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Grafting Enhances the Photosynthesis and Nitrogen Absorption of Tomato Plants Under Low-Nitrogen Stress

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Abstract

Nitrogen is an essential macronutrient required for plant growth. In this study, the relationships between plant growth, photosynthetic capability, chlorophyll fluorescence characteristics and nitrogen forms in response to low-nitrogen stress were studied in tomato grafted with different nitrogen-use-efficiency tomato seedlings. Using tomato plants grafted with different rootstocks, we found that, under low-nitrogen stress, plant growth, chlorophyll contents, net photosynthetic rate (Pn), maximal photochemical efficiency (F_v/F_m), the total nitrogen (TN) concentration, and nitrate reductase (NR) activity were significantly inhibited but that the ammonium-nitrogen (NH_4^+-N) content in the roots significantly increased. High-nitrogen-use-efficiency tomato grafted plants exhibited significantly enhanced plant growth but reduced Pn, F_v/F_m, TN content and NR activity under low-nitrogen conditions compared to the grafted seedlings of the low-nitrogen-use-efficiency genotype. In addition, tomato plants grafted onto high-nitrogen efficient rootstock presented reduced damage caused by excessive accumulation of NH_4^+-N in the roots under low-nitrogen stress. Our results indicate that tomato plants grafted onto high-nitrogen efficient rootstock presented enhanced absorption and utilization of nitrogen and maintained growth by promoting the use efficiency of light energy under low-nitrogen stress.

Keywords Abiotic stress · Grafting · Nitrogen metabolism · Photosynthesis

Abbreviations

F_v/F_m	Maximal photochemical efficiency
NH_4-N	Ammonium-nitrogen
NO_2-N	Nitrite-nitrogen
NO_3-N	Nitrate-nitrogen
Pn	Net photosynthetic rate
ΦPSII	The effective PSII quantum yield
TN	The total nitrogen
Tr	Transpiration rate

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Introduction

Nitrogen is the most demanding mineral element in the process of plant growth and crop yield formation (Scagel et al. 2008). Nitrogen is mainly absorbed by plants in two forms: the main inorganic nitrogen form, which includes ammonium (NH_4^+) nitrogen and nitrate (NO_3^-) nitrogen, and organic nitrogen such as urea (Uscola et al. 2014; Yuan et al. 2017). Nitrogen is a major component of proteins, nucleic acids, and phospholipids and is an important component involved in the formation of cellular structures (Valentine et al. 2017; Rinaldoet al. 2018). Nitrogen also plays an important role in the metabolism of matter and energy by participating in the formation of enzymes, coenzymes and prosthetic groups (Cao et al. 2017). In addition, nitrogen is one of the components of chlorophyll, which is closely related to photosynthesis (Dordas 2017; Roca et al. 2018). Deficit nitrogen fertilization not only leads to the degradation of the synthesis of proteins, nucleic acids, phospholipids, etc.; inhibits chlorophyll formation; and modifies microbial soil biodiversity but also causes plant growth to be hindered, in which the leaves become small, thin and yellow and the fruits and seeds are not fully developed, eventually

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leading to reduced plant yields (Li et al. 2012; Qin et al. 2013; Wang et al. 2019).

One way to avoid or reduce losses in production caused by low-nitrogen conditions and promote nitrogen-use efficiency (NUE) in low-nitrogen-use-efficiency genotypes is to graft those plants onto rootstocks capable of improving the NUE of the scion (Nawaz et al. 2017; Suchoff et al. 2018). Grafting is an artificial propagation method for plants that involves grafting a branch or bud of a plant onto the stem or root of another plant such that the two parts that are joined together grow into a complete plant (Bie et al. 2017). According to the literature, grafting technology has been practiced for more than 2300 years in China (Zhou 1994). Today, grafting not only is used to offset soil pathogens (Ke and Saltveit 1988; Lee et al. 2010) but also promotes horticultural improvement and development by creating new genetic variability to improve the resistance of plants to adversity and increase plant yields (Nawaz et al. 2016; Huang et al. 2016). For example, grafting has been used to induce resistance against low- and high-temperatures (Muneer et al. 2016), improve water-use efficiency (Pagliarani et al. 2017), enhance nutrient uptake (Arzani et al. 2005), improve alkalinity and salt tolerance (Colla et al. 2010a; Ruiz et al. 2010), reduce the uptake of persistent organic pollutants from agricultural soils (Schwarz et al. 2010), and improve fruit quality (Kyriacou et al. 2017; Petropoulos et al. 2018). In addition, Keller et al. (2001) research on grapevines fund that rootstocks-induced differences in scion performance and stimulated reproductive growth and yield, which was largely independent of soil nitrogen level. The research by Sugiura et al. (2017) showed that the maximum photosynthetic rate was highly correlated with the leaf nitrogen content in *Raphanus sativus*, but the difference in it among the grafting combinations was small. However, Fullana-Pericàs et al. (2020) reported that grafting can be a useful technique to improve plant photosynthetic performance, crop yield and WUE, and so that the rootstock selection for a target environment is determinant for the variations in photosynthesis.

Tomato (*Solanum lycopersicum* L.) constitutes one of the largest vegetable crops cultivated worldwide and is a model plant for horticultural crop species; tomato plants have a large growth capability, are high yielding and have a large demand for nitrogen during their growth. According to reports, the application rate of nitrogen fertilizer is high in tomato cultivation, and the rate is much higher than that of corn, rice, wheat and other field crop species, although the rate of nitrogen fertilizer loss in soils cultivated with tomato is high (Li et al. 2017). However, there are few reports on the absorption and utilization of nitrogen by grafted tomatoes. Therefore, to study the effects of different rootstocks on nitrogen absorption and utilization, this paper investigated the photosynthesis and nitrogen forms of tomato plants grafted onto seedlings of high-nitrogen-use-efficiency (H) and low-nitrogen-use-efficiency (L) plants, then provide theoretical basis for low-nitrogen and high-yield of tomato cultivation.

Materials and Methods

Plant Material and Experimental Design

This experiment was carried out in a solar greenhouse located at Shandong Agricultural University in Tai'an (36° 09′ N, 117° 09′ E), eastern China. The following environmental conditions were applied: natural light conditions (photosynthetic photon flux density (PPFD) of 1400–1800 μmol m^{-2} s^{-1} (noon at sunny days) and temperatures of 28–37/18–21 °C (day/night). Two different tomato genotypes selected from 25 tomato genotypes, a high-nitrogen-use-efficiency ('TMS-150', H) genotype and a low-nitrogen-use-efficiency ('0301111', L) genotype, were used as plant materials (Zhang et al. 2021). The experimental design was a split plot, with the main plots being grafting involving either self-grafted ('H/H' and 'L/L') or reciprocal grafting ('H/L' and 'L/H') and the subplots being different nitrogen levels of either 15 mM (N+) or 1.5 mM (N−) treatments. When three true leaves of the seedlings were fully expanded (about 25 days after sowing), an 'oblique cutting grafting method' was performed. When the grafted plants were well-established, they were transferred into plastic pots (diameter 110 mm, height 120 mm; one seedling per pot) filled with vermiculite. After seedling survival for a week, treatment with different nitrogen levels was started. The grafted plants were watered daily with complete Hoagland's nutrient solution (N+) or low-nitrogen Hoagland's nutrition (N−), respectively. Each treatment included thirty grafted plants.

Growth Analysis

After 15 days of different nitrogen levels treatment, three plants were randomly selected from each treatment. The impact on the growth of the tomato plants of the different treatments was assessed by determining the leaf and root fresh and dry weight. In addition, the total leaf area was measured with an LI-3100 leaf area meter (LI-3100C; Lincoln, USA), and the chlorophyll content was assayed by the Arnon (1949) method.

Analysis of Photosynthetic Chlorophyll Fluorescence

The photosynthetic and chlorophyll fluorescence parameters were measured at 0, 5, 10, and 15 days after the different nitrogen levels were applied. The photosynthetic parameters

of fully expanded leaves, including the net CO_2 assimilation (Pn) and transpiration (Tr), were determined within the time period from 8:30 a.m. to 10:30 a.m. using an LI-6800 Portable Photosynthesis System (LI-6800; LI-COR, USA). The fluorescence parameters, which included the maximum photochemical efficiency of photosystem II (PSII) (F_v/F_m) and the effective PSII quantum yield (ΦPSII), were measured using an imaging-PAM chlorophyll fluorimeter equipped with a computer-operated PAM control unit (IMAG-MIN/B; Heinz Walz, Effeltrich, Germany).

Analysis of Nitrogen Content in Plants

Plant tissues (leaves and roots), derived from the grafted plants after 15 days of treatment, were heated at 105 °C for 20 min, dried for 48 h at 75 °C and then ground separately in a Wiley mill to pass through a 20-mesh screen. The dried plant tissues were then analyzed for the following nitrogen contents: total nitrogen (TN), nitrate-nitrogen (NO_3-N), ammonium-nitrogen (NH_4-N), and nitrite-nitrogen (NO_2-N).

For the TN content, 0.2 g of dried plant sample was digested with 5 ml of sulfuric acid overnight, after which 2 ml of hydrogen peroxide was added. The sample was then placed in a 370 °C digestion furnace for 7 min, after which it was cooled. Afterward, 2 ml of hydrogen peroxide was added, and the sample was then heated at 370 °C for 1 h until it was transparent, after which it was cooled to room temperature. The digested solution was diluted with water, brought to volume in a 50 ml volumetric flask, and then filtered. The TN content was subsequently determined by a discrete auto analyzer (Smart Chem 200, AMS Alliance, France) at a wavelength of 660 nm using ammonium sulfate colorimetry.

For the NO_3^--N, NO_2^--N and NH_4^+-N contents, 0.2 g of dried plant sample was digested with 50 ml of deionized water, after which two spoon fuls of activated carbon were added. The samples were sealed in a boiling water bath and shaken for 30 min. Afterward, they were cooled to room temperature and then filtered, after which the NO_3^--N, NO_2^--N and NH_4^+-N contents were determined by a discrete auto analyzer at wavelengths of 550, 550 and 630 nm, respectively, using ammonium sulfate colorimetry.

Analysis of Nitrate Reductase Activity in Plants

Five hundred milligrams of each sample were added to a test tube to which 9 ml of a 0.1 mol/l KO_3 solution was added, after which the mixture was evacuated for 1 h and placed in darkness at 25 °C for 30 min. Afterward, 1 ml of a 30% trichloroacetic acid solution was added to each tube, and after the sample color became yellow, 1 ml of the supernatant was aspirated into a new tube. Then, 2 ml of 1% sulfonamide and 2 ml of 0.02% naphthylamine solution were added to monitor the color for 20 min, and then colorimetry at 540 nm.

Statistical Analysis

The data are presented as the means of three replications and the corresponding standard errors. All the data were statistically analyzed by ANOVA using the DPS software package (DPS for Windows, 2009). The differences between the means were tested by Duncan's multiple range test at $P < 0.05$.

Results

Impact of Grafting on Tomato Plant Growth Traits

To study whether plant nitrogen-use efficiency is altered by the rootstock or scion, we performed reciprocal grafting between high-nitrogen-use-efficiency (H) and low-nitrogen-use-efficiency (L) seedlings. As shown in Fig. 1A, B, after 15 days, plant growth was inhibited, and the leaf color turned yellow in various grafting combinations under nitrogen limitation. Under high-nitrogen treatment, the plant height was different in different treatment, and H/H plants showed higher valve than other plants, while all the plants had similar leaf areas per plant except L/L plants (Fig. 1C). However, when all the plants were exposed to nitrogen limitation, the plants exhibited significant reductions in height and leaf area per plant. The reduction levels of plant height and leaf area of L/L and H/L were significantly higher than those of H/H plants.

Moreover, under high-nitrogen conditions, plants showed no significant differences in leaf fresh and dry weight or in root fresh and dry weight (Fig. 2). In contrast, nitrogen limitation-induced marked decreases in the fresh and dry weights of the leaves and roots of all grafted plants. Under nitrogen-limiting conditions, the biomass of the H/H plants, except for root dry weight, were significantly higher than that of the L/L and H/L plants, suggesting less suppression of growth for H/H plants.

Impact of Grafting and Nitrogen on Chlorophyll and Carotenoid Contents in the Leaves

According to multiple comparisons, the chlorophyll and carotenoid contents in the tomato leaves significantly differed under the four grafting combinations and nitrogen limitation with increasing treatment time (Fig. 3).

With the prolonging of low-nitrogen stress, the contents of chlorophyll and carotenoids decreased gradually, and the contents of chlorophyll and carotenoids in the H/H grafted plants were always significantly higher

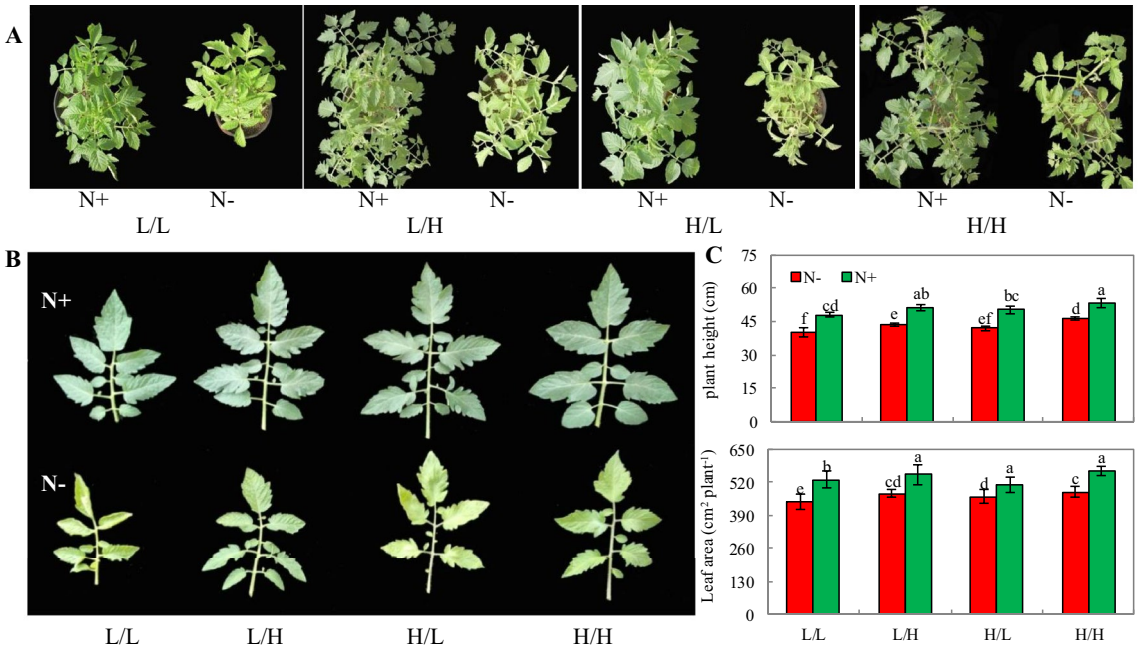

Fig. 1 Response of different grafting tomato seedlings to nitrogen limitation condition. **A** Grafted plants were under well (N+) and low (N−) nitrogen condition by treatment for 15 days. **B** The fourth leaf from top to bottom of different grafted plants. **C** Plant height and leaf area per plant. The data are mean ± SD and the different letters indicate a significant difference at $P < 0.05$ according to Duncan's test

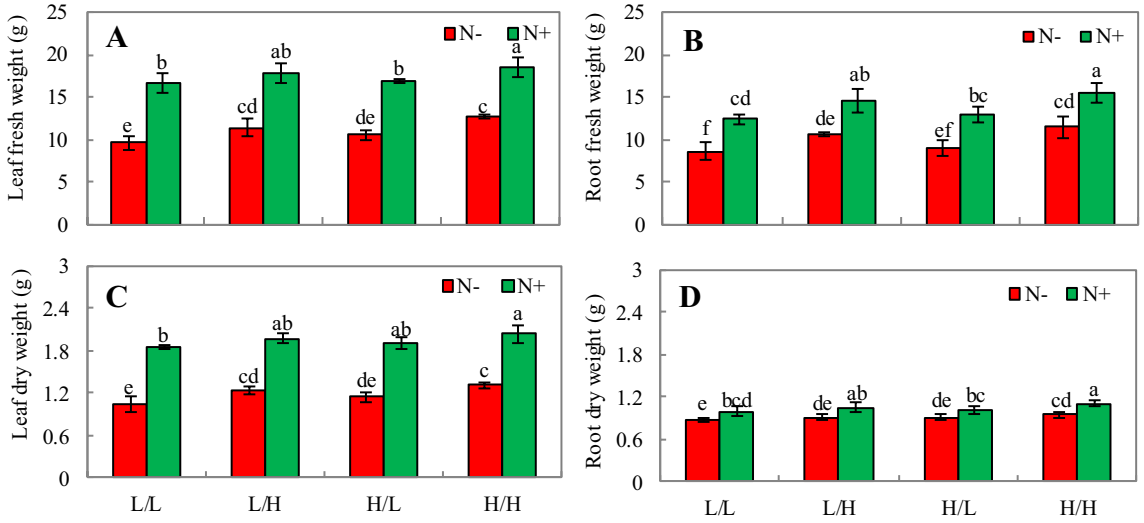

Fig. 2 Impact of grafting and nitrogen on plant growth. **A** The leaf fresh weight. **B** Root fresh weight. **C** Leaf dry weight. **D** Root dry weight. All data were determined 15 days after different nitrogen treatment. The data are mean ± SD and the different letters indicate a significant difference at $P < 0.05$ according to Duncan's test

than those in the L/L plants. In addition, there was no significant difference in chlorophyll or carotenoid content between the H/L and L/H grafted plants at the initial stage of nitrogen restriction, but the difference gradually increased: on the 15th day of stress treatment, L/H was significantly higher than H/L (Fig. 3A, C, E). Under the different nitrogen treatments, the chlorophyll a and b and carotenoid contents were similar at different periods under high-nitrogen conditions (N+). Under low-nitrogen-supply conditions (N−), the chlorophyll a and b and carotenoid contents showed a sharp decline with increased duration (Fig. 3B, D, F).

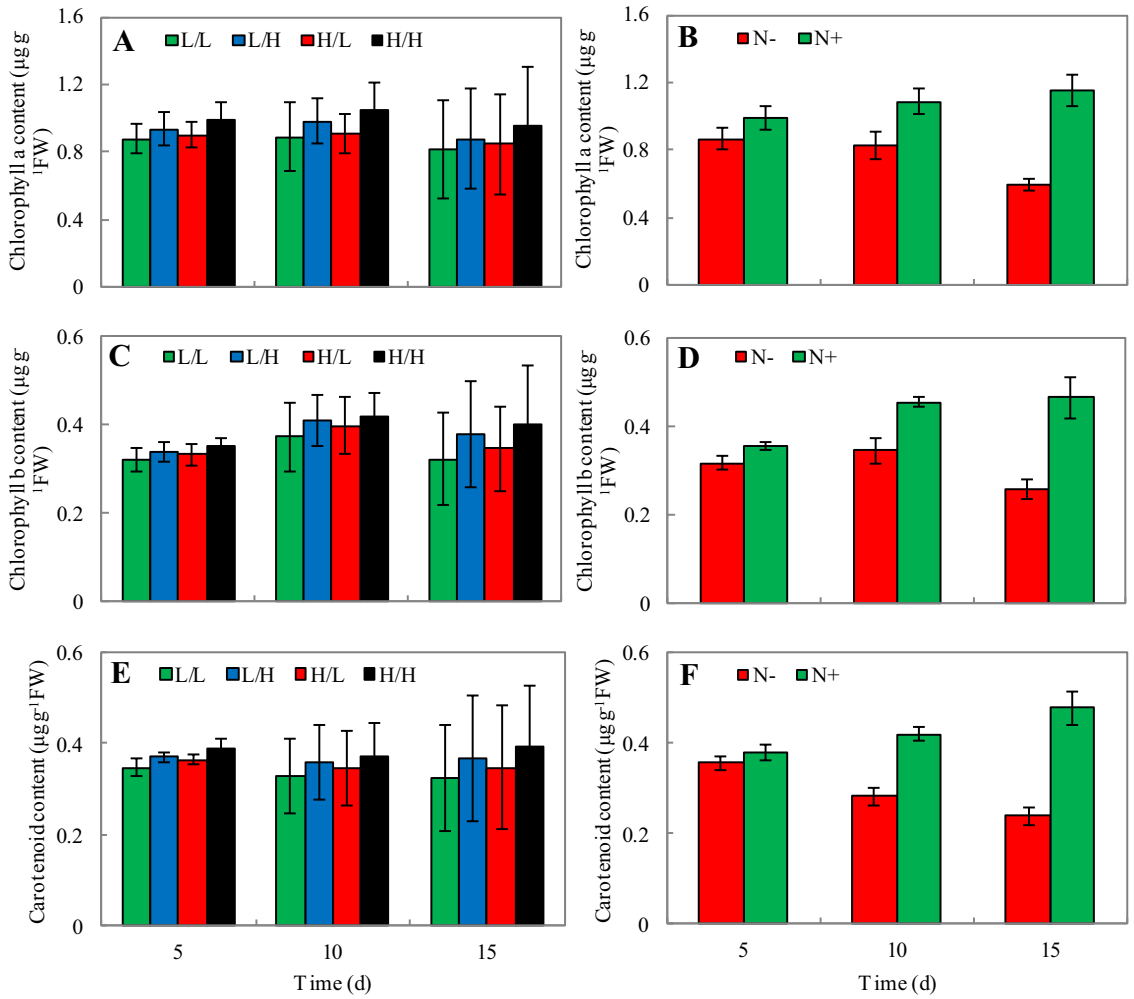

Fig. 3 Dynamic changes of chlorophyll and carotenoid content in different grafting tomato leaves during 15 days of nitrogen treatment. Chlorophyll *a* content (**A**, **B**), chlorophyll *b* content (**C**, **D**) and carotenoid content (**E**, **F**) of tomato grafted plants under well and low-nitrogen conditions. Data in **A**, **C** and **E** are presented as the leaf pigment content in different grafting combination and **B**, **D** and **F** are grafted tomato plants under well and low-nitrogen conditions. The data are mean of multiple comparison ± SD

Impact of Grafting on Photosynthesis and Chlorophyll Fluorescence

Similar to the chlorophyll data analysis method, the photosynthetic and chlorophyll fluorescence parameters of tomato were also analyzed according to multiple comparisons, the results of which are shown in Figs. 4 and 5.

Further investigation of the underlying photoprotection mechanisms in the different grafted tomato plants that were exposed to nitrogen-limiting conditions showed similar Pn and Tr trends (a gradual decrease) with increased processing time (Fig. 4A, C). However, nitrogen limitation resulted in a more significant decrease in Pn and Tr for L/L and H/L plants compared to H/H and L/H plants. The Pn of different L/L, L/H, H/L and H/H grafted plants decreased abruptly during the first stage of nitrogen limitation and reached the minimum values at the end of the experiment, with inhibition ratios of 40.05%, 30.67%, 32.75% and 29.75%, respectively, compared with the Pn before the stress. In addition, compared with the Pn of the different grafted plants at 15 days, the Pn of the L/H grafted plants was higher than that of the L/L plants and lower than that of the H/H plants by approximately 17.56% and 7.1%, respectively, while the Pn of the H/L plants was higher than that of the L/L plants and lower than that of the H/H plants by approximately 9.7% and 18.43%, respectively. Similarly, under the different nitrogen treatments conditions, the Pn and Tr significantly decreased and showed similar trends of sharp decline with increased duration (Fig. 4B, D). At the end of the experimental period, the Pn and Tr in the N− treatment decreased by 67.17% and 53.11%, respectively, compared to those in the N+ treatment.

Corroborating the previously described results for Pn, the L/L plants displayed a significant decrease in the F_v/F_m

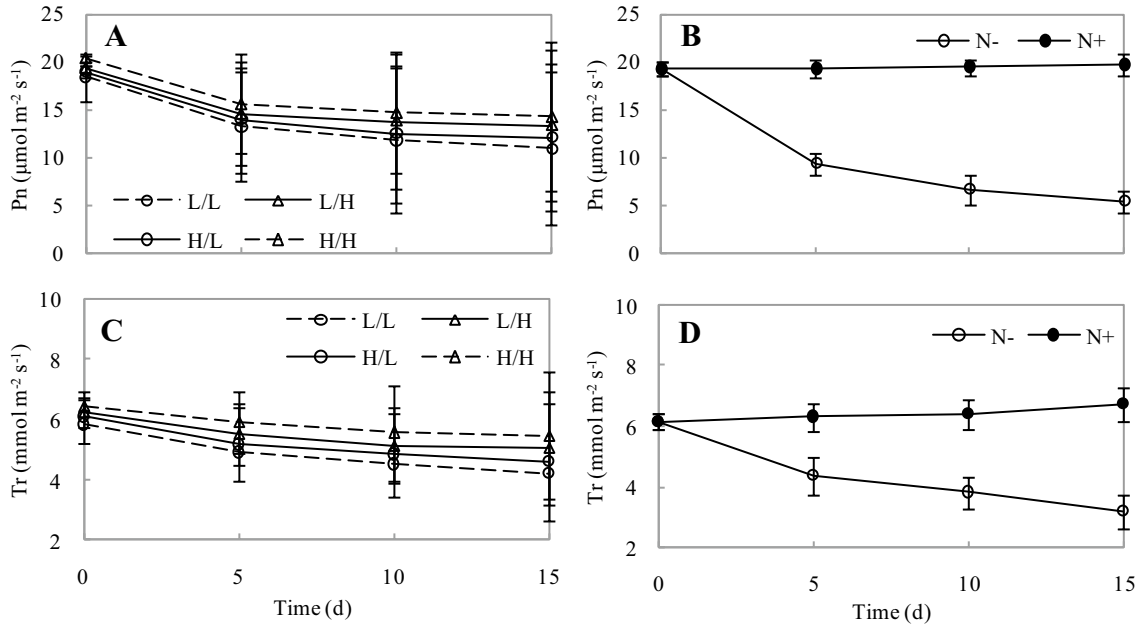

Fig. 4 Dynamic changes of photosynthesis and transpiration in different grafting tomato leaves during 15 days of nitrogen treatment. Net photosynthetic rate (Pn, **A**, **B**) and transpiration rate (Tr, **C**, **D**) of tomato grafted plants under well and low-nitrogen conditions. Data in **A** and **C** are presented as the Pn and Tr in different grafting combination and **B** and **D** are grafted tomato plants under different nitrogen conditions. The data are mean of multiple comparison ± SD

and ΦPSII compared to those of H/H plants throughout the whole experiment (Fig. 5). These plants also exhibited a strong decrease in F_v/F_m and ΦPSII with longer processing time. At 15 days of treatment in the different grafting combinations, the F_v/F_m and ΦPSII were correspondingly higher in L/H and H/L than in L/L by approximately 10.28% and 7.43% for F_v/F_m, respectively, and by 19.16% and 9.58% for ΦPSII, respectively. Furthermore, the F_v/F_m and ΦPSII were correspondingly lower in the L/H and H/L plants than in the H/H plants by approximately 4.23% and 6.67% for F_v/F_m, respectively, and by 6.32% and 14.28% for ΦPSII, respectively (Fig. 5B, D). Moreover, the results in Fig. 5C, E showed that the F_v/F_m and ΦPSII decreased significantly with further increasing treatment time under high-nitrogen conditions compared to normal-nitrogen-supply conditions. This finding demonstrated that, under low-nitrogen stress, the absorption and utilization of light energy by PSII in tomato leaves was unbalanced and that the photochemical efficiency was reduced. However, the grafting of 'H' rootstocks effectively restored the physiological function of PSII under nitrogen limitation and maintained high photosynthetic capability.

Impact of Grafting on Nitrogen Content and Nitrate Reductase Activity

To evaluate the nitrogen uptake efficiency of tomato grafted with different rootstocks, the effects of different grafting combinations on NO_3^--N, NO_2^--N, NH_4^+-N and TN contents and nitrate reductase (NR) activity in the leaves and roots under low-nitrogen stress were studied (Figs. 6, 7).

The results showed that there were significant differences in the NO_3^--N, NO_2^--N and NH_4^+-N contents in the tomato leaves and roots in the different grafted plants under different nitrogen conditions (Fig. 6). The three forms of nitrogen content were significantly higher in the H/H and L/H grafted plants than in H/L and L/L plants. For instance, the H/H and L/H grafted plants contained significantly elevated levels of NO_3^--N (2.80 and 2.55 mg g^{-1} DW, respectively), whereas the H/L and L/L plants contained relatively low levels of NO_3^--N content (2.32 and 2.15 mg g^{-1} DW, respectively) in the roots under low-N conditions. Compared with the high-nitrogen treatment, except for the significant increase in NH_4^+-N in the roots, the content of other nitrogen forms in the different grafted plants showed a sharply declining trend under low-nitrogen conditions. In addition, the contents of NO_3^--N and NO_2^--N were higher in the roots than in the leaves. The content of NO_3^--N was the highest among the three nitrogen forms. Under high-nitrogen conditions, the content of NO_3^--N in the roots was approximately 20 times that in leaves, while under low-nitrogen conditions, NO_3^--N in the roots was approximately 40 times that in leaves.

However, in contrast with the results in Fig. 6, the TN content and NR activity were higher in the leaves than in the roots in the different grafting treatments (Fig. 7). Under high-nitrogen conditions in plant leaves, except for H/H, which was

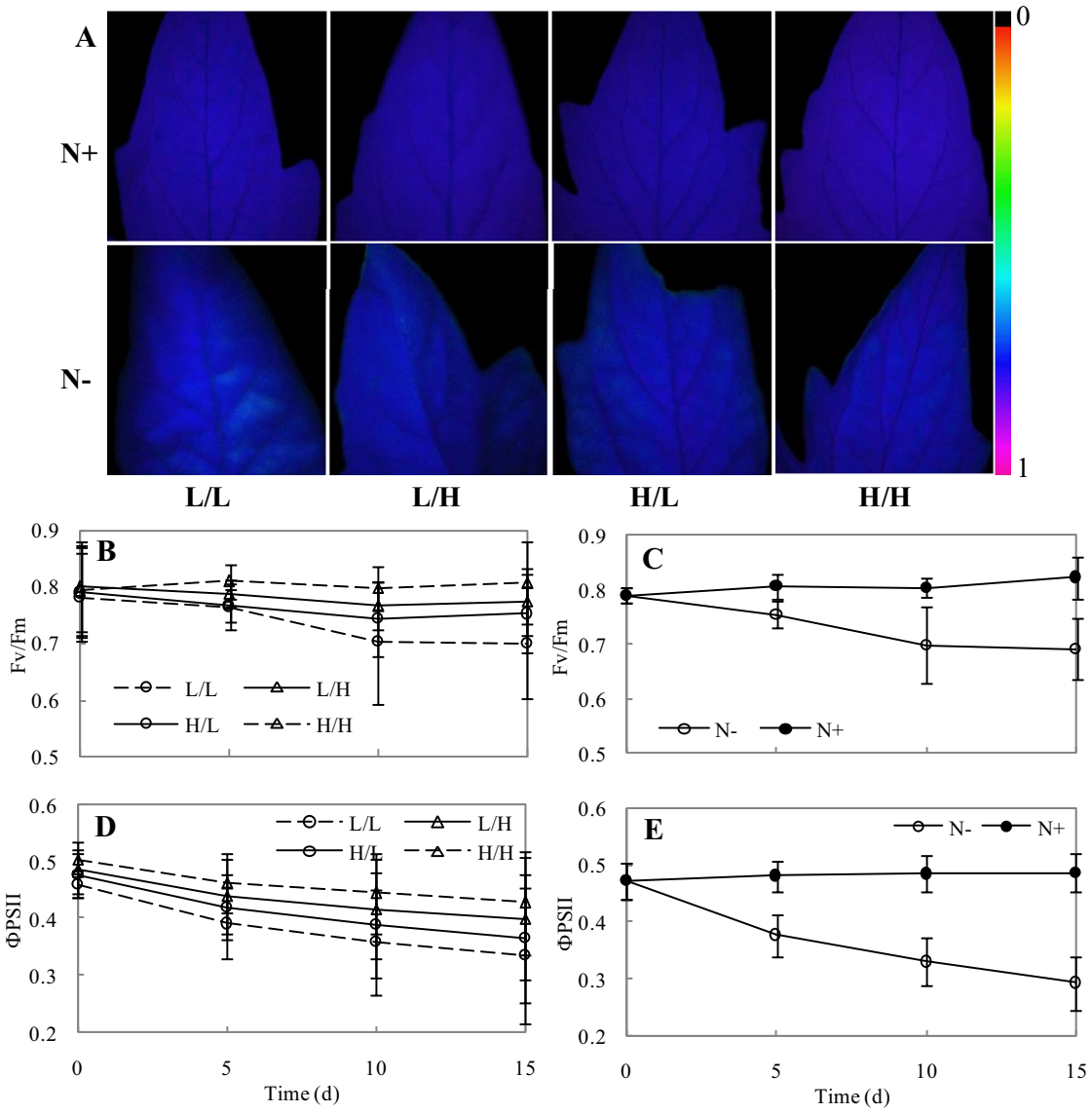

Fig. 5 Response of chlorophyll fluorescence parameters in various grafting tomato seedlings to nitrogen treatment. **A** The maximum photochemical efficiency of PSII (F_v/F_m) was determined 15 days after different nitrogen treatment. The underneath colour code depicted in the image, ranges from 0 (black) to 1 (purple). **B**, **C** The maximal photochemical efficiency (F_v/F_m). **D**, **E** The effective PSII quantum yield (ΦPSII). Data are mean of multiple comparison ± SD

significantly higher, there was no significant difference in TN content in response to the grafting treatments. Under low-nitrogen conditions, the TN was significantly reduced, and there were significant differences in the different grafting treatments. The TN content in the leaves was correspondingly higher in the L/H and H/L plants than in the L/L plants (by approximately 4.98% and 3.30%, respectively) and was correspondingly lower in the L/H and H/L plants than in the H/H plants (by approximately 3.14% and 3.46%, respectively) (Fig. 7A, B). Similar to the TN trend, the NR activity was significantly reduced under low-nitrogen stress, and there were significant differences in the different grafting treatments. Under nitrogen limitation stress, the H/H plants showed greater activities of NR than did the L/L, L/H and H/L plants. Even though the roots and leaves showed similar changes in NR activity under low-nitrogen stress conditions, the activities were higher in the roots than in the leaves.

Discussion

The sensitivity of many species to nitrogen imitation stress is manifested as a suppression of growth and physiological disorders. Low-nitrogen stress causes well-known effects by

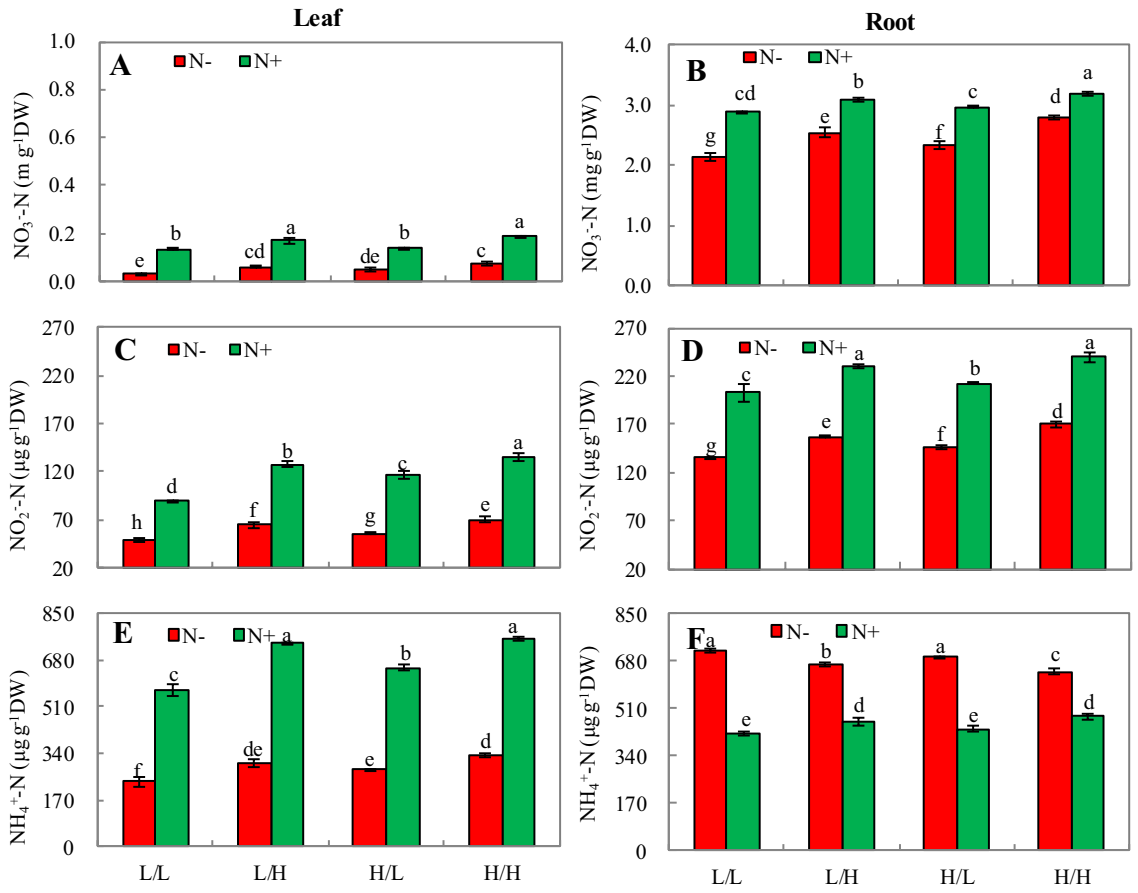

Fig. 6 Response of different nitrogen forms content in different grafting tomato leaves and roots under nitrogen treatment. **A**, **B** Nitrate nitrogen (NO_3^--N). **C**, **D** Nitrite-nitrogen (NO_2^--N). **E**, **F** Ammonium-nitrogen (NH_4^+-N). All data were determined 15 days after different nitrogen treatment. The data are mean ± SD and the different letters indicate a significant difference at $P < 0.05$ according to Duncan's test

which plants show reduced growth, small leaves and stunted root systems, and in severe cases, this leads to a sharp reduction in plant yield (Peng et al. 2017). Our results in this study showed that tomato plants grafted onto high-nitrogen efficient rootstock exhibited an effective strategy to cope with nitrogen imitation, as indicated the high biomass accumulation to enlarge leaf area, and the maintenance of good status of the whole plant, these increase photosynthetic area and provide material basis for photosynthesis under low-nitrogen stress. These results are in line with those of Colla et al. (2010b), who reported that, based on the higher NR activity in grafted plants under low-nitrogen conditions in melon, grafted plants potentially had the greatest uptake efficiency of nitrogen and the subsequent use of this nutrient for shoot biomass production. In addition, in grafted plants, control of growth is the result of a complex signaling system established between two different genotypes (i.e., the rootstock and scion). Among the various processes, rootstocks assure the mineral nutrition of the scion and alter its development (Cochetel et al. 2018). Nawaz et al. (2018b) found that grafting onto some wild rootstocks can improve nitrogen-use-efficiency of watermelon, and this improved nitrogen-use-efficiency could be attributed to better N uptake efficiency of wild watermelon rootstocks. Thus, rootstocks can alter the tolerance of shoots against stress (Schwarz et al. 2010; Li et al. 2014).

Nitrogen is one of the essential elements that composes chlorophyll; therefore, both the chlorophyll content decreased and the leaves turned yellow when the plants were exposed to low-nitrogen stress (Nawaz et al. 2018a). Therefore, nitrogen limitation can reduce the photosynthesis and chlorophyll fluorescence rate of plants, which is not conducive to the normal progress of carbon assimilation, inhibiting the accumulation of carbon-containing compounds and thus limiting plant growth (Bassi et al. 2018; Bascuñán-Godoy et al. 2018). In the present work, we found a significant decrease in chlorophyll content and the photosynthesis rate in the plants in the four grafting combinations

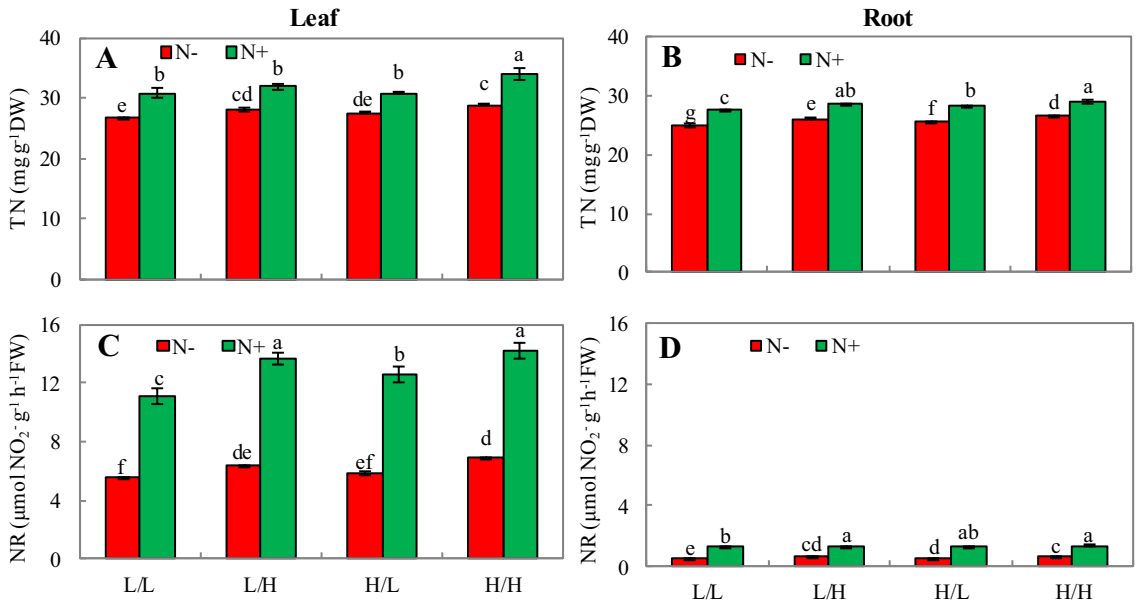

Fig. 7 Response of total nitrogen content and nitrate reductase activity in different grafting tomato leaves and roots under nitrogen treatment. **A**, **B** Total nitrogen (TN). **C**, **D** Nitrate reductase (NR). All data were determined 15 days after different nitrogen treatment. The data are mean ± SD and the different letters indicate a significant difference at $P < 0.05$ according to Duncan's test

under low-nitrogen stress, whereas in the 'H' grafted plants, especially in H/H, these values were higher under low-nitrogen conditions than other grafted plants. These results are in accordance with the findings of Liu et al. (2011), who observed that grafting muskmelon onto interspecific rootstocks enhances photosynthesis and the translocation of sugars in muskmelon leaves. These results are also in line with our previous observations (Zhang et al. 2019), as we observed that tomato grafted onto drought-tolerant seedlings reduced the loss of photosynthetic capability under water-deficit conditions. Our results also showed that the F_v/F_m and ΦPSII decreased with prolonged nitrogen limitation, and the value of the plants grafted with 'H' seedlings was higher than that of the other plants. These findings indicate that low-nitrogen led to a decrease in the PSII reaction center opening, the conversion efficiency of light and the energy used for carbon assimilation accumulation, while grafted plants with high-nitrogen-use-efficiency rootstocks can significantly improve the chlorophyll fluorescence efficiency of plants under low-nitrogen conditions, which is conducive to the energy accumulation from carbon assimilation.

Among the different forms of nitrogen, NO_3^- is the main source of nitrogen in the majority of agricultural soils, despite its concentration of which can vary from micromolar to millimolar amounts in soils (Crawford 1995). Therefore, most plants have two types of NO_3^- absorption systems: low-efficiency transport systems (LATS), which function when the external NO_3^- concentration is relatively high (> 0.5 mM), and high-efficiency transport systems (HATS), which function when the external NO_3^- concentration is relatively low (< 0.5 mM) (Wang et al. 2012; Frungillo et al. 2014). Once NO_3^- is absorbed into the plant, it is reduced to NO_2^- by NR. Then, a portion of NO_2^- will be enzymatically reacted with glutathione and NH_4^+, and the other portion of NO_2^- will be transported to the chloroplast to form NH_4^+. The NH_4^+ is then assimilated into amino acids in a series of reactions facilitated by a suite of enzymes (Frungillo et al. 2014). In our experiment, plants in the four grafting combinations were grown under low-NO_3^--content soil conditions. The results showed that a significant decrease in NO_3^--N, NO_2^--N, TN and contents as well as NR activity occurred both in the leaves and roots, whereas the plants grafted with 'H' as rootstock maintained relatively high N contents and NR activity. These data suggest that nitrogen limitation does restrict NO_3^- uptake and reduction in both leaves and roots, which is in agreement with the decrease in NR activity under low-nitrogen conditions found in durum wheat plants (Vicente et al. 2017), whereas the grafted plants with 'H' as rootstocks contained high levels of N content and NR activity. Although nitrogen is always converted to NH_4^+ in plants, the excessive assimilation of the NH_4^+ product is harmful to plants. High levels of NH_4^+ can inhibit the activity of NR and cause a decrease in cytokinins in xylem sap (Walch-Liu et al. 2000). In addition, the assimilation of NH_4^+ can damage membrane configuration and uncouple photophosphorylation and nonphotophosphorylation (Guo et al. 2006). Moreover, the assimilation of NH_4^+ requires substantial amounts of 2-oxoglutarate obtained from glucose, thus reducing sugar

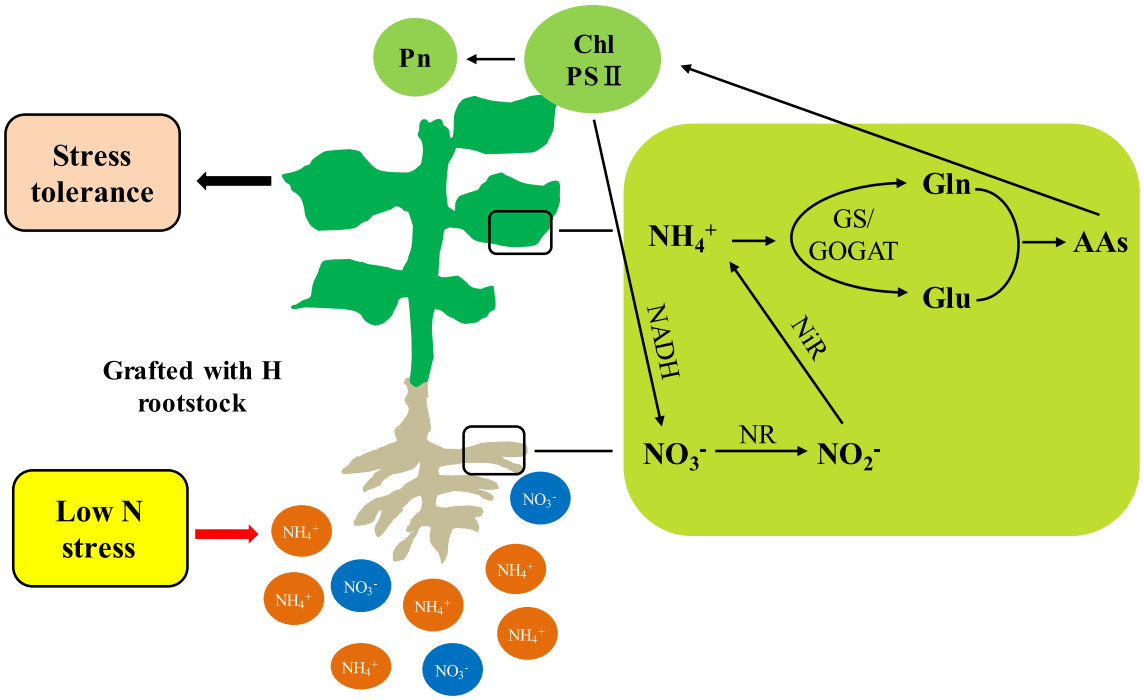

Fig. 8 The possible model for the nitrogen metabolism in tomato plants under low-N stress. *AAs* amino acid; *Chl* chlorophyll; *PSII* photosystem II; *Pn* net photosynthetic rate; *GS* glutamine synthetase; *GOGAT* glutamate synthase; *Gln* glutamine; *Glu* glutamate; *NADH* nicotinamide adenine dinucleotide; *NiR* nitrite reductase; *NR* nitrate reductase; NH_4^+ ammonium; NO_2^- nitrite; NO_3^- nitrate. Under nitrogen deficiency conditions, NO_3^- and NO_2^- mainly undergo reduction reactions in the root system, and then produced NH_4^+ that can be assimilated into Gln and Glu acid together with the NH_4^+ absorbed by the plant. Gln and Glu form other amino acids through a series of reactions, and then form proteins and chlorophyll to participate in the photosynthetic reaction

contents (Schortemeyer et al. 1997). In our study, the results showed that a significant decrease in leaf NH_4^+-N content occurred, whereas the content in the roots increased under nitrogen limitation. These data indicated that nitrogen limitation restricted the accumulation of assimilated NH_4^+ in the leaves, promoted the accumulation of NH_4^+ in the roots, and damaged the roots, while the grafted plants with 'H' as rootstocks were relatively little affected (Fig. 8).

Conclusion

In grafted tomato plants, rootstocks are the key factor that determine the nitrogen-use efficiency. Our results showed that, under nitrogen limitation stress, grafting onto both high- and low-nitrogen-use-efficiency genotypes reduced carbon assimilation and light energy utilization, inhibited the absorption and utilization of nitrogen by plants, and limited plant growth. However, under nitrogen deficiency conditions, plants grafted with high-nitrogen-use-efficiency tomato seedlings exhibited higher NR activity and N content induces accumulated more NH_4^+, then results in reducing the damage on chlorophyll and the reaction center of PSII, thus to enhancing photosynthesis and improve stress tolerance. The present study also showed the complexity of nitrogen metabolism in leaves and roots, and further investigation is still needed to understand the mechanisms by which the interactions between the rootstock and scion improve nitrogen utilization.

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Author Contributions ZZ and KX designed experiments; BC and ZC assisted in completing the experiments; ZZ and KX wrote the manuscript.

Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

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