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ORIGINAL ARTICLE

Effects of external supplied sucrose on the uptake and metabolism of glycine by pakchoi (*Brassica chinensis* L.)

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Abstract Sucrose is crucial for plant growth, but research about the effect of sucrose on the uptake of different nitrogen (N) and the metabolism of glycine is lacking. The uptake of glycine by pakchoi, when it was in a mixture of glycine, nitrate, and ammonium or acted as the single N source under different sucrose levels were detected in a sterilized environment. The optimal sucrose level for pakchoi growth varied with N supply; it was 6 μM in the single N source of glycine, while it was 15 μM in the mixture. The N contribution of glycine increased under the optimal sucrose, while the N contribution of ammonium decreased. The effect of exogenously supplied sucrose on the uptake and metabolism of glycine is dependent on the N supply. With the single N source, the metabolism of glycine to serine in roots rather than uptake was the limiting step under a high sucrose level (300 μM). In the mixed nitrogen, active uptake and the metabolism of glycine to serine are the limiting steps under high sucrose level. Externally supplied sucrose affects the absorption and metabolism of glycine by pakchoi greatly, and this effect varied with nitrogen supply.

Keywords Sucrose · Nitrogen · Glycine: uptake and metabolism · *Brassica chinensis* L.

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Introduction

Nitrogen is the principal limiting micronutrient for plant growth. Synthetic N fertilizers are widely used in the form of urea, ammonium, and nitrate. Though they remove the N limitation in plants, they increase the reactive N levels in the biosphere significantly, which results in severe environmental problems. In China, a 37-fold increase of N fertilizer just achieved 3.4-fold increase in Chinese agricultural food production, but the environmental cost has been very high (Zhang et al. 2012). Numerous data from individual sites and long-term monitoring have shown that the soil pH declined significantly in major Chinese croplands from the 1980s to the 2000s and that the overuse of N fertilizers has contributed to it substantially (Guo et al. 2010). Nitrate and ammonium are the most important inorganic N sources used in agriculture and are studied in depth. In addition to inorganic N, organic N can be absorbed by plants directly, bypassing the decomposition by microorganisms, which includes amino acids, quaternary ammonium compounds, nucleic acids, and proteins (Chanyarat et al. 2008; Torgny et al. 2009; Warren 2013, 2014). Amino acids act as the most important sources of organic N because of their high concentration in the

soil and bioavailability for plant growth (Jones et al. 2005; Warren 2014). Organic N may be a choice for agriculture and ecosystem services to improve nutrient use efficiency while reducing environmental risk and increasing crop productivity.

Sugars are important in plant biochemical and physiological process (Ruan 2014). Sucrose is the preferred photosynthate for phloem long-distance translocation (Bloom 2015); it not only functions as a carbon source for building blocks and energy but also important in long-distance signaling to coordinate plant growth (Mason et al. 2014). Many sugars have been detected in the xylem sap (Francesca and Zwieniecki 2012), which indicates the up-transportation of sugars from the roots to shoots. Moreover, lower sucrose (29 mM) and glucose (55 mM) were found to increase the growth of *Arabidopsis thaliana*, while a high concentration inhibited the growth severely (Schofield et al. 2009). For example, seedling growth on agar is inhibited by high levels of sugars, particularly by 263 mM sucrose (Francozorrilla et al. 2005) or 333 mM glucose (Bi et al. 2005).

Carbon (C) and N assimilation are vital processes for plant growth, and they interact with each other. Carbon skeletons are necessary for the assimilation of inorganic N to amino acids, which are further converted to proteins and nucleic acid, and therefore N uptake is closely connected with the status of sugars (Ruffel et al. 2014). Furthermore, N assimilation is an energy consuming process (Bloom 2015), i.e., enhancing C supply increases N uptake, and vice versa (Cross et al. 2007; Schofield et al. 2009). Studies have shown that C signaling pathways regulates N-responsive genes (Gutiérrez and Crawford 2007; Nero et al. 2009). This suggests that the reaction of plants to external N availability is closely related to C acquisition. For example, sugars increase the translation and activity of nitrate reductase and reduce its degradation (Cross et al. 2007). Sugar supply alters the expression of vast genes involved in nitrate uptake, ammonium metabolism, amino acid and protein synthesis (Bläsing et al. 2005). However, the signaling mechanisms between N and C remain mostly obscure (Ruffel et al. 2014). Better understanding of these interconnections may help regulate the nutrient, especially N uptake, and improve the crop quality.

However, detailed information on how different concentrations of sucrose affect the absorption of ammonium, nitrate, and amino acids is lacking. Noteworthy, amino-N is the required state for plant metabolism, which saves the energy needs of anabolic metabolism compared to ammonium and nitrate, and the "byproduct" of amino-C can reduce the supply of C skeleton from photoassimilates, which results in lower construction costs for plants growth (Zerihun et al. 1998). Considering the great difference between organic N and inorganic N, externally supplied sucrose may have a different effect on amino acid uptake and metabolism.

Pakchoi is an important vegetable in China, which is cultivated over a large area. Amino acids can be decomposed by microorganisms very fast, so we cultivated pakchoi seedlings under a sterilized environment. ^{15}N-labeling was used to detect (1) how different concentrations of sucrose affected pakchoi growth, (2) how externally supplied sucrose affected the N contribution of glycine, nitrate, and ammonium, and (3) how externally supplied sucrose affected the N contribution of amino acids from the perspective of uptake and metabolism.

Materials and methods

Pakchoi was cultured in a sterile environment as described by Ma et al. (2016). Briefly, pakchoi seeds were soaked for 24 h in purified water, and sterilized by the procedures described in Wu et al. (2005). Then, they were placed in sterilized culture dishes for 3 days, with 12-h light (360 μmol m^{-2} s^{-1}), a day/night temperature of 25/20 °C, and humidity of 60/40%. Later on, one seedling was planted in a 50 mL centrifuge tube fulling with 0.3% cooling-off agar, and placed in a sterilized culture room. One day later, the seedlings grew out from the holes on the tube cap, and silicone rubber (Nanda 704) was used to seal the hole. Then, the seedlings were transferred together with the tube cap to a new centrifuge tube filling with culture solution; this was defined as day 1 for each experiment. Nitrogen mixtures in different experiments were added to the nutrient solution before use and sterilized by passing through the 0.22 μm membrane filter (Millipore, PES Membrane, Ireland). The materials and culture solution without N were sterilized by steam under high pressure of 121 °C for 30 min. The culture solution was replaced every 3 days in a clean bench.

Experiment 1: effect of sucrose on pakchoi growth and N uptake

The seedlings were precultured as stated above with 3 mM mixed N source (1 mM ammonium + 1 mM glycine + 1 mM nitrate) for 6 days; then, 48 similar seedlings were chosen. The concentration of sucrose was 0, 1, 3, 15, 30, 300, 3,000, and 15,000 μM, and it was added to the nutrient solution with N and sterilized by passing through the membrane filter. Then, the seedlings were cultured in the same environment and N supplies for 15 days as stated above, and the solution was changed every 3 days. Then, the roots and shoots were separately harvested; the roots were washed by an ultrasonic cleaner for 1 min, followed by 50 mM $CaCl_2$, and washed three times by purified water. The above-ground and root parts were freeze-dried (Labconco Freezen System, USA) and a ball mill was used

to ground them to a fine powder (Retsch MM301, Germany). The N contents were determined by the Micro-Kjeldahl method (six replicates for each treatment).

Meanwhile, 63 similar seedlings precultivated for 18 days were selected, and the roots and the centrifuge tube were washed with purified and sterilized water. Then, the pakchoi seedlings were cultivated with 1 mM 10.0% ^{15}N-glycine under 0, 0.3, 0.9, 2.7, 6, 15, 30, and 300 μM sucrose for 8 days. Then, the roots and shoots were separately harvested, and three pakchoi seedlings were combined to one sample to reduce the differences in plants, and three replicates were done for each treatment. Pakchoi roots were washed, roots and shoots were dried and ground as described above, and the N content and ^{15}N enrichment of the powder were detected by an elemental analysis-stable isotope mass spectrometer (IsoPrime100, UK). Pakchoi growth were retarded or death under higher sucrose level (>3000 μM) with a single N source of glycine, so we set up different sucrose level according to the N supply in this part. For each treatment, additional three "blank" seedlings were reserved by supplying unlabeled N at the same composition with the ^{15}N-treated seedlings in Experiment 1, 2, 3, and 5.

Experiment 2: effect of sucrose on the N contribution of nitrate, glycine, and ammonium

Experiment 1 showed that externally supplied sucrose changed the growth and N uptake of pakchoi, whether it changed the relative uptake of different N? Pakchoi was precultivated as stated in Experiment 1 for 6 days, and 81 similar seedlings were picked. The sucrose concentration was 0, 15 (optimal concentration for pakchoi growth under mixed N), and 300 μM (over-high concentration inhibited pakchoi growth), meanwhile, three N mixtures were prepared at a sucrose level. The three N mixtures were same in composition (NO_3^-/NH_4^+/glycine (in mM) = 1:1:1), but only one N form was labeled with ^{15}N (5.0% $^{15}NH_4^+$, 5.0% $^{15}NO_3^-$, or 5.0% ^{15}N-glycine). There were nine treatments (3 N × 3 sucrose), the cultivation solution was changed every 3 days, and pakchoi was harvested on day 21. The roots and shoots were separately harvested. Three seedlings were combined into one sample, and each treatment had three replicates. The samples were treated and the ^{15}N contents were determined as stated in Experiment 1.

Experiment 3: effect of sucrose on glycine short-term uptake

Experiment 1 and 2 showed that the uptake of glycine was increased under low concentration of sucrose while decreased under high sucrose levels in the single or mixed N sources, whether this results was caused by root uptake? Pakchoi were precultivated for 25 days, and 180 homogeneous seedlings were picked for the short-term test. The centrifuge tube and seedling roots were washed with purified, sterilized water. The seedlings were "hungrily" cultivated for one night deprived of N, after which the short-term test was performed. Forty-five seedlings were cultivated under 1 mM 98.10% ^{15}N-glycine at 0, 6, and 300 μM sucrose for 4 h (360 μmol m^{-2} s^{-1} light, 60% humidity, 25 °C). Another 45 seedlings were cultivated under 1 mM 98.10 ^{15}N-glycine + 1 mM NH_4^+ + 1 mM NO_3^- with 0, 15, and 300 μM sucrose for 4 h. The optimal sucrose concentration was 6 μM for pakchoi growth under a single N source of glycine, while it was 15 μM under the mixed nitrogen; we set the sucrose concentration by N sources.

Furthermore, the protonophore carbonyl cyanide m-chlorophenylhydrazone (CCCP) (Persson and Näsholm 2002) was applied to determine the effect of sucrose on the passive and active absorption of glycine. Ninety pakchoi seedlings were "hungrily" cultivated and pretreated with CCCP (50 μM) for 1 h. Then, 45 pakchoi seedlings were cultivated under 1 mM 98.10%-^{15}N labeled glycine at 0, 6, and 300 μM sucrose for 4 h. Another 45 seedlings were supplied with 1 mM 98.10% ^{15}N-glycine + 1 mM NH_4^+ + 1 mM NO_3^- at 0, 15, and 300 μM sucrose for 4 h as described previously. The uptake test of the CCCP-treated and untreated seedling was conducted simultaneously. The shoots and roots were separately harvested; three seedlings of one treatment were combined, and the samples were detected as stated in Experiment 1.

Experiment 4: effect of sucrose on N metabolism enzymes activity

After root uptake, glycine was metabolized by several enzymes, whether the process of glycine metabolism changed the N contribution of glycine? Ninety pakchoi seedlings were precultured for 22 days, washed, and "hungrily" cultivated for one night as described in Experiment 3. Later, 45 seedlings were cultivated by 1 mM glycine with 0, 6, and 300 μM sucrose for 4 days. Another 45 seedlings were cultivated with 0, 15, and 300 μM sucrose in a mixed N source of 1 mM glycine + 1 mM NH_4^+ + 1 mM NO_3^-. Then, the activities of glutamic oxalocetic transaminase (GOT), and glutamic-pyruvic transaminase (GPT) (Lianghuan et al. 1998), glutamine synthetase (GS) (Horchani et al. 2010) in the roots and leaves were measured.

Experiment 5: effect of sucrose on the metabolism of glycine

Glycine as the single N, glycine uptake was inhibited in the long-term uptake test (Experiment 2) under 300 μM

sucrose, but in the short-term test (4 h), the uptake of glycine was significantly higher compared with 6 μM sucrose, which indicating that root metabolism rather than root uptake inhibits the N contribution of glycine. In the mixed N, glycine uptake in the short-term uptake test was inhibited, which is consistent with the short-term test, which indicating that root uptake limits glycine N contribution, but whether glycine metabolism limits its N contribution and which glycine metabolism process was inhibited under high sucrose level? To research the mechanism of high sucrose level on glycine metabolism, ^{15}N labeling and gas chromatography mass spectrometry (GC–MS) were applied to determine the ^{15}N labeled amino acids in pakchoi roots and shoots. One hundred and twenty similar seedlings were picked after precultured for 25 days as stated in Experiment 1, and the roots were washed with sterilized and purified water several times, then, the seedlings were "hungrily" cultivated for 12 h. Later, 60 seedlings were cultivated with 1 mM 98.1% ^{15}N-glycine under 6 μM and 300 μM sucrose for 12 h, and another 60 seedlings were cultivated with ^{15}N-glycine + 1 mM NH_4^+ + 1 mM NO_3^- under 15 μM and 300 μM sucrose for 12 h (360 μmol m^{-2} s^{-1} light, 60% humidity, 25 °C). Subsequently, the shoots and roots of pakchoi were separately harvested, five seedlings in a treatment were combined to a sample, and each treatment possess six replicates. The shoots and roots were dried and ball milled as stated above. The ^{15}N labeled amino acids were detected by GC–MS as stated by Thornton and Robinson (2005) with little modifications. In brief, 20 mg root or shoot samples were extracted for 1 h with 3 ml 80% ethanol, with a slight shaking every 10 min. Later, the extracted solution was centrifuged for 15 min at the speed of 3500*g*. The supernatant was reserved, and the residue was extracted and centrifuged as described above again. The supernatant was later combined with the reserved amount, and dried by a rotary evaporator (EYELA, SB-1100) at 25 °C, and re-suspended by 0.1 M hydrochloric acid (1 ml). Then, the re-suspended extract was centrifuged for 15 min at 12,000*g*, and added the supernatant to the cation exchange columns (Dowex 50WX8-200H^+ form, 2 ml bed volume,). Ultrapure water (20 ml) was used to wash the cation exchange columns, and the amino acids were wash outed by 20 ml 4 M ammonia solution. To remove the NH_3 in the eluate, it was blown with N_2 for 8 h, and freeze-dried (Labconco Freezen System, U.S.A.). *N*-Methyl-*N*-*tert*-butyldimethylsilyl-trifluoroacetamide (10 μL) was used to derivatize the amino acids in the resultant extracts to *t*-butyldimethylsilyl. Ultimately, GC–MS was used to detect the ^{15}N labeled amino acids in the shoots and roots (Ma et al. 2017a).

Calculations and statistics

The uptake of one N form was calculated by the ^{15}N abundance in the treated samples relative to the ^{15}N abundance in "blank" samples provided with the unlabeled N. The amount of NH_4^+, glycine, and NO_3^- absorbed by pakchoi seedling was calculated by Eq. (1) (Sauheitl et al. 2009):

$$N_{uptake} = N_{Total-N}\frac{A_s - A_c}{A_f}, \quad (1)$$

where N_{uptake} is the amount of a given N source taken up into the roots or shoots, $N_{Total-N}$ is the total N content of pakchoi shoots or roots, A_s is the ^{15}N atom% in pakchoi shoots or roots, A_c is the ^{15}N atom% in the "blank" seedlings supplied with unlabeled N, and A_f is the ^{15}N atom% of the labeled-N source [glycine (10.0%) in Experiment 1; NH_4^+ (5.0%), NO_3^- (5.0%), or glycine (5.0%) in Experiment 2; glycine (98.10%) in Experiments 3 and 5].

$$N_{contribution} = \frac{N_{uptake}}{N_{total-N}} \times 100, \quad (2)$$

where $N_{contribution}$ is the contribution of NH_4^+, glycine, or NO_3^- to the total N taken up by pakchoi seedlings; N_{uptake} is the absorption amount of a given N source in pakchoi shoots or roots calculated by Eq. (1); and $N_{total-N}$ is the total mass of N contained in the seedlings.

$$Tr = \frac{N_{shoot}}{N_{(shoot+root)}} \times 100, \quad (3)$$

where Tr is the transportation rate (in %), N_{shoot} is the amount of glycine-^{15}N in the shoots, and $N_{(shoot+root)}$ is the total glycine-^{15}N in the roots and shoots, as calculated by Eq. (1).

Statistical analysis

One-way analysis of variance combined with Duncan's multiple range method ($p < 0.05$) was applied to evaluate the differences between treatments. All statistical analyses were done by SAS 8.2 (SAS Institute Inc., Cary, NC), and the figures were made by Origin 8.1 (OriginLab, Northampton, MA).

Results

Pakchoi biomass and long-term N uptake

Externally supplied sucrose had a great effect on pakchoi growth and N uptake. The shoots' and roots' biomass and the total N uptake increased by lower sucrose, while they

were inhibited by higher sucrose (>300 μM) both in multiple N or a single N source of glycine. The optimum level of sucrose for pakchoi growth and N uptake under multiple N source was 15 μM (Fig. 1a, b), which resulted in 20.6 and 16.7% increase of biomass and N uptake, respectively, compared to when no sucrose was applied. However, the optimum sucrose level was 6 μM in a single N source of glycine (Fig. 1c, d).

Long-term uptake of ammonium, nitrate, and glycine from the mixed N source

Externally supplied sucrose affected the uptake of different N from in the mixed N source (Fig. 2). Compared to the control level (0 μM), the uptake of nitrate, glycine, and ammonium in roots and shoots was enhanced with the optimum sucrose level (15 μM), while they were hampered severely under a high sucrose level (300 μM) (Fig. 2a, c). The N contributions of glycine under the optimum sucrose level in roots were higher than under control and high levels (Fig. 2d). The uptake of ^{15}N-ammonium showed a similar pattern with ^{15}N-glycine, but the N contribution of ammonium was higher in the control level. In contrast, the N contribution of nitrate was lowest in shoots and roots under the high sucrose level. Besides, the N contributions of glycine were lower than nitrate in shoots, which were 27.0–30.9 and 31.7–36.1%, respectively, while glycine was much higher than nitrate in roots, which were 34.9–44.3 and 17.9–23.1%, respectively.

Short-term uptake and transportation of glycine

Sucrose had a great effect on the short-term absorption and transportation of ^{15}N-glycine, but the effect was dependent on the N status (Fig. 3). Glycine as the single N source, the active uptake of glycine under high sucrose (300 μM) was

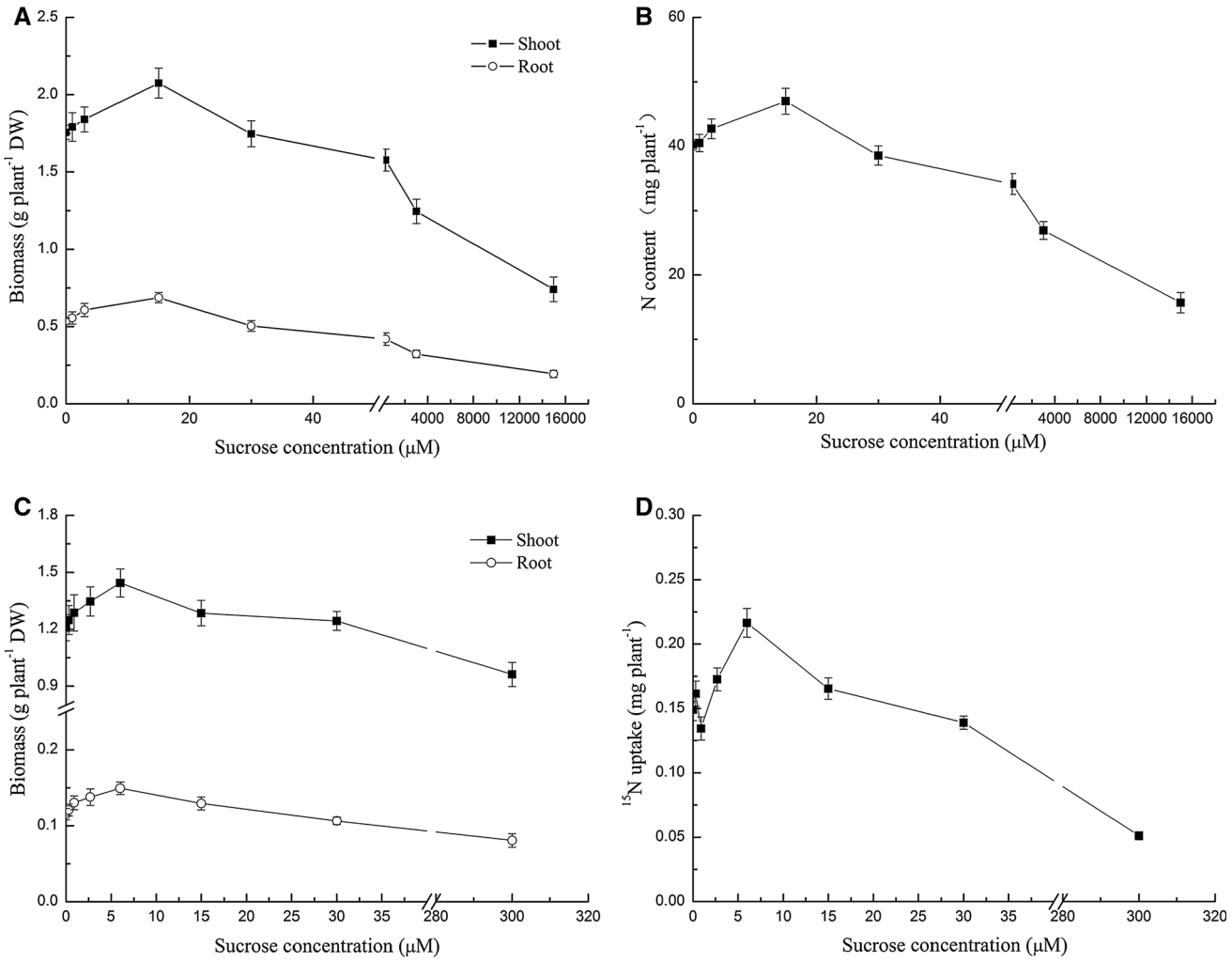

Fig. 1 Biomass and N uptake under different concentrations of sucrose. The shoots and roots biomass (**a**), N content (**b**) with a mixed N, and the shoots and roots biomass (**c**), ^{15}N-glycine uptake (**d**) with a single N source of glycine. Bars show mean values ± SE; $n = 6$ for **a** and **b**, $n = 3$ for **c** and **d**

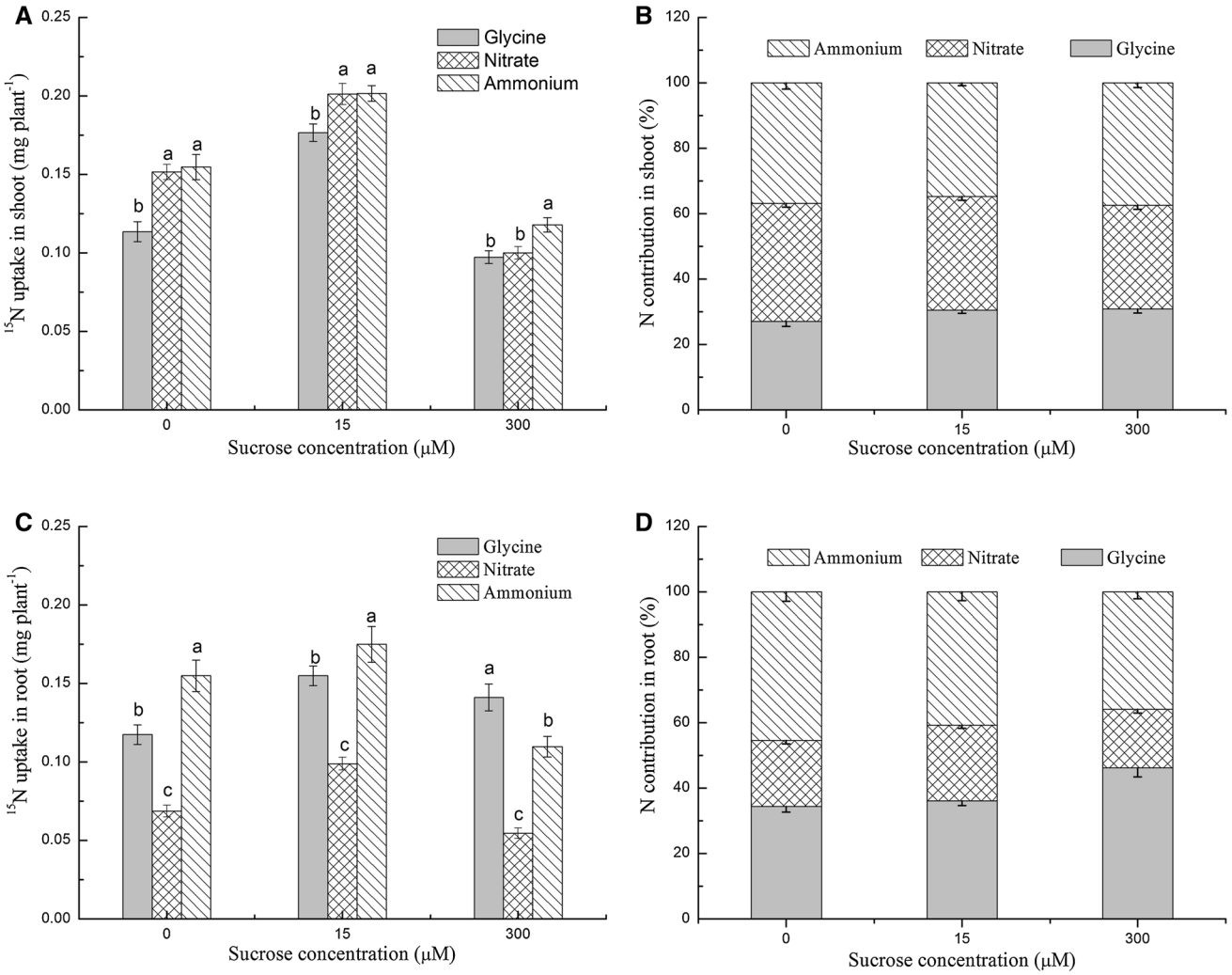

Fig. 2 Effect of sucrose on the ^{15}N uptake in mixed N sources. The uptake of glycine, nitrate, and ammonium in shoots (**a**) and roots (**c**), and the N contribution of each form of N to total N uptake (%) in shoots (**b**) and roots (**d**). Bars show mean values ± SE, $n = 3$

significantly higher than under optimum sucrose (6 μM) and control level ($p < 0.05$), while the transportation rate from the root to the shoot was much lower; the passive uptake of glycine under high sucrose level was much lower than the optimum level in shoots (Fig. 3a–c). In mixed N, the active uptake of ^{15}N-glycine under high sucrose level (300 μM) was much lower than the optimum (15 μM) and control levels, while no difference was detected in passive uptake; even the uptake was low at high sucrose level, and the transportation rate was higher than the optimum and control levels (Fig. 3d–f).

Activities of glycine metabolism enzymes

In the single N source treatments, the activities of GS, GPT, and GOT in roots under the optimum sucrose (6 μM) were significantly higher than those under high sucrose level, while little difference was found in the shoots. In multiple N sources, the GS and GPT activities in roots under optimal sucrose (15 μM) level were increased by 32.2 and 47.4% compared to those under high sucrose level, respectively (Table 1).

^{15}N labeled amino acids in roots and shoots

High sucrose level affects greatly on the content of ^{15}N labeled amino acids under single N or mixed N sources (Figs. 4, 5), and ^{15}N labeled amino acids vary between roots and shoots greatly. Glycine as the single N, the ^{15}N-glycine in roots under high sucrose level was significantly higher than it under optimal sucrose level, while the content of serine, glutamine, and asparagine was significantly lower; in shoots, the content of glutamine and asparagine was significantly higher under optimal sucrose level

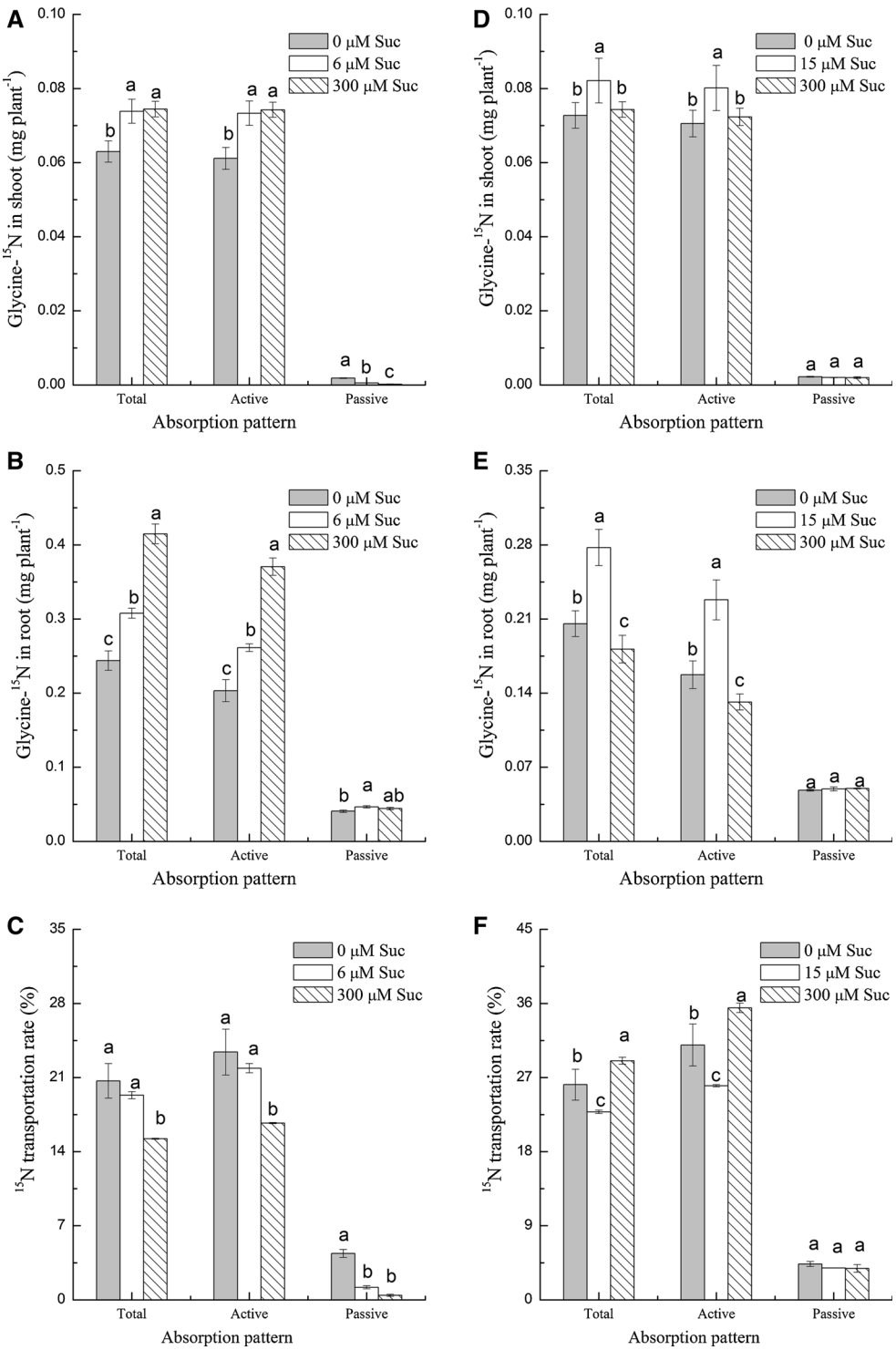

Fig. 3 Effect of sucrose on the short-term uptake of glycine-^{15}N. Glycine-^{15}N in shoots (**a**), roots (**b**), and transportation rate (**c**) under a single N source of glycine; glycine-^{15}N in shoots (**d**) and roots (**e**) and transportation rate (**f**) under a mixed N. Bars show mean values ± SE, $n = 3$. *Different letters* indicate significant differences between sucrose levels ($p < 0.05$)

(Fig. 4). The composition of ^{15}N-amino acids under the mixed N was similar with it under the single N of glycine, but the content was much lower (Fig. 5). In addition, glycine, serine, glutamic acid, glutamine, and asparagine took the main part of the ^{15}N labeled amino acids in roots, while it was asparagine, glutamic acid, gamma-aminobutyric acid, and glutamine in shoots.

Discussion

Effects of externally supplied sucrose on pakchoi growth and N uptake

Sugars are important in plant growth, and the large amounts of sugars in the xylem sap indicating that the

Table 1 Effect of sucrose on the activity of N metabolic enzymes in *Brassica chinensis* L.

Sucrose concentration (μM)	GS (A mg^{-1} protein h^{-1})		GOT (μmol g^{-1} 30 min)		GPT (μmol g^{-1} 30 min)	
	Shoot	Root	Shoot	Root	Shoot	Root
0	19.1 ± 0.7b	23.5 ± 0.6ab	9.8 ± 0.2a	12.5 ± 0.2a	3.8 ± 0.4a	7.8 ± 0.6a
6	22.3 ± 0.2a	25.7 ± 1.7a	10.5 ± 0.8a	12.4 ± 0.4a	3.3 ± 0.1a	8.8 ± 0.8a
300	22.1 ± 0.2a	20.9 ± 1.4b	10.5 ± 0.1a	10.8 ± 0.1b	4.3 ± 0.4a	3.5 ± 0.5b
0	19.2 ± 0.4b	24.7 ± 2.0a	12.1 ± 0.2a	13.8 ± 0.4a	4.5 ± 0.9a	8.7 ± 0.8b
15	21.8 ± 0.3a	23.0 ± 0.6a	12.2 ± 0.6a	12.9 ± 0.4ab	3.9 ± 0.4a	14.0 ± 2.0a
500	22.6 ± 0.1a	17.4 ± 0.5b	11.7 ± 0.3a	12.0 ± 0.2b	3.4 ± 0.2a	9.5 ± 0.6b

Values represent the mean ± SE ($n = 3$). Different letters in each column indicate significant differences between treatments at the $p < 0.05$ level. The first three lines are enzymes under a single N source of glycine, and the last three lines are enzymes under a mixed N

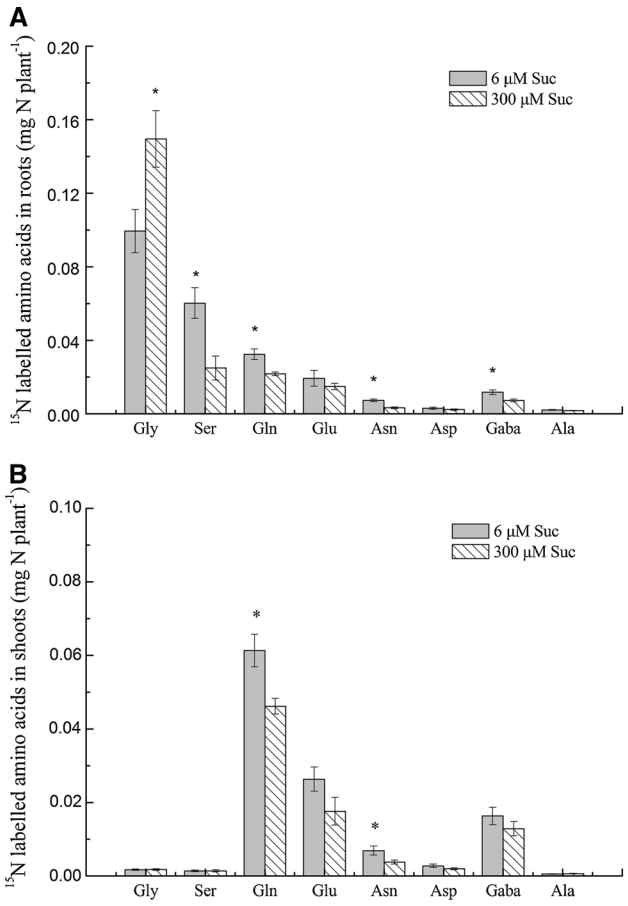

Fig. 4 Effect of sucrose on the content of ^{15}N-labeled amino acids in pakchoi roots (**a**) and shoots (**b**) after a 12-h uptake of 1 mM 98.1% ^{15}N-glycine. Bars indicate mean values ± SE; $n = 6$; *asterisk* indicates significant differences between sucrose levels ($p < 0.05$ level)

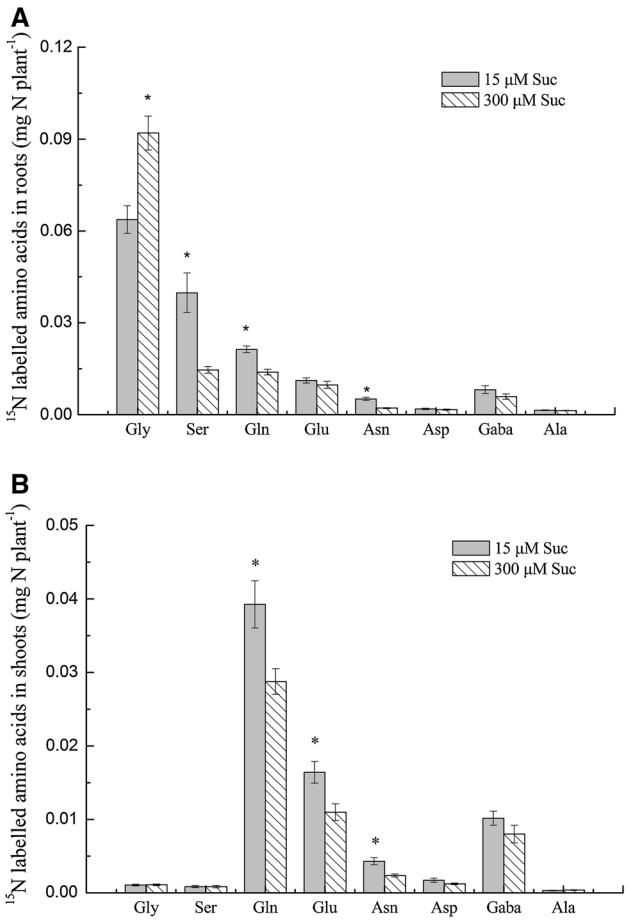

Fig. 5 Effect of sucrose on the content of ^{15}N-labeled amino acids in pakchoi roots (**a**) and shoots (**b**) after a 12-h uptake of 1 mM NO_3^- + 1 mM NH_4^+ + 1 mM 98.1% ^{15}N-glycine. Bars indicate mean values ± SE; $n = 6$; *asterisk* indicates significant differences between sucrose levels ($p < 0.05$ level)

sucrose in roots can be transported to shoots (Secchi and Zwieniecki 2012; Wang and Ruan 2015). Sucrose is the energy and carbon resource for plant growth, and it is a rapid signal to regulate plant development (Wang and Ruan 2015). We have shown that sucrose can enhance plant growth under lower levels, while, when the sucrose level was 300 μM, retardation was seen (Fig. 1). The optimum sucrose level was dependent on the N supply, which was 6 μM in 1 mM glycine, and 15 μM in 3 mM multiple N. Bi

et al. (2005) showed that the nitrate level could affect the glucose sensitivity of wild-type *Arabidopsis* germination, with an inhibitory effect appearing at 3% glucose under 0.1 mM nitrate, but at 5% glucose under either 1 or 5 mM nitrate. Since the N uptake and assimilation are coupled with C, higher N supply may increase the C demand and increase the sucrose uptake and the tolerance of high sucrose. Remarkably, the optimal sucrose level was shown to be 29 mM for *Arabidopsis thaliana* seedlings growth in agar media, which is much higher than 6 or 15 μM as we observed (Schofield et al. 2009). This great difference may be due to the cultivation condition difference, because the previous study was done with agar media, and sucrose dissolved in water is more active and available than in agar media (solid state).

Effect of sucrose on the relative uptake of ammonium, nitrate, and glycine

Plants show great flexibility in the uptake of different N compounds to face the environmental and autologous changes (Ma et al. 2017b). In this work, we found that optimal sucrose increased pakchoi growth and the absorption of glycine, ammonium and nitrate, but the N contribution of nitrate (except in roots) and ammonium was decreased, indicating that the increasing range of glycine was much higher than that of nitrate and ammonium (Fig. 2). Most studies have shown that an increased supply of sugars can increase the rate of nitrate uptake and assimilation. Sugars increase nitrate reductase activity and translation and decrease NR degradation (Cross et al. 2007), and alter the expression of numerous genes involved in nitrate uptake, ammonium metabolism, and amino acid synthesis (Bläsing et al. 2005). De et al. (2014) shown that sucrose in root affects not only NO_3^- transporters but also assimilatory genes. Nitrate is inorganic N without C, and its metabolism to amino acids needs more C supply compared to glycine. But, we showed that optimal sucrose decreased the N contribution of nitrate, which may be because (1) compared to the sugars in the plant itself, the optimal concentration of sucrose (6–15 μM) is very low, which might play an important role in signaling or regulating, rather than as an C source, and (2) the absorption of one form of nitrogen changes the uptake of other forms (Miller and Bowman 2003; Thornton and Robinson 2005), ammonium inhibits the absorption of nitrate, and the existence of amino acids inhibits the absorption of both nitrate and ammonium (Thornton 2004); Most of the above-stated studies were done under a single N source of nitrate, which might neglect the effect of ammonium and amino acids.

Pakchoi possesses the ability to absorp and metabolize a large number of amino acids. Glycine constituted more than 27.0% of the total N absorption under the mixed N, which indicating that plants possess great ability to utilize amino acids. However, this work was done under a sterilized environment, bypassing the effect of microorganisms, which are regarded as stronger competitors for organic N. This result may have overestimated the N contribution of amino acids, but it shows that plants possess the ability to use it, and organic N nutrition under natural environment warrants further research.

Furthermore, the N contribution of nitrate and glycine in roots and shoots were greatly different. The N contribution of glycine in shoots was 27.0–30.9% in shoots, whereas it was 34.4–46.2% in roots. The N contribution of nitrate in shoots was 31.7–36.1%, while it was just 17.9–23.1% in roots (Fig. 2b, d). Most of the root uptake of nitrate was uptransported and metabolized in the shoots (Xu et al. 2011), which led to the high N contribution in the shoots. Studies have shown that the rate of plants uptake amino acids is faster than nitrate (Kielland et al. 2006; Persson et al. 2006; Thornton and Robinson 2005), but most of them are metabolized in roots (Warren 2012) and transported to shoots in a slower rate compared with nitrate (Persson et al. 2006; Schmidt and Stewart 1999). So, the significant differences in their N contributions in shoots and roots are resulted from the differences in the nitrate and glycine metabolism, and N partitioning to shoots and roots reflect differences in metabolism of the different N sources.

The mechanism of sucrose on the absorption and metabolism of glycine

Environmental factors affect plant N status by regulating root N uptake, metabolism, transportation, storage, and reallocation (Susanne et al. 2014). Studying the signaling pathways and bottlenecks of amino acid metabolism under different environments can help to improve the plants N use efficiency (Tegeder 2012).

In the single N source of glycine, the process of glycine to serine in root limited glycine-N contribution under high sucrose levels. In the long-term test, the uptake amount of glycine was inhibited under high sucrose level compared to the optimal level (Fig. 1d). However, short-term uptake test showed that the uptake of glycine under high sucrose level was significantly higher than the optimal level (Fig. 3a, b). So, the roots uptake was not the limiting step under the high sucrose level. Most glycine are taken up by amino acids transporters, after uptake into roots, glycine is converted to serine and ammonium catalyzed by serine hydroxymethyltransferase, and serine will be translated to other amino acids. The ammonium produced by glycine can be converted to be glutamine catalyzed by GS (Xu et al. 2011). Studies have shown that glycine metabolism rather than uptake was the limiting step under some environmental stresses (Ma et al. 2016; Thornton and Robinson

2005). Under high sucrose level, the activities of GS, GOT, and GPT in roots were much lower than at the optimal level (Table 1), which indicated that the metabolism in roots was the limiting step for glycine under high sucrose levels. According to the content of ^{15}N-amino acids, the ^{15}N-glycine under high sucrose level was significantly higher than it under optimal sucrose level, while the contents of serine, glutamine, and asparagine was significantly lower, which indicating that the metabolism process of glycine to serine was the limiting step for glycine-N contribution under the single N supply.

In mixed nitrogen, limited active uptake and metabolism process of glycine to serine inhibited glycine-N contribution under high sucrose levels. In contrast with the single N source case, root active uptake of glycine was severely inhibited under high sucrose levels (Fig. 3d, e). Furthermore, the GS, GOT, and GPT activities in roots under high sucrose level were much lower than the optimum level (Table 1). Most amino acids absorbed by plants are through a series of co-transporters that are driven by H^+-ATPase (Bush 2003). This is consistent with our results because glycine absorption in plants not treated with CCCP was more than five-fold greater than that in CCCP-treated plants, as CCCP inhibits active uptake of glycine. Externally supplied sucrose had little effect on the passive uptake in the mixed N, which indicates that the sucrose concentration in our test was not so high to affect the root osmotic pressure. This may indicate that sucrose was acting as a signal to regulate N uptake and metabolism from another aspect. In addition, the ^{15}N-glycine under high sucrose level was significantly higher than it under optimal sucrose level, while the content of serine, glutamine, and asparagine was significantly lower, which indicating that the metabolism process of glycine to serine was the another limiting step for glycine uptake under the mixed N supply. So, the limited root active uptake and the metabolism of glycine to serine were the factors inhibited the glycine-N contribution under the high sucrose level under the mixed N.

Furthermore, glycine uptake under high sucrose level was increased in the single N source, but decreased in mixed N source compared with the optimal level (Fig. 3). This indicates that N supply can affect plants' corresponding strategies for exogenously supplied sucrose. In mixed N, high sucrose inhibited glycine uptake, but it could enhance the absorp ratio of nitrate, which is a compensatory strategy for N demand. But in the single N, no N existed without glycine; so even though it was not suitable for glycine absorption under the high sucrose level, pakchoi has to take up a large amount of glycine to satisfy its N demand. This highlights the fact that plants show great flexibility in response to environmental stress.

Ecological significance to study the sucrose on the uptake of N

Plants transfer 20–50% of leaf fixed C to their roots (Kuzyakov and Domanski 2000; Lynch and Whipps 1990), and 2–70% of it is released to the soil as rhizodeposition (Jones et al. 2004; Kuzyakov and Domanski 2000; Lynch and Whipps 1990). Furthermore, the exudation is regulated by innate and environmental factors such as nutrient availability (Pearse et al. 2006), soil type (Mimmo et al. 2011), and root system architecture (Lambers and Veneklaas 2006). Sucrose and glucose are important part of root rhizodeposition, and the concentration of sucrose may change constantly, which may further affect the uptake of different N sources in the rhizosphere. The amino acid content is high in rhizosphere and they are more accessible for plant absorption (Jones et al. 2005). So, the sucrose is important in regulating plants uptake and metabolism of free amino acids in soil. However, this work was done under the sterilized environment, bypassing the effect of microorganisms, so, research of how sucrose affects the uptake of different N in the natural soil environment is needed.

Author contribution statement Q.X.M. and L.H.W. designed the experiments and wrote the manuscript. X.Y., J.Z.M., J.Q.C., and Y.S performed the experiments and analyzed the data. All authors reviewed the manuscript.

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