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Full Length Research Paper

The contribution of *bnnrt1* and *bnnrt2* to nitrate accumulation varied according to genotypes in Chinese cabbage

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Two Chinese cabbage cultivars (SYM and HGQGC) with significant difference in nitrate accumulation were used in the experiment by real-time PCR technique. The expression level of *BnNRT1* and *BnNRT2,* nitrate uptake rate and nitrate reductase activity (NRA) were detected under 0.2 mM and 2 mM NO_3^- treatments. The results showed that the nitrate accumulation in Chinese cabbage varied according to genotype, and high accumulator SYM got significant higher nitrate concentration and nitrate uptake rate than low accumulator HGQGC. The difference between cultivars became more obvious with high nitrate in growing medium especially in root; and the SYM was more sensitive to nitrate enhancement in growing medium than HGQGC. The different expression pattern of *BnNRT1* and *BnNRT2* may partly explain the different nitrate concentration between SYM and HGQGC. Under 2 mM nitrate treatment, *BnNRT2* may be the key factor resulting in higher nitrate concentration and higher nitrate uptake rate, in SYM than HGQGC. The higher nitrate accumulator SYM posses higher NRA than HGQGC, which means stronger ability to assimilate absorbed nitrate in SYM than the low accumulator, HGQGC.

Key words: Chinese cabbage, *NRT*, nitrate-transporter, genotypes-difference.

INTRODUCTION

Nitrate in vegetable is harmful to human health because of its poisonous deoxidize product nitrite. Previous reports have proved that 80% of nitrate in human body come from vegetable (Sharat et al., 1994). Many scientists studied the mechanism of nitrate accumulation in plant and found out the approach to reduce nitrate in vegetable since 1960's. Research reported that the nitrate in vegetable can be reduced by appropriate nitrogen fertilizer, harvest and preservation (Zerulla et al., 2001; Pasda et al., 2001). All these measures can keep the nitrate in vegetable to some extent, but cannot solve the problem radically. Most recently, the emphases of work have been transferred to screen out the low nitrate accumulator, and then, investigate the molecular mechanism of nitrate accumulation especially the difference in relation to genotypes, finding out the key gene and breeding new cultivars through molecular technology.

Uptake of NO_3^- in plants is mediated by *NRT1* (low-affinity transport systems, LATS) and *NRT2* (high-affinity transport systems, HATS) family of nitrate transporters in the plasma membranes of root cells (Tsay et al., 2007; Glass and Siddiqi, 1996). A gene considered to encode the LATS (*AtNRT1*, originally *CHL1*) was the first higher plant NO_3^- transporter gene to be cloned from Arabidopsis (Tsay et al., 1993). Then the *NRT* was cloned in tomato, rice and other plant one after the other (Lin et al., 2000; Lauter et al., 1996). Later report defined seven *AtNRT1* genes and four *AtNRT2* genes in *Arabidopsis* (Okamoto et al., 2003), and recent research suggested there were 53 *AtNRT1*genes and seven *AtNRT2* genes in Arabidopsis *thaliana* with different characteristic and functions in nitrate assimilation (Anabel et al., 2008). *AtNRT1.1* was found involved in both low-and high-affinity nitrate uptake

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Abbreviations: NRA, Nitrate reductase activity; **LATS,** low-affinity transport systems; **HATS,** high-affinity transport systems; **NRA,** nitrate reductase activity; **RT-PCR,** real time polymerase chain reaction.

(Liu et al., 1999), then *AtNRT1.2*, *AtNRT1.4,* and *AtNRT1.5* are pure low-affinity transporters (Huang et al., 1999). *AtNRT2.1* and *AtNRT2.2* are involved in the inducible phase of high-affinity nitrate uptake (Li et al., 2007) while *AtNRT2.7* is involved in seed nitrate storage (Chopin et al., 2007). Beside Arabidopsis, the *HvNRT2.1* characterized in *Xenopus laevis* oocytes were shown to be high-affinity nitrate transporters in barley (Tong et al., 2005; Siddiqi et al., 1990). *BnNRT1* and *BnNRT2* have been cloned in *Brassica napus* (Zhou et al., 1998; Sandrine et al., 2002; Antonin et al., 2008), but there are few reports on their expression pattern as well as their contribution to nitrate accumulation with relation to genotypes. Chinese cabbage *(Brassica campestris* ssp*. Chinensis (L) Makino)* is popular vegetable that tends to absorb nitrate, therefore, the reduction of its nitrate is important. Our research made two Chinese cabbage cultivars differ significantly in nitrate accumulation as materials. Focus was then on the different expression patterns of *BnNRT* in relation to genotypes and the role of *BnNRT* in nitrate accumulation in relation to different genotypes. Foundation for breeding low-nitrate cultivars was then made.

MATERIALS AND METHODS

Plant materials and growth conditions

Hydroponically grown Chinese cabbage plants (*B. campestris* ssp. *chinensis (L.) Makino)* were used for all experiments. The cultivars used in our experiment were HGQGC (low-accumulator) and SYM (high-accumulator) as shown in Table 1, which had been proved in our previous research (not published). Seeds were surface-sterilized in 1% NaOCl for 30 min, rinsed with deionized water several times and left to germinate at 26°C for 24 h in the dark. The germinating seeds were then placed into quartz sand in aperture disk. The aperture disk were placed in a controlled-environment growth room with a temperature of 25 ± 2°C, RH 70% and irradiance of 300 $Uem^{-2}s^{-2}$ under fluorescent lighting on a cycle of 14 h of light and 10 h of dark. Ten days later, the plant was transplant into Hoagland solution in plastic container, with cover board and the plant were placed in the hole on cover board by sponge. There were 36 holes on the cover board and 18 l Hoagland solution in plastic container. Generally, 1 l of nutrients solution was supplied in the morning per day and renewed every 3 days. The pH of growth media was maintained at 6.0 ± 0.5 by adding diluted NaOH or HCl once or twice daily. Fifteen-day-old cabbage plants were used in our experiment. The composition of Hoagland solution was as follows: $MgSO_4$ = 493 mgl^{-1}; KH_2PO_4 = 136 mgl^{-1}; NH_4NO_3 = 80 $mg{\cdot}L^{-1}$; KNO_3 = 506 mgl^{-1}; $Ca(NO_3)_2$ = 945 $mg{\cdot}L^{-1}$; $FeSO_4{\cdot}7H_2O$ = 5.57 mgl^{-1}; H_3BO_3 = 2.86 μgl^{-1}; $CuSO_4{\cdot}5H_2O$ = 0.08 μgl^{-1}; $ZnSO_4{\cdot}7H_2O$ = 0.22 μgl^{-1}; $MnCl_2{\cdot}4H_2O$ = 1.81 μgl^{-1}; $H_2MoO{\cdot}4H_2O$, = 0.09 μgl^{-1}.

Plants for detection of nitrate accumulation, nitrate reductase activity (NRA), *BnNRT1* and *BnNRT2*

Fifteen-day-old cabbage seedlings of uniform size were divided into two portions; one portion was grown hydroponically in revised Hoagland solution with 0.2 mM $NaNO_3$ as the only source of N, while the other portion was grown in revised Hoagland solution too, but containing 2 mM $NaNO_3$ as the source of N. Three replicates were arranged for each portion. Ten days later, the roots stem and leaves of the plants were harvested and frozen in liquid nitrogen, stored afterwards at -20°C for the detection of nitrate concentration and NRA. At the same time, the samples were stored at -80°C partly, for the determination of expression levels of *BnNRT1* and *BnNRT2*. The composition of revised Hoagland solution was as follows: $MgSO_4$ = 493 mgl^{-1}; KH_2PO_4 = 136 mgl^{-1}; KCl = 370 mgl^{-1}; $CaCl_2$ = 426 mgl^{-1}; NH_4Cl = 53 mgl^{-1}; $FeSO_4{\cdot}7H_2O$ = 5.57 mgl^{-1}; H_3BO_3 = 2.86μgl^{-1}; $CuSO_4{\cdot}5H_2O$ = 0.08 μgl^{-1}; $ZnSO_4{\cdot}7H_2O$ = 0.22μgl^{-1}; $MnCl_2{\cdot}4H_2O$ = 1.81μgl^{-1}; $H_2MoO{\cdot}4H_2O$, = 0.09μgl^{-1}; PH = 6.0 ± 0.5; nitrification inhibitor $C_2H_4N_4$ 5.89 mgl^{-1} was also added to prevent conversion of ammonium into nitrate by nitrification.

NRA and nitrate analysis

Each sample was extracted in duplicate in 4 ml phosphate (Ph 8.7, $C_4H_9NO_4S$ 1.211 gl^{-1}, ethylenediaminetetraacetic acid (EDTA) 0.327 $g{\cdot}L^{-1}$, Na_2HPO_4 8.8640 $g{\cdot}L^{-1}$, K_2HPO_4 0.0507 L^{-1}) at 4°C. The extracts were centrifuged at 4000 rpm for 15 min and supernatants assayed immediately. The reaction mixture contain 0.4 ml supernatants earlier describe, 1.2 ml 0.1M KNO_3, 0.4 ml 2 $g{\cdot}L^{-1}$ NADH. Assays were conducted at 27°C. The reactions were stopped after 30 min by the addition of 0.5 ml each of 1% sulphanilamide in 3 N HCl and 0.02% naphthyl ethylenediamine dihydrochloride and the absorbance at 540 nm were determined.

For nitrate analysis, sample 5.0 g and 2.5 ml saturated solution of $Na_2B_4O_7{\cdot}10H_2O$, were incubated in boiled water for 15 min. After cooling, 5 ml 0.25M $C_6FeK_4N_6{\cdot}3H_2O$, 5 ml 1M Zn $(CH_3COO)_2$ and 2 g active carbon were added, then the mixture were filtered and 10 ml solution were assayed by the addition of 2 ml 1M HCl and 0.1 ml 0.8% sulphanilamide, and the absorbance at 210 nm was determined.

Total RNA extraction, cDNA synthesis, preparation of primers and verification of RT-PCR products

Total RNA samples were isolated using the guanidine isothioyanate method. 5 μg of total RNA was used to synthesize cDNA by reverse transcriptase powerscript™ following the manufacturer's protocol. The cDNA samples were used as template to quantify the target gene expression levels. We designed the primers for each gene according to information in GenBank database, (NCBI/GenBank accession number AJ278966 for *BnNRT1*, AJ293028 for *BnNRT2*). The products of cDNA segments for *BnNRT1* and *BnNRT2* were 1155 ~ 1223 and 1162 ~ 1224 on full length cDNA sequence, and the PCR product sizes for the two genes were 69 and 63 bp, respectively. The specific primers in Real-time PCR for the two genes were as follows: for *BnNRT1* forward: 5'- CTATATCGGTGG CCTCCTCCTA-3' and reverse: 5'- AGCTTTTTGCATAAGGGAA TCG-3'; for *BnNRT2* forward: 5'- GGAGCACAAGCCGCTTGT-3' and reverse: 5'- AAGGGCTCGCCGAGAAAC-3'; and for *18s rRNA* forward: 5'- AAACGGCTACCACATCCA-3' and reverse: 5'- CACCA GACTTGCCCTCCA-3'. The taqman probe used in real-time PCR for *BnNRT1*is fam + CCACCGCCGTCTACGACCGTCTC + tamra; for *BnNRT2* is fam + AGCCACCTTCGCAATCGTTCCCTT + tamra, and for 18s rRNA is fam + AGCAGGCGCGCAAATTACC + tamra. All specific RT-PCR products were cloned, sequenced and compared with the sequence of the respective *BnNRT* genes to confirm the specificity of the RT-PCR products.

Treatments of plants for determination of the rate of nitrate absorption

Fifteen-day-old cabbage plants were cultivated in Hoagland solution for another 10 days, and then the 25-day-old plants were

Table 1. The different nitrate concentration in Chinese cabbage with different genotypes.

Tissue	0.2 mM NO_3^- treatment		2 mM NO_3^- treatment	
	HGQGC	SYM	HGQGC	SYM
Root	9346.0±820 c A	19814±952 b A	6299.9±430 d C	25509±1899 a A
Leaves	6574.6±503 c B	6417.9±600 c C	8886.7±739 b B	17194±1221 a B
Stem	6902.6±625 c B	9852.8±935 b B	10025±1001 b A	13724±1165 a C

Two Chinese cultivars HGQGC and SYM were cultured in Hoagland solution with 0.2 and 2 mM NO_3^- respectively after 15 days growing, and the nitrate concentration (mg NO_3- kg-1 fr wt) in root, leaves and stem were measured ten days later. Each value in the table was an average of three replicates ± SE (standard errors); the difference in lowercase letters following the data in the same row indicates significant difference among the treatments or cultivars at 5% level; and the difference in capital letters in the same column indicates significant difference among tissues at 5% level.

transplanted in Hoagland solution without nitrogen supplied for 48 h. After nitrogen starvation, the plants of uniform size were divided into four groups and provided 30 ml absorption solution: 0.2, 2, 1, 5, 10 and 20 mM KNO_3 with 0.2 mM $CaSO_4$ as solvent, respectively. The total weight of plant and solution was weighed quickly, and then place into controlled-environment growth room as earlier described. Five hours later, the total weight of plant and solution were weighed quickly again, then the plant was collected for root weighting and sampled the solution to determine the concentration of KNO_3 remaining. The rate of nitrate absorption was calculated as according to the follows:

$$V = \{C1 \times 30 - C2 \times [30 - (M1 - M2)]\} / M3$$

Where, V = nitrate uptake rate; 30 = volume of absorption solution; C1 = nitrate concentration in solution at the beginning; C2 = concentration of nitrate in absorption solution after 5 h; M1 = total weight of absorption and plant at the beginning; M2 = total weight of absorption and plant after 5 h; M3 = weight of plant root.

Statistical analysis

Statistical analysis was conducted using Excel software (Microsoft Office Excel 2003). The mean comparison was calculated according to the Duncan multiple range test using the statistical analysis system (SPSS 10.0).

RESULTS

The different nitrate accumulation between SYM and HGQGC

SYM had higher significant difference in nitrate concentration under 2 and 0.2 mM NO_3^- treatment in root, leaves and stem than HGQGC except for leaves with 0.2 mM NO_3^- treatment (Table 1). The data in Table 1 suggested that the difference between cultivars was enlarged with higher NO_3^- supply treatment. Significant differences exist between SYM and HGQGC in root, leaves and stem with 2 mM NO_3^- treatment only. According to the absolute value of nitrate concentration, SYM is 4.05, 1.94 and 1.37 times as high as HGQGC in root, leaves and stem under 2 mM NO3- treatment, while only 2.12, 0.97 and 1.43 under 0.2 mM NO_3^- treatment, and the difference between cultivars in root is the most remarkable. All previous report proved that the concentration of nitrate in SYM is higher than HGQGC, especially in root or in high-nitrate-growing medium.

When the NO_3^- in growing medium increased from 0.2 to 2 mM, the NO_3^- concentration in root, leaves and stem was increased by -32%,35% and 45% in HGQGC while 29, 168 and 39% in SYM; that means that the SYM was more sensitive to nitrate content enhancement in growing medium than HGQGC, especially in leaves (Table 1).

According to the different tissue in plant, we notice that the root gets higher concentration in SYM under 0.2 or 2 mM nitrate supply, while in HGQGC, the tissue accumulate nitrate from root to stem; following the nitrate supplied, it increases from 0.2 to 2 mM. It indicates that the HGQGC transfer more absorbed nitrate to plant above ground than SYM (Table 1).

The different nitrate uptake rate between SYM and HGQGC

When the nitrate concentration in growing medium varied from 0.2 to 20 mM, the rate of nitrate uptake is higher in SYM than HGQGC (Figure 1). This is corresponding to the higher nitrate concentration in SYM as shown in Table 1 and there is the same tendency that the difference between SYM and HGQGC is enhanced by the higher nitrate concentration in the growing medium. The nitrate uptake rate of SYM is higher than HGQGC significantly at 1% level of probability when supplied with 2, 5, 10 and 20 mM nitrate (Figure 1). We focus on the nitrate uptake rate when treated with 0.2 and 2 mM nitrate solution; the value is 1.56 and 12.98 for HGQGC, and 4.19 and 36.31 for SYM, respectively. Totally, the uptake rate of SYM is 2.68 and 2.80 times of HGQGC.

The expression *BnNRT1* and *BnNRT2*

In our result, the gene BnNRT1 and BnNRT2 was 99.6 and 99.2% homologous to AJ278966 and AJ293028, respectively. These confirm the exact genes of *BnNRT1* and *BnNRT2* detected by Real-time PCR technique. Our result showed the different express patterns of *BnNRT*

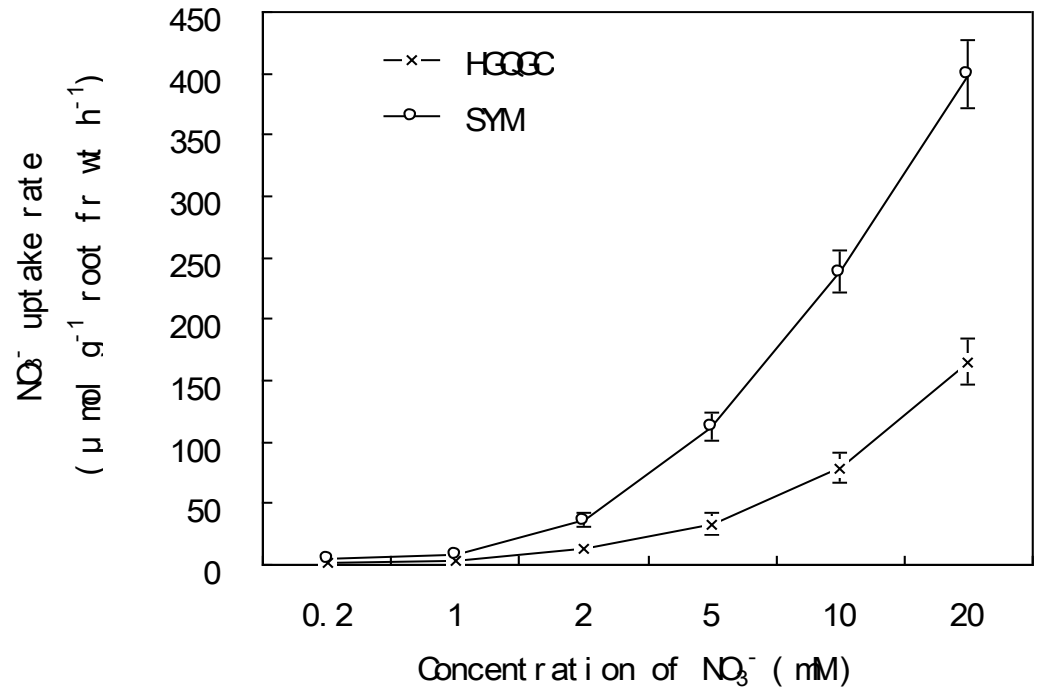

Figure 1. The different nitrate uptake rate of the two Chinese cabbage cultivars. After 48-h nitrogen starvation, 25-day-old Chinese cabbage young plants were exposed for 5 h to different NO_3^- nutrient solutions. The mean values and standard errors are presented (n = 3).

between different cultivars (Figure 2).

With 2 mM nitrate treatment, the expression of *BnNRT2* in root, leaves and stem was higher in SYM than HGQGC significantly at 5% level of probability (Figure 2D, E and F) and this corresponded to the higher nitrate concentration and higher nitrate uptake rate in SYM than HGQGC (Table 1 and Figure 1). While the expression of *BnNRT1* is higher significantly in SYM than HGQGC only in root, no significant difference between SYM and HGQGC in leaves or stem was observed. The different expression patterns of *BnNRT1*and *BnNRT2* may mean that the higher nitrate concentration of SYM in root, leaves and stem can be mainly attributed to the higher expression gene *BnNRT2* and partly because of the *BnNRT1* in root. Meanwhile, we cannot ignore the contribution of *BnNRT1* in leaves and stem, which is 32.58 and 16.54% higher in expression level in SYM than that in HGQGC, respectively.

With 0.2 mM nitrate treatment, the same expression patterns for *BnNRT1*and *BnNRT2*, that is the expression of *BnNRT1*or *BnNRT2* was not significantly different between SYM and HGQGC in root, while higher significance in SYM than HGQGC in leaves or stem were observed. We noticed that the expression patterns of *BnNRT1* and *BnNRT2* all disagree with the nitrate concentration in SYM and HGQGC except for stem (Table 1 and Figure 2). Apparently, there maybe some gene involved and dominated in nitrate absorption when supplied with 0.2 mM nitrate treatment especially in root and leaves.

The NRA in SYM and HGQGC

The nitrate reductase is the key factor in nitrate accumulation, and in most case the concentration of nitrate in plant tissue lies on NRA. In our result, the NRA in SYM is significantly higher than that of HGQGC in root, leaves and stem independent of the different tissue and nitrate concentration in growing medium (Figure 3). The high NRA in SYM means strong ability to utilize the absorbed nitrate.

DISCUSSION

The expression of *BnNRT* in SYM and HGQGC

Nowadays, researches on *NRT* gene family mainly focused on the model plant such as rice and Arabidopsis, and most researches emphased on the express patterns and gene function (Orsel et al., 2006; Fan et al., 2009). Before now, genes of *NRT* family were not clearly known and much less reports about the genotype different in *NRT* characteristic were available. In our research, the expression of *BnNRT2* in high nitrate accumulator SYM was higher significantly than that in lower accumulator HGQGC with 2 mM nitrate treatment, and this was supported with the higher nitrate concentration in SYM than HGQGC (Figure 2 and Table 1), and the higher nitrate uptake rate as shown in Figure 1. We thus confer that

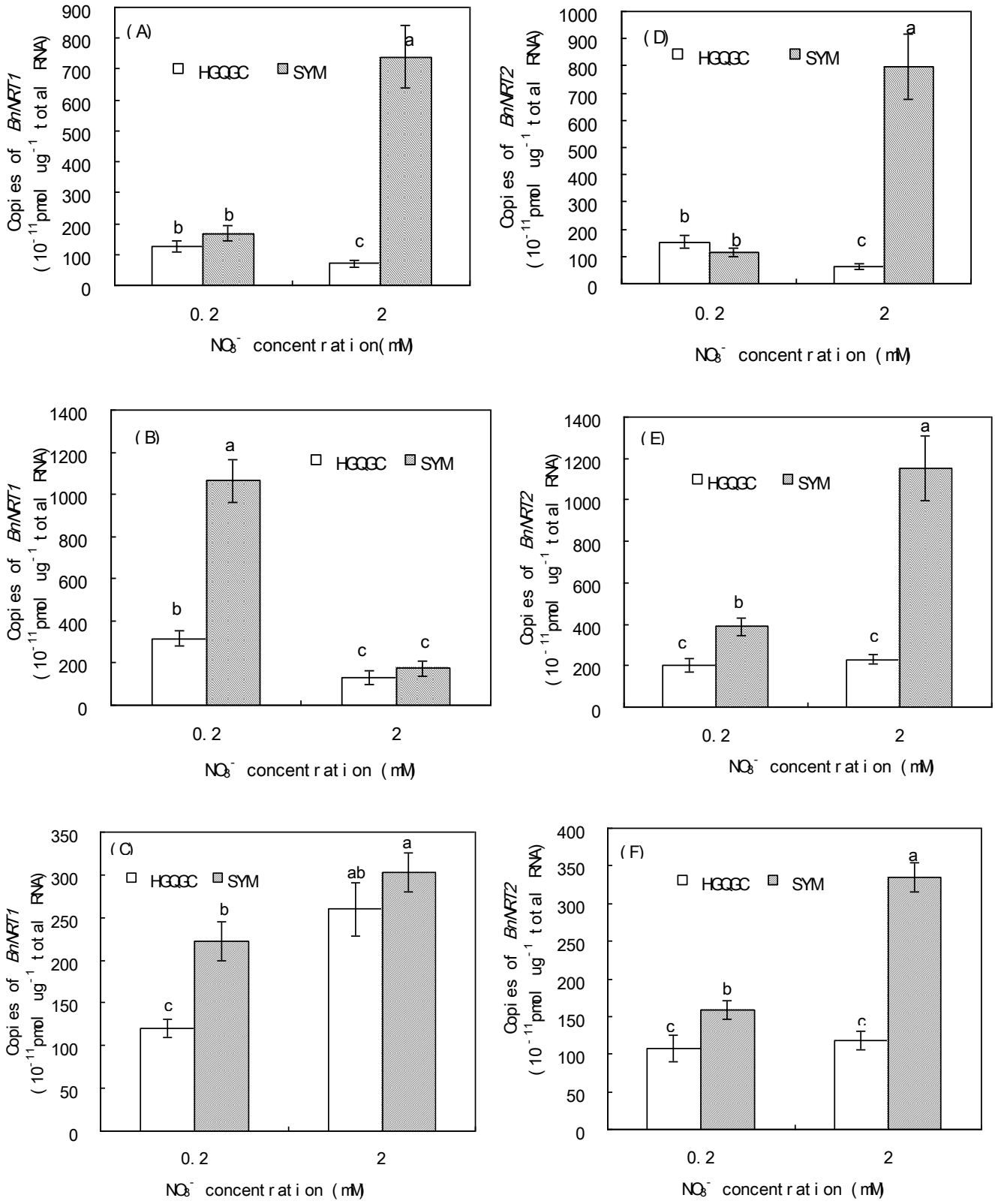

Figure 2. The expression levels of *BnNRT1* and *BnNRT2* in root (A and D), leaves (B and E), and stem (C and F) in Chinese cabbage. 15-day-old Chinese cabbage young plant were cultured in nutrient solution with NO_3^- 2 and 0.2 mM respectively, and 10 days later the expression level of *BnNRT1* and *BnNRT2* were detected by real-time PCR. The different letters on the bar indicate the significant difference at 5% level of probability; the line on the bar means standard errors and each bar was the average of three replicates.

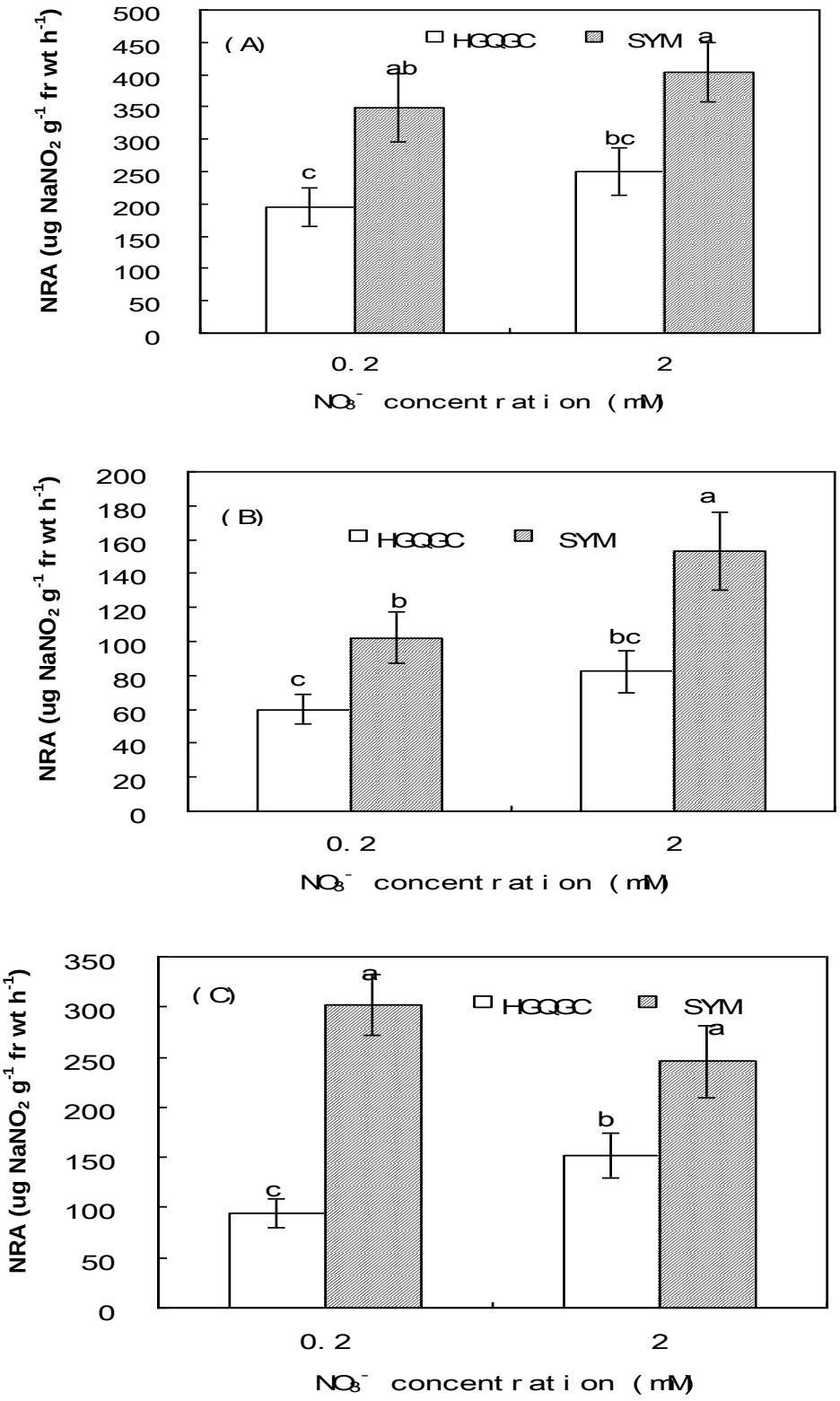

Figure 3. Comparison of nitrate reductase activity (NRA) in root (A), leaves (B), and stem (C) between different genotypes of Chinese cabbage cultivars. 15-day-old Chinese cabbage young plant was cultured in nutrient solution with NO_3^- 2 and 0.2 mM, respectively for 10 days. The nitrate reductase activity was detected. The different letters on the bar indicate the significant difference at 5% level of probability; the line on the bar means standard errors and each bar was the average of three replicates.

BnNRT2 may be the reason for higher nitrate concentration in SYM. At the same time, the express level of *BnNRT1* was higher in SYM than HGQGC significantly at 5% probability level only in root (Figure 2A). Considering the different significant characteristic between *BnNRT1*and *BnNRT2*, we suggested that *BnNRT2* may be the dominant factor in higher nitrate concentration in SYM than HGQGC with 2 mM nitrate treatment especially in leaves and stem, while *BnNRT1* may play partial role in root.

Difference occur when treated with 0.2 mM nitrate in growing medium, the *BnNRT1*and *BnNRT2* posses the same expression patterns, that is the expression levels of *BnNRT1* and *BnNRT2* was significant higher in SYM than HGQGC in leaves and stem (Figure 2), which did not correspond to the different nitrate concentration in root and leaves as showed in Table 1. Many researches have shown that there are several genes involved in nitrate accumulation in plant (Anabel et al., 2008; Tsay et al., 2007), such as the *NAR* gene in barley and Arabidopsis among others (Tong et al., 2005; Okamoto et al., 2006). Researches have also shown that there are not only one member in the *NRT1* or *NRT2* family in plant. For instance, in Arabidopsis there are 53 *AtNRT1* members and 7 *AtNRT2* members which differ in function as well as in nitrate absorption and assimilation (Anabel et al., 2008). In our experiment, it is possible that the *BnNRT1* and *BnNRT2* were not all the reason for higher nitrate concentration in SYM with 0.2 mM nitrate treatment at least in root and leaves. Maybe there were other unknown key genes involved in nitrate accumulation in Chinese cabbage and these need further research. In the present paper, focus was on only the cloned gene in Chinese cabbage (Sandrine et al., 2002; Antonin et al., 2008).

Considering the nitrate uptake rate, Figure 1 shows that the SYM posses higher nitrate uptake rate than HGQGC especially in high NO_3^- solution. In root, only with 2 mM NO_3^- treatment, the expression levels of *BnNRT1*and *BnNRT2* were significantly higher in SYM than HGQGC (Figure 2A and D), which led to the significant higher nitrate uptake rate and nitrate concentration in root (Figure1 and Table 1). The above proved the role of *BnNRT2* in higher nitrate absorption and accumulation in SYM than HGQGC including the partial contribution of *BnNRT1* in root when treated with 2mM NO_3^-. What is noticeable is that, we defined the role of *BnNRT1*and *BnNRT2* in nitrate accumulation relation to different genotype only through nitrate concentration, absorption rate and gene expression level determined by real-time PCR technique and the affirmation of gene function still need more deep research.

The NRA in SYM and HGQGC

Despite many document about the toxicity of nitrate in vegetable, previous research reported that the deposition of nitrate in plant vacuole could be a means of storage for nitrogen required during nitrogen deficient circumstance (Ferrari et al., 1973). The nitrate in vacuole can be transported to cytoplasm and participate in nitrogen assimilations, and this is very important for plant growing normally as it helps to keep the balance of nitrogen assimilation (Jackson and Volk, 1981). However, the toxicity of nitrate is controversial while nitrite toxicity is well accepted by researchers. Nitrate mainly lies in the vacuole where there is no nitrate reductase, and thus, only in cytoplasm can nitrate cn be reduce to nitrite by NR (Heimer and Filner, 1971). The control of nitrite in plant should be limited to the process of nitrate reductive reaction by NR to nitrite in the cytoplasm.

Our data showed that the SYM posses higher nitrate reductase activity (NRA) than HGQGC in plant tissue (Figure 3) which means that there is higher efficiency in nitrate assimilation in SYM than HGQGC. Many researchers reported that the imbalance of nitrate and NRA was the main reason for nitrate accumulation (Datta and Sharma, 1999) with the high accumulator possessing lower NRA and higher nitrate concentration than low accumulator, and eventually accumulate more nitrate (Scheible et al., 2000; Matt et al., 2001a). Different observations were made in the present study, the high accumulator, SYM, got higher nitrate concentration and higher NRA than HGQGC (Table 1 and Figure 3). The difference from previous research may be partial due to the different cultivars used in the experiments and may be partial because of the super-high nitrate concentration in SYM. With 2 mM nitrate treatment, the nitrate concentration in root, leaves and stem were 4.05, 1.93 and 1.37 times in SYM as high as that in HGQGC, respectively, and the NRA was 1.61, 1.68 and 1.62 times, respectively (Table 1). Maybe the super-high nitrate concentration killed the effect of high NRA in SYM, and the NRA was not high relative to their super-high nitrate concentration in SYM, while the NRA was high relative to their low nitrate concentration in HGQGC relatively. Although, the absolute value of NRA was higher in SYM than in HGQGC, it could not change the higher concentration of nitrate accumulation in SYM which is as a result of the significant stronger ability to absorb nitrate from the growing medium (Figure 1). Another possible reason for this was the inducible characteristic of NRA (Hoff et al., 1994) as higher nitrate concentration lead to the higher NRA in SYM than HGQGC. Based on the present result, we suggest that the NRA is not the reason of nitrate accumulation but could be the combination of NRT and NRA which resulted in the different nitrate accumulation among cultivars.

Conclusion

The nitrate accumulation in Chinese cabbage varied in

relation to genotype, and high accumulator SYM gets significant higher nitrate concentration and nitrate uptake rate than HGQGC. Under 2 mM nitrate treatment, *BnNRT2* may be the key factor for higher nitrate concentration, higher nitrate uptake rate in SYM than HGQGC. The higher nitrate accumulator SYM posses higher NRA than HGQGC, which means stronger ability to metabolize absorbed nitrate in SYM than the low accumulator, HGQGC.

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