Effects of different nitrogen forms on the nutritional quality and physiological characteristics of Chinese chive seedlings

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ABSTRACT

Nitrogen plays a vital role in the growth, development and nutritional quality of Chinese chive, which is an important leafy vegetable. The effects of improved Hoagland nutrient solutions on the nutritional quality and physiological characteristics of three Chinese chive cultivars (Saisong, Pingjiu No. 4 and Pingjiu No. 8) were investigated by modulating the ratio of nitrite nitrogen (NO_3^--N) to ammonia nitrogen (NH_4^+-N). Improved Hoagland nutrient solutions with NO_3^--N to NH_4^+-N ratios of 50:50 and 75:25 could effectively promote the accumulation of soluble protein, soluble sugar, vitamin C and proline; decrease the malondialdehyde content; and induce the superoxide dismutase and peroxidase activities of the three cultivars. It is strongly suggested that NO_3^--N to NH_4^+-N ratios of 50:50 and 75:25 improve nutritional quality and promote growth and are thus suitable for Chinese chive growth under hydroponic culture.

Keywords: *Allium tuberosum* Rottler; NO_3^--N to NH_4^+-N ratio; soluble protein; soluble sugar and vitamin C; antioxidant metabolism

Nitrogen (N) is one of the most important nutrients affecting the growth, development, yield and fruit quality of plants. Two main nitrogen forms are used by plants in natural conditions: nitrite nitrogen (NO_3^--N) and ammonia nitrogen (NH_4^+-N) (Tschoep et al. 2009). In addition to the amount supplied, the form of the available nitrogen has a significant effect on the growth, photosynthesis (Ali et al. 2013), yield and quality (Lošák et al. 2008) of plants. In general, most plants use NO_3^--N preferentially as an N source, whereas plant roots can absorb NH_4^+-N when soils are deficient in NO_3^--N. When NH_4^+-N is used as the only N source, most plant species show reduced growth, smaller leaves and a stunted root system on exposure to high NH_4^+-N concentrations and in severe cases, this leads to chlorosis. NH_4^+-N toxicity can be alleviated and a synergistic growth response can be induced in many plants by co-provision of NO_3^--N (Tabatabaei et al. 2008). For many plants, mixed NO_3^--N and NH_4^+-N has been documented to be superior over individual NO_3^--N or NH_4^+-N (Zhou et al. 2011). The optimal proportion of NO_3^--N to NH_4^+-N for plant growth depends on the plant species, environmental conditions, developmental stage and the total concentration of supplied nitrogen (Tang et al. 2011, Guo et al. 2012).

Chinese chive (*Allium tuberosum* Rottler) is a hardy perennial plant that originated in China and is widely cultivated in East and Southeast Asia. N is vital for the growth, development and nutritional quality of Chinese chive; however, few reports are available regarding the effect of the proportions of different nitrogen forms on the growth, nutritional quality and physiological characteristics of Chinese chive under hydroponic culture. The exact mechanism underlying nitrogen metabolism is unclear at this stage and the optimal nutrient solution and NO_3^--N to NH_4^+-N ratio remain to be identified. In the present study, the effects of improved Hoagland nutrient solution with different NO_3^--N to NH_4^+-N ratios (25:75, 50:50, 75:25

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and 100:0) on nutritional quality and physiological characteristics were studied for the three cultivars, Saisong, Pingjiu No. 4, and Pingjiu No. 8, under a total N concentration of 14.3 mmol/L.

MATERIAL AND METHODS

Plant materials and treatments. Fresh plant material from Chinese chive was selected randomly from the experimental plots of the School of Horticulture and Landscape Architecture of the Henan Institute of Science and Technology at Xinxiang, China, on October 8, 2012. Fresh samples with some rhizospheric soil were placed into plastic bags and transported to the greenhouse under natural light conditions at 28–30°C and 60–70% relative humidity. The root systems were washed in tap water to remove soil and surface-dried with tissue paper. The seedlings were then cultured in troughs filled with a 2:1 (v/v) mixture of vermiculite and perlite and irrigated with half-strength Hoagland nutrient solution every 4 days. Fifteen days later, uniform seedlings were selected and transferred to plastic pots with full-strength Hoagland nutrient solution, which were covered with a polystyrol plate containing six evenly spaced holes (with three seedlings in each hole). A month later, the leaves were excised and rhizomes and roots were placed in troughs and supplied with full-strength Hoagland nutrient solution containing NO_3^--N and NH_4^+-N at one of four ratios. The composition of the macroelements in the nutrition solution under different treatments was shown in Table 1. The composition of micronutrients (μmol/L: 140 H_3BO_3, 100 $CuSO_4 \cdot 5\ H_2O$, 36 $MnCl_2 \cdot 4\ H_2O$, 46 $ZnSO_4 \cdot 7\ H_2O$, 30 Fe-EDTA and 1 H_2MoO_2) was the same in all nutrient solutions. The total N concentration in both systems was 14.3 mmol/L. All treatments were conducted with three replicates.

Determination of physiological indexes. Eighteen days later, Chinese chive leaves were excised randomly, rinsed in deionized water and blotted carefully with tissue paper. The fresh samples were then rapidly frozen in liquid nitrogen for determination of physiological indexes. Soluble protein content and vitamin C (Vc) content were assayed according to the method of Shi and Sun (2011). Soluble sugar content was quantified by the anthrone sulfuric acid method (Fales 1951). Malondialdehyde (MDA) content was determined using the thiobarbituric acid reaction, as described by Sudhakar et al. (2001). Proline content was determined following the method of Xiang et al. (2013). Superoxide dismutase (SOD) and peroxidase (POD) activities were measured according to the method of Xu et al. (2011).

Statistical analysis. All data were subjected to the analysis of variance (ANOVA) using the SPSS version 10.0 statistical package (Chicago, USA) for Windows. When the *F*-test showed significant differences among means, Duncan's multiple range tests were applied at the 0.05 level of probability to separate the means.

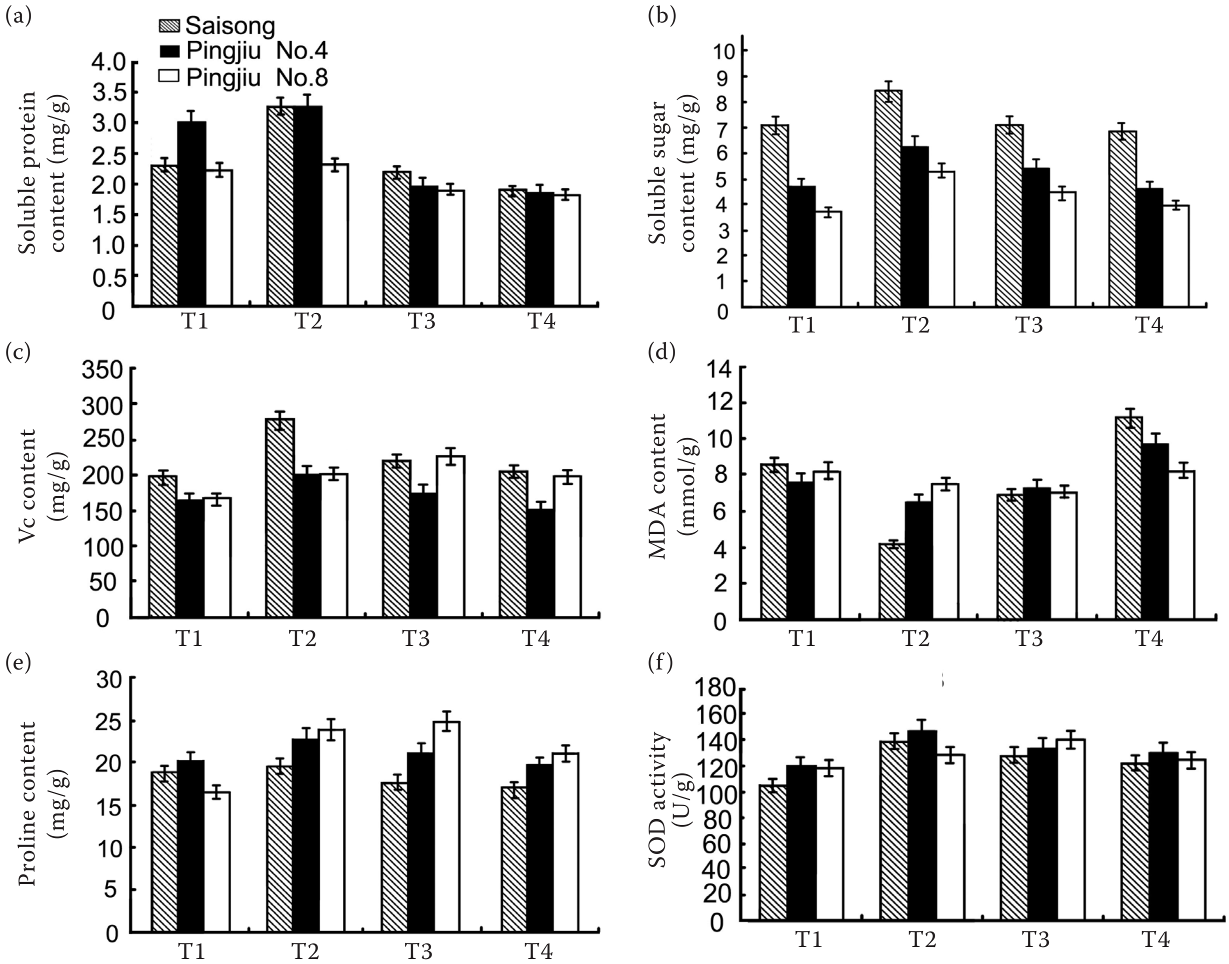
RESULTS AND DISCUSSION

Soluble protein content. Nutritional quality indicators for vegetables include soluble protein, soluble sugar, Vc and other factors. In the present study, different nitrogen forms were associated with different levels of soluble protein content (Figure 1a). The soluble protein contents in the leaves of Saisong, Pingjiu No. 4 and Pingjiu No. 8 were highest in the T2 nitrogen solution at 3.28, 3.32 and 2.34 mg/g, respectively. The leaves of the three cultivars treated with T4 nitrogen solution all maintained the lowest levels of soluble protein content, which were 1.92, 1.92 and 1.83 mg/g, respectively. Therefore, the ratio of 50:50 (NO_3^--N to NH_4^+-N) promoted the accumulation of soluble protein for the three cultivars.

Soluble sugar content and Vc content. To assess the effect of different nitrogen forms on the accumulation of soluble sugar, we compared the

Table 1. Components of macroelements in the nutrition solution under different treatments (mmol/L)

Treatments	NO_3^--N:NH_4^+-N	$Ca(NO_3)_2$	KNO_3	$MgSO_4$	$NH_4H_2PO_4$	KH_2PO_4	KCl	NH_4Cl	$CaCl_2$
T1	25:75	1.8	0	2	1	0	7.7	9.7	2.4
T2	50:50	3.6	0	2	1	0	7.7	6.1	0.7
T3	75:25	2.7	5.4	2	1	0	2.3	2.5	1.5
T4	100:0	4.3	5.7	2	0	1	1	0	0



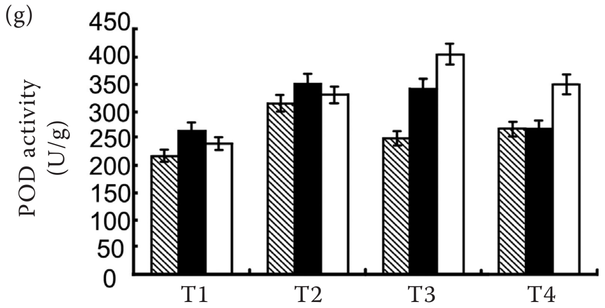


Figure 1. Effects of different nitrate and ammonia ratios on (a) soluble protein content; (b) soluble sugar content; (c) Vc content; (d) malondialdehyde (MDA) content; (e) proline content; (f) superoxide dismutase (SOD) activities and (g) peroxidase (POD) activities. T1 – NO_3^--N to NH_4^+-N ratio of 25:75; T2 – NO_3^--N to NH_4^+-N ratio of 50:50; T3 – NO_3^--N to NH_4^+-N ratio of 75:25; T4 – NO_3^--N to NH_4^+-N ratio of 100:0

sugar levels in the leaves when the plants were incubated in solutions with different NO_3^--N to NH_4^+-N ratios (Figure 1b). The T2 nitrogen solution greatly promoted the accumulation of soluble sugar and in all three cultivars, the leaves had the highest levels of soluble sugar. In contrast, the leaves of cultivars treated with the T4 nitrogen solution had the lowest soluble sugar contents, which were decreased by 18.74, 26.10 and 24.90%, respectively, in comparison to the T2 nitrogen solution.

The effects of different nitrogen forms on the Vc content were shown in Figure 1c. The different NO_3^--N to NH_4^+-N ratios significantly affected the Vc contents for the three cultivars. The Vc contents in the leaves of Saisong and Pingjiu No. 4 were maximized in T2 nitrogen solution, whereas that in Pingjiu No. 8 was maximized in T3 nitrogen solution.

The effects of different nitrogen forms on soluble sugar and Vc content in different plant seedlings were not the same. Zhang et al. (1990) reported that the contents of soluble sugar and Vc of spinach were increased when the NO_3^--N to NH_4^+-N ratio in the nutrient solution was reduced, and the ratio of 50:50 (NO_3^--N to NH_4^+-N) provided a higher sugar and Vc contents. Zhu et al. (1998) observed that the Vc content of tobacco was increased by increased light intensity but decreased by NH_4^+-N. Yang et al. (2003) found that the contents of soluble sugar and Vc in the fruit of tomato increased with increased NH_4^+-N. In the present study, the highest contents of soluble sugar and Vc were obtained at NO_3^--N to NH_4^+-N ratios of 50:50 and 75:25 for the three cultivars, which suggests that NO_3^--N to NH_4^+-N ratios of 50:50 and 75:25 are suitable for improving the nutrient quality of Chinese chive seedlings.

MDA content. Membrane permeability is a sensitive test for determining abiotic stress and tolerance. Higher membrane stability may be correlated with abiotic stress tolerance (Meloni et al. 2003). MDA is the product of biomembrane lipid peroxidation, and its content in plants reflects the degree of membrane injury. In this study, the MDA contents were lower in leaves treated with T2 and T3 nitrogen solutions and reached the maximum level in treatment with the T4 nitrogen solution (Figure 1d). In comparison to treatment with the T4 nitrogen solution, the MDA contents of the three cultivars decreased by 23.18, 21.13, and 0.12%, respectively, in treatment with the T2 nitrogen solution. These results indicate that the T2 and T3 nitrogen solutions protect seedlings against oxidative membrane damage.

Proline content and enzyme activity. Proline is the primary substrate for osmotic adjustment in plant cells. Verbruggen and Hermans (2008) found that proline accumulation plays adaptive role in plant stress tolerance in crops. Reactive oxygen species (ROS) are easily produced in plant cells by osmotic stress and can injure the plant cells if they are not eliminated (Zhang et al. 2005). An efficient antioxidant system is important for combating abiotic stress. An increase in the activity of antioxidant enzymes under abiotic stress could be indicative of increased production of ROS and thus suggests escalation of a protective mechanism to reduce oxidative damage triggered by stress. SOD and POD are protective enzymes for the cell membrane and the most important antioxidative enzymes for scavenging ROS. In the present study, the proline content and enzyme activities for SOD and POD were assayed in leaves of the three cultivars receiving different nitrogen forms (Figures 1e,f). The proline content and enzyme activity in leaves of Saisong and Pingjiu No. 4 were both significantly higher in the T2 nitrogen solution than in the other solutions, whereas the proline content and enzyme activity of Pingjiu No. 8 were highest in the T3 nitrogen solution. Therefore, the optimum ratios of NO_3^--N to NH_4^+-N for protecting Chinese chive against oxidative stress are 50:50 and 75:25.

In conclusion, improved Hoagland nutrient solutions with NO_3^--N to NH_4^+-N ratios of 50:50 and 75:25 could effectively promote the accumulation of soluble protein, soluble sugar, Vc, and proline; decrease the MDA content; and induce the SOD and POD activities of the three cultivars. It is strongly suggested that NO_3^--N to NH_4^+-N ratios of 50:50 and 75:25 could improve the nutritional quality and promote growth and are thus suitable for Chinese chive growth under hydroponic culture.

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