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Expression and analysis of *StNR* and *StNiRs*, key enzyme genes of nitrogen assimilation in potato (*Solanum tuberosum* L.) with different nitrogen efficiencies

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Abstract: The potato is a pivotal food crop on a global scale. Nitrate reductase (NR) and nitrite reductase (NiR) are the key enzymes in nitrogen assimilation. In previous research, we found that the nitrogen assimilation process was effectively regulated by StNR and StNiRs in potato and that there were significant differences in nitrogen utilisation efficiency between different potato varieties. In this study, three potato variants with different nitrogen use efficiency (NUE) were subjected to various nitrogen supply levels and photoperiod treatments. The results indicated that the relative expression levels of *StNR* and *StNiRs* in their leaves and roots, along with the enzyme activity of NR and NiR, were proportional to the nitrogen supply levels and photoperiod. This study further clarified the expression patterns of *StNR* and *StNiRs*, as well as the enzyme activity changes of NR and NiR in leaves and roots under different nitrogen supply levels and different photoperiod treatments. This provides a theoretical basis for further in-depth exploration of the specific functions related to nitrogen absorption and assimilation efficiency in potato.

Keywords: enzyme activity; gene expression level; nitrogen efficiency; nitrogen metabolism

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Potato (*Solanum tuberosum* L.) is the fourth largest food crop globally (Ezekiel et al. 2013). In agricultural production, nitrogen fertilisation is a common method to increase crop yield. However, there are great differences in nitrogen uptake and utilisation in different variants of potato. Some studies have shown that the nitrogen use efficiency (NUE) of potato could be improved to a limited extent by selection of high-yielding varieties or soil management practices (Hu et al. 2020). Therefore, the molecular and physiological approaches were necessary to study the role of key genes in regulating the nitrogen metabolic pathway under nitrogen stress in potato, and thus to improve NUE in potato to achieve high and stable yields.

In recent studies on nitrogen utilisation in potato, the different NUE types have been identified in different genetic and phenotypic variations. For instance, Zebarth et al. (2004) evaluated 20 commercial potato cultivars under low (0 kg N/ha) and high (100 kg N/ha) nitrogen regimes, revealing that late-maturing variants exhibited significantly higher NUE while early-maturing cultivars showed lower NUE. Kassie (2020) identified 52 quantitative trait loci (QTLs) associated with NUE and related traits in a potato population under different nitrogen levels (40 vs. 120 kg/ha). These QTLs were also found to be associated with potato maturity and nitrogen utilisation efficiency. Ferrante et al. (2017) discovered that two nitrate transporters (NRT1.1, NRT2.1) were regulated by nitrogen availability and explored the molecular mechanisms of nitrogen uptake.

Recent studies have employed the biophysiological and omics approaches to further discover the geneotype-specific responses to N stress. Rawal et al. (2024) used visible and ner-infrared spectroscopy to predict the leaf N content, supplying a non-destructive NUE monitor. Lu et al. (2022) identified 13 differentially expressed miRNAs, and discovered that the miR396-5p negatively regulated StNiR via luciferase assays, highlighting miRNA networks in N assimilation. Han et al. (2022) found N-efficient variants exhibit higher nitrate reductase, glutamine synthase, and glutamate synthase activities under low N. Xie et al. (2024) identified the upregulated nitrate transporter (NRT), nitrate reductase (NR), and nitrite reductase (NiR) in N-efficient XS6, by using integrated transcriptomics and metabolomics methods. They also discovered that trehalose acted as a key metabolite, mitigating N deficiency via carbon-nitrogen metabolic coordination. However, the expression patterns of NR and NiR under different light treatments were not clear. In order to further clarify the expression of *StNR* and *StNiRs* in potato varieties with different NUE under different nitrogen supply levels and different photoperiod treatments, we conducted in-depth studies.

In this study, we used potato varieties with different NUE as materials, focuses on the key enzymes NR and NiR in the nitrogen assimilation process, and deeply explores the expression changes of the key enzyme genes *StNR*, *StNiRs*, and the key enzymes NR and NiR in the roots and leaves of tissue-cultured seedlings under different nitrogen supply levels and different photoperiod treatments. The aim is to clarify their expression characteristics at the gene-enzyme activity level, and further elucidate the regulatory role of *StNR* and *StNiRs* in nitrogen assimilation of potato. Our research will provide a theoretical basis for research on nitrogen absorption and utilisation efficiency in potato.

MATERIAL AND METHODS

Plant material. nine potato varieties with varying NUE were chosen as experimental specimens (Table 1). These varieties were sourced as tissue-culture seedlings and grouped into three distinct NUE categories. The materials were provided by the potato innovation team of Jilin Agricultural University.

Test treatment. Three NUE variants with different NUE were selected, and a total of nine potato varieties were selected as experimental materials. Sterile potato tissue culture seedlings with good plant growth and strong stems were selected. Approximately three cuttings were made in each bottle, each treatment was inoculated with one bottle, repeated nine times.

Table 1. Names and IDs of three nitrogen use efficiency (NUE) variants of potato

Three NUE variants	Nine potato varieties	ID
Nitrogen efficient potato	Yanshu 4	1
	Kexin 1	2
	Helan 14	3
Nitrogen moderate-efficient potato	Shepody	4
	Chunshu 4	5
	Helan 7	6
Nitrogen inefficient potato	Zaodabai	7
	Chunshu 5	8
	Atlantic	9

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The tissue culture bottles were marked with variety names, inoculation time and inoculation information (Table 1). Three nitrogen supply levels were applied: normal nitrogen application (N) was cultured with conventional MS medium (including large amount of elemental solution, trace element solution, iron salt solution, organic solution) (NH_4NO_3 concentration was 33 g/L), small amount of nitrogen application (N1) was cultured with 1/2 nitrogen MS medium (NH_4NO_3 concentration was 16.5 g/L), and excessive nitrogen application (N2) was cultured with 2× nitrogen MS medium (NH_4NO_3 concentration was 66 g/L). Three different photoperiods were applied: normal photoperiod was 14 h/day light and 10 h/day dark (A), short photoperiod was 8 h/day light and 16 h/day dark (B), completely dark condition (C).

In this study, the culture temperature was 23 °C. Three colour plant LED lamps were used for lighting, and the light intensity was 2 500 lx. The leaves (L) and roots (R) of potato were collected from tissue culture plantlets with three to four weeks, and then immediately frozen in liquid nitrogen and subsequently transferred to a –80 °C refrigerator. Three biological repeats and three technical repeats were set.

RNA extraction and cDNA synthesis. TransZol® Up Plus RNA Kit (TRANS ER501) was used to extract total RNA from nine potato varieties' leaves and roots. cDNA synthesis was performed using the TransScript® One-Step gDNA Removal and cDNA Synthesis SuperMix kit (TRANS AH311). The reaction system included 1 μg of total RNA, 1 μL of Anchored Oligo (dT) 18 Primer (0.5 μg/μL), 2× TS Reaction Mix of 10 μL, RT/RI Enzyme Mix of 1 μL, gDNA Remover of 1 μL, variable RNase-free water, The total volume was adjusted to 20 μL. After mixing, the reaction conditions were as follows: 42 °C for 30 min, 85 °C for 30 s.

Reverse transcription-quantitative PCR (RT-qPCR). PerfectStart® Green qPCR SuperMix kit (TRANS AQ601) was used to perform RT-qPCR. The reaction system included: 1 μg of cDNA, 0.4 μL of forward primer (10 μM), 0.4 μL of reverse primer (10 μM), 10 μL of 2× PerfectStart® Green qPCR super mix, 0.4 μL of passive reference dye (50×). Nuclease-free water was adjusting the total volume to 20 μL. The RT-qPCR reaction was performed under the following reaction conditions: 95 °C for 30 s, 40 cycles of 95 °C for 15 s and 60 °C for 60 s. Finally, the fold change in expression was calculated using the $2^{-\Delta\Delta Ct}$ method. RT-qPCR primers and their sequences used in this study are detailed in Table 2.

Determination of NR and NiR enzyme activity. The determination of NR enzyme activity referred to Sunder et al. (2024) experimental technique of plant physiology. Briefly, 0.5 g fresh samples were weighed to extract crude enzyme. 0.1 mol/L KNO_3 phosphate buffer and 0.4 mL NADH solution were added to 0.4 mL crude enzyme solution for the enzyme reaction. At the end of the reaction, 1 mL sulfonamide solution was added to terminate the enzyme reaction, followed by 1 mL naphthylvinylamine solution for colour development. Finally, the colourimetric determination at 540 nm was carried out for absorbance measurement.

The determination of NiR enzyme activity referred to Wu's test method (Wu et al. 2012). 0.2 g of fresh samples were weighed to extract crude enzyme. Potassium phosphate buffer solution, potassium nitrate solution and methyl viologen solution were added to 0.1 mL crude enzyme solution, and then sodium dithionite was added to start the reaction. At the end of the reaction, 1 mL sulfamide solution was added to stop the enzyme reaction, and then 1 mL sulfamide was added for colour development. Finally, we carry out a colourimetric determination at 540 nm for absorbance.

Statistical analysis. Statistical analysis was performed using SPSS 20.0 and Graphpad Prism 9. Data were presented as the mean ± SE with three replicates for each data. A two-way analysis of variance

Table 2. RT-qPCR primers used and their respective forward and reverse sequences

Primer name	Forward primer (5'–3')	Reverse primer (5'–3')
*StEF1α**	GATGGTCAGACCCGTGAACA	CCTTGGAGTACTTCGGGGTG
StNR	ACGCTGAACTTGCTAACGCTGAA	GCTGAGTAGTCCACGCATTGATAGG
StNiR5823	ACATTCCAGTGGGTCGTGTC	CACAGTCAGCCGTAGCTCTC
StNiR8262	GGAAGGCGCCGATGTTTTCT	TTCATCACACGGAACGGCTT

**StEF1α* is utilised as the reference gene in this study; this is due to the fact that *StEF1α* shows relatively high stability under diverse experimental conditions, including salt stress, late blight, and cold stress (Tang et al. 2017)

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(ANOVA) was conducted to evaluate the quantitative data across different groups. To further determine the differences between specific groups, Duncan's multiple range test was then conducted. Person's correlation analyses the relationship between continuous quantitative variables. Results were visualised with graphs, using different lowercase letters or asterisks to mark significant differences.

RESULTS

Effects of different nitrogen supply levels on the relative expression of *StNR* and *StNiRs* in potato variants under normal photoperiod. Figure 1 shows the relative expression levels of *StNR*, *StNR8262*, and *StNiR5823* in leaves and roots of three NUE variants under normal photoperiod and varying nitrogen supplies (N1: 1/2 normal, N: normal, N2: 2× normal). All genes exhibited significant responses to nitrogen levels and variants patterns linked to NUE.

The *StNR8262* gene showed a similar pattern. In the leaves (Figure 1C), its expression level increased with the increase in nitrogen supply, and the expression level of the different NUE variants was generally higher than that of other variants at various nitrogen levels. In the roots (Figure 1D), as the nitrogen sup-

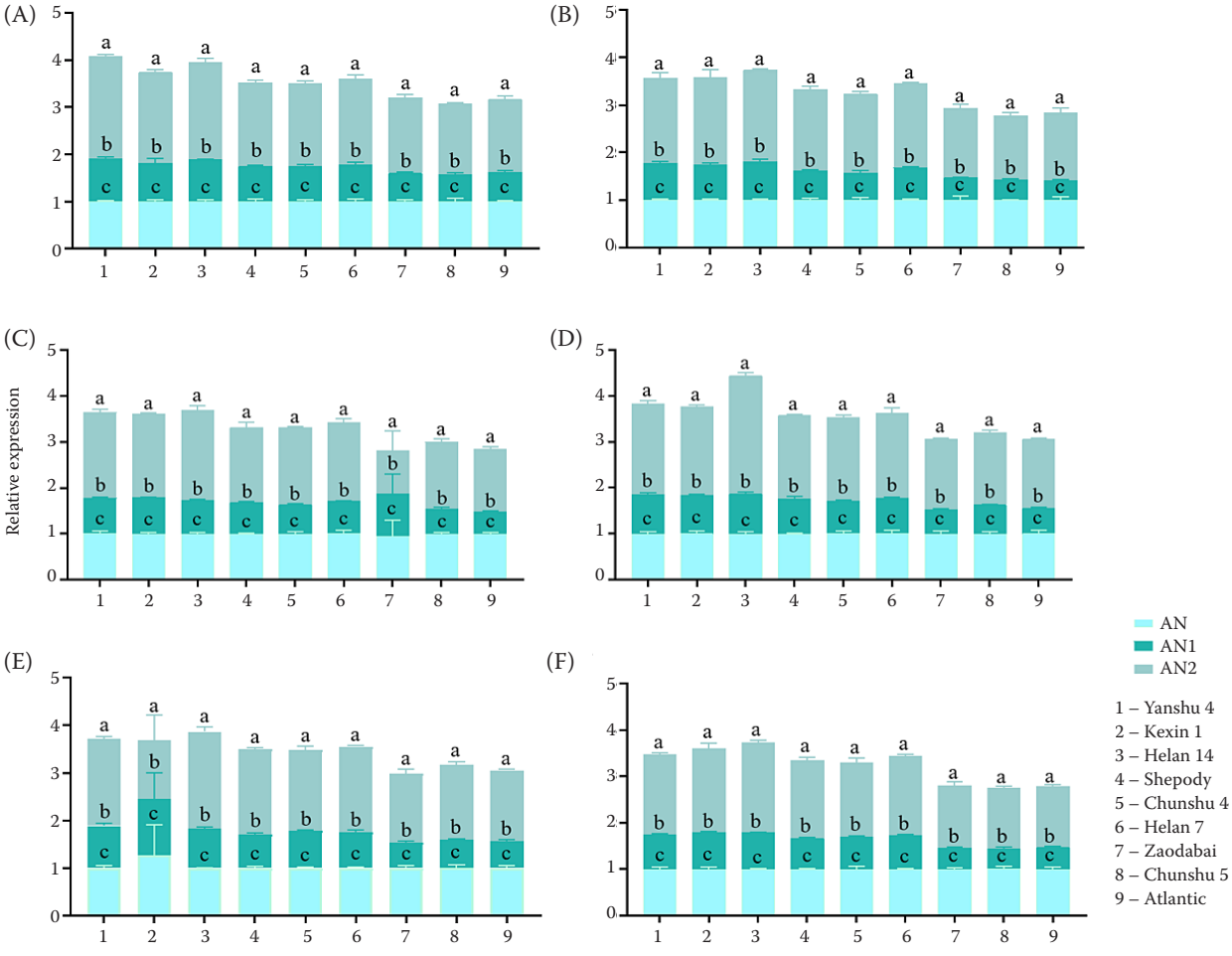

Figure 1. Relative expression of *StNR* (A), (B), *StNR8262* (C), (D) and *StNiR5823* (E), (F) in leaves and roots of three nitrogen use efficiency (NUE) variants under normal photoperiod treatment with different nitrogen supply levels
(A), (C), (E) – leaves; (B), (D), (F) – roots; A – normal photoperiod condition: 14 h/d light, 10 h/d dark; N – normal nitrogen application; N1 – small amount of nitrogen application, which is 1/2 of the normal nitrogen application level; N2 – excessive nitrogen application, which is 2 times the normal nitrogen application level; the nine varieties contain three NUE variants with different nitrogen efficiency; different lowercase letters represent the significance of the relative expression of the same variants of potato under different nitrogen supply levels ($P < 0.05$)

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ply increased from N1 to N2, the expression level also increased, and the nitrogen-efficient variants had an obvious expression advantage.

The *StNiR5823* gene followed the same trend. In the leaves (Figure 1E), the expression level of this gene increased with the increase in nitrogen supply, and the expression level of the nitrogen-efficient variants was higher than that of other variants. In the roots (Figure 1F), the expression level increased with the increase in nitrogen supply, and the nitrogen-efficient variants had a relatively high expression level at various nitrogen levels.

Overall, in the leaves and roots of different potato varieties, the expression levels of these three genes were all affected by the nitrogen supply level and all showed a trend of higher expression levels in nitrogen-efficient variants. This indicates that the three genes have similar patterns in responding to nitrogen supply and in relation to NUE and may jointly participate in the process of nitrogen response and utilization in potatoes.

Effect of light conditions on the relative expression of *StNR* and *StNiRs* in three NUE variants under normal nitrogen supply. It can be seen from Figure 2, under normal photoperiod treatment, the relative expression levels of *StNR* and *StNiRs* in the three NUE variants exhibited disparities across normal photoperiod, short photoperiod and completely darkness conditions.

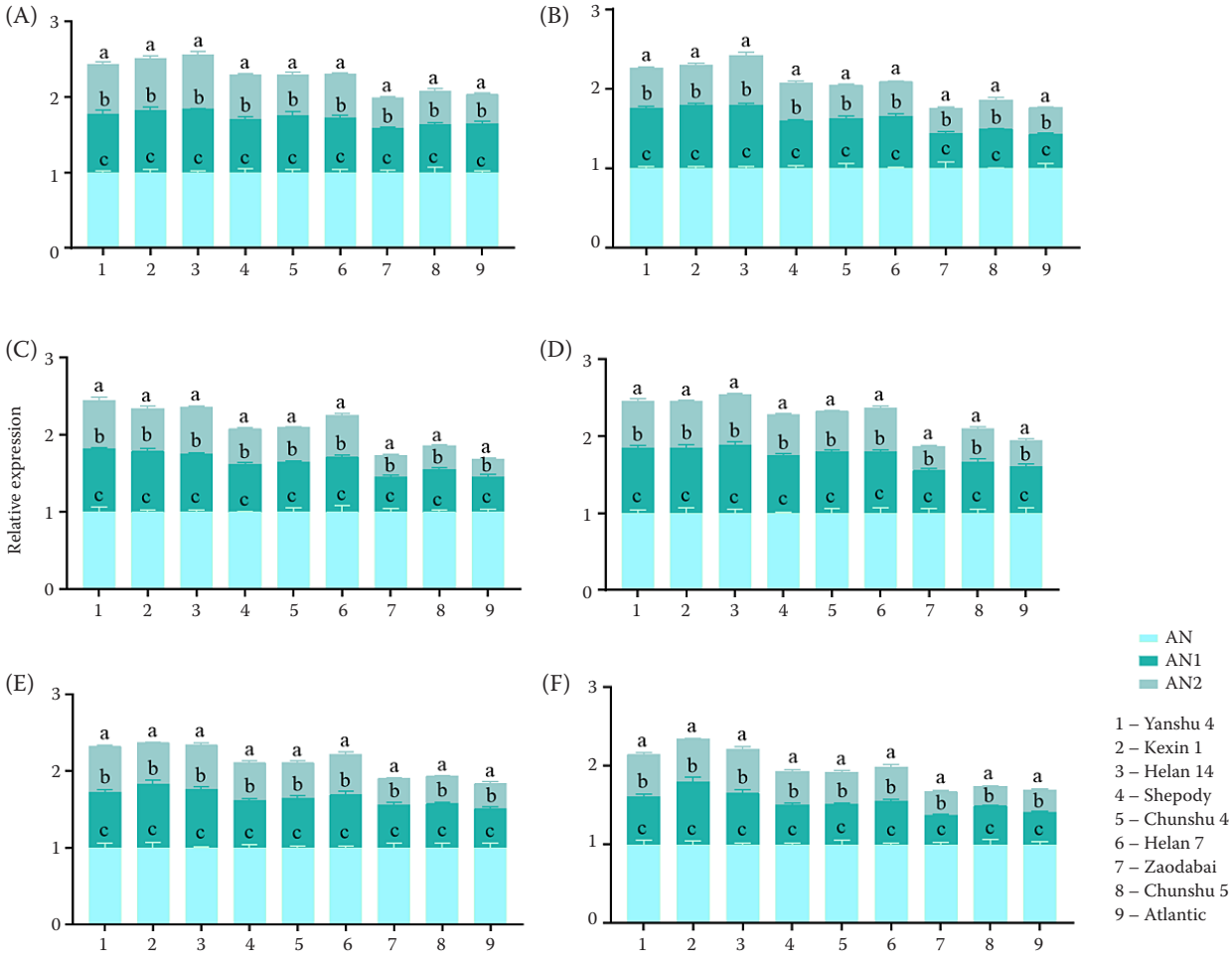

Figure 2. Relative expression of *StNR* (A), (B), *StNR8262* (C), (D) and *StNiR5823* (E), (F) in leaves and roots of three potato variants under normal nitrogen supply level with different photoperiod treatments

(A), (C), (E) – leaves; (B), (D), (F) – roots; A – normal photoperiod condition: 14 h/d light, 10 h/d dark; N – normal nitrogen application; B – short photoperiod treatment: 8 h/d light, 16 h/d dark; C – completely darkness condition; the nine varieties contain three nitrogen use efficiency (NUE) variants with different nitrogen efficiency; different lowercase letters represent the significance of the relative expression of the same variants potato under different photoperiod treatments ($P < 0.05$)

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In terms of the relative expression of *StNR* and *StNiRs* under different photoperiod treatments, an overall pattern emerged: normal photoperiod > short photoperiod > completely darkness condition. Among the three NUE variants, the nitrogen efficient potato demonstrated higher expression levels than the nitrogen-efficient potato demonstrated higher expression levels than the nitrogen moderate-efficient potato, which in turn exceeded the nitrogen inefficient potato.

In summary, this result indicates that both photoperiod and potato variants play crucial roles in regulating the relative expression of *StNR* and *StNiRs* genes.

Effect of nitrogen supply on the relative expression of *NR* and *NiR* in three NUE variants under normal photoperiod. Figure 3 illustrates that, under normal photoperiod treatment, there existed highly significant differences in the activity of NR among three potato variants under different nitrogen supply levels, both in the leaves and roots.

Regarding the activity of NiR, highly significant differences in NiR activity were observed in roots among the three NUE variants of potato. However, in the leaves, more significant differences in NiR activity were only detected in the nitrogen efficient potato and nitrogen moderate-efficient potato under 1/2 nitrogen supply and 2× nitrogen supply levels ($P < 0.001$).

The activity of NR and NiR increased significantly with the increase in nitrogen supply levels, following an overall trend of 2× nitrogen supply > normal nitrogen supply > 1/2 nitrogen supply. In terms of the activity in the leaves and roots of the three potato variants, the order was nitrogen efficient potato > nitrogen moderate-efficient potato > nitrogen inefficient potato.

Effect of different nitrogen supply levels on NR and NiR activity in leaves and roots of three potato variants under short photoperiod treatment. As depicted in Figure 4, under short photoperiod treatment and varying nitrogen supply levels, the activity of NR and NiR activity in three NUE variants exhibited notable discrepancies.

With the increment of nitrogen supply, a significant up-regulation in NR and NiR activities was observed ($P < 0.01$), following the order of 2× nitrogen supply > normal nitrogen supply > 1/2 nitrogen supply. In terms of the variants, the activities of NR and NiR demonstrated the sequence of nitrogen-efficient potato > nitrogen moderate-efficient potato > nitrogen inefficient potato, both in leaves and roots.

Effect of different nitrogen supply levels on NR and NiR activity in leaves and roots of three potato variants under completely dark conditions. Under completely dark conditions and varying nitrogen supply levels, the activity of NR and NiR in three NUE variants was investigated (Figure 5).

For NR activity, significant differences were observed among normal nitrogen, 1/2 nitrogen and 2× nitrogen supply. Across the three NUE variants, an increase in nitrogen supply levels led to a notable rise in NR activity in leaves, following the order 2× nitrogen supply > normal nitrogen supply > 1/2 nitrogen supply (Figure 5A). In roots, NR activity exhibited significant differences only between 1/2 nitrogen and 2× nitrogen supply ($P < 0.001$, Figure 5B).

Regarding NiR activity, under normal nitrogen supply, 1/2 nitrogen and 2× nitrogen supply, significant genotypic differences were found in roots (Figure 5C). In leaves, more pronounced differences in NiR activity were evident only under 1/2 nitrogen and 2× nitrogen supply for nitrogen-efficient and nitrogen-inefficient potato variants ($P < 0.01$).

Overall, for both NR and NiR activities in leaves and roots of three NUE variants, the pattern was nitrogen efficient potato > nitrogen moderate-efficient potato > nitrogen inefficient potato.

Effect of different photoperiod treatments on NR and NiR activity in leaves and roots of three NUE variants under normal nitrogen supply. As depicted in Figure 6 (A, B), under normal nitrogen supply and varying photoperiod treatments, significant differences in NR activity were observed among the three NUE variants.

Notably, there were substantial differences between the normal photoperiod and the completely dark condition, as well as between the short photoperiod and the completely dark condition. This was particularly evident in nitrogen-inefficient potato varieties. With an increase in light exposure duration, NR activity rose significantly, following the order: normal photoperiod > short photoperiod > completely dark condition. NR activity of roots was significantly different only under normal photoperiod and completely dark condition ($P < 0.001$).

It can be seen from Figure 6 (C, D) that the activity difference of NiR in the three NUE variants was similar to that of NR. Marked differences were found between the normal photoperiod and the completely dark condition, and between the short photoperiod and the completely dark condition,

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Figure 3. NR (A), (B) and NiR (C), (D) activity in leaves and roots of nine potato varieties under normal photoperiod treatment with different nitrogen supply levels (A), (C) – leaves; (B), (D) – roots; NR – nitrate reductase; NiR – nitrite reductase; A – normal photoperiod: 14 h/d light, 10 h/d dark; N – normal nitrogen application; N1 – small amount of nitrogen application; N2 – excessive nitrogen application; the nine varieties contain three potato variants with different nitrogen efficiency; *, **, ***significant at P <0.05, 0.01, 0.001

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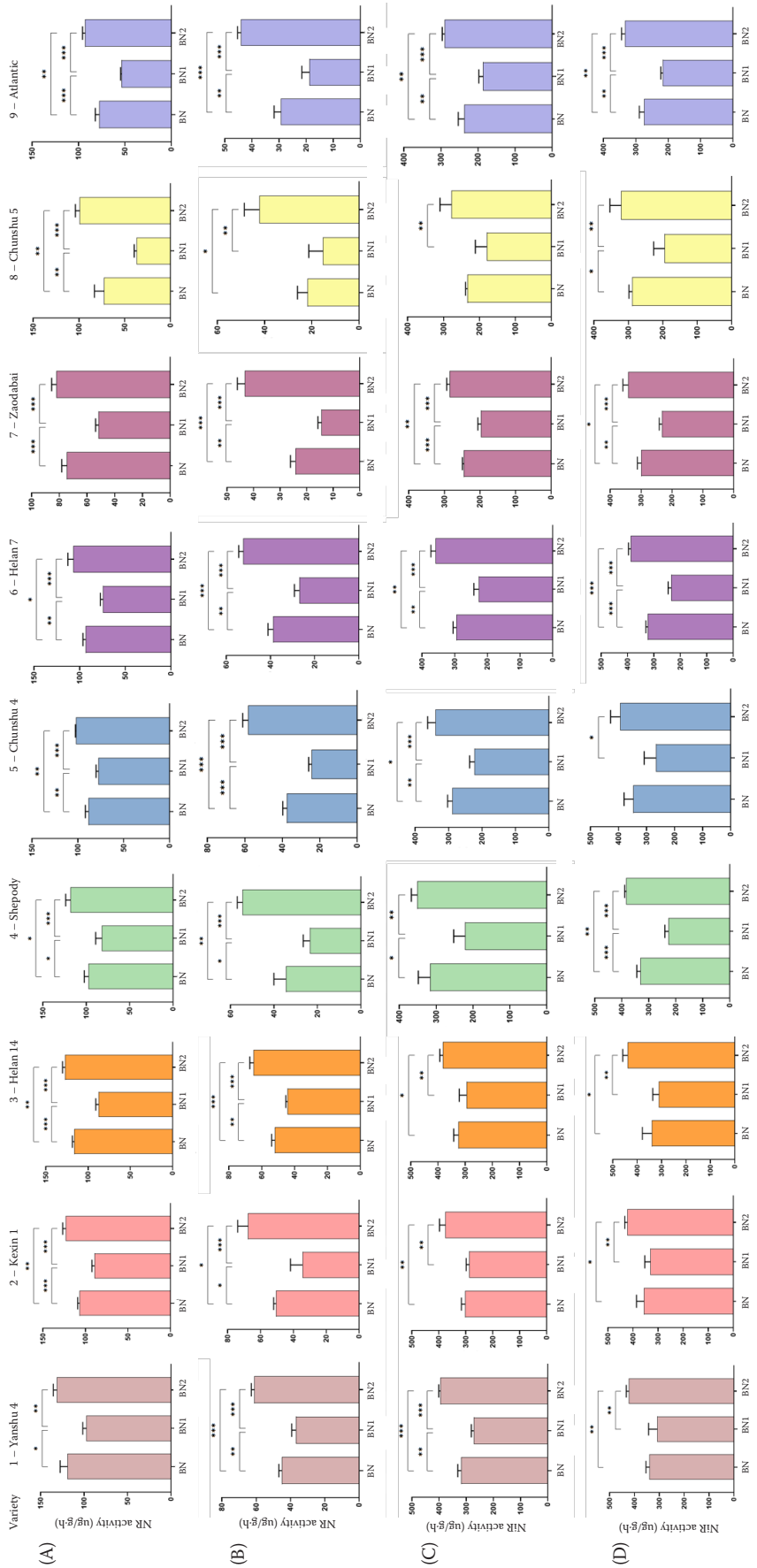

Figure 4. NR (A), (B) and NiR (C), (D) activity in leaves and roots of nine potato varieties under short photoperiod treatment with different nitrogen supply levels (A), (C) – leaves; (B), (D) – roots; NR – nitrate reductase; NiR – nitrite reductase; B – short photoperiod, 8 h/d light, 16 h/d dark; N – normal nitrogen application; N1 – small amount of nitrogen application; N2 – excessive nitrogen application; the nine varieties contain three nitrogen use efficiency (NUE) variants with different nitrogen efficiency; *, **, ***significant at $P < 0.05$, 0.01, 0.001

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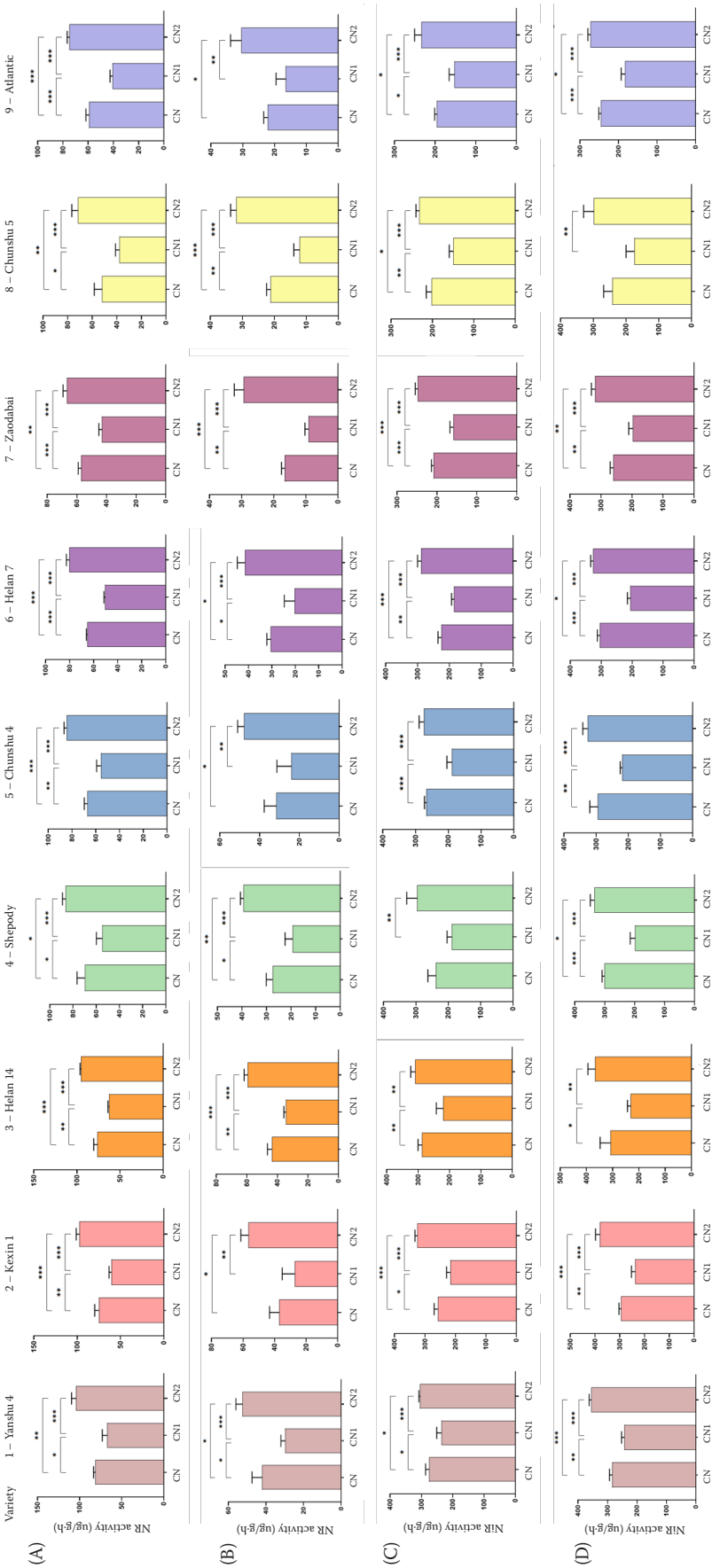

Figure 5. NR (A), (B) and NiR (C), (D) activity in leaves and roots of nine potato varieties under completely dark treatment with different nitrogen supply levels
(A), (C) – leaves; (B), (D) – roots; NR – nitrate reductase; NiR – nitrite reductase; C – completely dark condition; N – normal nitrogen application; N1 – small amount of nitrogen application; N2 – excessive nitrogen application; the nine varieties contain three nitrogen use efficiency (NUE) variants with different nitrogen efficiency; *, **, ***significant at $P < 0.05$, 0.01, 0.001

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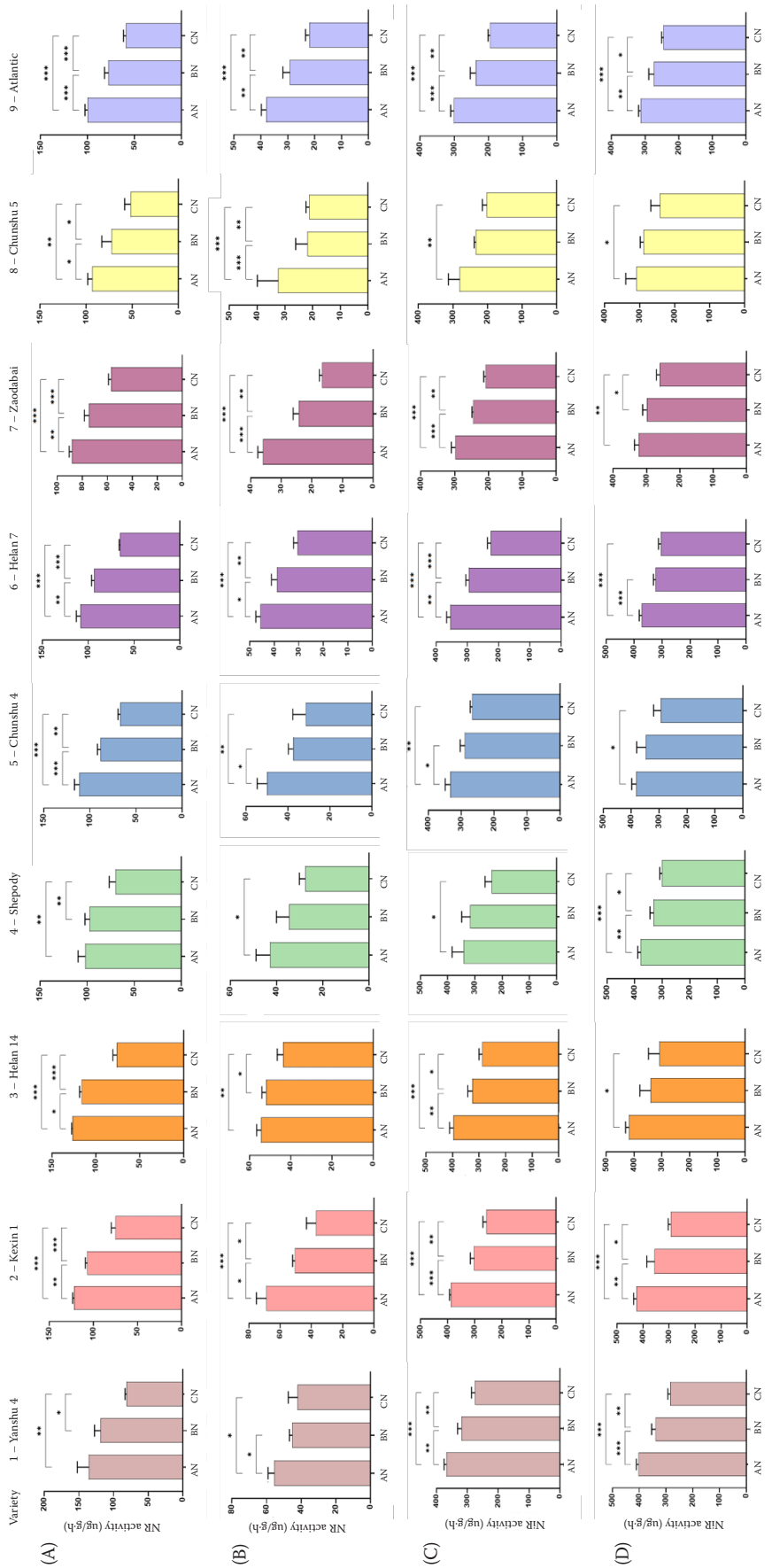

Figure 6. NR (A), (B) and NiR (C), (D) activity in leaves and roots of nine potato varieties under normal nitrogen supply level with different photoperiod treatments (A) – leaves; (B) – roots; NR – nitrate reductase; NiR – nitrite reductase; N – normal nitrogen application; A – normal photoperiod, 14 h/d light, 10 h/d dark; B – short photoperiod, 8 h/d light, 16 h/d dark; C – completely dark condition; the nine varieties contain three nitrogen use efficiency (NUE) variants with different nitrogen efficiency; *, **, ***significant at $P < 0.05$, 0.01, 0.001

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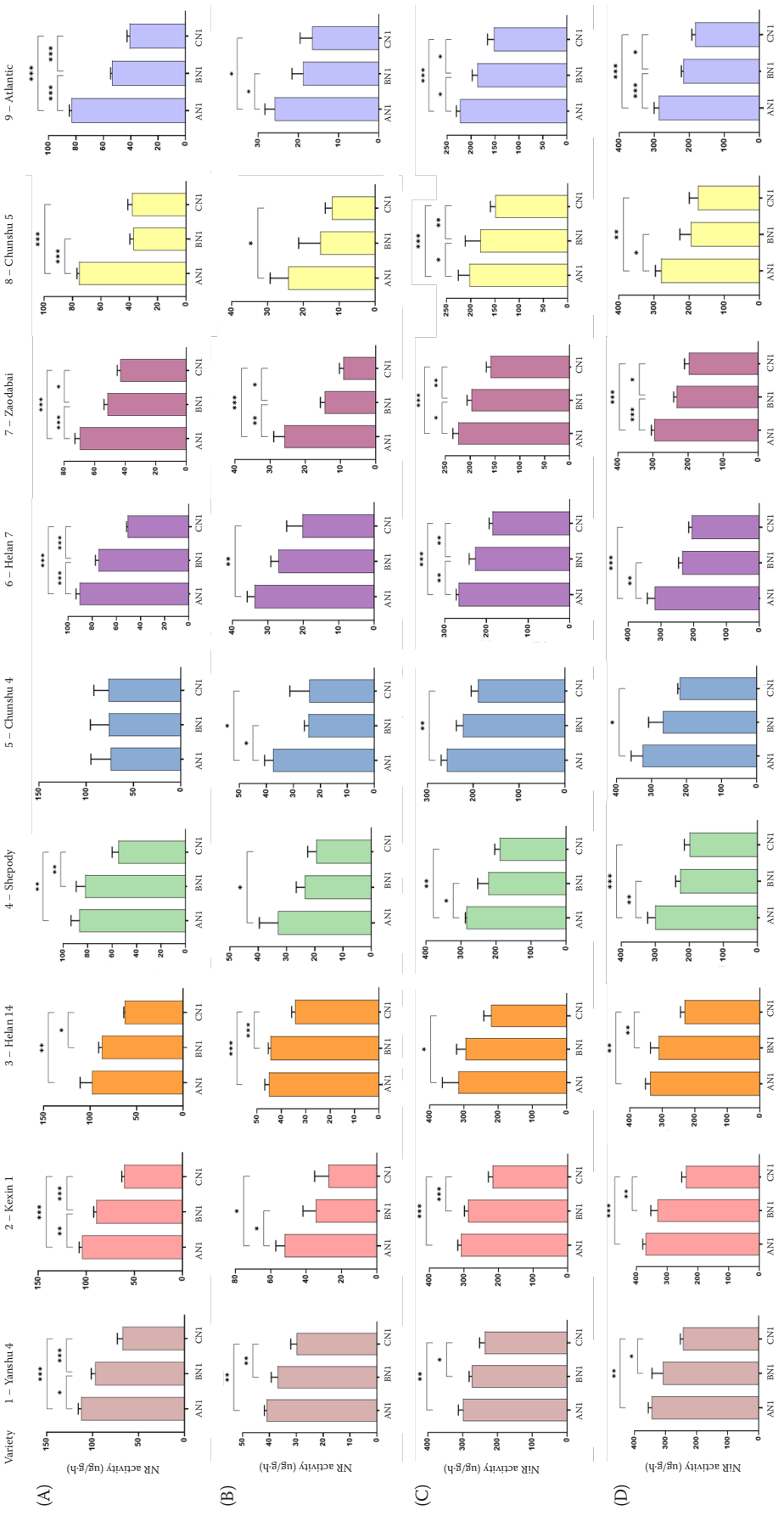

Figure 7. NR (A), (B) and NiR (C), (D) activity in leaves and roots of nine potato varieties under low nitrogen supply level with different photoperiod treatments (A), (C) – leaves; (B), (D) – roots; NR – nitrate reductase; NiR – nitrite reductase; N1 – small amount of nitrogen application; A – normal photoperiod, 14 h/d light, 10 h/d dark; B – short photoperiod, 8 h/d light, 16 h/d dark; C – completely dark condition; the nine varieties contain three nitrogen use efficiency (NUE) variants with different nitrogen efficiency; *, **, ***significant at $P < 0.05$, 0.01, 0.001

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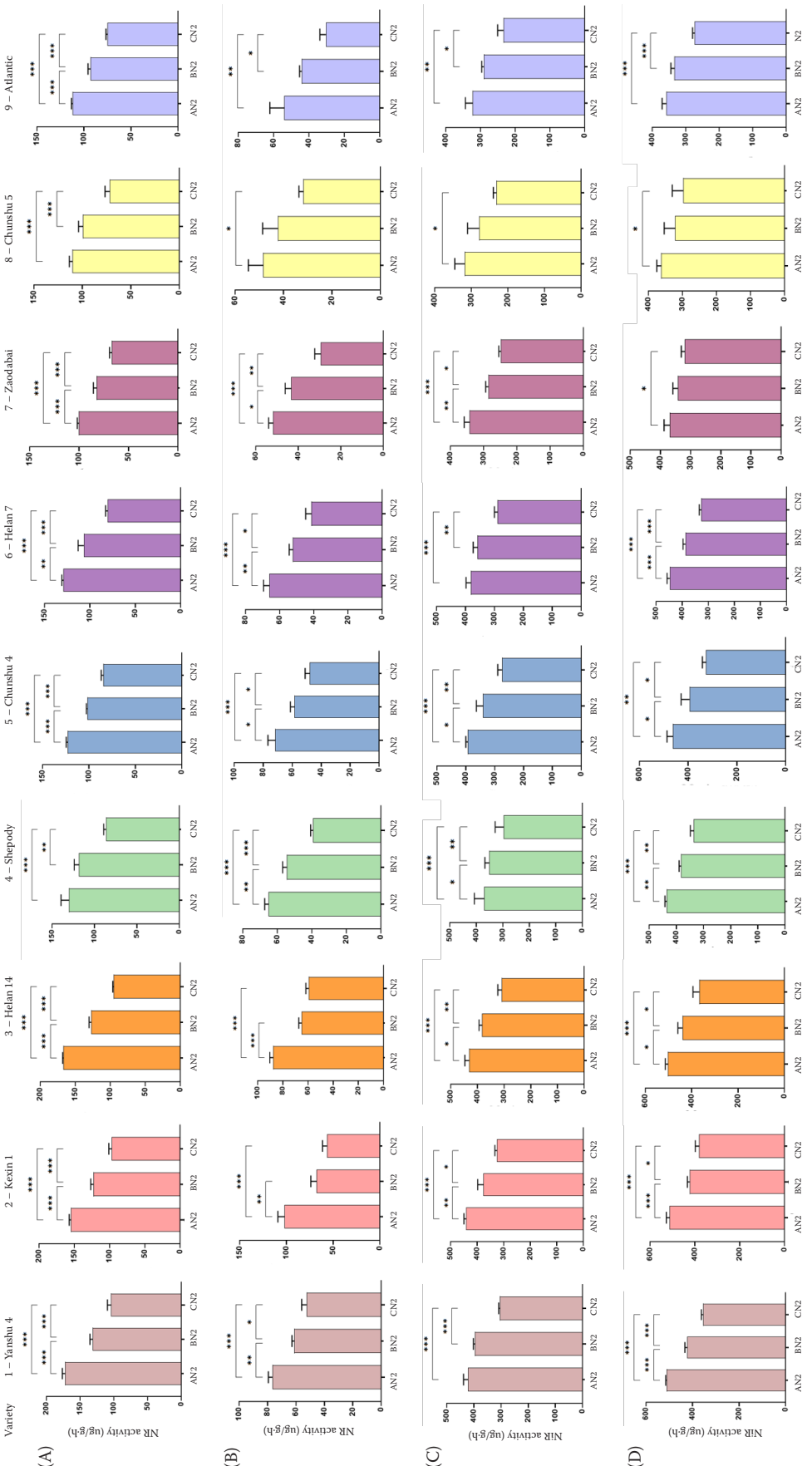

Figure 8. NR (A), (B) and NiR (C), (D) activity in leaves and roots of nine potato varieties under high nitrogen supply level with different photoperiod treatments (A), (C) – leaves; (B), (D) – roots; NR – nitrate reductase; NiR – nitrite reductase; N2 – excessive nitrogen application; A – normal photoperiod, 14 h/d light, 10 h/d dark; B – short photoperiod, 8 h/d light, 16 h/d dark; C – completely dark condition; the nine varieties contain three nitrogen use efficiency (NUE) variants with different nitrogen efficiency; *, **, ***significant at $P < 0.05$, 0.01, 0.001

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with a more pronounced effect in nitrogen efficient potato ($P < 0.001$).

Overall, for both NR and NiR activities in the three NUE variants of potato, the activity levels followed the pattern: nitrogen-efficient potato > nitrogen moderate-efficient potato > nitrogen-inefficient potato.

Effect of different photoperiod treatments on NR and NiR activity in leaves and roots of three potato variants under 1/2 nitrogen supply. As shown in Figure 7 (A, B), under 1/2 nitrogen supply and different photoperiod treatments, significant differences in the activity of NR were observed in both leaves and roots of the three NUE variants.

Notably, there were highly significant discrepancies in NR activity between the normal photoperiod and the completely dark condition, as well as between the short photoperiod and the completely dark condition ($P < 0.01$). As the duration of the light exposure increased, the enzyme activity rose significantly, following the order normal photoperiod > short photoperiod > completely dark condition.

Regarding the activity of NiR (Figure 7C, D) in leaves and roots of the three NUE variants, as well as that of NR, the overall pattern was: nitrogen-efficient potato > nitrogen moderate-efficient potato > nitrogen inefficient potato.

Effect of different photoperiod treatments on NR and NiR activity in leaves and roots of three NUE variants under 2× nitrogen supply. As illustrated in Figure 8 (A, B), under 2× nitrogen supply and various photoperiod treatments, significant differences in NR activity were detected. There were highly significant differences in NR activity between the normal photoperiod and the completely dark condition, between the short photoperiod and the completely dark condition, as well as between the normal photoperiod and the short photoperiod. As the duration of light exposure increased, NR activity increased significantly, following the order: normal photoperiod > short photoperiod > completely dark condition.

For NiR activity significant differences were observed in the leaves and roots of nitrogen-efficient potato and nitrogen moderate-efficient potato under the normal photoperiod and completely dark conditions, as well as under the short photoperiod and completely dark conditions. In nitrogen inefficient potato, significant differences in NiR activity were noted only between the normal photoperiod and the completely dark condition ($P < 0.001$).

Overall, across the leaves and roots of the three NUE variants, the activity of NR and NiR followed the pattern: nitrogen efficient potato > nitrogen moderate-efficient potato > nitrogen inefficient potato.

Effect of different photoperiod treatments on NR and NiR activity in leaves and roots of three NUE variants under different nitrogen supply levels. As illustrated in Figure 9–10, under different nitrogen supply levels with different photoperiod treatments, overall, across the leaves and roots of the three NUE variants, the activity of NR and NiR followed the pattern: nitrogen-efficient potato > nitrogen moderate-efficient potato > nitrogen inefficient potato. And the activity in leaves is higher than that in roots.

DISCUSSION

StNR and *StNiRs* are key enzyme genes in the uptake and assimilation of nitrogen in potato.

We found in this study that three NUE variants with distinct nitrogen use efficiencies (NUE) exhibited significant differences in StNR and StNiR expression across tissues and treatments. Our findings align with prior studies, where NR and NiR expression positively correlated with enzyme activity. For example, under red-blue-purple LED light, leaf NR gene expression of Chinese cabbage was significantly higher than root expression. Besides, the activity of leaf enzyme was tightly linked to nitrate uptake and amino acid accumulation (Li et al. 2024). In tea plants, low nitrogen upregulated TAR2/ARF2, increasing lateral root density by 46.15% via auxin biosynthesis (Hu et al. 2020). This research demonstrated that StNR relative expression and NR enzyme activity were consistently higher in leaves than roots across all variants. For StNiR isoforms, StNiR8262 showed preferential root expression, while StNiR5823 was more abundant in leaves. Furthermore, expression levels and enzyme activities of both genes correlated positively with nitrogen supply levels and photoperiod duration, with high-NUE variants displaying the strongest responses.

In this study, we found that the leaf expression levels and enzymatic activities of StNR and StNiR5823 in high-NUE potato variants were significantly higher than those in low-NUE variants. This finding is consistent with the theory that NR and NiR drive nitrate reduction to ammonium, a process crucial for nitrogen assimilation; their elevated expression in high-NUE variants underscores their role in enhancing nitrogen

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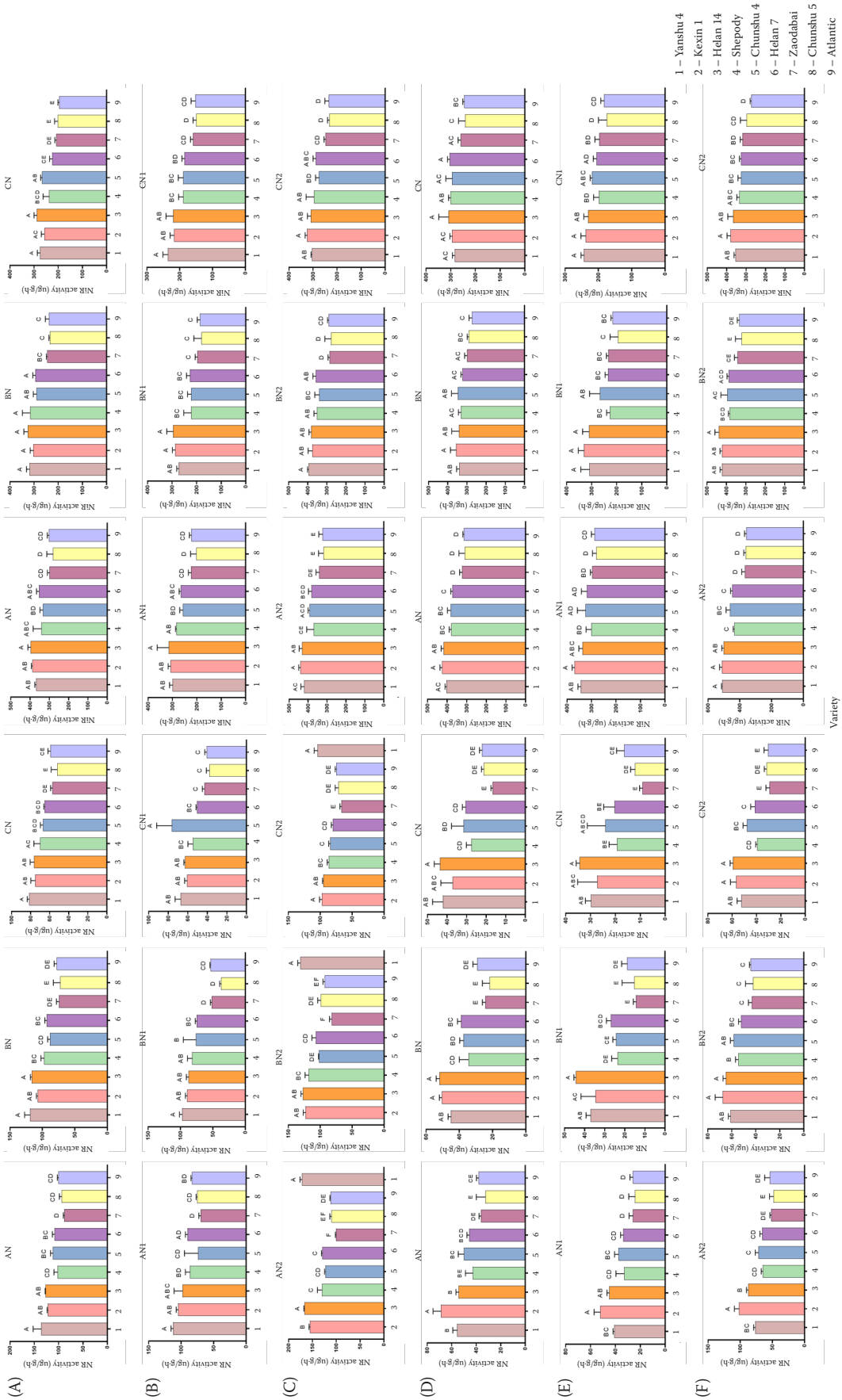

Figure 9. Nitrate reductase (NR) and nitrite reductase (NiR) activity in leaves and roots of three potato NUE variants with different nitrogen supply levels
(A), (B), (C) – leaves; (D), (E), (F) – roots; (A), (D) – N; (B), (E) – N1; (C), (F) – N2; N –normal nitrogen application; N1 – small amount of nitrogen application, which is 1/2 of the normal nitrogen application level; N2 – excessive nitrogen application, which is 2 times the normal nitrogen application level; A – normal photoperiod condition: 14 h/d light, 10 h/d dark; B – short photoperiod treatment: 8 h/d light, 16 h/d dark; C – completely darkness condition; the nine varieties contain three nitrogen use efficiency (NUE) variants with different nitrogen efficiency; different letters represent the significance of the relative expression of the same variants of potato under different nitrogen supply levels ($P < 0.05$)

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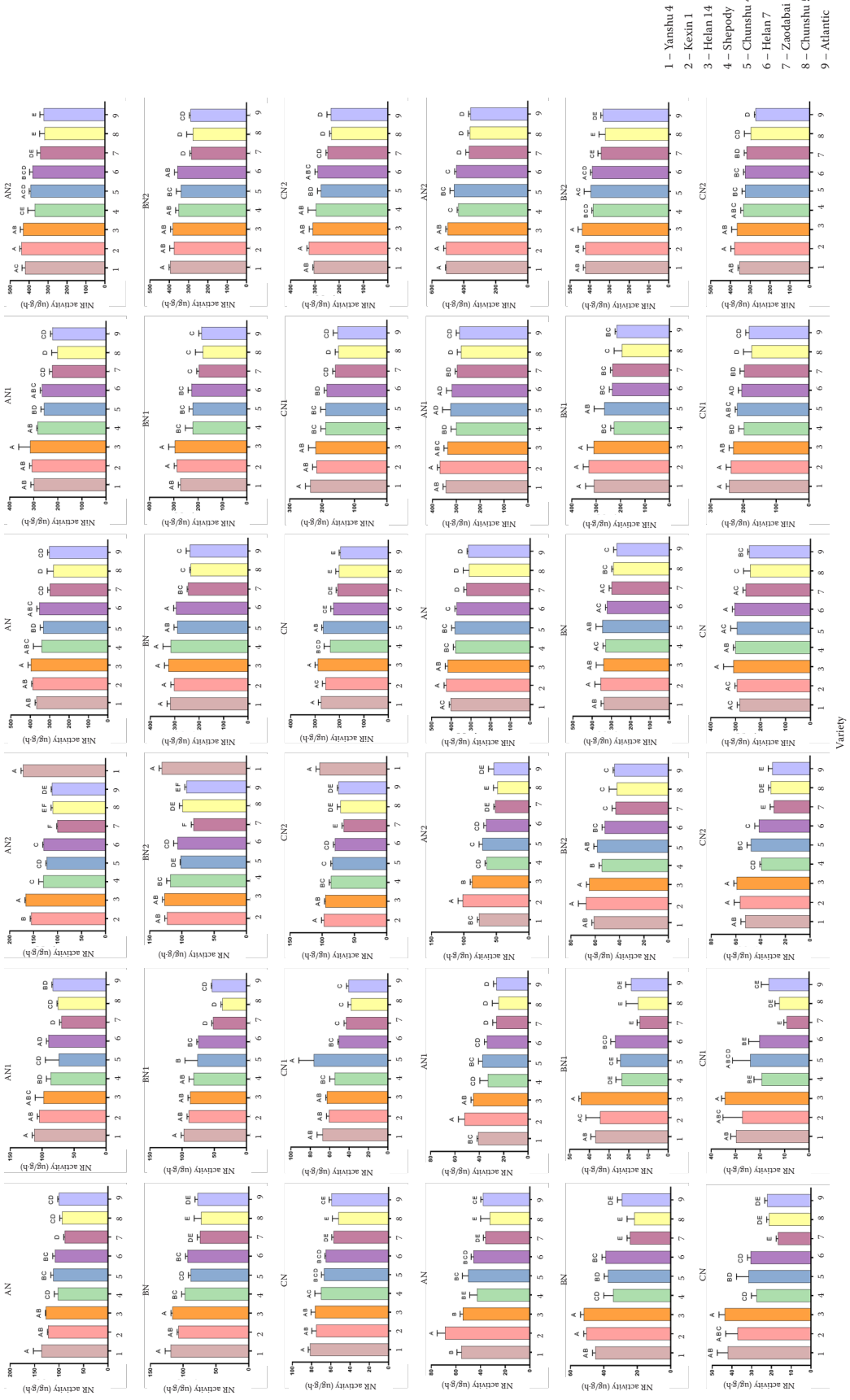

Figure 10. Nitrate reductase (NR) and nitrite reductase (NiR) activity in leaves and roots of three potato nitrogen use efficiency (NUE) variants with different photoperiod treatments

(A), (B), (C) – leaves; (D), (E), (F) – roots; (A), (D) – A; (B), (E) – B; (C), (F) – C; A –normal photoperiod condition: 14 h/d light, 10 h/d dark; B – short photoperiod treatment: 8 h/d light, 16 h/d dark; C – completely darkness condition; N – normal nitrogen application; N1 – small amount of nitrogen application, which is 1/2 of the normal nitrogen application level; N2 – excessive nitrogen application, which is 2 times the normal nitrogen application level; the nine varieties contain three NUE variants with different nitrogen efficiency; different letters represent the significance of the relative expression of the same variants of potato under different nitrogen supply levels ($P < 0.05$)

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assimilation efficiency (Luo et al. 2023). Notably, the specific high expression of StNiR8262 in roots may expand the function of NR/NiR in local nitrogen metabolism within roots, suggesting a unique mechanism by which potato optimises NUE through tissue-specific regulation.

Potato variants with differing NUE showed distinct N absorption and gene expression patterns under varying N supplies. Han et al. reported higher NR/NiR expression in nitrogen-efficient potato than that in nitrogen-inefficient potato under similar nitrogen supply (Han et al. 2022), consistent with our observation that high NUE potatoes exhibited stronger N responsiveness, followed by moderate NUE, and low NUE variants.

Some studies have demonstrated that the activity of NR and NiR are closely linked to nitrogen absorption, assimilation, and photoperiod regulation. For example, in soybean (*Glycine max*), extended photoperiods after flowering enhanced nitrogen accumulation by 31%–76%. During this reaction, both biological nitrogen fixation and soil nitrogen uptake were also increased (Kelly et al. 2021). In tomato (*Solanum lycopersicum*), continuous light disrupted the circadian rhythms of NR and NiR, leading to a 1.5–3.5-fold accumulation of toxic nitrite in susceptible cultivars. However, reducing nitrogen supply restored NiR activity and alleviated photoperiodic injury by 40%–60% (Kelly et al. 2021). Our study further reveals that under identical photoperiod treatments, the activities of potato NR/NiR-related enzymes were significantly higher under high nitrogen treatments than that under normal or low nitrogen conditions. Conversely, at the same nitrogen supply level, these enzyme activities were significantly higher under normal photoperiods than under short photoperiods or complete darkness, indicating a synergistic interaction between nitrogen availability and photoperiod in regulating nitrogen metabolic enzymes.

The expression of *StNR* and *StNiRs* in potato is influenced by many factors during nitrogen uptake and assimilation. In this study, the highest activity was found in nitrogen-efficient potato under normal photoperiod, indicating that these varieties exhibit a stronger physiological response compared to nitrogen-moderate-efficient and nitrogen-inefficient potato varieties. This finding resonates with previous research on nitrogen-related molecular mechanisms.

Previous studies have indicated that nitrogen availability is a key regulator of gene expression in nitrogen-related pathways. For instance, it has been shown that when nitrogen supply is adequate, specific transcription factors bind to the regulatory regions of genes involved in nitrogen metabolism (M'hamdi et al. 2016). In the context of our study, it is likely that in nitrogen-efficient potato varieties, a similar regulatory mechanism exists. These varieties may have evolved a more refined nitrogen-sensing system, enabling them to promptly upregulate the expression of StNR and StNiR genes upon nitrogen availability. This would enhance the rate of nitrogen reduction and assimilation, which is consistent with our observation of higher enzyme activity in these varieties. Regarding enzymatic reactions, light and substrate concentration are known to affect the activity of nitrate reductase and nitrite reductase (Chen et al. 2024). Nitrogen-efficient potato varieties may possess a more efficient photosynthetic electron-transfer chain. This would ensure a better supply of reducing power (such as NADH) to nitrate reductase under normal photoperiod conditions, thereby enhancing its activity (Krapp et al. 2014).

The above research will help us better understand *StNR* and *StNiRs*, provide some insights for molecular breeding of nitrogen efficient genes, and lay the foundation for the functional research of *StNR* and *StNiRs* in the process of nitrogen absorption and assimilation.

CONCLUSION

In this study, we utilised three potato variants with varying nitrogen-use efficiency (a total of nine biological materials) as experimental materials. These were subjected to different nitrogen supply levels and different photoperiod treatments. We found notable differences in nitrogen absorption and utilisation among these potato variants. The relative expression of *StNR* and *StNiRs* and the enzyme activity of NR and NiR were proportional to nitrogen supply levels and photoperiod in leaves and roots of three NUE variants with different nitrogen efficiencies. This research clarified the expression of *StNR* and *StNiRs* and the enzyme activity of NR and NiR in leaves and roots under different conditions. It lays a theoretical foundation for exploring their roles in nitrogen absorption and assimilation efficiency, and provides a theoretical basis for potato nitrogen absorption and utilisation.

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