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Overexpression of Cytosolic Glyceraldehyde-3-Phosphate Dehydrogenase 1 Gene Improves Nitrogen Absorption and Utilization in Potato

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Abstract: Nitrogen is one of the most important elements for improving potato yield. However, excessive application of nitrogenous fertilizer not only produces river and other environmental pollution but also increases agricultural production costs. In recent years, to explain the molecular mechanisms of nitrogen metabolites, some vital genes involved have been reported; however, only limited success has been achieved in potato. Here, we report that the expression of cytosolic glyceraldehyde-3-phosphate dehydrogenase 1 (*StGAPC1*) is increased under low-nitrogen stress. *StGAPC1*-overexpressing potato seedlings had more biomass and a significant increase in total nitrogen content and root nitrate influx rate compared to the wild type. The overexpression of *StGAPC1* also increased the expression of nitrate transporters and increased ROS system activity to reduce hydrogen peroxide content under low-nitrogen stress. Our results provide a foundation for further research on StGAPC1 function in nitrogen absorption and utilization mechanisms in potato.

Keywords: potato; *StGAPC1*; nitrogen; absorption; ROS system

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1. Introduction

Potatoes (*Solanum tuberosum* L.) are the world's fourth largest food crop after rice, wheat, and corn, and have the advantages of strong adaptability, high yield, and rich nutritional value. Nitrogen (N) is one of the most important elements in plant nutrition, and its application plays a key role in improving crop yield. Although applying nitrogen fertilizer can improve crop yields, excessive application not only increases agricultural production costs, but also causes environmental pollution and ecological degradation [1,2]. Many studies have indicated that plants absorb nitrate (NO_3^-) and ammonium (NH_4^+) as the two main nitrogen forms [3], with the nitrate peptide transporter family/transporters (NPF/NRTs) playing a role in NO_3^- absorption and ammonium transporters (AMTs) absorbing NH_4^+ in roots [4,5]. In addition, NO_3^- and NH_4^+ are recognized as signalling molecules that interact with a variety of phytohormone signalling pathways to regulate N uptake, transport, and assimilation in plants [6]. Yang et al. reported that the N, potassium (K), phosphorus (P), sodium (Na), and iron (Fe) contents were reduced, meanwhile, magnesium (Mg), calcium (Ca), zinc (Zn), and copper (Cu) contents were increased in blueberry under nitrogen deficiency stress [7].

In recent years, some vital genes involved in N nutrition have been reported; for example, Dof daily fluctuations 1 (RDD1) improved N uptake and accumulation to increase grain productivity in rice [8]. Jia et al. reported that mild N deficiency induces key genes

for BR biosynthesis; for example, the overexpression of *DWF1* increases N accumulation to promote plant growth [9]. RNA-sequencing analysis results suggested that bHLH transcription factors, phosphatase 2C, sugar transporters, high-affinity nitrate transporters, proline-rich proteins, etc., probably play crucial roles in enhancing nitrogen use efficiency (NUE) in potato [10]. In potato, differences in N metabolism among different varieties were found using transcriptome analysis of three potato cultivars, "Yanshu 4", "Xiabodi", and "Chunshu 4". The upregulated DEGs in "Yanshu 4" related to N metabolism were closely related to N-utilization efficiency [11]. In rice, Luo et al. reported that the co-overexpression of genes for nitrogen transport, assimilation, and utilization improved rice grain yield and nitrogen use efficiency [12]. For example, the overexpression of an important ammonium transporter from flowering Chinese cabbage (BcAMT1;5) increased the NH_4^+ and NO_3^- contents compared to those in the wild type under low N concentrations. The findings indicate that BcAMT1;5 may be vital for the N metabolic process [13].

A previous study reported that cytosolic glyceraldehyde-3-phosphate dehydrogenase (GAPC) mainly regulated the glycolysis process in the cytoplasm [14]. Further studies revealed that GAPC was vital for normal fertility and root development in *Arabidopsis* [15,16]. Anoman et al. reported that a lack of GAPCp activity influenced nitrogen, carbon, and mineral nutrition metabolism using transcriptomic and metabolomics analyses [17]. Interestingly, the downregulation activities of phosphorylating GAPC in transgenic potato also decreased the 3-phosphoglycerate content and increased the sucrose and UDP glucose content in leaves [18]. Liu et al. reported increased sucrose and glucose content in potato tubers, resulting in more and longer buds in *StGAPC1* gene-silenced transgenic potato [19]. Further research suggested that the overexpression of *StGAPC1* in potato delayed tuber sprouting [20]. Zhang et al. reported that reducing the use of N in field production significantly prolonged the dormancy period of tubers [21]. Therefore, these data indicate that N content is closely related to sprouting in potatoes, but few studies have reported the role of *StGAPC1* in nitrogen nutrition in potatoes. Therefore, we wanted to explore whether *StGAPC1* functions in the N absorption and assimilation processes in potato.

In our study, we found that the expression of *StGAPC1* was inducible under N starvation. The overexpression of *StGAPC1* promoted potato seedling growth and N accumulation under N starvation stress. Further research suggested that the overexpression of StGAPC1 increased the root nitrate influx rate and the expression of nitrate transporters. Finally, we report that the overexpression of *StGAPC1* increases ROS system activity to reduce hydrogen peroxide content under N starvation stress, implying that *StGAPC1* is a valuable candidate for developing crops with more efficient N use.

2. Materials and Methods

2.1. Preparation and Detection of Transgenic Potato

The coding region of *StGAPC1* (PGSC0003DMG400017433) was obtained using an RT-PCR experiment. Then, *StGAPC1* was inserted into the PBI1221 vector with XbaIand SmaIendonucleases; the map of the constructed vector is provided in Figure S1. Then, the *StGAPC1*-PBI1221 vector was transformed into *Agrobacterium tumefaciens* GV3101. 'Chuanyu 10' potato cultivar plants were propagated in MS medium at 24 °C in a growth chamber under a 16 h light (100 $\mu mol \cdot m^{-2} \cdot s^{-1}$)/8 h dark cycle [22]. Microtubers with diameters of ~5 mm were cut into slices (~1–2 mm) to conduct gene transformation following a previously described method [22]. Three *StGAPC1* transgenic potato lines were identified using real-time reverse transcription–PCR (qRT-PCR) experiments. The primers used in this study are listed in Table S1.

2.2. Potato Seedling Culture

Seedlings of the wild type (WT, Chuanyu 10) and the transgenic lines OE1, OE2, and OE3 were used in the hydroponic culture experiments. The seedling growth conditions and nutrient solution composition were described previously [23]. To analyse the expression patterns of *StGAPC1* in response to nitrate, WT seedlings and transgenic potato lines

(grown on MS medium for 20 d) were grown in nutrient solution that contained 1 mM $Ca(NO_3)_2$ (N-sufficient conditions) or 0.1 mM $Ca(NO_3)_2$ (low-N treatment, LN) for 15 d. The nutrient solution composition was described previously [24]. At least three replicates were used for each experiment. Seedlings from the WT and transgenic lines were obtained for physiological index measurements and gene expression analysis.

2.3. Measurement of Physiological Parameters and Enzyme Activity

Potato seedlings were collected and dried, and a semiautomated Kjeldahl method was used to measure the N content [24]. The activities of GS and NR were tested according to a previously described protocol [25]. The activities of POD and SOD were determined according to previously described methods [26,27]. H_2O_2 and superoxide anion radical (O^{2-}) contents were determined according to a previously described method [28]. The GAPDH activity of seedlings was assayed according to the method described in [15].

2.4. Determination Method for Ion Flux Rate

The flux of NO_3^- and NH_4^+ in potato seedling tips was measured using a noninvasive micrometer system (NMT) according to a method described previously [29]. A nitrogen concentration of 1.44 mM NH_4NO_3 was used as a control, and 0.24 mM NH_4NO_3 was used as the low-nitrogen concentration. Each sample was measured for 10 min.

2.5. Real-Time Reverse Transcription–PCR Analysis of Gene Expression

Total RNA from potato seedlings was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Then, a reverse transcriptase kit (Thermo, Tokyo, Japan) was used to create cDNA. qRT-PCR experiments were conducted using a 7500 Real Time PCR System (Bio-Rad, Hercules, CA, USA). The elongation factor gene in potato was used as an internal control, and the formula $2^{-\Delta\Delta Ct}$ was used to calculate gene expression level.

2.6. Data Processing

In this study, all original data were obtained from at least three biological replicates. The data were analysed using SPSS 14.0 (IBM Corporation, Armonk, NY, USA) and Excel 2016 statistical software (Microsoft Corporation, Redmond, WA, USA). Significant statistical differences between treatments were determined using One Way ANOWA ($p \leq 0.01$ and $p \leq 0.05$).

3. Results

3.1. StGAPC1 Is a Nitrate-Inducible Gene

Examining the expression patterns of *StGAPC1* in different organs, the qRT-PCR experimental results suggested that the expression of *StGAPC1* was higher in tuber pith and roots than in flowers and seedlings (Figure 1A). Then, the mRNA levels of *StGAPC1* were determined under LN conditions ranging from 0 to 24 h in roots; the expression of *StGAPC1* increased over time, reaching the highest level at 12 h (Figure 1B). Our results indicated that *StGAPC1* was nitrate inducible.

3.2. Overexpression of StGAPC1 Promotes Seedling Growth under LN Stress

StGAPC1-overexpressing transgenic potatoes were obtained in our previous studies [20]. The expression levels of *StGAPC1* and GAPDH activity were higher in the three selected transgenic seedlings than in the wild type (WT) (Figures S2 and S3). Then, the growth of the WT and transgenic lines in solution culture at the seedling stage was examined under N-sufficient and LN-stress conditions. Compared to the wild type, the three transgenic lines grew significantly better under both N-sufficient and LN conditions for 15 days (Figure 2A). At the same time, the three transgenic lines seedling had higher dry weights and N concentrations than the WT (Figure 2B,C). In summary, the transgenic lines grew better and had greater dry weights than the WT under both N-sufficient conditions and LN stress.

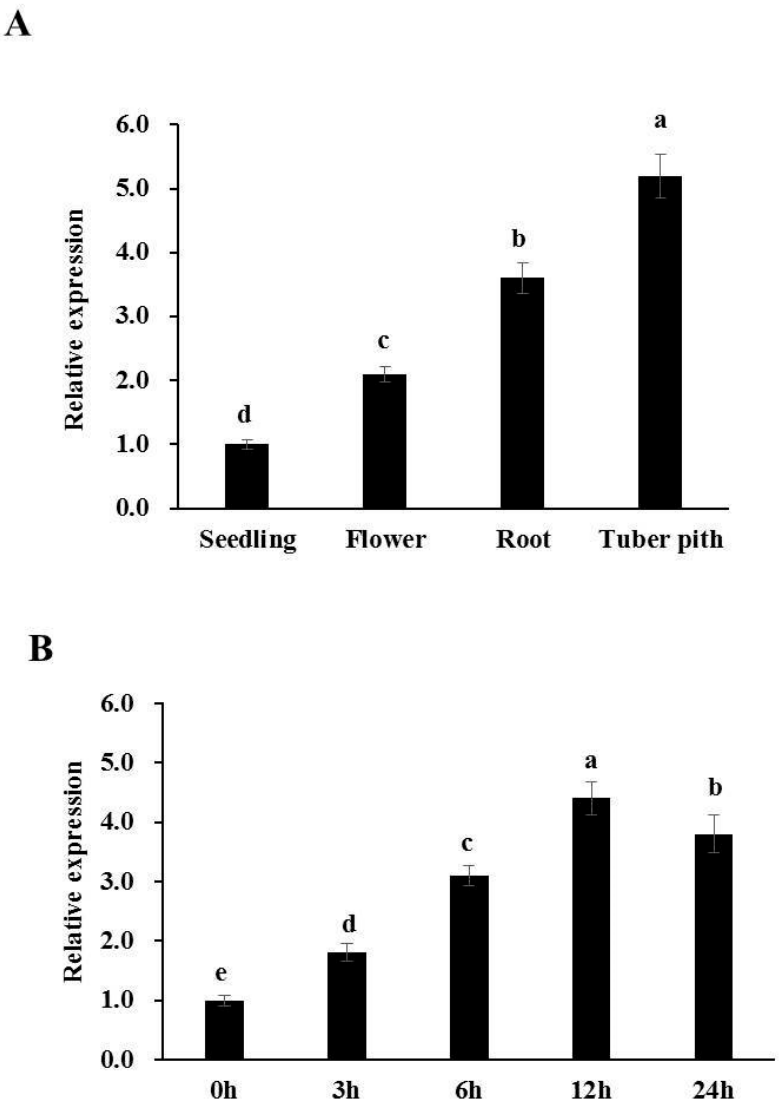

Figure 1. Expression pattern analysis of *StGAPC1* in organs and under LN conditions. (**A**) Expression of *StGAPC1* in different organs of potato plants. (**B**) *StGAPC1* expression was increased in roots under LN stress. Potato seedlings were grown in nutrient solutions that contained 0.2 mM nitrate (low N) for 3, 6, 12, and 24 h, and nutrient solutions that contained 2 mM nitrate (N-sufficient conditions) were used as controls (0 h). The data are the means ± SEs of three replicates. Different lowercase letters represent a significant difference at $p < 0.05$.

3.3. Overexpression of StGAPC1 Increases Enzyme Activity and Expression Level

To study the responses of transgenic lines to N concentration changes, seedlings of the WT and transgenic potato lines OE1, OE2, and OE3 were cultured in nutrient solution (N-sufficient conditions and LN conditions) for 15 days. First, the nitrate reductase (NR) and glutamine synthetase (GS) activities were obviously higher in the transgenic lines than in the WT under LN conditions; on the other hand, there were no significant differences between the transgenic lines and the WT under N-sufficient conditions (Figure 3A,B). Further analysis suggested that the expression levels of two enzymes were also increased in the transgenic lines compared to the WT under LN conditions. Similarly, there were no differences between the transgenic lines and the WT under N-sufficient conditions (Figure 3C,D). Therefore, it can be inferred that the *StGAPC1*-overexpressing transgenic seedlings were more adaptable to LN stress due to the promotion of NR and GS enzyme activity and gene expression level.

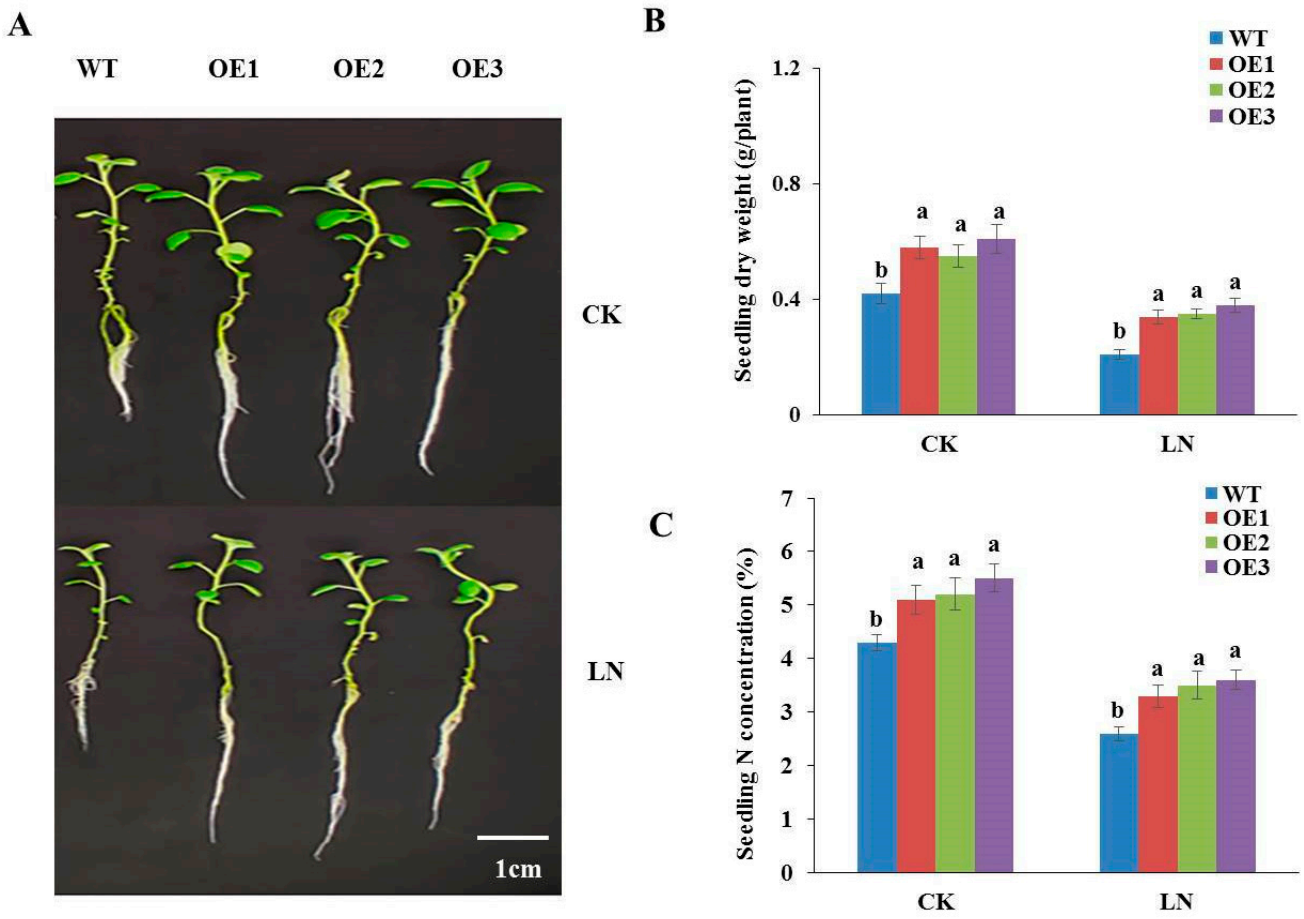

Figure 2. Plant growth and dry weight analysis of the wild type and transgenic lines. (**A**) Seedlings of the WT and the transgenic lines (OE1, OE2, and OE3) grown under N-sufficient conditions and LN conditions. (**B**) Dry weight of seedlings. (**C**) N concentration of seedlings. Different lowercase letters represent a significant difference at $p < 0.05$.

3.4. Overexpression of StGAPC1 Increases the Root NO_3^- Influx Rates

The noninvasive microtest technique (NMT) is widely used for the study of NO_3^- and NH_4^+ influx and efflux in plants. In our study, there was no significant difference in NO_3^- influx in the roots of the OE1 transgenic line and the WT under N-sufficient conditions (Figure 4A), but under LN conditions the NO_3^- influx of the transgenic lines increased obviously compared to that of the WT (Figure 4B). On the other hand, there was no significant difference in NH_4^+ efflux between the OE1 transgenic line and the WT under both N-sufficient conditions and LN stress (Figure 4C,D). The results indicated that the overexpression of *StGAPC1* increased the uptake of NO_3^-, which coincided with the increased N content in transgenic line seedlings.

3.5. Overexpression of StGAPC1 Upregulates the Expression of Nitrate Transporters in Roots

Based on the above NO_3^- and NH_4^+ flux experimental results, we analysed the effects of overexpressing *StGAPC1* on the expression of 10 nitrate transporter genes. The expression of NRT1.1, NRT2.1, NRT2.4, NRT2.5, NRT4.5, and NRT4.6 was obviously higher in the OE1 transgenic line compared to the WT under LN stress. In contrast, the expression of NRT1.5 and NRT2.7 was lower in the OE1 transgenic line root than the WT (Figure 5). Therefore, we concluded that the overexpression of StGAPC1 resulted in strong NO_3^- uptake, possibly due to the higher expression of the six NRT genes involved in nitrate transport.

3.6. Overexpressing StGAPC1 Increases ROS System Elimination

To study the effect of overexpressing *StGAPC1* on the ROS system under LN stress, first, the seedlings of the WT and transgenic potato lines OE1, OE2, and OE3 were cultured in nutrient solution (N-sufficient conditions and LN conditions) for 15 days. Then, we measured the POD activity, SOD activity, and H_2O_2 and O^{2-} contents. The results suggested that overexpressing *StGAPC1* significantly increased the activity of SOD and POD (Figure 6A,B). On the other hand, the contents of both H_2O_2 and O^{2-} in the transgenic lines were also decreased compared to those in the WT (Figure 6C,D). All the results demonstrated that overexpressing *StGAPC1* increases antioxidant enzyme activity to reduce H_2O_2 and O^{2-} accumulation under LN stress.

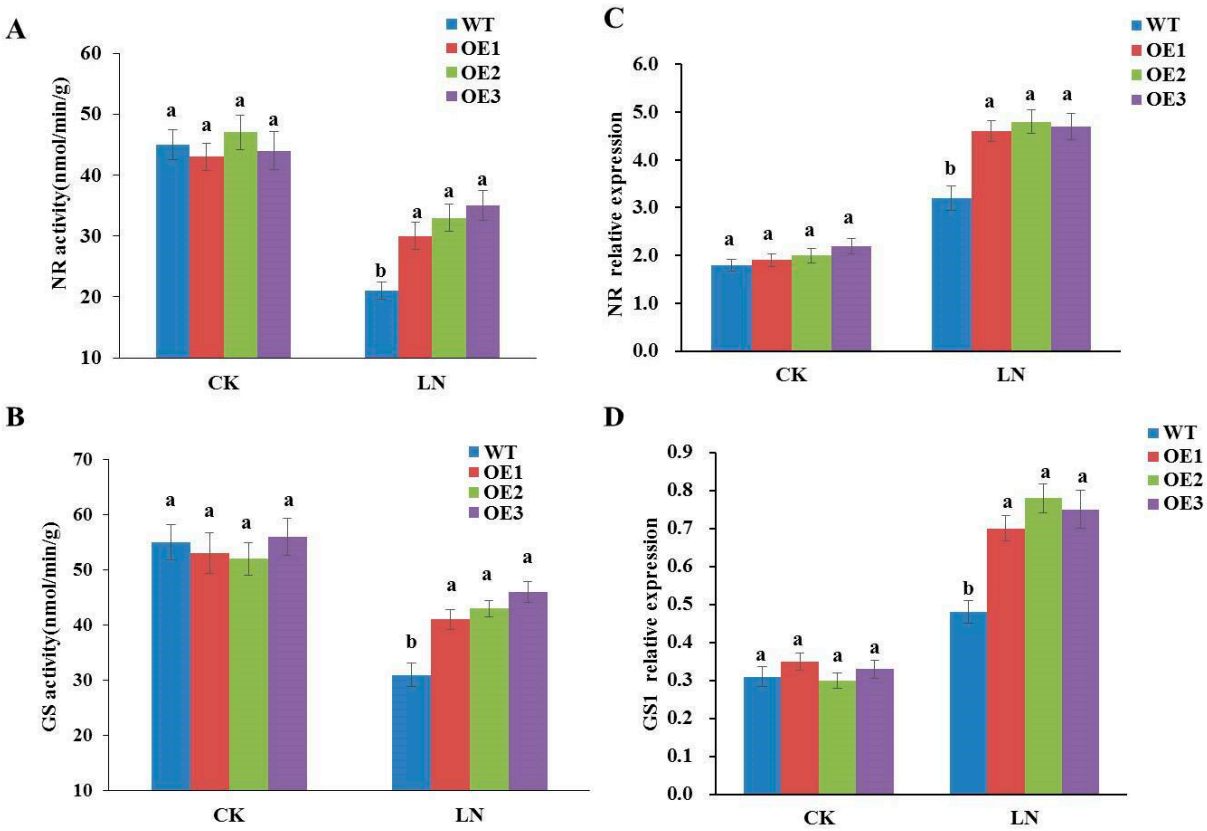

Figure 3. Analysis of enzyme activity and expression level of the transgenic lines. (**A**) NR activity of seedlings; (**B**) GS activity of seedlings; (**C**) NR expression level in seedlings; (**D**) GS expression level in seedlings. WT means wild type, OE1–OE3 means the transgenic lines. The data are the means ± SEs of three replicates. Different lowercase letters represent a significant difference at $p < 0.05$.

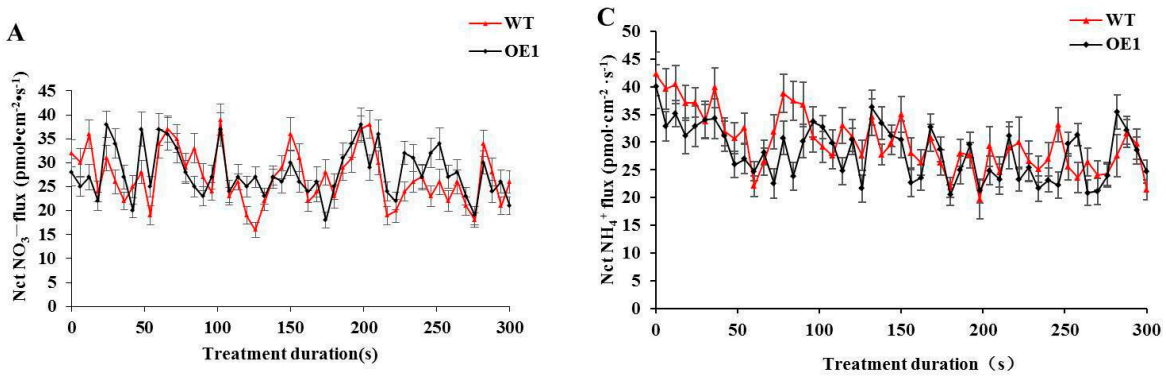

Figure 4. *Cont.*

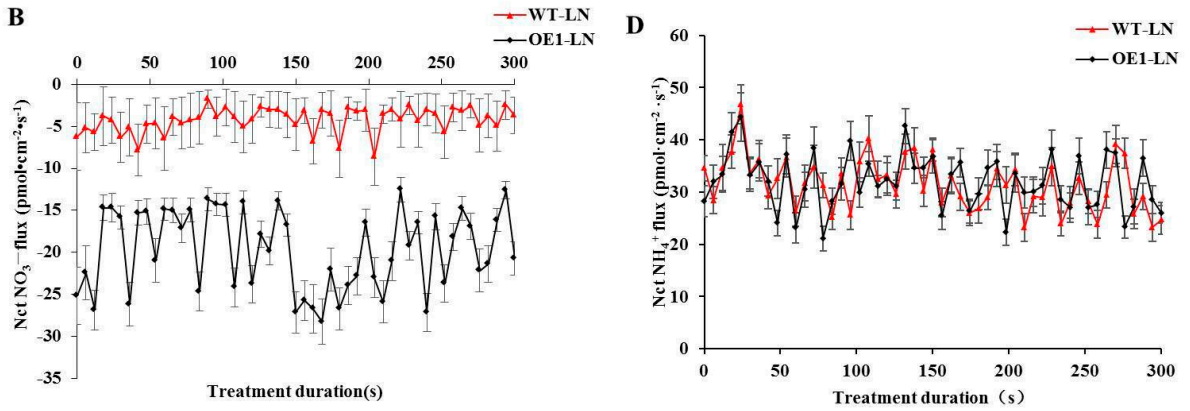

Figure 4. NO_3^- and NH_4^+ fluxes of potato roots under different N concentration treatments. (**A**) NO_3^- flux under N-sufficient conditions; (**B**) NO_3^- flux under LN conditions; (**C**) NH_4^+ flux under N−sufficient conditions; (**D**) NH_4^+ flux under LN conditions. A positive value indicates efflux, a negative value indicates influx. WT means wild type; OE1 means the transgenic lines.

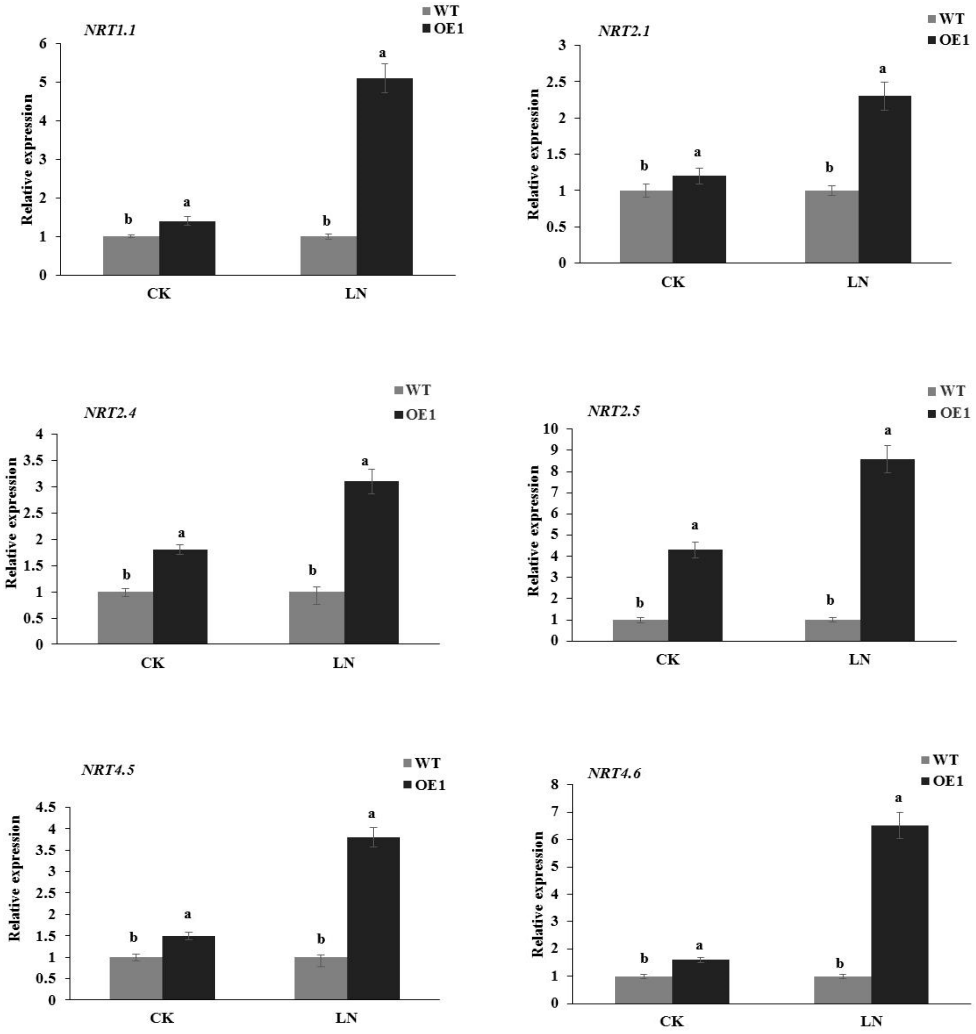

Figure 5. *Cont.*

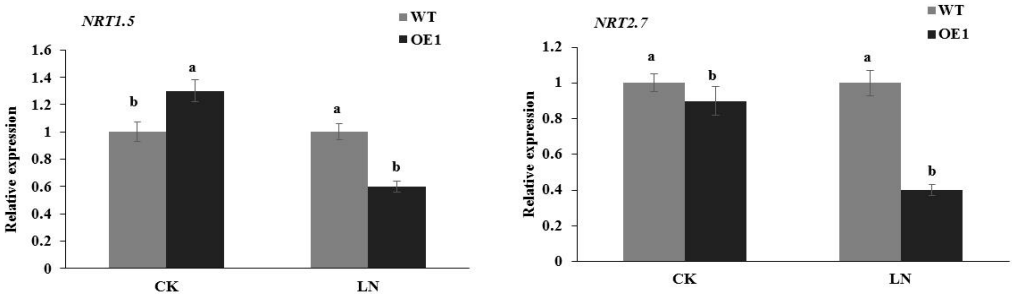

Figure 5. Expression analysis of nitrate transporters in transgenic plants. WT means wild type; OE1 means the transgenic lines. Different lowercase letters represent a significant difference at $p < 0.05$.

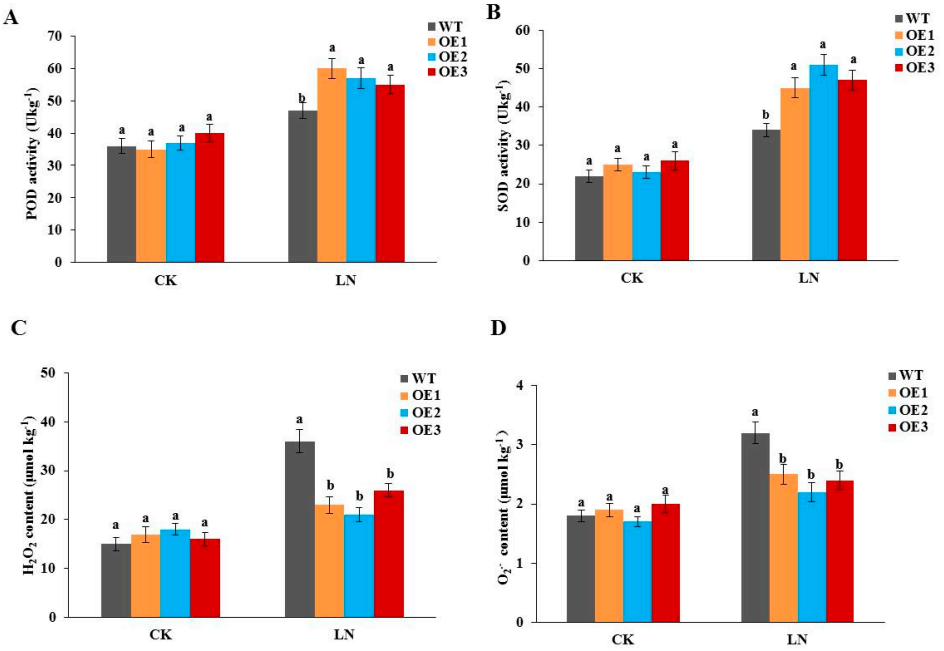

Figure 6. Effect of *StGAPC1* overexpression on the ROS system. (**A**) SOD activity; (**B**) POD activity; (**C**) H_2O_2 content; (**D**) O^{2-} content. WT means wild type, OE1–OE3 means the transgenic lines. Different lowercase letters represent a significant difference at $p < 0.05$.

4. Discussion

Applying N fertilizer can significantly promote photosynthesis, increase dry matter accumulation, and significantly increase tuber yield [30]. Soualiou et al. reported that a high nitrogen treatment significantly improved maize photosynthesis under cold stress and enhanced the activity of nitrogen metabolism enzymes and nitrogen transport from roots to aboveground parts, therefore, promoting a significant increase in maize root and aboveground biomass compared to a low-nitrogen treatment [31]. Up to now, many important N-efficiency genes such as GRF4 and MYB61 and gene loci such as TCP19 and NAC42 have been identified in rice [32], but important N-efficiency genes in potato need further exploration.

Nishizawa et al. reported that cytosolic GAPDH had a role in cell death and interacted with ubiquitin ligase EL5 under high-nitrogen conditions to prevent root meristematic cell death [33]. Downregulated GAPCp activity affected nitrogen and carbon metabolism, especially glycerate and glutamine metabolites, and the ammonium assimilation pathway in *Arabidopsis* [14]. In this study, *StGAPC1* was expressed in tuber pith, roots, flowers, and seedlings (Figure 1A). The expression of *StGAPC1* increased continuously after the LN treatment, reaching its peak at 12 h in seedlings. After the LN treatment for 15 d, the StGAPC1-overexpressing lines had higher biomass and N concentration accumulation than

the WT under N-sufficient conditions, but there was no significant difference between them under N-sufficient conditions (Figure 2A–C). The findings suggest that the overexpression of *StGAPC1* can promote nitrogen accumulation, which may be similar to TaNAC2-5A as a positive regulator [24].

As key enzymes in the N assimilation process, nitrate reductase (NR) and glutamine synthetase (GS) activities were higher in the transgenic lines than in the WT under LN conditions (Figure 3A,B). Similarly, the expression levels of two enzymes were also increased in the transgenic lines compared to the WT under LN conditions (Figure 3C,D), suggesting that the overexpression of *StGAPC1* enhances N assimilation under low-nitrogen stress, similar to PLDe function. Lu et al. reported that the overexpression of PLD lines increased the activity of nitrite reductase under nitrogen deficiency [34]. Khan et al. reported that transgenic rice overexpressing calcineurin B-like interacting protein kinase 2 (OsCIPK2) absorbed more NO_3^- and Ca^{2+} compared to the WT under LN [30]. Further research reported that the influx of NO_3^- increased significantly in the roots of the StGAPC1-overexpressing line compared to that in the roots of the WT plants under LN stress (Figure 4A,B). The results suggested that the upregulation of StGAPC1 expression promoted the uptake of NO_3^- in roots under LN conditions. A similar result was found for apple bHLH130 [35].

There are 39 StNRT gene members in the potato genome, and different members display different expression patterns in different tissues [36]. The transcript levels of eight TaNRTs were significantly increased after N starvation treatment in wheat [37]. In maize, the transcript expression of four N transporters was increased to promote N uptake after brassinosteroid treatment [38]. In rice, six N transporters (OsNRT1.1A, OsNRT1.1B, OsNPF6.1, OsNRT2.1, OsNRT2.3b, and OsNAR2.1) could increase N uptake and NUE [39]. Ma et al. reported that the expression of the OsNRT2s gene was regulated by ARE4 and exhibited a clear rhythmic pattern in response to the diurnal fluctuations of soluble sugar content, and nitrogen utilization was reduced in the *are4* mutant, resulting in obvious defects in growth and development [40]. The expression of eight NRTs was higher in *StGAPC1*-overexpressing roots than in CK roots after 12 h of LN (Figure 5). These results suggested that *StGAPC1* promoted the uptake of NO_3^- and NH_4^+ in roots through enhancing NRT function.

On the other hand, Krouk et al. reported that some N transporters transported hormones; for example, NPF6.3/NRT1.1 transported auxin in addition to transporting NO_3^- in *Arabidopsis* [41]. Four NO_3^- transporters had been found to transport abscisic acid in yeast or insect cells [42]. Similarly, NPF3 and NPF2.10 were reported to transport gibberellin in *Arabidopsis* [43]. Therefore, we speculated that the increased expression of NRT transporters in *StGAPC1*-overexpressing lines changed the auxin, ABA, and GA signalling pathways to improve low-nitrogen tolerance.

Previous studies suggested that LN stress promoted the accumulation of ROS, especially increased hydrogen peroxide (H_2O_2) content [44]. Similarly, cytosolic ascorbate peroxidase 1 (CsAPX1) might, together with nitrogen regulatory protein P-II (CsGLB1), promote ascorbic acid accumulation compared to the WT under N deficiency solution condition in the tea plant [45]. In *Arabidopsis*, Chu et al. reported that transcription factor HOMOLOG OF BRASSINOSTEROID ENHANCED EXPRESSION2 INTERACTING WITH IBH1 (HBI1) was a vital regulator of nitrate signalling; meanwhile, it induced the expression levels of some antioxidant genes, then decreased the content of reactive oxygen species (ROS) under LN conditions [46].

Hancock et al. reported that GAPDH might also have a similar role in mediating ROS signalling, and H_2O_2 activated the interaction of GAPC and PLD to regulate growth and stress responses together in *Arabidopsis* [47]. In rice, the overexpression of the *OsGAPC3* gene improved salt tolerance through increasing the activity of catalase and eliminating oxidative free radicals [48]. Zhang et al. found that the overexpression of TaGAPC1 promoted H_2O_2 detoxification to increase drought tolerance in transgenic *Arabidopsis* [49]. The activities of POD and SOD were obviously higher in the *StGAPC1* overexpression line seedlings than in the WT seedlings (Figure 6A,B), and in contrast, the contents of both

H_2O_2 and O^{2-} in the transgenic lines were also decreased compared to those in the WT (Figure 6CD), suggesting that the overexpression of *StGAPC1* may contribute to enhanced ROS pathway function under LN stress.

Many studies had reported that GAPC and interacting proteins worked together in some important physiological processes. Han et al. reported that the overexpression of GAPCs inhibited autophagy in tobacco, and oxidative stress inhibited the interaction between GAPCs and autophagy-related protein 3 (ATG3) [50]. Zhang et al. reported that glyceraldehyde 3-phosphate dehydrogenase (GAPDH) interacted with OsSRT1 to regulate cell redox states and that OsSRT1 depressed GAPDH lysine acetylation and nuclear accumulation under oxidative stress in rice [51]. In potato, StGAPC1 interacted with snakin-2 to decrease its oxidative modification to inhibit sprout growth [20]. Summarising the above results, we speculate that *StGAPC1* may interact with some proteins to regulate N nutrition pathways. GAPC also plays a role in gene transcription regulation, and GAPDH directly regulates the transcriptional activation function of a series of glycolytic enzyme-encoding genes in rice [51]. Kim et al. reported that in *Arabidopsis*, AtGAPC bound to the transcription factor NF-YC10 to induce the expression of heat-tolerance genes and enhance plant heat tolerance [52]. We believe that *StGAPC1* may directly regulate the genes involved in N absorption and assimilation, which requires more research in the future.

5. Conclusions

The results of this study indicated that the expression of *StGAPC1* was increased after low-nitrogen treatment. The *StGAPC1*-overexpressing transgenic potato seedlings had higher shoot and root dry weights than the WT potato seedlings after LN treatment for 15 days. Meanwhile, the transgenic potato seedlings also had higher N accumulation, NR and GS enzyme activity, and nitrate transporter expression. In addition, the activity of SOD and POD increased; in contrast, the contents of H_2O_2 and O^{2-} were also decreased after the low-nitrogen treatment in the transgenic lines. In summary, our study provides a foundation for the thorough analysis of StGAPC1 function in potato N signalling pathways and improving the utilization rates of potato N fertilizer.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9101105/s1, Figure S1: The map of constructed vector; Figure S2: Expression level analysis of *StGAPC1* in transgenic lines; Figure S3: The activity analysis of GAPDH in transgenic lines; Table S1: The primer sequences of ten genes.

Author Contributions: L.L. (Liqin Li) wrote the first draft of the manuscript; J.L., J.S. and X.Z. performed the experiments; Y.L. and S.Y. analysed the data; Q.W. and L.L. (Liming Lu) edited the manuscript; X.W. amended the manuscript. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data presented in this study are available within the article.

Conflicts of Interest: The authors declare no conflict of interest.

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