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Low Nitrogen Stress Promotes Root Nitrogen Uptake and Assimilation in Strawberry: Contribution of Hormone Networks

Wenjie Zhang [1,†], Ting Zhang [1,†], Jia Zhang [1,2], Weiwei Lei [3], Lin Zhao [1], Shuai Wang [1], Mengyun Shi [1] and Meng Wei [1,2,*]

[1] Xuzhou Institute of Agricultural Sciences of the Xuhuai District of Jiangsu Province, Xuzhou 221131, China
[2] Tongshan Test Station, Xuzhou Institute of Agricultural Sciences of the Xuhuai District of Jiangsu Province, Xuzhou 221121, China
[3] Beijing Changping District Agricultural Technology Extension Station, Beijing 102200, China
* Correspondence: weimeng@jaas.ac.cn
† These authors contributed equally to this work.

Abstract: Low nitrogen stress severely impedes crop growth and productivity. There has been substantial research on root adaptation to low nitrogen conditions in many plant species. However, the mechanism underlying the morphological response of the strawberry (*Fragaria* × *ananassa* Duch.) root to low-NO_3^- or low-NH_4^+ stress remains poorly understood. Strawberry plants were hydroponically cultivated under 1 mM NO_3^-, 1 mM NH_4^+, and control (15 mM NO_3^-) conditions to assess the physiological responses of their roots to low nitrogen stress. As a result, low nitrogen stresses increased the fresh weight of root, lateral root density, and root surface area, as well as enhanced the accumulation of indole-3-acetic acid and jasmonic acid while significantly reducing salicylic acid in the roots. Correspondingly, low nitrogen stresses increased PM H^+-ATPase activity. Low-NO_3^- stress enhanced the activities of nitrate reductase and glutamine synthetase, whereas low-NH_4^+ treatment led to higher glutamine synthetase and glutamate synthase activities. Collectively, the present results demonstrate that low nitrogen stresses enhance nitrogen uptake of strawberry roots by regulating hormones (indole-3-acetic acid, jasmonic acid, and salicylic acid) and thereby mediating PM H^+-ATPase activity, while promoting nitrogen metabolism by upregulating the activities of nitrate reductase, glutamine synthetase, and glutamate synthase. In conclusion, low nitrogen conditions may facilitate more efficient acquisition of available N from the soil by strawberry root system.

Keywords: low nitrogen; auxin; jasmonic acid; salicylic acid; lateral root; root architecture; root remodeling

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1. Introduction

Nitrogen (N) is one of the most important nutrient elements involved in many key physiological and metabolic reactions in crop growth [1]. Low N supply limits crop growth and therefore reduces its yield [2]. In pursuit of higher crop yields, growers tend to apply excessive N, which far exceeds the actual demand of crops. Globally, about 100 million tons of N is consumed for agricultural production every year [3]. However, there has been significant loss of N due to the low N use efficiency (25–50%) [4], which further causes the significant loss of economic and environmental benefits [5]. Therefore, it is urgent to develop crops with low N demand and high N use efficiency for environment-friendly and sustainable agriculture.

Nitrate (NO_3^-) and ammonium (NH_4^+) are the two major sources of N in soil, and act as important signaling molecules to modulate various physiological reactions of plants, including gene expression and root architecture modifications [6,7]. The root is a primary organ for nutrient uptake in plants. It has been well documented that root architecture

remodeling is a main strategy for plants to adapt to changing environments such as soil nutrient fluctuations [8]. For instance, maize roots respond to low N stress by enhancing root horizontal and vertical extension [9,10], and the formation of lateral roots can be stimulated in response to phosphorus deficiency in rice [11]. Therefore, understanding the mechanisms by which crops respond to nutrient stress through root plasticity can help advance the breeding targets for improving crop nutrient use efficiency via root phenotypes.

Many studies have demonstrated the pivotal role of hormones in regulating root growth under N deficiency. For example, auxin has been widely recognized as a pivotal basis for other hormones to affect root development [12]. Jasmonic acid (JA) is a critical phytohormone regulating crop growth and various defense responses [13,14]. Previous studies have demonstrated that JA can suppress primary root development [15], stimulate lateral root initiation [16], and induce root regeneration [17]. In addition to indole-3-acetic acid (IAA) and JA, salicylic acid (SA) is also an essential component in the complex network of hormonal regulation during plant resistance response. It has been reported that SA can significantly inhibit the development of primary roots, but promote lateral root growth [18] and the formation of root primordia [19]. Several studies have established that roots respond to N fluctuations under the synergistic effect of multiple hormones [20], and the response is largely dependent on the genotypes, environmental conditions, and intensity or duration of low N stress.

Before utilization by plants, NO_3^- and NH_4^+ must undergo an assimilation process regulated by a series of enzymes. After absorption by the roots, NH_4^+ is converted into amino acid by glutamine synthetase (GS) and glutamate synthase (GOGAT). For NO_3^-, after being absorbed through the root, it will be reduced into NO_2^- by nitrate reductase (NR), and then into NH_4^+ by nitrite reductase (NiR), which is finally assimilated to organic N. Moreover, extensive research has demonstrated the critical role of NO_3^- and NH_4^+ as signaling molecules in inducing the expression of many genes associated with N assimilation [21]. For example, NH_4^+ can induce GOGAT gene expression in root tips [22].

As a significant ion pump in cell membranes, plasma membrane (PM) H^+-ATPase can establish a transmembrane chemical gradient of H^+ [23], which drives the acidification of the cell wall and thereby promotes root growth [24,25]. Several reports have noted that PM H^+-ATPase activity is modulated by various environmental signals, including some classical plant hormones and N (reviewed in [23,26]).

Strawberry is a popular fresh fruit widely grown in most parts of the world. N acts as a major contributor to strawberry growth and fruit quality [27]. Low N severely inhibits strawberry growth, resulting in leaf chlorosis [28] and a decrease in leaf area, dry matter [29], and amino acids content in fruit [30]. Although there have been some reports about the morphological changes of roots in response to N deficiency in strawberry, the regulatory mechanism of hormones on strawberry root remodeling under N deficiency remains poorly understood. Our hypothesis is that low N stimulates root remodeling and enriches root structure for better N acquisition, which is regulated by hormonal interactions. Therefore, this study aims to evaluate the physiological and morphological responses of strawberry roots to low N stress, and preliminarily elucidates the possible regulatory mechanism. The findings are expected to afford a theoretical basis for the genetic improvement and more efficient N use in strawberry production.

2. Materials and Methods

2.1. Cultivation Conditions

Strawberry stolons (Miaoxiang 3) were selected from the greenhouse, soaked in fungicide, and then transferred into distilled water. When new leaves appeared, uniform seedlings were cultured in plastic basins containing nutrient solution (five seedlings per pot). The seedlings were grown in an artificial climate chamber at 25/20 °C (day/night) with a light cycle of 16/8 h (light/dark), 70% relative humidity, and an average light intensity of 350 $\mu mol\, m^{-2}\, s^{-1}$ PPFD.

The nutrient solution contained 2.05 mM KCl, 2.001 mM $K_2HPO_4 \cdot 3H_2O$, 1.0 mM $MgSO_4 \cdot 7H_2O$, 0.03 mM EDTA-Fe, 0.025 mM H_3BO_4, 2.0 µM $MnSO_4 \cdot H_2O$, 0.78 µM $CuSO_4$, 3.21 µM $ZnSO_4 \cdot H_2O$, and 0.5 µM Na_2MoO_4. Strawberry seedlings were supplied with a half-strength nutrient solution containing 15.0 mM NO_3^- for 2 d [31], and then cultured in a solution containing 1 mM NO_3^- (LN treatment), 1 mM NH_4^+ (LA treatment), or 15 mM NO_3^- (Control). NO_3^- was added as Ca $(NO_3)_2$; NH_4^+ was added as NH_4Cl; and $CaCl_2$ was used to maintain the same Ca^{2+} concentration for all treatments. The pH of the nutrient solution was held at 6.5 with KOH and HCl. The nutrient solution was renewed every two days and the pots were randomly positioned.

At each sampling time, seedlings were first washed with distilled water and dried with absorbent paper, and then were divided into two parts. The fresh sample was used for biomass, root morphology, and lateral root density determination. The other part was treated with liquid nitrogen and stored at −80 °C for the determination of soluble proteins, enzyme activities, and hormones.

2.2. Biomass Determination

Strawberry seedlings were washed with distilled water and dried with absorbent paper at 24, 48, and 96 h after treatment. The stems and roots were weighed separately.

2.3. Root Morphology Examination

At 0, 48, and 96 h after treatment, the roots were washed with distilled water and floated in a PVC tray containing 2–3 mm of water, and then scanned with an EPSON V850 PRO scanner (EPSON, Beijing, China). Total root length, root surface area, average diameter, and the number of root tips per plant in the images were analyzed using WinRHIZO 2017a (Regent Instruments Inc., Quebec, QC, Canada).

2.4. Lateral Root Density Determination

After washing the adventitious roots with distilled water at 48 h after treatment, the number of emerged lateral roots and the length of the adventitious root were counted under a microscope. Lateral root density (LRD) was recorded as the ratio of the number of emerged lateral roots to the distance, which was expressed as cm^{-1} root length [32].

2.5. Enzyme Activity Assay

Strawberry roots were washed with distilled water and dried with absorbent paper at 12 and 48 h after treatment. After being ground into powder, these roots were used for the determination of enzyme activity.

NR activity was determined by reference to the method of Majláth et al. [33]. Strawberry roots (1 g) were well ground in 1 mL 0.2 M phosphate buffer (pH 7.5) and centrifuged at 12,000× *g* for 5 min at 4 °C. About 0.5 mL 0.1 M KNO_3 and 0.3 mL 2.0 mg mL^{-1} NADH were added into 0.2 mL supernatant and the mixture reaction was conducted in a water bath at 25 °C for 30 min. Then, the reaction was terminated by the addition of 1 mL 30% trichloroacetic acid. The reaction mixture was added with 2 mL 1% sulfonamide and 2 mL 0.2% α-naphthylamine and mixed well. After standing for 15 min, colorimetry was performed at a 520 nm wavelength by using a Multiskan GO 1510 spectrophotometer (Thermo Fisher Scientific, Vantaa, Finland). One unit of NR activity (U g^{-1}) was defined as the amount of 1 µmol NADH consumed per gram of sample per hour.

NiR activity was assayed under the conditions adapted from the study of Caroline et al. [34]. Briefly, 0.1 g strawberry root and 1 mL buffer (pH 8.0) were ground in a frozen mortar and centrifuged at 12,000× *g* for 2 min at 4 °C. The buffer was a mixture of 50 mM Tris-HCl and 3 mM EDTA. About 0.1 mL of supernatant was added to a 1.2 mL reaction solution (including 100 mM potassium phosphate buffer (pH 7.5), 10 mM KNO_2, 15 mg mL^{-1} methyl viologen, H_2O, and 5% sodium dithionite). The mixture reaction was conducted in a water bath at 25 °C for 30 min. Then, 1 mL 1% sulfonamide and 1 mL 0.2% α-naphthylamine were added and shaken well, and the absorbance was measured

at 540 nm after standing for 30 min. One unit of NiR activity (U g^{-1}) was defined as the amount of reduction of 1 µmol NO_2^- g^{-1} h^{-1}.

GOGAT activity was measured according to Singh et al. [35], with minor modifications. Strawberry roots (0.1 g) were well ground in 1 mL 25 mM Tris-HCl buffer and centrifuged at 8000× *g* for 10 min at 4 °C. The supernatant was mixed thoroughly with 20 mM glutamine, 100 mM α-ketoglutarate, and 3 mM NADH. Then, the activity of GOGAT was measured at 340 nm, and expressed as the amount of 1 nmol NADH consumed g^{-1} min^{-1}.

GS activity was determined according to the method described by Wang et al. [36]. Strawberry roots (1 g) and 4 mL 0.05 mM phosphate buffer (containing 0.4 M sucrose and 4 mM L-cysteine) were thoroughly ground in a frozen mortar. After centrifugation at 12,000× *g* and 4 °C for 15 min, 1 mL of supernatant was added to 3 mL enzyme reaction solution, which was composed of 50 mM L-glutamate sodium, 4 mM ATP-2Na, 40 mM hydroxylamine, 20 mM magnesium sulfate, 10 mM L-cysteine, and 40 mM phosphate buffer. The reaction was carried out at 30 °C for 15 min. Then, 30% trichloroacetic acid, 5.5 M HCl, and 8% $FeCl_3$ were added to the mixture to terminate the reaction. After standing for 10 min, the absorbance was measured at 540 nm. GS activity was expressed as the amount of 1 nmol of γ-glutamylhydroxamate generated g^{-1} min^{-1}.

2.6. Soluble Protein Content Determination

Soluble protein content was determined according to the method described by Bradford et al. [37], with minor modifications. At 12 h and 48 h after treatment, strawberry roots (0.2 g) were fully ground and centrifuged at 5000× *g* for 10 min at 4 °C. About 0.1 mL supernatant was mixed with 5 mL of Komas Brilliant Blue G-250 solution (consisting of 100 mg L^{-1} Komas Brilliant Blue G-250, 4.7% ethanol (*v*/*v*) and 8.5% (*w*/*v*) phosphoric acid), and the absorbance was measured at 595 nm after standing for 2 min. The soluble protein content was calculated using a standard curve based on bovine serum protein.

2.7. Total N Content Measurement

The total N content in strawberry roots was determined using the Kjeldahl method [38]. At 48 h after treatment, dried strawberry roots (0.1 g) were digested in a Kjeldahl flask with sulfuric acid (5 mL) and hydrogen peroxide. After the mixture was clear, the distilled solution was obtained using a Kjeldahl apparatus (FOSS Kjeltec™ 8400, Hilleroed, Denmark) and titrated with 0.01 mol L^{-1} 1/2 H_2SO_4.

2.8. PM H^+-ATPase Activity Assay

PM H^+-ATPase activity was determined according to Zhang et al. [39]. At 12 h and 48 h after treatment, strawberry roots were homogenized in buffer (containing 250 mM sucrose, 4 mM DTT, 7.2 µg mL^{-1} PMSF, 50 mM Tris, 8 mM EDTA, and 1.5% PVP). After centrifugation at 10,000× *g* for 15 min at 4 °C, the supernatant was centrifuged again at 10,000× *g* for 30 min at 4 °C. The precipitate was re-solubilized in a buffer containing 250 mM sucrose, 2 mM DTT, and 5 mM Pipes. After treatment with sucrose gradient solution and KCl, the obtained precipitate was added into 0.5 mL of reaction solution consisting of 250 mM HEPES-Tris, 25 mM ATP-Na_2, 3 mM Na_2MoO_4, 1 mM NaN_3, 1 mM EDTA, and 0.02% Triton X-100. After the reaction was terminated, 50 µL 10% ascorbic acid was added, and the absorbance was measured at 660 nm after standing for 40 min.

2.9. Determination of Plant Hormones

Hormones in strawberry roots were determined according to Yang et al. [40], with slight modifications. At 12 h and 48 h after treatment, strawberry roots (0.1 g) were ground in liquid nitrogen and added to 1 mL of extraction solution (consisting of 0.4 mL methanol, 0.4 mL acetonitrile, and 0.2 mL water), and then extracted for 12 h at 4 °C with protection from light before centrifugation at 14,000× *g* for 10 min. About 0.8 mL supernatant was dried using a nitrogen evaporator, then re-dissolved in 0.2 mL 50% methanol and centrifuged at 14,000× *g* for 10 min to obtain the supernatant.

The supernatant was separated by an ExionLC™ AD series high-performance liquid chromatography system (AB SCIEX, Framingham, MA, USA) and a Kinetex® C18 column (1.7 µm, 150 × 2.1 mm; Phenomenex, Torrance, CA, USA). Distilled water containing 0.04% (*v*/*v*) formic acid was used for mobile phase A, and methanol was used for mobile phase B. The elution gradients were as follows: 0–5.5 min, mobile phase B varied linearly from 10% to 95%; 5.6–7 min, mobile phase B remained at 95%; 7.1–7.5 min, mobile phase B varied linearly from 95% to 10%; 7.6–10 min, mobile phase B remained at 10%. Mass spectrometry in the positive/negative ion mode was completed using an AB Sciex Triple Quad 3500 (AB SCIEX, Framingham, MA, USA). Multi Quant software was used to extract the peak areas and retention time of the chromatograms. Hormone contents in strawberry roots were calculated from the standard curve.

2.10. Statistical Analysis

Data analysis was performed using SPSS V.26 (SPSS Statistics, Armonk, NY, USA). Duncan's test was used to detect significant differences at the 95% probability level. If the difference is significant between treatments, a different letter is used.

3. Results

3.1. Biomass of Strawberry Roots

Compared with the control, low N stresses showed no significant effect on shoot fresh weight at 24, 48, and 96 h (Figure 1a), but significantly increased the fresh weight of strawberry roots at 48 h (Figure 1b).

3.2. Morphology of Strawberry Roots

Low N stresses altered the root structure of strawberry. The total root length under LN treatment increased by 30.34% at 48 h and 27.71% at 96 h in comparison with that under the control but by 23.67% at 48 h and 8.5% at 96 h under LA treatment (Figure 2a).

At 48 h, compared with that of the control, the root surface area under LN and LA treatments increased by 21.23% and 22.93%, respectively. However, both LN and LA treatments resulted in no significant difference in the root surface area relative to the control at 96 h (Figure 2b).

The number of root tips increased significantly under low N stresses (Figure 2c). Compared with that of the control, the number of root tips under LN and LA treatments increased by 15.63% and 8.26% at 48 h, respectively, and a similar trend was observed at 96 h.

However, the average root diameter decreased by 9.02% and 7.46% under LN and LA treatments, respectively, compared with that of the control at 48 h (Figure 2d). At 96 h, compared with that of the control, the average root diameter decreased by 11.34% and 6.40%, respectively.

3.3. Lateral Root Density

LN and LA treatments significantly promoted the formation of lateral roots at 48 h (Figure 3). Compared with that of the control (4.28 ± 0.14 cm^{-1} root length), LRD increased by 36.69% under LN treatment (5.86 ± 0.32 cm^{-1} root length) and 24.82% under LA treatment (5.35 ± 0.38 cm^{-1} root length).

3.4. Hormone Contents in Strawberry Roots

Compared with the control, the LN and LA treatment increased the IAA concentration in strawberry roots by 42.37% and 49.51% at 12 h, respectively (Figure 4a). However, no significant difference was detected in root IAA level at 48 h among the three treatments.

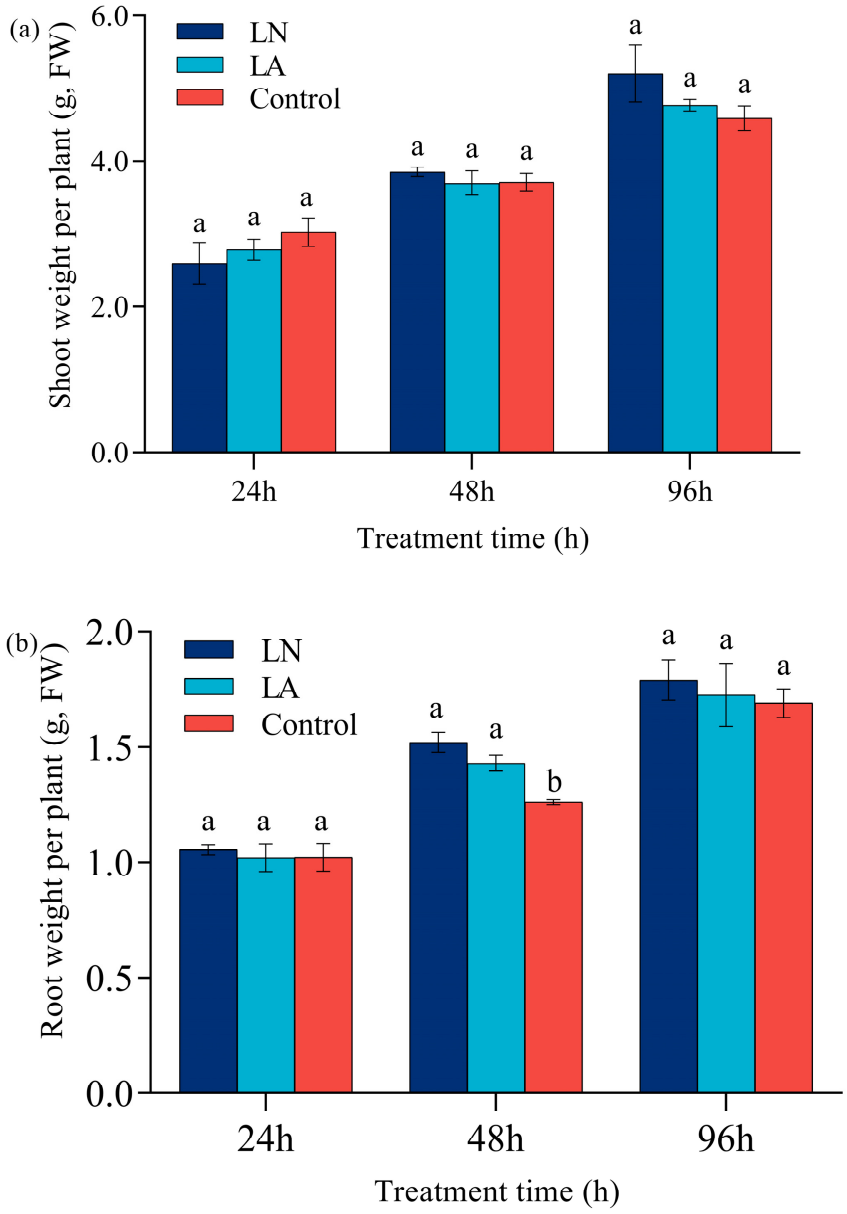

Figure 1. Effect of low N stress on the fresh weight of strawberry shoots and roots at 24 h, 48 h, and 96 h. (**a**) Shoot fresh weight. (**b**) Root fresh weight. Values represent mean ± SEM of five biological replicates. Different letters indicate significant differences at $p < 0.05$.

The JA concentration increased significantly under low N stresses (Figure 4b). Compared with that of the control, the JA concentration under LN and LA treatments increased by 64.49% and 36.68% at 12 h, respectively, and a similar trend was observed at 48 h. Moreover, the root JA concentration under LN treatment was 49.13% higher than that under LA treatment.

However, the root SA concentration was significantly lower under low N stresses compared with that under the control (Figure 4c). The root SA concentration decreased by 35.55% and 47.69% under LN and LA treatments, respectively, compared with that of the control at 12 h, and a similar trend was observed at 48 h. However, there was no significant difference between LN and LA treatments at 12 h and 48 h.

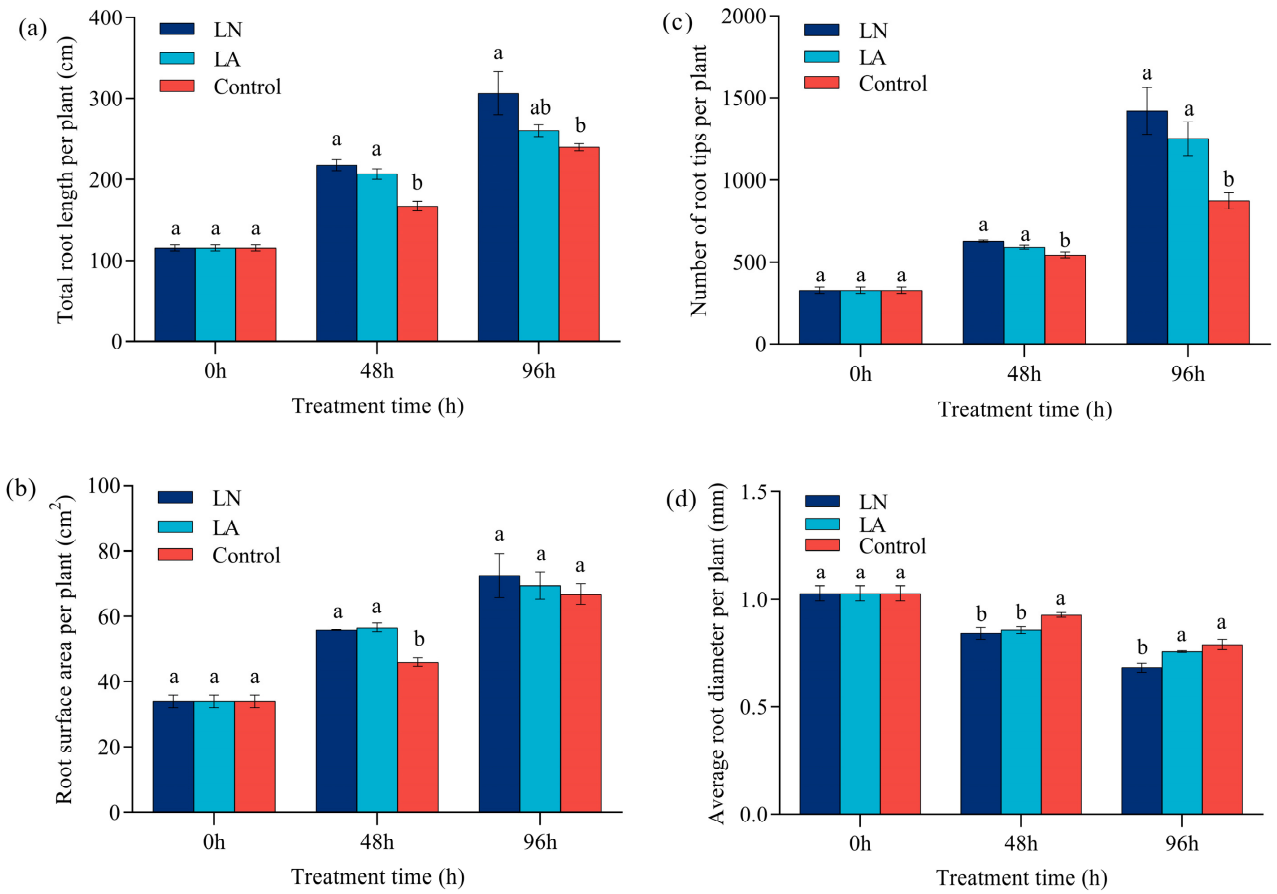

Figure 2. Root morphology of strawberry at 0 h, 48 h, and 96 h under different N treatments. (**a**) Total root length. (**b**) Total surface area. (**c**) Total number of root tips. (**d**) Average root diameter. Values represent mean ± SEM of three biological replicates. Different letters indicate significant differences at $p < 0.05$.

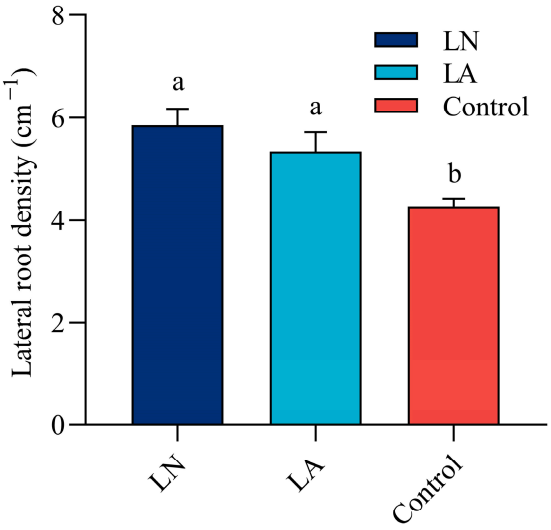

Figure 3. Lateral root density under three treatments at 48 h. Values represent mean ± SEM of five biological replicates. Different letters indicate significant differences at $p < 0.05$.

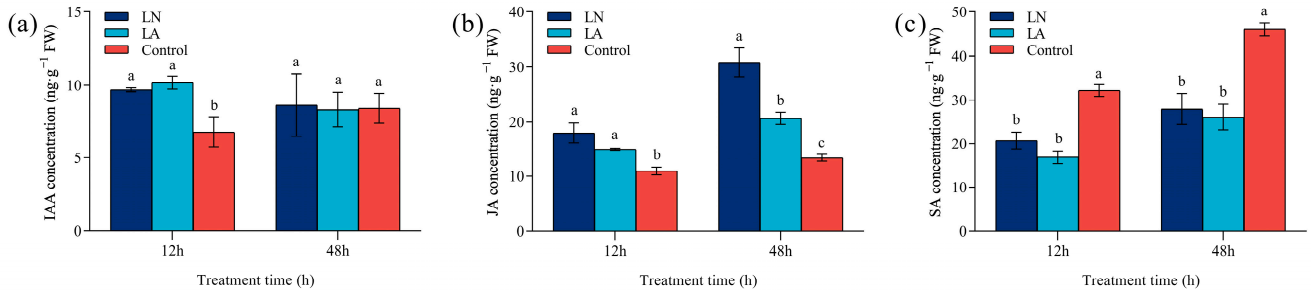

Figure 4. IAA concentration (**a**), JA concentration (**b**), and SA concentration (**c**) in strawberry roots at 12 h and 48 h under different N treatments. Values represent mean ± SEM of three biological replicates. Different letters indicate significant differences at $p < 0.05$.

3.5. PM H⁺-ATPase Activity

At 12 h, compared with that of the control, the PM H⁺-ATPase activity was significantly higher under the LN treatment, but only slightly increased under LA treatment with no significant difference (Figure 5). The PM H⁺-ATPase activity was significantly increased at 48 h under both LN and LA treatments compared with that of the control.

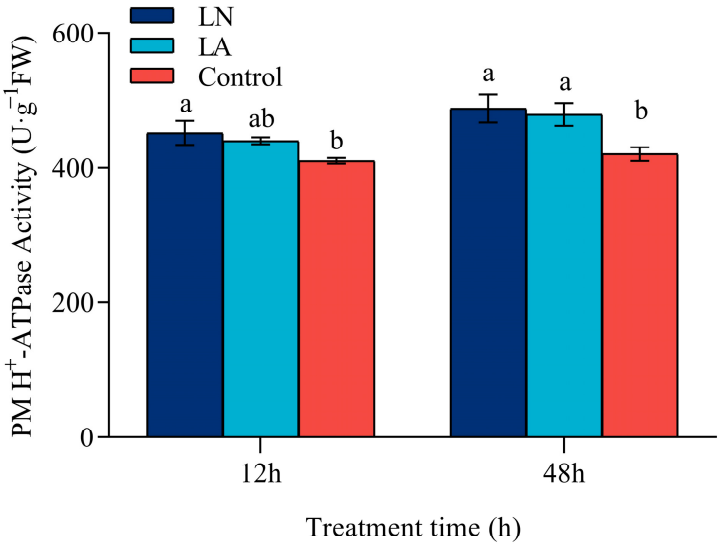

Figure 5. Effect of different N treatments on H⁺-ATPase enzyme activity in strawberry roots at 12 h and 48 h. Values represent mean ± SEM of three biological replicates. Different letters indicate significant differences at $p < 0.05$.

3.6. Enzyme Activity Related to Nitrogen Assimilation

There was no significant difference in the root NR activity of strawberry between low N treatments and the control at 12 h (Figure 6a). At 48 h, the LN and LA treatments increased the NR activity by 19.83% and 10.48%, respectively. However, both LN and LA treatments resulted in no significant difference in NiR activity relative to the control at 12 h and 48 h (Figure 6b).

Compared with the control, the LN treatment significantly increased the GS activity at 12 h and 48 h, while the LA treatment only significantly increased the GS activity at 48 h (Figure 6c).

The LN treatment resulted in similar GOGAT activity to the control at 12 h and 48 h (Figure 6d). However, the LA treatment increased the GOGAT activity by 15% and 14.85% at 12 h and 48 h, respectively, compared with the control.

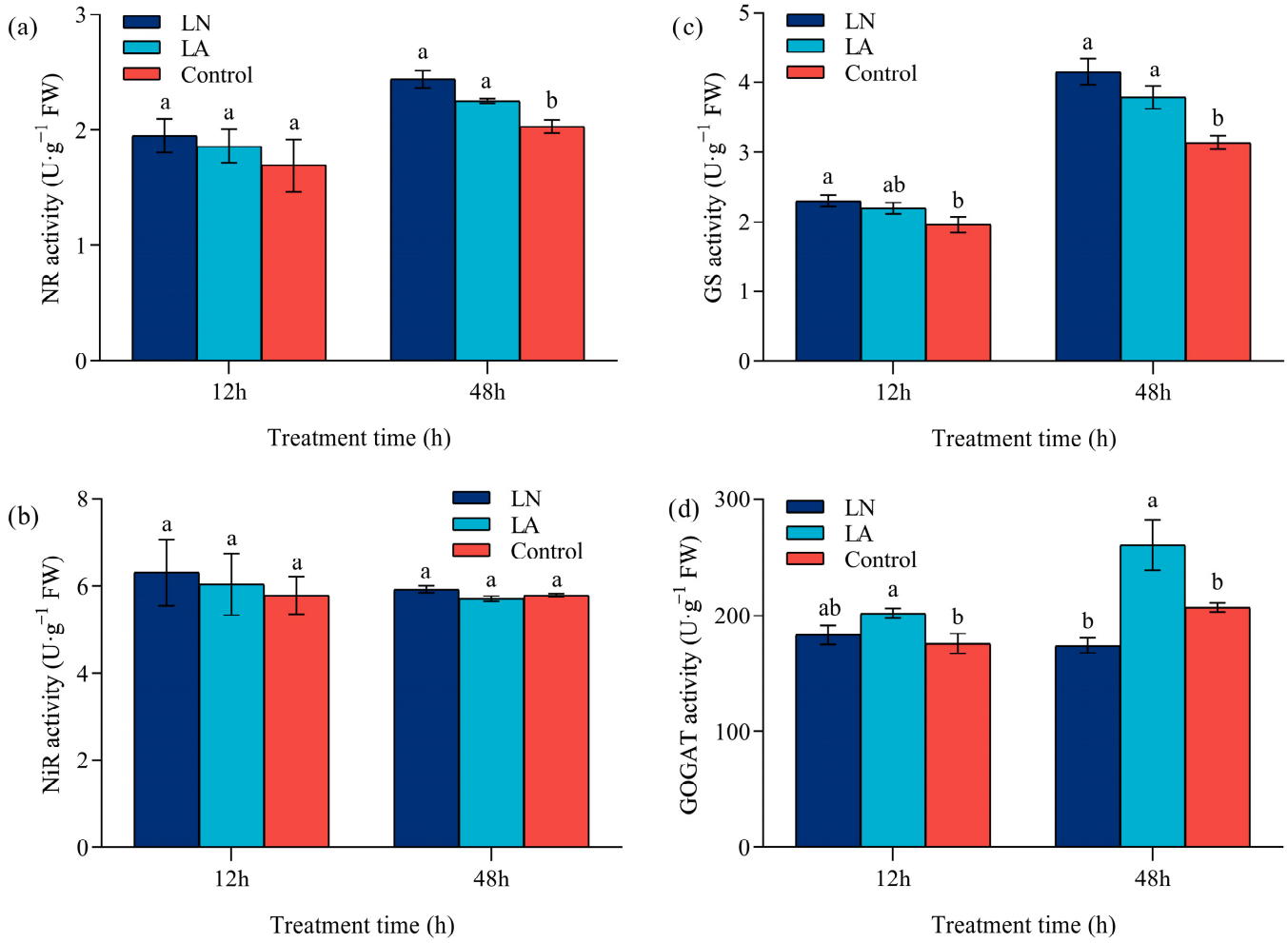

Figure 6. Effects of low N stresses on NR (**a**), NiR (**b**), GS (**c**), and GOGAT (**d**) activity in strawberry roots at 12 h and 48 h. Values represent mean ± SEM of three biological replicates. Different letters indicate significant differences at $p < 0.05$.

3.7. Soluble Protein Content in Strawberry Roots

Low N stresses showed no significant effect on the soluble protein content in strawberry roots at 12 h (Figure 7). In contrast, at 48 h, the LN and LA treatment increased the soluble protein content by 96.97% and 84.25%, respectively, compared with the control. At 12 h and 48 h, the LN treatment led to a slightly higher soluble protein content than the LA treatment without significant difference.

3.8. Total Nitrogen Content in Strawberry Roots

At 48 h, both LN and LA treatments significantly reduced the total N content in strawberry roots (Figure 8). Compared with the control, the LN and LA treatment reduced the total N content by 17.86% and 24.4%, respectively.

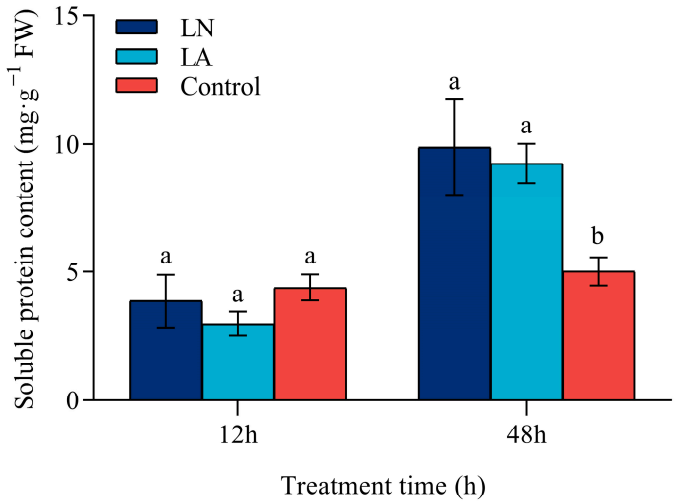

Figure 7. Soluble protein content at 12 h and 48 h under different N treatments. Values represent mean ± SEM of three biological replicates. Different letters indicate significant differences at $p < 0.05$.

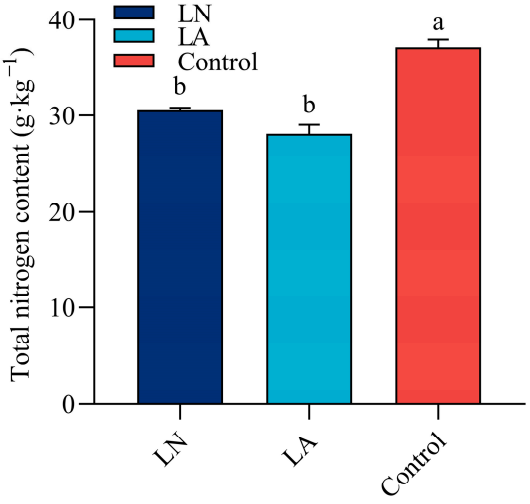

Figure 8. Total N content at 48 h under different N treatments. Values represent mean ± SEM of three biological replicates. Different letters indicate significant differences at $p < 0.05$.

4. Discussion

4.1. Root Architecture Changes for Better N Uptake

Root structural plasticity is critical to plant adaptive response to complex and varying living environments. To date, numerous studies have demonstrated that changes in root architecture can dramatically improve crop nutrient efficiency [41]. Correspondingly, root structure can be influenced by nutrient conditions such as N supply [42]. The results of this experiment showed a significant increase in the root fresh weight at 48 h under low N conditions. Therefore, we focused more on the analysis of the roots. Further examination of root architecture revealed that low N treatments significantly increased the total root length, root surface area, and total root tip number, but obviously reduced the average root diameter at 48 h compared with the control. These results are consistent with previous studies, which reported that crops would develop a deeper root system and larger root surface area for more efficient acquisition of N to better adapt to N deficiency [43–45].

Plant hormones play important roles in controlling root development, including lateral root growth and root hair formation [46], and have significant interactions with N [47]. Jia et al. [48] revealed that low N stress could upregulate the transcription of YUC8 and its homologs and the TAA1 gene to enhance local IAA biosynthesis in Arabidopsis roots.

Some recent studies have demonstrated that NO_3^- and NH_4^+ signaling can mediate the shoot-to-root transport of auxin [49], and regulate its accumulation in the epithelial cells of lateral root primordia, which in turn stimulates the emergence and growth of lateral roots [50]. Sun et al. [51] reported that low N stress could increase auxin accumulation in roots, enhancing root development through several auxin-mediated pathways. We observed a significant increase in IAA concentration at 12 h under low N treatments in this study. Based on the above results, it could be speculated that low-NO_3^- and low-NH_4^+ signaling could induce local IAA biosynthesis in strawberry plants and enhance its intercellular transport, thereby increasing root IAA concentration and promoting root growth.

As mentioned above, JA and SA are also closely associated with signaling pathways regulating root structure. Wang et al. [52] and Sun et al. [53] suggested that jasmonate application could increase the lateral root number in rice and Arabidopsis. Gutierrez et al. [54] reported that JA negatively affected adventitious root formation. Moreover, extensive research has revealed a close correlation between JA and auxin. Several studies have suggested that the JA signaling pathway is positively associated with auxin homeostasis by regulating the expression of the auxin-related gene [16,55]. Xu et al. [12] revealed that JA promotes auxin transport by regulating PIN genes, while auxin regulates JA homeostasis by modulating GH3.3/5/6 genes. Our study revealed significant increases in LRD, IAA, and JA concentration under low N stresses in strawberry. Therefore, it can be inferred that there is a synergistic association between JA and IAA to facilitate lateral root formation under N deficiency.

Previous research has highlighted the role of SA in reducing the number of lateral roots in Arabidopsis seedlings [18,19,56]. Moreover, several studies have indicated that SA promotes or inhibits lateral root growth depending on its own concentration [57] and the IAA concentration [58]. Research in this area has documented the interaction between SA and IAA. Kitakura et al. [59] observed that a high level of SA would interfere with auxin distribution. Llorente et al. [60] found that SA could improve the stability of auxin repressor proteins in the process of auxin signal transduction by binding to them, thereby short circuiting the auxin signal network. Similarly, the antagonistic relationship between SA and JA in regulating root morphology has been widely investigated [61,62]. SA-induced expression of ANAC032 and GRX480 can inhibit JA signaling in plant immunity [63,64]. Interestingly, JA signaling also blocks SA biosynthesis by modulating the activities of multiple NAC transcription factors (NAM, ATAF, and CUC transcription factors) [65]. In this study, we observed a significant decrease in SA concentration in strawberry roots under low N stresses, which was contrary to the trend of IAA and JA. Hence, it is reasonable to speculate that SA may negatively regulate lateral roots by interfering with the signal transduction of IAA and JA.

Cell wall loosening, which is facilitated by H^+ efflux provided by PM H^+-ATPase [66], is the direct reason for cell expansion and plant growth [67]. Sperandio et al. [68] assessed the importance of PM H^+-ATPase activity in the adaptation to N deficiency in rice. Further research revealed that the starvation and resupply of N could promote PM H^+-ATPase activity and root growth [69]. It is widely acknowledged that IAA can induce PM H^+-ATPase activity according to the acid growth theory [24]. Furthermore, recent research has confirmed that PM-ATPase activity is related to auxin-binding protein 1 (ABP1), which can be activated by auxin accumulation [70]. In this study, we observed the same increasing trend of LRD, IAA concentration, and H^+-ATPase activity in strawberry under low N stresses. In general, it seemed that the accumulation of IAA increased PM H^+-ATPase activity, which in turn promoted lateral root initiation and primordium development. This speculation is consistent with findings in the studies of maize and wheat by Sun et al. [51] and Lv et al. [71], who reported that an increase in IAA in maize and wheat roots under low N stress led to an increase in H^+ efflux and acidification of apoplastic space, and ultimately boosted lateral root growth. Moreover, the addition of sodium orthovanadate (Na_3VO_4, an

inhibitor of PM H^+-ATPase activity) or 2,3,5-triiodobenzoic acid (TIBA, an inhibitor of the polar transport of auxin) eliminated the enhancing effect of N deficiency on root elongation.

Soaking with methyl jasmonate could significantly increase the H^+-ATPase activity and H^+ transmembrane transport in ginger rhizomes [72]. Chen et al. [73] reported the enhancement of H^+-ATPase activity mediated by JA signaling under multiple stresses (herbivore stress and salt stress). In addition, SA is involved in the regulation of PM H^+-ATPase. Recent research has demonstrated that H^+-ATPase activity can be upregulated in SA-pretreated seedlings, resulting in an enhanced tolerance to salt stress [74]. Interestingly, previous studies of temperature stress have shown that SA pretreatments can also stimulate PM H^+-ATPase activity in grapes [75] and peas [76]. In this study, significant increases in JA concentration and H^+-ATPase activity while a significant decrease in SA concentration were observed under low N stress in strawberry. Taking into account the synergistic and/or antagonistic effects among IAA, JA, and SA, it can be speculated that low N stress stimulates an asymmetric activation of the crosstalk network between hormones and contributes to a final balance of interaction, which may lead to increases in H^+-ATPase activity and H^+ efflux, thereby promoting lateral root formation and growth.

Lv et al. [71] observed significant decreases in root NO_3^- and NH_4^+ influx under low N conditions, which was consistent with the decrease in root total N content. This finding is generally in agreement with our study. Moreover, root weight under low N conditions was significantly higher than that of the control, which may be related to the redistribution of photosynthate [77,78].

4.2. Improving N Utilization via Enzyme Activities Changes

After NO_3^- is absorbed by roots, NR and NiR initiate the first stage of N assimilation, followed by the GS/GOGAT cycle responsible for the conversion of inorganic N into organic N [79]. NR is the first and also a rate-limiting enzyme in N assimilation [80]. GS is a multifunctional enzyme, and its level can reflect the strength of N assimilation [81]. Li et al. [82] observed that NR activity in the growth medium of *Pseudochlorococcum* sp. gradually increased with N depletion. In contrast to NR, NiR does not appear to be affected by N level and form [83]. Xiong et al. [83] demonstrated that a low N level stimulates but a high N level inhibits GS activity in citrus. Zhou et al. [84] showed that lower N treatments induced higher GOGAT activity in lettuce. Here, we found that low NO_3^- resulted in higher NR and GS activities, whereas low NH_4^+ led to higher GS and GOGAT activities. Considering the significant increase in soluble protein content in strawberry roots at 48 h under low N stresses, we speculate that low N promotes N assimilation mainly by inducing NR, GS, and GOGAT activities, thereby promoting protein biosynthesis.

It has been well documented that the activity of enzymes related to N assimilation is also regulated by phytohormones. In a previous study, with exogenous IAA application under copper (Cu) stress, spinach seedlings exhibited higher NR, GS, GOGAT activities, and soluble protein content [85]. Similarly, Parihar et al. [86] observed that the application of methyl jasmonate could increase the NR, GS, and GOGAT activities in luffa. Several studies have suggested that low concentrations of SA could increase the NR activity in wheat [87] and maize [88]; on the other hand, an inhibitory effect was observed at a high SA concentration [89]. In this study, compared with the control, low N stress significantly changed the NR, GS, and GOGAT activities as well as IAA, JA, and SA concentrations. Therefore, it is very possible that the crosstalk between hormones may increase the activities of N assimilation-related enzymes, which help the strawberry roots to maintain higher levels of N assimilation and protein biosynthesis under low N conditions.

5. Conclusions

This study elucidates the central role of hormones in the complex regulatory network under N deficiency in strawberry. There are two possible mechanisms for the changes in root architecture of strawberry plants for better N acquisition. First, a larger root surface area is formed to enhance the ability of root to explore the soil for more N. This

process may be mediated by the final balance of hormonal interactions (including IAA, JA, and SA), which can increase PM H^+-ATPase activity and ultimately accelerate cell wall acidification, thereby enhancing lateral root formation and growth. Second, NR, GS, and GOGAT activities are enhanced to improve root N assimilation and protein biosynthesis, thus promoting lateral root growth. This process may also be associated with the regulation of hormonal networks. In conclusion, strawberry roots can better adapt to the N-deficient environment by increasing N absorption area and N assimilation.

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References

1. Urban, A.; Rogowski, P.; Wasilewska-Debowska, W.; Romanowska, E. Understanding Maize Response to Nitrogen Limitation in Different Light Conditions for the Improvement of Photosynthesis. *Plants* **2021**, *10*, 1932. [CrossRef] [PubMed]
2. Quan, X.Y.; Qian, Q.F.; Ye, Z.L.; Zeng, J.B.; Han, Z.G.; Zhang, G.P. Metabolic Analysis of Two Contrasting Wild Barley Genotypes Grown Hydroponically Reveals Adaptive Strategies in Response to Low Nitrogen Stress. *J. Plant Physiol.* **2016**, *206*, 59–67. [CrossRef] [PubMed]
3. Glass, A.D.M. Nitrogen Use Efficiency of Crop Plants: Physiological Constraints Upon Nitrogen Absorption. *Crit. Rev. Plant Sci.* **2003**, *22*, 453–470. [CrossRef]
4. Undurraga, S.F.; Ibarra-Henriquez, C.; Fredes, I.; Alvarez, J.M.; Gutierrez, R.A. Nitrate Signaling and Early Responses in Arabidopsis Roots. *J. Exp. Bot.* **2017**, *68*, 2541–2551. [CrossRef] [PubMed]
5. Zhang, Y.T.; Wang, H.Y.; Lei, Q.L.; Luo, J.F.; Lindsey, S.; Zhang, J.Z.; Zhai, L.M.; Wu, S.X.; Zhang, J.S.; Liu, X.X.; et al. Optimizing the Nitrogen Application Rate for Maize and Wheat Based on Yield and Environment on the Northern China Plain. *Sci. Total Environ.* **2018**, *618*, 1173–1183. [CrossRef]
6. Forde, B.G. Local and Long-Range Signaling Pathways Regulating Plant Responses to Nitrate. *Annu. Rev. Plant Biol.* **2002**, *53*, 203–224. [CrossRef]
7. Krouk, G.; Ruffel, S.; Gutierrez, R.A.; Gojon, A.; Crawford, N.M.; Coruzzil, G.M.; Lacombe, B. A Framework Integrating Plant Growth with Hormones and Nutrients. *Trends Plant Sci.* **2011**, *16*, 178–182. [CrossRef]
8. Wang, X.; Shen, J.; Liao, H. Acquisition or Utilization, Which Is More Critical for Enhancing Phosphorus Efficiency in Modern Crops? *Plant Sci.* **2010**, *179*, 302–306. [CrossRef]
9. Gaudin, A.C.M.; McClymont, S.A.; Holmes, B.M.; Lyons, E.; Raizada, M.N. Novel Temporal, Fine-Scale and Growth Variation Phenotypes in Roots of Adult-Stage Maize (*Zea Mays* L.) in Response to Low Nitrogen Stress. *Plant Cell Environ.* **2011**, *34*, 2122–2137. [CrossRef]
10. Liu, J.; Li, J.; Chen, F.; Zhang, F.; Ren, T.; Zhuang, Z.; Mi, G. Mapping Qtls for Root Traits under Different Nitrate Levels at the Seedling Stage in Maize (*Zea Mays* L.). *Plant Soil* **2008**, *305*, 253–265. [CrossRef]
11. Kirk, J.G.D.; Du, L.V. Changes in Rice Root Architecture, Porosity, and Oxygen and Proton Release under Phosphorus Deficiency. *New Phytol.* **1997**, *135*, 191–200. [CrossRef]
12. Xu, P.; Zhao, P.X.; Cai, X.T.; Mao, J.L.; Miao, Z.Q.; Xiang, C.B. Integration of Jasmonic Acid and Ethylene into Auxin Signaling in Root Development. *Front. Plant Sci.* **2020**, *11*, 271. [CrossRef] [PubMed]
13. Glazebrook, J. Genes Controlling Expression of Defense Responses in Arabidopsis—2001 Status. *Curr. Opin. Plant Biol.* **2001**, *4*, 301–308. [CrossRef]
14. Turner, J.G.; Ellis, C.; Devoto, A. The Jasmonate Signal Pathway. *Plant Cell* **2002**, *14*, S153–S164. [CrossRef] [PubMed]
15. Chen, Q.; Sun, J.Q.; Zhai, Q.Z.; Zhou, W.K.; Qi, L.L.; Xu, L.; Wang, B.; Chen, R.; Jiang, H.L.; Qi, J.; et al. The Basic Helix-Loop-Helix Transcription Factor MYC2 Directly Represses *PLETHORA* Expression During Jasmonate-Mediated Modulation of the Root Stem Cell Niche in *Arabidopsis*. *Plant Cell* **2011**, *23*, 3335–3352. [CrossRef] [PubMed]
16. Cai, X.T.; Xu, P.; Zhao, P.X.; Liu, R.; Yu, L.H.; Xiang, C.B. *Arabidopsis* Erf109 Mediates Cross-Talk between Jasmonic Acid and Auxin Biosynthesis During Lateral Root Formation. *Nat. Commun.* **2014**, *5*, 5833. [CrossRef] [PubMed]
17. Zhang, G.F.; Zhao, F.; Chen, Y.Q.; Pan, Y.; Sun, L.J.; Bao, N.; Zhang, T.; Cui, C.X.; Qiu, Z.Z.; Zhang, Y.J.; et al. Jasmonate-Mediated Wound Signalling Promotes Plant Regeneration. *Nat. Plants* **2019**, *5*, 491–497. [CrossRef]
18. Conesa, C.M.; Saez, A.; Navarro-Neila, S.; de Lorenzo, L.; Hunt, A.G.; Sepulveda, E.B.; Baigorri, R.; Garcia-Mina, J.M.; Zamarreno, A.M.; Sacristan, S.; et al. Alternative Polyadenylation and Salicylic Acid Modulate Root Responses to Low Nitrogen Availability. *Plants* **2020**, *9*, 251. [CrossRef]

19. Pasternak, T.; Groot, E.P.; Kazantsev, F.V.; Teale, W.; Omelyanchuk, N.; Kovrizhnykh, V.; Palme, K.; Mironova, V.V. Salicylic Acid Affects Root Meristem Patterning Via Auxin Distribution in a Concentration-Dependent Manner. *Plant Physiol.* **2019**, *180*, 1725–1739. [CrossRef]
20. Petricka, J.J.; Winter, C.M.; Benfey, P.N. Control of Arabidopsis Root Development. *Annu. Rev. Plant Biol.* **2012**, *63*, 563–590. [CrossRef]
21. Patterson, K.; Cakmak, T.; Cooper, A.; Lager, I.; Rasmusson, A.G.; Escobar, M.A. Distinct Signalling Pathways and Transcriptome Response Signatures Differentiate Ammonium- and Nitrate-Supplied Plants. *Plant Cell Environ.* **2010**, *33*, 1486–1501. [CrossRef]
22. Ishiyama, K.; Kojima, S.; Takahashi, H.; Hayakawa, T.; Yamaya, T. Cell Type Distinct Accumulations of Mrna and Protein for Nadh-Dependent Glutamate Synthase in Rice Roots in Response to the Supply of Nh_4^+. *Plant Physiol. Biochem.* **2003**, *41*, 643–647. [CrossRef]
23. Falhof, J.; Pedersen, J.T.; Fuglsang, A.T.; Palmgren, M. Plasma Membrane H^+-Atpase Regulation in the Center of Plant Physiology. *Mol. Plant* **2016**, *9*, 323–337. [CrossRef]
24. Rayle, D.L.; Cleland, R.E. The Acid Growth Theory of Auxin-Induced Cell Elongation Is Alive and Well. *Plant Physiol.* **1992**, *99*, 1271–1274. [CrossRef]
25. Rober-Kleber, N.; Albrechtova, J.T.P.; Fleig, S.; Huck, N.; Michalke, W.; Wagner, E.; Speth, V.; Neuhaus, G.; Fischer-Iglesias, C. Plasma Membrane H^+-Atpase Is Involved in Auxin-Mediated Cell Elongation During Wheat Embryo Development. *Plant Physiol.* **2003**, *131*, 1302–1312. [CrossRef] [PubMed]
26. Duby, G.; Boutry, M. The Plant Plasma Membrane Proton Pump Atpase: A Highly Regulated P-Type Atpase with Multiple Physiological Roles. *Pflug. Arch. Eur. J. Physiol.* **2009**, *457*, 645–655. [CrossRef] [PubMed]
27. Menzel, C.M. Changes in the Concentration of Leaf Nitrogen over the Season Affect the Diagnosis of Deficiency or Sufficiency in Strawberries in the Subtropics. *Agriculture* **2018**, *8*, 126. [CrossRef]
28. Nestby, R.; Lieten, F.; Pivot, D.; Lacroix, C.R.; Tagliavini, M. Influence of Mineral Nutrients on Strawberry Fruit Quality and Their Accumulation in Plant Organs: A Review. *Int. J. Fruit Sci.* **2005**, *5*, 139–156. [CrossRef]
29. Yoshida, Y.; Goto, T.; Hirai, M.; Masuda, M. *Anthocyanin Accumulation in Strawberry Fruits as Affected by Nitrogen Nutrition*; International Society for Horticultural Science: Leuven, Belgium, 2002; pp. 357–360.
30. Ojeda-Real, L.A.; Lobit, P.; Cárdenas-Navarro, R.; Grageda-Cabrera, O.; Farías-Rodríguez, R.; Valencia-Cantero, E.; Macías-Rodríguez, L. Effect of Nitrogen Fertilization on Quality Markers of Strawberry (Fragaria× Ananassa Duch. Cv. Aromas). *J. Sci. Food Agric.* **2009**, *89*, 935–939. [CrossRef]
31. Kun, C.; Yimin, C.; Hong, Z.; Tongyong, L.; Qing, X.; Lei, W. Effects of Different No_3^- Concentrations on Growth and Photosynthetic Characteristics in Strawberry Seedling. *Chin. Agric. Sci. Bull.* **2012**, *28*, 221–224.
32. Placido, D.F.; Sandhu, J.; Sato, S.J.; Nersesian, N.; Quach, T.; Clemente, T.E.; Staswick, P.E.; Walia, H. The Lateral Root Density Gene Regulates Root Growth During Water Stress in Wheat. *Plant Biotechnol. J.* **2020**, *18*, 1955–1968. [CrossRef]
33. Majláth, I.; Darko, E.; Palla, B.; Nagy, Z.; Janda, T.; Szalai, G. Reduced Light and Moderate Water Deficiency Sustain Nitrogen Assimilation and Sucrose Degradation at Low Temperature in Durum Wheat. *J. Plant Physiol.* **2016**, *191*, 149–158. [CrossRef] [PubMed]
34. Bowsher, C.G.; Emes, M.J.; Cammack, R.; Hucklesby, D.P. Purification and Properties of Nitrite Reductase from Roots of Pea (Pisum Sativum Cv. Meteor). *Planta* **1988**, *175*, 334–340. [CrossRef]
35. Singh, R.P.; Srivastava, H.S. Increase in Glutamate Synthase Nadh Activity in Maize Zea-Mays Cultivar Ganga-Safed-2 Seedlings in Response to Nitrate and Ammonium Nitrogen. *Physiol. Plant.* **1986**, *66*, 413–416. [CrossRef]
36. Yuefu, W.; Zhenwen, Y.; Shangxia, L.; Songlie, Y. Effect of Nitrogen Nutrition on the Change of Key Enzyme Activity During the Nitrogen Metabolism and Kernel Protein Content in Winter Wheat. *Zuo Wu Xue Bao* **2002**, *28*, 743–748.
37. Bradford, M.M. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Anal. Biochem.* **1976**, *72*, 248–254. [CrossRef]
38. Saez-Plaza, P.; Jose Navas, M.; Wybraniec, S.; Michalowski, T.; Garcia Asuero, A. An Overview of the Kjeldahl Method of Nitrogen Determination. Part Ii. Sample Preparation, Working Scale, Instrumental Finish, and Quality Control. *Crit. Rev. Anal. Chem.* **2013**, *43*, 224–272. [CrossRef]
39. Zhang, M.; Huang, L.; Ji, Y.; Fang, Y. Effects of Nacl Stress on Activity and Expression of Plasma Membrane H^+-Atpase in Broussonetia Papyrifera. *J. Beijing For. Univ.* **2011**, *33*, 21–26.
40. Yang, L.; Jon, C.-S.; Wang, L.; Zou, Y.; Liu, L.; Ri, H.-C.; Zhao, J.; Cui, M.; Shang, H.-B.; Li, D. Analysis of Multiple-Phytohormones During Fruit Development in Strawberry by Using Miniaturized Dispersive Solid-Phase Extraction Based on Ionic Liquid-Functionalized Carbon Fibers. *J. Food Compos. Anal.* **2022**, *106*, 104262. [CrossRef]
41. van der Bom, F.J.; Williams, A.; Bell, M.J. Root Architecture for Improved Resource Capture: Trade-Offs in Complex Environments. *J. Exp. Bot.* **2020**, *71*, 5752–5763. [CrossRef]
42. Kenobi, K.; Atkinson, J.A.; Wells, D.M.; Gaju, O.; De Silva, J.G.; Foulkes, M.J.; Dryden, I.L.; Wood, A.T.A.; Bennett, M.J. Linear Discriminant Analysis Reveals Differences in Root Architecture in Wheat Seedlings Related to Nitrogen Uptake Efficiency. *J. Exp. Bot.* **2017**, *68*, 4969–4981. [CrossRef] [PubMed]
43. Gao, K.; Chen, F.; Yuan, L.; Zhang, F.; Mi, G. A Comprehensive Analysis of Root Morphological Changes and Nitrogen Allocation in Maize in Response to Low Nitrogen Stress. *Plant Cell Environ.* **2015**, *38*, 740–750. [CrossRef]

44. Ju, C.; Buresh, R.J.; Wang, Z.; Zhang, H.; Liu, L.; Yang, J.; Zhang, J. Root and Shoot Traits for Rice Varieties with Higher Grain Yield and Higher Nitrogen Use Efficiency at Lower Nitrogen Rates Application. *Field Crops Res.* **2015**, *175*, 47–55. [CrossRef]
45. Mi, G.; Chen, F.; Wu, Q.; Lai, N.; Yuan, L.; Zhang, F. Ideotype Root Architecture for Efficient Nitrogen Acquisition by Maize in Intensive Cropping Systems. *Sci. China-Life Sci.* **2010**, *53*, 1369–1373. [CrossRef] [PubMed]
46. Lopez-Bucio, J.; Cruz-Ramirez, A.; Herrera-Estrella, L. The Role of Nutrient Availability in Regulating Root Architecture. *Curr. Opin. Plant Biol.* **2003**, *6*, 280–287. [CrossRef]
47. Kiba, T.; Kudo, T.; Kojima, M.; Sakakibara, H. Hormonal Control of Nitrogen Acquisition: Roles of Auxin, Abscisic Acid, and Cytokinin. *J. Exp. Bot.* **2011**, *62*, 1399–1409. [CrossRef]
48. Jia, Z.; Giehl, R.F.H.; von Wiren, N. Local Auxin Biosynthesis Acts Downstream of Brassinosteroids to Trigger Root Foraging for Nitrogen. *Nat. Commun.* **2021**, *12*, 5437. [CrossRef]
49. Meier, M.; Liu, Y.; Lay-Pruitt, K.S.; Takahashi, H.; von Wiren, N. Auxin-Mediated Root Branching Is Determined by the Form of Available Nitrogen. *Nat. Plants* **2020**, *6*, 1136–1145. [CrossRef]
50. Liu, Y.; von Wiren, N. Integration of Nutrient and Water Availabilities Via Auxin into the Root Developmental Program. *Curr. Opin. Plant Biol.* **2022**, *65*, 102117. [CrossRef]
51. Sun, X.; Chen, H.; Wang, P.; Chen, F.; Yuan, L.; Mi, G. Low Nitrogen Induces Root Elongation Via Auxin-Induced Acid Growth and Auxin-Regulated Target of Rapamycin (Tor) Pathway in Maize. *J. Plant Physiol.* **2020**, *254*, 153281. [CrossRef]
52. Wang, S.C.; Ichii, M.; Taketa, S.; Xu, L.L.; Xia, K.; Zhou, X. Lateral Root Formation in Rice (*Oryza Sativa*): Promotion Effect of Jasmonic Acid. *J. Plant Physiol.* **2002**, *159*, 827–832. [CrossRef]
53. Sun, J.; Xu, Y.; Ye, S.; Jiang, H.; Chen, Q.; Liu, F.; Zhou, W.; Chen, R.; Li, X.; Tietz, O.; et al. Arabidopsis Asa1 Is Important for Jasmonate-Mediated Regulation of Auxin Biosynthesis and Transport During Lateral Root Formation. *Plant Cell* **2009**, *21*, 1495–1511. [CrossRef] [PubMed]
54. Cheng, Y.; Dai, X.; Zhao, Y. Auxin Biosynthesis by the Yucca Flavin Monooxygenases Controls the Formation of Floral Organs and Vascular Tissues in Arabidopsis. *Genes Dev.* **2006**, *20*, 1790–1799. [CrossRef] [PubMed]
55. Hentrich, M.; Boettcher, C.; Duechting, P.; Cheng, Y.; Zhao, Y.; Berkowitz, O.; Masle, J.; Medina, J.; Pollmann, S. The Jasmonic Acid Signaling Pathway Is Linked to Auxin Homeostasis through the Modulation of Yucca8 and Yucca9 Gene Expression. *Plant J.* **2013**, *74*, 626–637. [CrossRef] [PubMed]
56. Kong, X.P.; Zhang, C.L.; Zheng, H.H.; Sun, M.; Zhang, F.; Zhang, M.Y.; Cui, F.H.; Lv, D.P.; Liu, L.J.; Guo, S.Y.; et al. Antagonistic Interaction between Auxin and Sa Signaling Pathways Regulates Bacterial Infection through Lateral Root in Arabidopsis. *Cell Rep.* **2020**, *32*, 108060. [CrossRef]
57. Echevarria-Machado, I.; Escobedo-Gm, R.M.; Larque-Saavedra, A. Responses of Transformed Catharanthus Roseus Roots to Ferntomolar Concentrations of Salicylic Acid. *Plant Physiol. Biochem.* **2007**, *45*, 501–507. [CrossRef] [PubMed]
58. Bagautdinova, Z.Z.; Omelyanchuk, N.; Tyapkin, A.V.; Kovrizhnykh, V.V.; Lavrekha, V.V.; Zemlyanskaya, E.V. Salicylic Acid in Root Growth and Development. *Int. J. Mol. Sci.* **2022**, *23*, 2228. [CrossRef]
59. Kitakura, S.; Vanneste, S.; Robert, S.; Lofke, C.; Teichmann, T.; Tanaka, H.; Friml, J. Clathrin Mediates Endocytosis and Polar Distribution of Pin Auxin Transporters in Arabidopsis. *Plant Cell* **2011**, *23*, 1920–1931. [CrossRef]
60. Llorente, F.; Muskett, P.; Sanchez-Vallet, A.; Lopez, G.; Ramos, B.; Sanchez-Rodriguez, C.; Jorda, L.; Parker, J.; Molina, A. Repression of the Auxin Response Pathway Increases Arabidopsis Susceptibility to Necrotrophic Fungi. *Mol. Plant* **2008**, *1*, 496–509. [CrossRef]
61. Hou, S.; Tsuda, K. Salicylic Acid and Jasmonic Acid Crosstalk in Plant Immunity. *Essays Biochem.* **2022**, *66*, 647–656. [CrossRef]
62. Chen, J.; Wang, Z.B.A.; Liu, S.D.; Zhang, S.P.; Ge, C.W.; Shen, Q.; Ma, H.J.; Zhang, X.M.; Dong, H.L.; Zhao, X.H.; et al. Nitrogen Stress Inhibits Root Growth by Regulating Cell Wall and Hormone Changes in Cotton (*Gossypium Hirsutum* L.). *J. Agron. Crop Sci.* **2021**, *207*, 1006–1023. [CrossRef]
63. Allu, A.D.; Brotman, Y.; Xue, G.P.; Balazadeh, S. Transcription Factor Anac032 Modulates Ja/Sa Signalling in Response to Pseudomonas Syringae Infection. *Embo Rep.* **2016**, *17*, 1578–1589. [CrossRef]
64. Ndamukong, I.; Al Abdallat, A.; Thurow, C.; Fode, B.; Zander, M.; Weigel, R.; Gatz, C. Sa-Inducible Arabidopsis Glutaredoxin Interacts with Tga Factors and Suppresses Ja-Responsive *Pdf1.2* Transcription. *Plant J.* **2007**, *50*, 128–139. [CrossRef] [PubMed]
65. Zheng, X.Y.; Spivey, N.W.; Zeng, W.Q.; Liu, P.P.; Fu, Z.Q.; Klessig, D.F.; He, S.Y.; Dong, X.N. Coronatine Promotes Pseudomonas Syringae Virulence in Plants by Activating a Signaling Cascade That Inhibits Salicylic Acid Accumulation. *Cell Host Microbe* **2012**, *11*, 587–596. [CrossRef] [PubMed]
66. Obroucheva, N.V.; Lityagina, S.V.; Sinkevich, I.A. Activation and Activity of Plasma Membrane H^+-Atpase: Key Events in Germinating Vicia Faba Seeds. *Seed Sci. Res.* **2021**, *31*, 76–82. [CrossRef]
67. Anderson, C.T.; Kieber, J.J. Dynamic Construction, Perception, and Remodeling of Plant Cell Walls. *Annu. Rev. Plant Biol.* **2020**, *71*, 39–69. [CrossRef]
68. Sperandio, M.V.L.; Santos, L.A.; Tavares, O.C.H.; Fernandes, M.S.; de Freitas Lima, M.; de Souza, S.R. Silencing the *Oryza Sativa* Plasma Membrane H^+-Atpase Isoform *OsA2* Affects Grain Yield and Shoot Growth and Decreases Nitrogen Concentration. *J. Plant Physiol.* **2020**, *251*, 153220. [CrossRef]
69. Sperandio, M.V.L.; Santos, L.A.; Bucher, C.A.; Fernandes, M.S.; de Souza, S.R. Isoforms of Plasma Membrane H^+-Atpase in Rice Root and Shoot Are Differentially Induced by Starvation and Resupply of No_3^- or Nh_4^+. *Plant Sci.* **2011**, *180*, 251–258. [CrossRef]
70. Vanneste, S.; Friml, J. Auxin: A Trigger for Change in Plant Development. *Cell* **2009**, *136*, 1005–1016. [CrossRef]

71. Lv, X.; Zhang, Y.; Hu, L.; Zhang, Y.; Zhang, B.; Xia, H.; Du, W.; Fan, S.; Kong, L. Low-Nitrogen Stress Stimulates Lateral Root Initiation and Nitrogen Assimilation in Wheat: Roles of Phytohormone Signaling. *J. Plant Growth Regul.* **2021**, *40*, 436–450. [CrossRef]
72. Li, C.; Zhang, J.; Wei, M.; Ge, Y.; Hou, J.; Cheng, Y.; Chen, J. Methyl Jasmonate Maintained Antioxidative Ability of Ginger Rhizomes by Regulating Antioxidant Enzymes and Energy Metabolism. *Sci. Hortic.* **2019**, *256*, 108578. [CrossRef]
73. Chen, Y.; Cao, C.; Guo, Z.; Zhang, Q.; Li, S.; Zhang, X.; Gong, J.; Shen, Y. Herbivore Exposure Alters Ion Fluxes and Improves Salt Tolerance in a Desert Shrub. *Plant Cell Environ.* **2020**, *43*, 400–419. [CrossRef] [PubMed]
74. Ghassemi-Golezani, K.; Abdoli, S. Improving Atpase and Ppase Activities, Nutrient Uptake and Growth of Salt Stressed Ajowan Plants by Salicylic Acid and Iron-Oxide Nanoparticles. *Plant Cell Rep.* **2021**, *40*, 559–573. [CrossRef]
75. Liu, Y.; Zhang, J.; Liu, H.; Huang, W. Salicylic Acid or Heat Acclimation Pre-Treatment Enhances the Plasma Membrane-Associated Atpase Activities in Young Grape Plants under Heat Shock. *Sci. Hortic.* **2008**, *119*, 21–27. [CrossRef]
76. Liu, Y.Y.; Liu, H.T.; Pan, Q.H.; Yang, H.R.; Zhan, J.C.; Huang, W.D. The Plasma Membrane H^+-Atpase Is Related to the Development of Salicylic Acid-Induced Thermotolerance in Pea Leaves. *Planta* **2009**, *229*, 1087–1098. [CrossRef]
77. Remans, T.; Nacry, P.; Pervent, M.; Girin, T.; Tillard, P.; Lepetit, M.; Gojon, A. A Central Role for the Nitrate Transporter Nrt2.1 in the Integrated Morphological and Physiological Responses of the Root System to Nitrogen Limitation in Arabidopsis. *Plant Physiol.* **2006**, *140*, 909–921. [CrossRef]
78. Sun, X.; Jia, X.; Huo, L.Q.; Che, R.M.; Gong, X.Q.; Wang, P.; Ma, F.W. Mdatg18a Overexpression Improves Tolerance to Nitrogen Deficiency and Regulates Anthocyanin Accumulation through Increased Autophagy in Transgenic Apple. *Plant Cell Environ.* **2018**, *41*, 469–480. [CrossRef] [PubMed]
79. Liu, X.J.; Hu, B.; Chu, C.C. Nitrogen Assimilation in Plants: Current Status and Future Prospects. *J. Genet. Genom.* **2022**, *49*, 394–404. [CrossRef] [PubMed]
80. Ding, S.T.; Shao, X.Q.; Li, J.X.; Ahammed, G.J.; Yao, Y.L.; Ding, J.; Hu, Z.J.; Yu, J.Q.; Shi, K. Nitrogen Forms and Metabolism Affect Plant Defence to Foliar and Root Pathogens in Tomato. *Plant Cell Environ.* **2021**, *44*, 1596–1610. [CrossRef]
81. Miflin, B.J.; Habash, D.Z. The Role of Glutamine Synthetase and Glutamate Dehydrogenase in Nitrogen Assimilation and Possibilities for Improvement in the Nitrogen Utilization of Crops. *J. Exp. Bot.* **2002**, *53*, 979–987. [CrossRef]
82. Li, Y.T.; Han, D.X.; Sommerfeld, M.; Hu, Q.A. Photosynthetic Carbon Partitioning and Lipid Production in the Oleaginous Microalga Pseudochlorococcum Sp (Chlorophyceae) under Nitrogen-Limited Conditions. *Bioresour. Technol.* **2011**, *102*, 123–129. [CrossRef]
83. Xiong, H.Y.; Ma, H.T.; Hu, B.; Zhao, H.Y.; Wang, J.; Rennenberg, H.; Shi, X.J.; Zhang, Y.Q. Nitrogen Fertilization Stimulates Nitrogen Assimilation and Modifies Nitrogen Partitioning in the Spring Shoot Leaves of Citrus (Citrus Reticulata Blanco) Trees. *J. Plant Physiol.* **2021**, *267*, 153556. [CrossRef]
84. Zhou, W.W.; Liang, X.; Li, K.J.; Dai, P.B.; Li, J.L.; Liang, B.; Sun, C.L.; Lin, X.Y. Metabolomics Analysis Reveals Potential Mechanisms of Phenolic Accumulation in Lettuce (Lactuca Sativa L.) Induced by Low Nitrogen Supply. *Plant Physiol. Biochem.* **2021**, *158*, 446–453. [CrossRef] [PubMed]
85. Gong, Q.; Li, Z.; Wang, L.; Dai, T.; Kang, Q.; Niu, D. Exogenous of Indole-3-Acetic Acid Application Alleviates Copper Toxicity in Spinach Seedlings by Enhancing Antioxidant Systems and Nitrogen Metabolism. *Toxics* **2020**, *8*, 1. [CrossRef]
86. Parihar, P.; Singh, R.; Singh, A.; Prasad, S.M. Role of Oxylipin on Luffa Seedlings Exposed to Nacl and Uv-B Stresses: An Insight into Mechanism. *Plant Physiol. Biochem.* **2021**, *167*, 691–704. [CrossRef]
87. Hayat, S.; Fariduddin, Q.; Ali, B.; Ahmad, A. Effect of Salicylic Acid on Growth and Enzyme Activities of Wheat Seedlings. *Acta Agron. Hung.* **2005**, *53*, 433–437. [CrossRef]
88. Gautam, S.; Singh, P.K. Salicylic Acid-Induced Salinity Tolerance in Corn Grown under Nacl Stress. *Acta Physiol. Plant.* **2009**, *31*, 1185–1190. [CrossRef]
89. Hayat, Q.; Hayat, S.; Irfan, M.; Ahmad, A. Effect of Exogenous Salicylic Acid under Changing Environment: A Review. *Environ. Exp. Bot.* **2010**, *68*, 14–25. [CrossRef]