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# Expression of Genes Associated with Nitrogen Uptake and Metabolism in Potato (Solanum tuberosum L.) under Nitrogen Fertilization and Bacterial Inoculation

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**ABSTRACT:** Potato (Solanum tuberosum L.) is one of the most important crops worldwide due to its nutritional value and adaptability to diverse agricultural and environmental conditions. Nitrogen use efficiency (NUE) is an essential determinant of potato productivity and nitrogen management. This study assessed the expression of genes involved in nitrogen uptake and assimilation in two potato cultivars, Arizona and Riviera, under different nitrogen fertilization protocols and bacterial inoculation treatments. A field experiment was performed during spring 2025 using three nitrogen concentrations (62.5, 125.0, and 187.5 kg N ha-1) combined with four bacterial inoculants: no inoculation, Azotobacter chroococcum, Azospirillum brasilense, and their combination. Expression of nitrogen-related genes was assessed using conventional PCR and RT-qPCR, with EF1α used as the reference gene, and relative expression was estimated using the 2-ΔΔCT method. Conventional PCR confirmed amplification of the expected products for NRT, NR, and EF1α in both cultivars. In contrast, amplification of AMT and GDH was not detected in Arizona, and these genes were excluded from subsequent RT-qPCR analysis. NRT and NR expression showed significant responses at the moderate nitrogen rate of 125 kg N ha-1 combined with bacterial inoculants. NRT expression reached 9.94-fold in Rivera and 8.05-fold in Arizona, while NR expression reached 6.74-fold in Rivera and 5.08-fold in Arizona. Conversely, the highest nitrogen rate generally resulted in reduced expression pr nearly control expression of both genes. These results demonstrated that moderate nitrogen fertilization with bacterial inoculation can enhance the expression of the essential enzyme involved in nitrogen uptake and assimilation. On the other hand, excessive nitrogen use may suppress this response. It has also been shown that combining biofertilizers with optimized nitrogen fertilization is an effective protocol to enhance NUE in potato.

## INTRODUCTION

Potato (*Solanum tuberosum* L.) is an important food crop that is the main contributor to global food security. It is cultivated in more than 150 countries and provides a main source of carbohydrate, dietary fiber, vitamins, minerals, and bioactive compounds for hundreds of millions of people around the globe (Devaux *et al.*, 2021). In addition, because of its nutritional importance, potato is a valuable industrial crop used in starch production, processed foods, and animal feed, making this crop important for sustainable agriculture (Hu *et al.*, 2025).

Tuber yield and quality depend mostly on effective nitrogen (N) management because N is a crucial component of nucleic acids, proteins, chlorophyll, and many enzymes that contribute to plant growth and metabolism (Zayed *et al.*, 2023). Potato has a high need for N but low nitrogen recovery, leading to significant fertilizer losses through leaching, denitrification, and

volatilization. Extreme nitrogen fertilization increases production costs and contributes to environmental pollution, which may affect tuber quality and soil health (Chatzitriantafyllou *et al*., 2026).

Improving nitrogen use efficiency (NUE) has become a main goal of sustainable potato production. NUE is determined by the ability of plants to obtain, assimilate, and use the N effectively (Majrashi, 2026). Complex interactions among genetic, biochemical, and physiological factors regulate it. Agronomic practices can enhance N management; however, understanding the molecular mechanisms behind nitrogen uptake and assimilation is vital to improve potato cultivars with enhanced NUE and optimize fertilizer application. Therefore, determining genes associated with N uptake and assimilation and assessing their expression under nitrogen stress has become an important method to improve N utilization and support potato production (Akhtar *et al*., 2024; Ali *et al*., 2025).

Plants, in general, mainly absorb nitrogen as nitrate ($NO_3^-$) and ammonium ($NH_4^+$) by nitrate transporter (NRT) and ammonium transporter (AMT) proteins. After uptake, nitrate is reduced to ammonium by nitrate reductase (NR) and nitrite reductase (NiR), which is incorporated into amino acids chiefly by the glutamine synthase/glutamate synthase (GS / GOGAT) cycle. Glutamate dehydrogenase (GDH) participates in nitrogen recycling and metabolic optimization under stress conditions (Goel and Singh, 2015; Muratore *et al*., 2021). Synchronizing the activities of these transporters and enzymes plays an essential role in N assimilation, plant growth, and productivity.

Recent advances in molecular approaches have established that the expression of the genes involved in N metabolism is affected by nitrogen availability, plant developmental stage, and genotype (Xie *et al*., 2024). Differential expression of the proteins and enzymes has been associated with nitrogen uptake, biomass accumulation, and tuber yield. These findings demonstrate the importance of these genes in N assimilation and emphasize their potential as molecular markers for assessing plant responses to different N management protocols (Qiao *et al*., 2026).

Nitrogen fixation bacteria have been used as a sustainable alternative to chemical nitrogen fertilizers due to their ability to improve nitrogen availability and enhance plant growth through biological nitrogen fixation (Aasfar *et al*., 2024). These include several bacterial genera, such as *Azotobacter*, *Derxia*, *Azomonas*, *Bacillus*, *Klebsiella*, *Clostridium*, *Enterobacter*, and *Azospirillum* (Sumbul *et al*., 2020; Ajeet Kumar, 2025).

Recent reports suggested that these bacteria can also affect the expression of genes involved in N metabolism, leading to improved nutrient uptake and plant production (Rangasamy and Saleh, 2026). However, the molecular response of potato to the mixed application of N fertilizers with N-fixing bacteria remains poorly understood.

The present study aimed to identify selected genes involved in nitrogen transport and assimilation in potato and to investigate their expression patterns under different nitrogen fertilization regimes supplemented with nitrogen-fixing bacteria. The findings are expected to improve our understanding of the molecular mechanisms underlying nitrogen use efficiency and contribute to the development of sustainable nutrient management strategies for potato production.

## MATERIALS AND METHODS

### Field Experiment

The field experiment was conducted during the spring growing season of 2025 at the research station of the College of Agricultural Engineering Sciences, University of Baghdad, Iraq, to evaluate two potato hybrids **(***Riviera* and *Arizona*) under different nitrogen fertilization programs.

The experiment included the following factors: Factor one (Potato Hybrids), $V_1$: *Arizona* (early-maturing hybrid) and $V_2$: *Riviera* (medium-early maturing hybrid). Factor two (Nitrogen Fertilization Program): Nitrogen fertilizer was applied in three split applications by placement in furrows approximately 10 cm deep alongside the plants according to the following nitrogen rates: $N_1$: 62.5 kg N $ha^{-1}$, $N_2$: 125.0 kg N $ha^{-1}$, $N_3$: 187.5 kg N $ha^{-1}$, as can be seen in Table 1.

**Table 1**. Fertilizer application schedule during the growing season

| Fertilizer application stage | 187.5 kg N $ha^{-1}$ | 125 kg N $ha^{-1}$ | 62.5 kg N $ha^{-1}$ | 200 kg $P_2O_5$ $ha^{-1}$ | 300 kg $K_2O$ $ha^{-1}$ |
|---|---|---|---|---|---|
| First application (after emergence) | 40% | 40% | 40% | 60% | 20% |

| | | | | | |
|---|---|---|---|---|---|
| Second application (one month after the first application) | 40% | 40% | 40% | 30% | 30% |
| Third application (at tuber initiation stage) | 20% | 20% | 20% | 10% | 50% |
| Total fertilizer applied | 100% | 100% | 100% | 100% | 100% |

Factor three (Biofertilizer Application): Biofertilizers were applied beneath the seed tubers at planting. The inoculants were carrier-based using peat moss and applied at a rate of 10 g tuber$^{-1}$ for each single inoculant treatment as well as for the combined inoculant treatment, according to the manufacturer's recommendation. The biofertilizer treatments were as follows: $B_0$: No biofertilizer (control), $B_1$: *Azotobacter chroococcum* inoculant, $B_2$: *Azospirillum brasilense* inoculant, and $B_3$: Combined inoculation with *Azotobacter chroococcum* + *Azospirillum brasilense*.

**Leaf Sampling**

Leaf samples were collected during the second nitrogen fertilizer application for molecular analysis. The fourth fully expanded leaf from the shoot apex was sampled from five plants per experimental unit. The collected leaves were used for the analysis and expression profiling of nitrogen-related genes in the two potato cultivars, Arizona and Riviera.

**Molecular analysis**

**Primer design and preparation**

The primers specific to AMT, GDH, NR, and NRT genes, as well as Elongation factor 1-alpha (EF1α) as a reference gene, were designed in this study (Table 2) using the free online websites Primer-BLAST (Ye *et al.*, 2012) and Primer3 Plus (Untergasser *et al.*, 2012). These primers were used to estimate gene expression under different combinations of biological and chemical nitrogen fertilizers. The primer sets were purchased from Macrogen, Korea. Each primer tube was centrifuged at 1000×g for 1 min. Then, Tris-EDTA buffer was used to dissolve the primers to get a final concentration of 100 µM. The stock solution was diluted 10 times by adding 90 µl of Nuclease-free water to 10 µl of the stock solution for each primer to get a final concentration of 10 µM.

**Table 2:** Primer sequences used for gene expression of AMT, GDH, NR, and NRT genes

| No. | Gene Name | Primer | Sequence (5'-----3') | Size (bp) |
|---|---|---|---|---|
| 1 | *AMT* | AMT-F | AGGGTTGGCTGTTCAGGATG | 109 |
| | | AMT-R | GCCCTTGGTCCAACCCAATA | |
| 2 | *GDH* | GDH-F | TGTCAAAAACGGCTGGATCG | 133 |
| | | GDH-R | ACAATTGTGCGCTCAGGTTC | |
| 3 | *NR* | NR-F | AACGGTGCGGTGTTCATAGT | 120 |
| | | NR-R | CGAGATGGATCCATGGCGAA | |
| 4 | *NRT* | NRT-F | GGCTAGTGAACGGAACAGCT | 116 |
| | | NRT-R | AGCAGTGAACGGAGTAGCAC | |
| 5 | *EF1a* | EF1α -F | CAAGTCTGTTGAGATGCACCAC | 113 |
| | | EF1α -R | AGGCAACAAAACCACGCTTC | |

**Gene expression analysis**

**RNA isolation and quantification**

RNA was isolated from leaves of potato plants using TransZol Up Plus RNA Kit (Transgen Biotech., China) according to the manufacturer's recommendations. The RNA quantity and purity were estimated using a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA).

**cDNA formation**

Purified RNA from potato leaves was converted into cDNA using the EasyScript Reverse Transcription System (Transgen Biotech., China) according to the manufacturer's recommendations. Briefly, RNA (5 µl) was mixed with Oligo dT18 primer (0.5 µg/reaction), incubated for 5 min at 65°C, then quickly placed on ice and left for at least 2 min. Then, the other reverse transcription mixture was added. The reverse transcription reaction was prepared by combining 10 µl 2X ES Reaction Mix, 1.0 µl EasyScript RT/RI Enzyme Mix,1 µl gDNA Remover, and Nuclease-Free Water to a final volume of 14 µl. RNA and primer mix was added to the reverse transcription mix and incubated for 15 min at 42°C; reverse transcriptase was inactivated at 85°C for 5 s in a thermal cycler.

**Amplification of cDNA using conventional PCR**

cDNA from the previous step was amplified for the five genes (*AMT, GDH, NR, NRT* and *EF1a*) using the primers designed in Table 2. Conventional PCR was used to amplify the cDNA to be tested in the following RT-qPCR. The PCR reaction mixture was prepared using GOTaq G2 Green Master Mix according to the manufacturer's instructions (Promega, USA) to a total volume of 20 µl per reaction. The mixture contained 10 µl of Master Mix (2X), 0.5 µl of each primer (10 µM), template DNA, and nuclease-free water and was placed in the thermal cycler (Techne, UK). Thermal cycling parameters were as follows: initial denaturation: 95°C for 2 min; 35 cycles of denaturation: 95°C for 30 s, annealing: 60°C for 30 s, extension: 72°C for 45 s, and a final extension: 72°C for 5 min. The PCR products were electrophoresed in a 1% agarose gel in 1X TBE buffer at 70 V for 70 min, stained with 0.5 µg/ml Ethidium bromide. The gel was photographed with the Gel Imaging System (BIO-RAD, USA).

**Gene expression and analysis**

Gene expression was determined using RT-qPCR with SYBR Green. The reaction was carried out using the PerfectStart Green qPCR SuperMix kit according to the manufacturer's instructions (Transgen Biotech, China). The total reaction mixture volume was 20 µl, composed of 10 µl of 2×PerfectStart Green qPCR SuperMix, 0.4 µl of each primer, 2 µl of cDNA, and completed to 20 µl with nuclease-free water. Cycling was performed in an Applied Biosystems 7300 thermocycler. They were as follows: initial denaturation at 94°C for 30 s, followed by 40 cycles of 94°C for 30 s, 60°C for 30 s, 72°C for 30 s, and a final melting step from 65-95°C. Gene expression analysis was performed according to the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001).

**Statistical analysis**

## RESULTS AND DISCUSSION

**Amplification of cDNA**

Complementary DNA from Potato RNA was used as a template for the amplification of five genes using conventional PCR. Results in Figure 1 show the PCR results of agarose gel electrophoresis of amplification of cDNA from the two potato cultivars, Arizona ($V_1$) and Riviera ($V_2$). The results revealed the presence of all five genes in both cultivars, except for the AMT and GDH genes in the $V_1$ cultivar, where no amplification bands corresponding to these genes were observed on the agarose gel. The amplified products were observed on the gel at the expected sizes for the five genes (AMT = 109 bp, GDH = 133 bp, NR = 120 bp, NRT = 116 bp, and EF1α = 113 bp). Therefore, *the AMT and GDH genes were excluded from the RT-qPCR assessment*. The absence of amplification bands of these genes in the V1 cultivar may be explained by the absence or reduced gene expression, indicating cultivar-dependent regulation of N metabolism. Previous studies have reported that potato cultivars differ substantially in their transcriptional regulation of genes that participate in N uptake and assimilation. Such variations are associated with differences in NUE (Han *et al.*, 2022). Additionally, both *AMT* and *GDH* are highly responsive to nitrogen status, tissue type, and developmental stage, which can make gene expression fall below the detection limit (Krpp, 2015).

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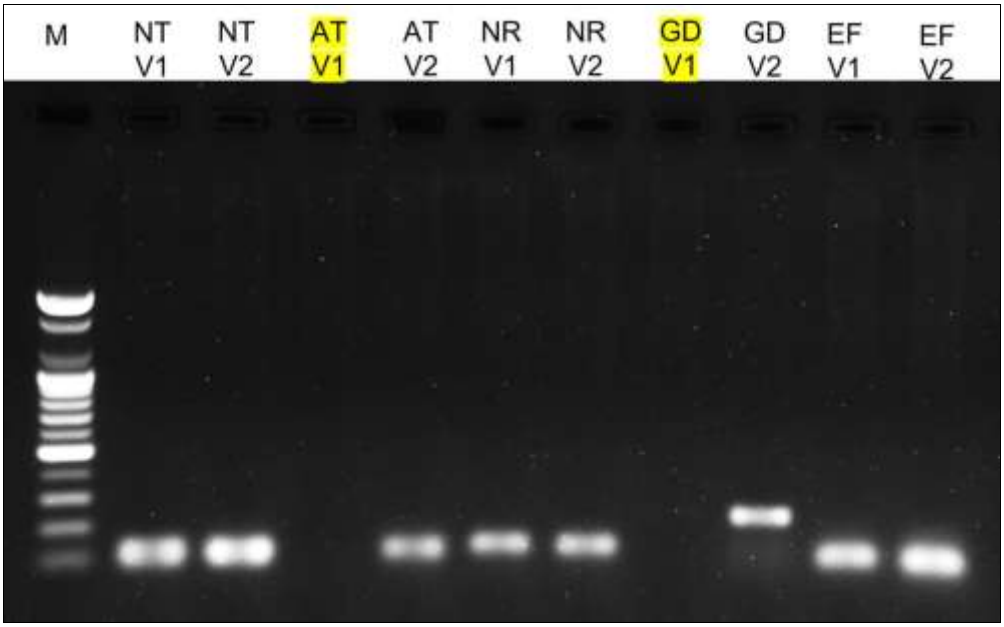



**Figure 1:** Agarose gel electrophoresis for PCR products of five nitrogen genes amplified for two potato varieties (Arizona ($V_1$) and Riviera ($V_2$). Gel electrophoresis was done in 1% agarose in 1X TBE and 70 V for 70 min—Lane M: 100 bp DNA marker.

**Gene Expression Analysis Using Quantitative Real-Time PCR (qPCR)**

The relative expression levels of the NRT gene varied significantly among bacterial inoculations and nitrogen levels in both potato cultivars, as shown in Table 3. Under the N1 and N2 levels, NRT expression increased gradually with increased bacterial inoculation (B1 < B2 < B3) in both cultivars. The highest expression was seen under B3N2 treatment, reaching 9.94-fold in V2 and 8.05–fold in V1 compared with the control (1-fold). Conversely, at the N3 level, NRT gene expression was significantly lower, remaining close to or below the control. V2 showed reduced expression under B1 (0.69- fold) and B3 (0.27 -fold), while V1 showed expression near the control (1.1-1.7-fold).

**Table 3:** NRT gene expression under different conditions of N levels and bacterial inoculations

| Expression fold change )NRT( | Treatments for V2 | Expression fold change) NRT( | Treatments for V1 |
|---|---|---|---|
| 1 | B0N1 | 1 | B0N1 |
| 2.56 | B1N1 | 1.94 | B1N1 |
| 5.5 | B2N1 | 4.36 | B2N1 |
| 6.32 | B3N1 | 5.2 | B3N1 |
| 1 | B0N2 | 1 | B0N2 |
| 5.3 | B1N2 | 4.24 | B1N2 |
| 7.2 | B2N2 | 6.54 | B2N2 |
| 9.94 | B3N2 | 8.05 | B3N2 |
| 1 | B0N3 | 1 | B0N3 |
| 0.69 | B1N3 | 1.1 | B1N3 |
| 1.59 | B2N3 | 1.1 | B2N3 |
| 0.27 | B3N3 | 1.7 | B3N3 |

Table (2) shows that the $B_3N_2$ treatment produced the highest relative expression of the Nitrate reductase (NR) gene in both potato cultivars. The relative expression level reached 5.08 in the Arizona ($V_1$) cultivar and 6.74 in the Riviera ($V_2$) cultivar. In contrast, the $B_1N_3$ and $B_2N_3$ treatments resulted in the lowest NR gene expression in both cultivars. In the Arizona cultivar, the lowest relative expression values were 0.80 and 0.86, respectively, whereas the corresponding values in the Riviera cultivar were 1.03 and 0.49, respectively.

The results in Table 2 showed the important role of nitrate transporters in regulating nitrogen management. Potato plants have a large NRT family, comprising 81 identified members showing nitrogen–dependent expression patterns (He *et al.*, 2025). The high NRT gene expression at N2 suggested that a moderate nitrogen concentration provided a suitable balance between nitrate supply and plants' nitrogen demand. Nitrate transport systems are tightly regulated according to nitrate availability at the transcriptional, post-transcriptional, and translational levels (Aluko *et al.*, 2023). Increased NRT expression with bacterial inoculation may be due to bacteria's capacity to promote root development, produce phytohormones, and improve nutrient availability, leading to enhanced nitrogen uptake and utilization (Reed and Glick, 2023). The expression of the NRT gene is higher in V2 than in V1 under N1 and N2 nitrogen concentrations. These differences suggest cultivar-dependent regulation of nitrate acquisition, which may be explained by genetic variation leading to differences in NUE among crop genotypes (Zhang *et al.*, 2021). Nitrate transporter expression was significantly reduced at N3 nitrogen concentration. This response is consistent with nitrate feedback regulation leading to the suppression of the nitrate management system. Nitrate signaling is also regulated by NRT1.1, which functions not only as a transporter but also as a nitrate sensor initiating signaling responses (Fang *et al.*, 2021).

**Table 4:** NR gene expression under different conditions of N levels and bacterial inoculations

| Expression fold change (NR) | Treatments for V2 | Expression fold change (NR) | Treatments for V1 |
|---|---|---|---|
| 1 | B0N1 | 1 | B0N1 |
| 1.41 | B1N1 | 1.6 | B1N1 |
| 1.26 | B2N1 | 1.2 | B2N1 |
| 2.25 | B3N1 | 2.33 | B3N1 |
| 1 | B0N2 | 1 | B0N2 |
| 3.73 | B1N2 | 2.9 | B1N2 |
| 2.87 | B2N2 | 1.74 | B2N2 |
| 6.74 | B3N2 | 5.08 | B3N2 |
| 1 | B0N3 | 1 | B0N3 |
| 1.03 | B1N3 | 0.8 | B1N3 |
| 0.49 | B2N3 | 0.86 | B2N3 |
| 1.2 | B3N3 | 1.04 | B3N3 |

Nitrate reductase gene expression showed significant variation depending on nitrogen level and the type of bacterial inoculation in both potato cultivars (Table 4). Under N1 nitrogen concentration, NR gene expression demonstrated a mild increase with bacterial treatment, reaching 2,25- fold in V2 and 2.33-fold in V1 with B3 inoculation. Under N2 nitrogen concentration, stronger gene expression was observed, with the highest expression shown under B3, reaching 6.74-fold in V2 and 5.08-fold in V1. On the other hand, under N3 nitrogen concentration, NR gene expression was almost the same as the control, ranging from 0.49-1.2-fold in V2 and 0.8-1.04-fold in V1.

The NR gene expression was almost consistent with NRT results but with some different fold responses. The synchronized increase in NR and NRT gene expression is important for the potato plant. NRT-mediated nitrate uptake and NR catalyze the reduction of nitrate to nitrite, enhancing both nitrogen acquisition and assimilation (Zayed *et al.*, 2023).

The results of both NRT and NR gene expression suggest that excessive nitrogen fertilization leads to downregulation of both NR and NRT gene expression. A recent review highlights the importance of optimizing nitrogen fertilization in potato to improve nitrogen use efficiency while reducing nitrate leaching and other nitrogen losses (Chatzitriantafyllou *et al.*, 2026).

## CONCLUSIONS

Fertilization using nitrogen combined with bacterial inoculation significantly affected the gene expression of nitrogen uptake and assimilation in potato, with different responses of the two tested cultivars. The NRT and NR genes were consistently expressed. They showed the highest increase in expression under moderate nitrogen with *Azotobacter chroococcum* and *Azospirillum brasilense*; conversely, higher nitrogen levels reduced their expression. Gene's expression was cultivar-dependent, and *AMT* and *GDH* were not detected in one Rivera cultivar, highlighting possible genetic differences. A combination of moderate nitrogen fertilization with bacterial inoculation was more effective in enhancing gene expression than nitrogen fertilization alone. This study supports the use of biofertilizers with nitrogen management; however, more work is needed to explore gene expression in relation to plant growth, yield, and NUE.

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