Effects of Amino Polysaccharides on Nitrogen Metabolism, Growth and Quality of Non-heading Chinese Cabbage

Yi JIANG#, Xiaolong SHI#, Lirong WANG, Huixian QIAN, Fengying CAO, Yuan GAO, Yingying QIAO, Zhangyu SHI, Xuqin REN*

Huaiyin Institute of Technology, Huaian 223003, China

Abstract [**Objectives**] This study was conducted to promote the high-yield and high-quality cultivation of non-heading Chinese cabbage. [**Methods**] Two non-heading Chinese cabbage varieties, 'Suzhouqing' and 'Huakaifugui', were used as experimental materials. Five concentrations of amino polysaccharides, A (1 000 times solution), B (500 times solution), C (100 times solution), D (10 times solution), and CK, were set up to investigate the effects of amino polysaccharides on nitrogen metabolism, chlorophyll synthesis, dry matter accumulation, and nutritional quality in non-heading Chinese cabbage leaves. [**Results**] Nitrate reductase and glutamine synthetase activities in the leaves of the two non-heading Chinese cabbage varieties were significantly enhanced, and the free amino acid content, chlorophyll content, soluble protein content, vitamin C content and plant dry weight increased significantly, while the nitrate nitrogen content decreased significantly. Amino polysaccharides facilitated nitrogen conversion and amino acid synthesis in non-heading Chinese cabbage, thereby promoting growth, increasing yield, and improving quality. The application effect of high concentration amino polysaccharide solutions (100 times and 10 times dilutions) was better. [**Conclusions**] This study provides a theoretical foundation for exploring the application potential of amino polysaccharides in high-yield and high-quality cultivation of non-heading Chinese cabbage.

Key words Amino polysaccharide; Non-heading Chinese cabbage; Nitrogen; Growth, Quality
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Non-heading Chinese cabbage (*Brassica rapa* ssp. *chinensis*), belonging to *Brassica* of the *Brassicaceae* family, is a subspecies of Brassica rapa. Originating in China[1]. It is widely cultivated in southern China and valued for its short growth cycle, strong adaptability, and plays an important role in year-round vegetable supply. To ensure increased yield and reduced occurrence of pests and diseases in non-heading Chinese cabbage, fertilization and pesticide application are commonly adopted in production. Over time, this can lead to soil degradation and the creation of new pollution sources. Against the backdrop of agricultural efforts to improve crop yields, reducing nitrogen fertilizer comsumption and enhancing nitrogen efficiency are of significant importance for achieving green and safe vegetable production that improves quality, increases efficiency, and reduces pollution.

Amino polysaccharides (APs) are the only natural cationic polysaccharides found in nature[2]. They primarily exist in the form of chitin and deacetylated chitin in the exoskeletons of arthropods and the cell walls of fungi, ranking as the second most abundant natural polymer after cellulose[3]. As marine bioactive substances, amino polysaccharides are colorless, odorless and nontoxic, and possess favorable physicochemical properties, making them applicable in agriculture, medicine, and food industries. In agriculture, amino polysaccharides can induce disease resistance and stress tolerance in crops, promote growth, improve quality, and simultaneously function as both a pesticidal and fertilizing agent with dual biological regulatory effects[4]. To promote high-yield and high-quality cultivation of non-heading Chinese cabbage, in this study, Suzhouqing and Huakaifugui were selected as experimental materials, which was analyzed for changes in indicators such as nitrate reductase activity, glutamine synthetase activity, nitrate nitrogen content, and free amino acid content after amino polysaccharide treatment, so as to investigate the effects of amino polysaccharides on nitrogen transformation and amino acid synthesis in non-heading Chinese cabbage. Furthermore, the application potential of amino polysaccharides in high-yield and high-quality cultivation of non-heading Chinese cabbage was explored by examining changes in indicators related to growth and quality.

Materials and Methods

Test materials

Two varieties of non-heading Chinese cabbage were selected: Suzhouqing and Huakaifugui.

An amino polysaccharide aqueous solution was provided by Jiangsu Shuanglin Ocean Biological Pharmaceutical Co., Ltd., with an amino polysaccharide content ≥100 g/L.

Experimental design

The experiment adoped plug seedling tray cultivation and pot

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Yi JIANG (1986 –), male, P. R. China, assitant experimentalist, devoted to research about cultivation physiology and laboratory management of horticultural plants.
Xiaolong SHI (2001 –), male, P. R. China, master, devoted to research about vegetable cultivation physiology and molecular breeding.
#These authors contributed equally to this work.
* Corresponding author. Xuqin REN (1973 –), female, P. R. China, professor, PhD, devoted to research about cultivation physiology of horticultural plants.

planting methods. Seeds were sown on October 3, and seedlings of the two varieties were transplanted into plastic pots on November 12, with five plants per pot. When the non-heading Chinese cabbage developed 4 to 5 true leaves, foliar application of the amino polysaccharide solution was started. The experiment included five treatments with three replicates each: amino polysaccharide solutions diluted at ratios of 1 : 1 000 (A), 1 : 500 (B), 1 : 100 (C), and 1 : 10 (D), and a control group sprayed with clean water (CK). The solutions were applied once every 5 d, for a total of 3 times. Sampling and measurements were conducted on December 25.

Measurement items and methods

Methods for determining physiological indicators: Chlorophyll content was measured using the ethanol extraction method[5]. Nitrate nitrogen content was determined by the salicylic acid method[5]. Free amino acid content was assessed via the ninhydrin solution colorimetric method[5]. Vitamin C (Vc) content was measured using the 2,6-dichlorophenolindophenol titration method[5]. Protein content was determined by the Coomassie Brilliant Blue G-250 method[5]. Nitrate reductase (NR) activity was determined using the *in-vitro* method[5]. The determination of glutamine synthetase (GS) activity adopted the spectrophotometric method[6].

The method for determining dry weight: Fresh non-heading Chinese cabbage was placed in an oven at approximately 105 ℃ for deactivation, followed by drying at 80 ℃ until a constant weight was achieved.

Results and Analysis

Effects of amino polysaccharides on nitrogen metabolism-related indicators

Nitrogen plays a crucial role in the physiological metabolism of plants. In the process of nitrogen metabolism within plants, key enzymes are involved, enabling inorganic nitrogen to be assimilated into organic nitrogen such as glutamine and glutamate for absorption and utilization by the plant. Specifically, nitrate nitrogen is first reduced to ammonium nitrogen under the action of nitrate reductase. Subsequently, with the involvement of nitrogen metabolism enzymes such as glutamine synthetase, amino acids and glutamine are synthesized[7]. These amino acids are primarily utilized for organ development or other physiological processes[8].

Nitrate reductase activity and glutamine synthetase activity As shown in Fig. 1, the amino polysaccharide treatment had a significant impact on the NR activity of Suzhouqing. With the treatment concentration increasing, the NR activity in the leaves gradually strengthened. Treatment D exhibited the highest NR activity, which was 98.4% higher than that of the CK. The NR activity did not reach a statistically significant difference between Suzhouqing and Huakaifugui. Compared with the CK, the NR activity in Huakaifugui also showed a gradual increasing trend, with treatment D showing a 78.5% increase in NR activity compared with the control.

As shown in Fig. 1, there was no significant difference in GS activity between the leaves of Suzhouqing and Huakaifugui. After treatment with amino polysaccharide, both varieties exhibited similar trends in GS activity changes. With the concentration of amino polysaccharide increasing, the GS activity of Suzhouqing and Huakaifugui significantly was enhanced. Treatment D resulted in the highest GS activity, which increased by 83.4% and 92.9% compared with the control, respectively.

Nitrate nitrogen content and amino acid content As shown in Fig. 2, the nitrate nitrogen content in Suzhouqing and Huakaifugui exhibited similar trends, with no significant differences between the two varieties except for the control group. Amino polysaccharides had a significant impact on the nitrate nitrogen content in the leaves of non-heading Chinese cabbage. As the concentration of amino polysaccharides increased, the nitrate nitrogen content in both Suzhouqing and Huakaifugui showed a decreasing trend. Compared with the CK, the nitrate nitrogen content in Suzhouqing leaves decreased by 21.9%, 32.5%, 42.3%, and 49.8%, respectively, while in Huakaifugui, it decreased by 10.5%, 21.1%, 30.3%, and 40.3%, respectively. All reductions reached statistically the significant level.

Although the free amino acid content in Suzhouqing leaves was significantly higher than that in Huakaifugui, amino polysaccharides had a similar impact on amino acid synthesis in both varieties. As shown in Fig. 2, after amino polysaccharide treatment, the free amino acid content in the leaves of both Suzhouqing and Huakaifugui was significantly higher than that in the CK. However, there were no significant differences in amino acid content among the various concentrations of amino polysaccharide treatment. Compared with the CK, the amino acid content in Suzhouqing increased by 48.4% to 61.2%, while in Huakaifugui, it increased by 52.4% to 76.2% after amino polysaccharide treatment.

NR and GS are involved in nitrogen metabolism and serve as key enzymes in nitrate assimilation and amino acid synthesis within plants. Their activity levels have certain influence on plant growth, development, yield, and quality. In summary, the foliar application of amino polysaccharide helped enhance NR and GS activity, promoted the reduction of nitrate nitrogen in non-heading Chinese cabbage, and provided the material basis for amino acid synthesis. After treatment with amino polysaccharide solution, the nitrate nitrogen content in the leaves of both non-heading Chinese cabbage varieties decreased, while the amino acid content increased. It indicates that amino polysaccharides facilitate nitrogen conversion and amino acid synthesis in non-heading Chinese cabbage.

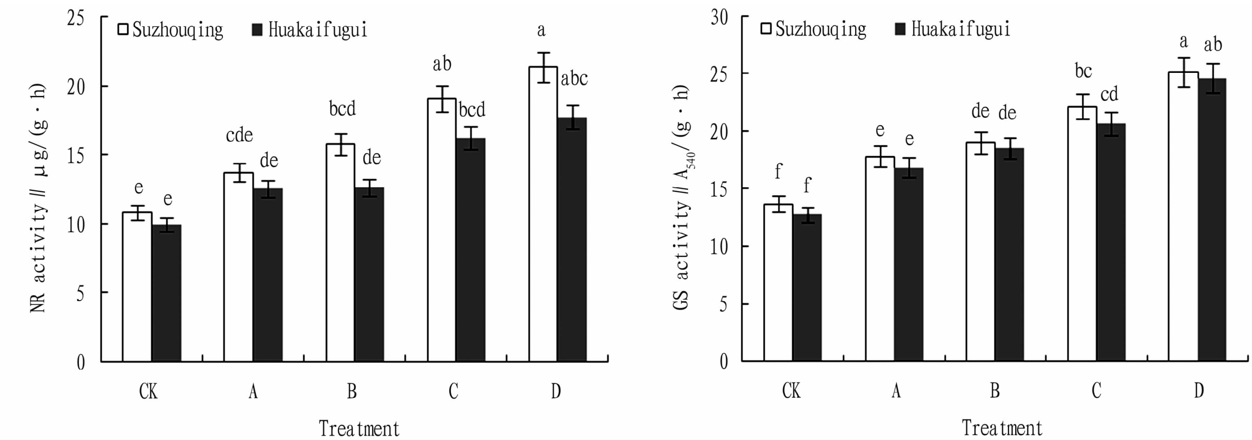

Fig. 1 Effects of amino polysaccharides on NR activity and GS activity in leaves of non-heading Chinese cabbage

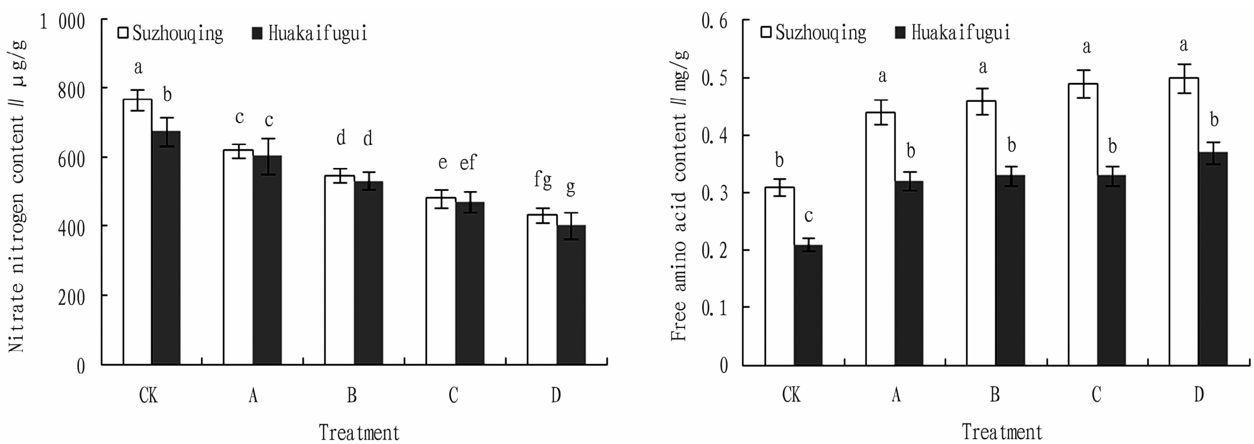

Fig. 2 Effects of amino polysaccharides on nitrate nitrogen content and free amino acid content in leaves of non-heading Chinese cabbage

Effects of amino polysaccharides on growth and quality indicators of non-heading Chinese cabbage

Chlorophyll content and plant dry weight Chlorophyll is essential for photosynthesis in plants and plays a significant role in enhancing the yield and quality of vegetables. As shown in Fig. 3, due to varietal differences, there were significant variations in chlorophyll a, chlorophyll b, and total chlorophyll content between the two non-heading Chinese cabbage varieties, Suzhouqing and Huakaifugui. The chlorophyll content of Suzhouqing was significantly higher than that of Huakaifugui. The amino polysaccharide solution also had a significant impact on the chlorophyll content of the two non-heading Chinese cabbage varieties. High concentrations of the amino polysaccharide solution (10 times and 100 times dilutions) significantly increased their chlorophyll content. Although the variation in chlorophyll a content in Huakaifugui was relatively small, the difference between treatment D and the CK still reached a statistically significant level.

As can also be seen from Fig. 3, there was a significant difference in the dry weight per plant between Suzhouqing and Huakaifugui. The dry weight per plant of Suzhouqing was significantly higher than that of Huakaifugui, and with the concentration of amino polysaccharides increasing, the dry weight showed a clear upward trend.

Vc content and soluble protein content As shown in Fig. 4, both the Vc content and protein content of Suzhouqing were higher than those of Huakaifugui. Foliar application of amino polysaccharide helped increase the Vc content and soluble protein content in non-heading Chinese cabbage. Compared with the CK, the Vc content in treatment A showed no significant change, while treatments B, C and D had significantly higher Vc content than the CK. No notable difference was found between treatments B and C. Different from the change of Vc content, after amino polysaccharide treatment, the soluble protein content of both varieties was significantly higher than that of the CK.

It could be concluded that foliar application of amino polysaccharides effectively promoted chlorophyll synthesis and dry matter accumulation in the leaves of non-heading Chinese cabbage, while increasing Vc content and soluble protein content. This demonstrated significant effects in promoting growth, enhancing yield, and improving quality, with particularly notable results observed at high concentrations of amino polysaccharides (100 times and 10 times dilutions).

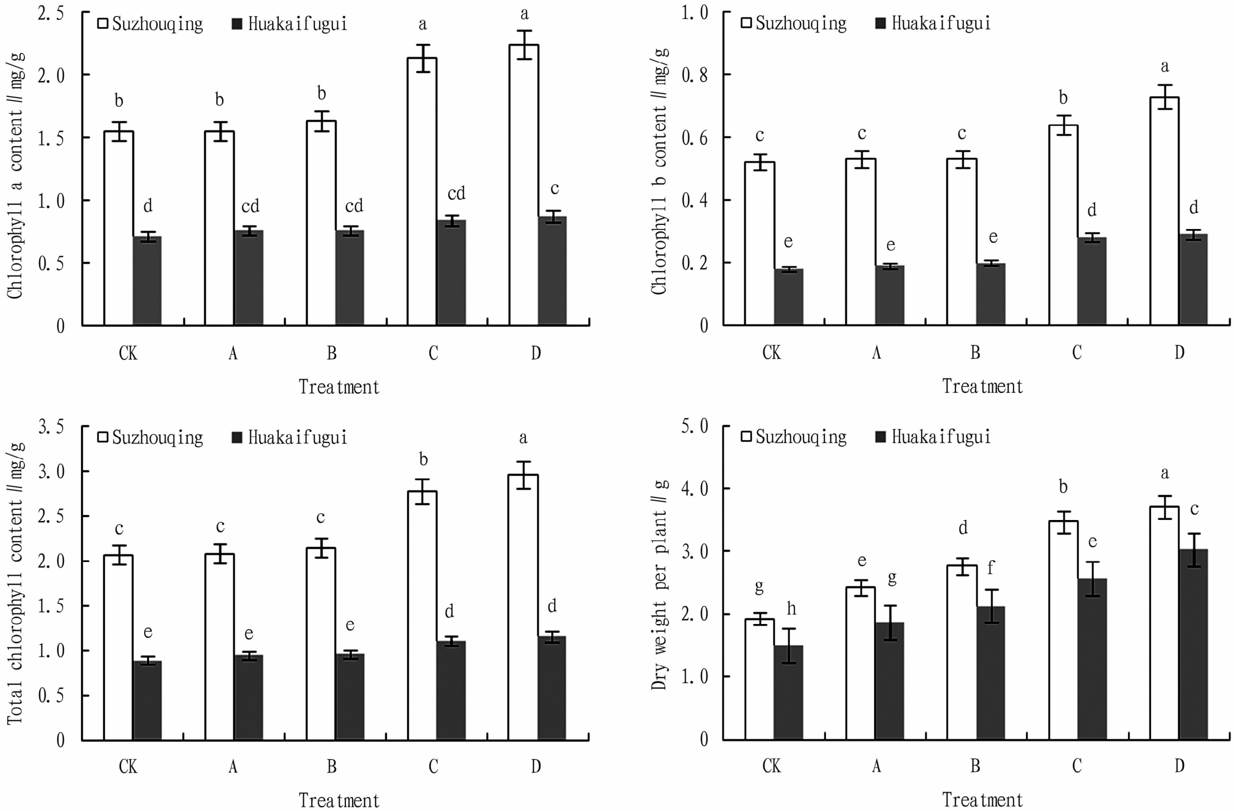

Fig. 3 Effects of amino polysaccharides on chlorophyll content and plant dry weight in leaves of non-heading Chinese cabbage

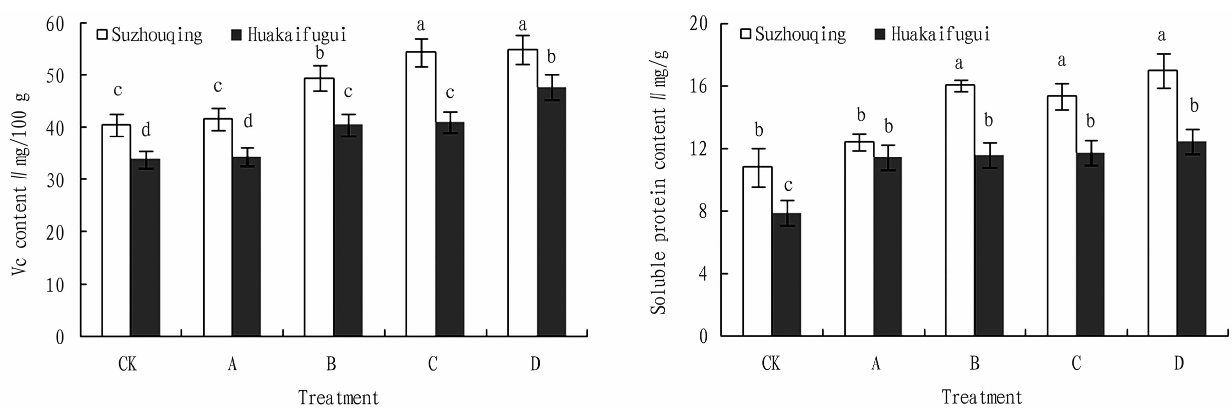

Fig. 4 Effects of amino polysaccharide on Vc content and soluble protein content in leaves of non-heading Chinese cabbage

Conclusions and Discussion

Nitrogen is one of the essential macronutrients for plant growth, playing a critical role in plant development, yield, and quality. Its contribution to final crop yield can reach as high as 40% – 50%[9]. Nitrate reductase (NR) is a key enzyme in the nitrate assimilation process in plants. The nitrogen assimilation capacity in plants is closely related to NR, and its activity level affects nitrate content in vegetables, thereby influencing plant growth, development, yield, and quality[10–11]. GS converts the reduced ammonia obtained from nitrate reduction in chloroplasts into amino acids and also catalyzes the synthesis of glutamine from glutamate, ammonia, and ATP. It is one of the key enzymes in nitrogen metabolism[12]. Its activity, along with total nitrogen content, chlorophyll content, and soluble protein content, can serve as indicators of nitrogen assimilation and recycling[13]. In this study, after treatment with amino polysaccharides, the activities of NR and GS in non-heading Chinese cabbage were significantly enhanced, free amino acid content markedly increased, while nitrate nitrogen content significantly decreased. These results indicated that amino polysaccharide treatment effectively improved nitrogen conversion and utilization efficiency in non-heading Chinese cabbage, demonstrating considerable potential for application in reducing nitrogen fertilizer application. Consistent with the conclusions of this study, Chen[14] reported that chitosan had a significant physiological regulatory effect on ammonia assimilation in non-heading Chinese cabbage. It could notably enhance the ability of non-heading Chinese cabbage to assimilate NH_4^+, promote amino acid formation, increase protein content, and improve the nutritional quality and traits of non-heading Chinese cabbage.

Amino polysaccharides have the effects of promoting plant growth, increasing chlorophyll content, enhancing stress resistance, and improving yield and quality[15-16]. Chang *et al.*[17] found that chitosan significantly increased the protein content in corn kernels, thereby providing material support for the physiological metabolism of corn. Ouyang *et al.*[18] suggested that chitosan could significantly improve the agronomic traits and quality of non-heading Chinese cabbage. Du *et al.*[2] demonstrated that amino polysaccharides could enhance the antioxidant capacity of wheat flag leaves, delay root system senescence, promote grain filling, and improve both grain yield and quality in wheat. Wang *et al.*[19] reported that amino polysaccharides increased the survival rate of parent Chinese cabbage plants after transplantation, boosted the activities of POD, SOD, and CAT in the leaves, and elevated the contents of chlorophyll, soluble sugars, and soluble proteins while significantly reducing MDA content. It also notably enhanced seed yield, thousand-grain weight of the parent plants, as well as the germination index and vigor index of the produced seeds. Furthermore, Wang *et al.*[20] also found that an appropriate concentration of amino polysaccharides effectively increased the pigment content in the leaves of fruit radishes, strengthened the material basis for photosynthesis, and enhanced the efficient absorption, utilization, and transfer of light energy by the leaves, thereby promoting the efficient operation of plant photosynthesis. In contrast, high concentrations of amino polysaccharides inhibited the increase in stomatal conductance and transpiration rate, reduced photochemical quantum efficiency and the primary light energy conversion efficiency, hindered the rise in leaf photosynthetic activity, and led to a significant decrease in net photosynthetic rate. Consistent with previous findings, in this study, it was concluded that 10 times and 100 times dilutions of amino polysaccharide solution could promote nitrogen absorption and conversion in non-heading Chinese cabbage, increase chlorophyll content and dry matter accumulation in the leaves, and elevate Vc and protein levels in the foliage. These treatments demonstrate significant improvements in growth, yield, and quality of the crop.

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