16 and 5%, respectively of "Lvbao 70","Youlv 80" and "Chixin NO.2" cultivars, and it was decreased by 42, 57 and 53% of T3 treatment, respectively of the former three cultivars.

Compared to the complete nitrate nutrition, the accumulation of N, P and K had been improved in low ammonium proportion (25%), but nutrient uptake was inhibited in high ammonium proportion (> 50%).

The main source of N uptake by plant is nitrate and ammonium, and plants showed maximum differences to adapt N environment (Lasa *et al.* 2002). For most of the higher plants, ammonium is preferably the N source, with less energy during the absorption. However, there were serious physiological and morphological disorders when ammonium acted as the sole N source (Bloom *et al*. 1992). Other forms of N might also have the disorder, e.g. glutamine conditions when compared to the NH_4NO_3 condition, carbon-nitrogen metabolic balance was interrupted and plant growth decreased significantly in rice (Li *et al*. 2016). While using nitrate as the sole N source, plant tissues could accumulate nitrate excessively, also much nitrate could leach to ground water (Demsar and Osvald 2003). Many reports demonstrated that, using the mixture of ammonium and nitrate as crop nutrition can obtain higher growth efficiency and yield (Wang *et al*. 2009, Helali *et al*. 2010, Wang and Shen 2011). In this study, moderate (25%) enhancement of ammonium treatment showed higher biomass and lower nitrate concentration in three cultivars of flowering Chinese cabbage.

Plant morphology and growth rate often depended on N metabolism, whereas the ability of absorbing ammonium and nitrate related to characteristic of root morphology and N flux from soil to root (Brix *et al*. 2002). Low enhancement of ammonium promoted the growth of flowering Chinese cabbage may be related to changing environmental conditions in rhizosphere. Nitrate could increase pH value in the rhizosphere, whereas ammonium could decrease pH (Helali *et al*. 2010). When both supplying ammonium and nitrate, the pH of nutrient solution could be stabilized at a suitably slight acidic range, which might result in growing better (Wang *et al*. 2009). In addition, the low enhancement of ammonium increases the root activity of flowering Chinese cabbage (Song *et al*. 2012c), thus promoting nutrient uptake. But too high ammonium proportion in nutrient solution (>50%) resulted in rhizosphere acidification, which could reduce the uptake and transportation ability of nutrients. Eventually the growth of root and whole plant was inhibited.

Previous study showed that the nitrate concentration of vegetable products was positively correlated with the ratio of nitrate in mixed nutrition, and a small amount of ammonium could significantly reduce the nitrate concentration in vegetables (Wang *et al*. 2009). It was noted that with increasing of ammonium proportion in nutrient solution, the nitrate concentration of leaves and flower stalks gradually decreased in three cultivars of flowering Chinese cabbages.

The amount of nitrate accumulation was actually the result of N absorption and reduction of plant (Ai *et al*. 2000). Nitrate assimilation was governed by plant growth (Rivas-Ubach *et al.* 2012). Therefore, when the uptake rate exceeded that of assimilation, nitrate accumulates in plant vacuoles (Anjana and Iqbal, 2007). In this experiment, the nitrate concentration in flower stalk was higher than that in leaf. The possible reason was that the reduction and assimilation of nitrate took place mainly in leaf, whereas flower stalk played main role of transportation and storage of nitrate.

Different forms of N fertilizer, affected not only on the uptake of N and P (Kronzucker *et al*. 2001), but also on the absorption of K (Helali *et al*. 2010). It was found that a moderate ratio of ammonium and nitrate was necessary to the nutrient uptake and accumulation of flowering Chinese cabbages (Fig. 4). With the increasing proportion of ammonium, the K concentration in *Arabidopsis* was reduced (Helali *et al*. 2010). In this experiment, K concentration was also significantly reduced, along with the increasing proportion of ammonium. Increasing ammonium concentration in plant may result in the accumulation of excess cations *in vivo*. Concurrently, the competitive absorption of other cations (e.g., K^+, Ca^{2+}) occurred and it was deterred the nutrient uptake, resulted by acidification of the rhizosphere. Thus the important cationic homeostasis of root system was damaged (Kronzucker *et al*. 2003). Ammonium inhibited the activity of HAK5 potassium transport protein, thereby plant K uptake was inhibited (Baňuelos *et al*. 2002). Whereas limitation of K^+ and Ca^{2+} by high ammonium enhancement may be involved in regulating the plant growth (Siddiqi *et al*. 2002, Helali *et al*. 2010).

In soilless production, an effective strategy to reduce the nitrate concentrations in leave vegetables is to withhold N several days before harvest (Albornoz 2016). But the method of withholding N in nutrient solution cost more labor in practice. Compared to the complete nitrate nutrient solution, plant growth, accumulation of N, P and K were significantly promoted by low (25%) ammonium enhancement treatment in three cultivars of flowering Chinese cabbage. But high proportion of ammonium treatment (75%) significantly reduced plant biomass and nutrient accumulation. Thus in practice, 25% ammonium enhancement in nutrient solution was appropriate in hydroponics for flowering Chinese cabbage, resulting in higher yield and lower nitrate concentration. And this nutrition formula (T1 in Table 1) might apply to other leafy green vegetables in hydroponic production.

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