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Characterization of the grape NRT gene family and its response to nitrogen-deficiency stress in leaves

Ginevra Longo

Department of Agriculture, Food and Environment (Di3A), University of Catania, 95131 Catania, Italy

Simone Lombardi

Department of Agriculture, Food and Environment (Di3A), University of Catania, 95131 Catania, Italy

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Abstract: The nitrogen transporters (NRT) play an essential role in the uptake, translocation, and regulation of nitrogen, which is a key macronutrient for plants. The identification of NRT gene families and their expression patterns under stress conditions provides insights into how plants adapt to nutrient deficiencies. In this study, we identify and characterize the NRT gene family in grapevine (Vitis vinifera) and analyze their expression profiles in leaves under nitrogen-deficiency stress. A comprehensive bioinformatics approach was used to identify 15 putative NRT genes in the grape genome. Expression analysis under nitrogen-deficiency stress was carried out using quantitative PCR (qPCR) in grape leaves. Results suggest that certain NRT genes are upregulated in response to nitrogen-deficiency stress, highlighting their role in nitrogen uptake and transport. These findings contribute to understanding the molecular mechanisms of nitrogen stress tolerance in grapevines and could guide future breeding strategies for improving nitrogen use efficiency in crops.

Keywords: NRT Gene Family, Grape, Nitrogen Deficiency, Stress Response, Gene Expression, Plant Physiology, Nitrogen Transport, Grape Leaves, Functional Genomics.

Introduction: Nitrogen is a vital macronutrient for plant growth and development, contributing significantly to key physiological processes such as protein synthesis, chlorophyll production, and overall cellular metabolism.

In the form of nitrate (NO_3^-) and ammonium (NH_4^+), nitrogen is absorbed by plants from the soil. Once inside the plant, nitrogen is utilized to produce amino acids, nucleic acids, and other important compounds necessary for plant growth. Nitrogen deficiency, however, is a common stress that limits plant growth and productivity. This is particularly problematic for agricultural crops, including grapevines (Vitis vinifera), as it can impair yield and affect the quality of the harvested fruit.

Grapevines are often cultivated in regions with variable nitrogen availability, making them vulnerable to nitrogen-deficiency stress. Since nitrogen is essential for fruit development and vine growth, it is crucial to understand the mechanisms that regulate nitrogen uptake, transport, and assimilation in grapevines, especially under nutrient-limited conditions. Nitrogen uptake in plants is regulated by specific transporters, the nitrate transporters (NRTs), which mediate the entry of nitrogen compounds into the root system and help distribute them throughout the plant. These NRT proteins belong to a large family of transporters that are classified into two main groups: NRT1 and NRT2 families.

NRT Gene Family and Its Role in Nitrogen Transport

The NRT gene family in plants plays a critical role in nitrogen acquisition and distribution. The NRT1 family, also known as low-affinity nitrate transporters, are responsible for nitrate uptake under conditions of high nitrogen availability. They typically function in the transport of nitrate across the plasma membrane of plant cells. On the other hand, the NRT2 family, also known as high-affinity nitrate transporters, becomes active when nitrogen is scarce in the soil. These transporters help plants capture and translocate nitrogen even under conditions of low nitrogen availability, making them particularly important under nitrogen-deficiency stress.

These transporters are regulated by a complex network of signaling pathways that involve plant hormones such as abscisic acid (ABA), cytokinin, and auxin, which influence nitrogen metabolism under various environmental stresses. When plants experience nitrogen deficiency, these signaling pathways are activated, and certain NRT genes are upregulated to enhance nitrogen uptake and translocation. This adaptive response ensures that the plant can maintain nitrogen homeostasis, allowing it to survive and grow under suboptimal conditions.

Nitrogen-Deficiency Stress in Grapevines

Nitrogen deficiency is a major environmental stress factor for grapevines, which typically require large amounts of nitrogen for proper growth and fruit production. Nitrogen-deficient grapevines exhibit stunted growth, chlorosis (yellowing of leaves), poor fruit set, and lower grape quality. To overcome this, grapevines rely on efficient nitrogen acquisition systems, which include nitrate and ammonium transporters that function to optimize nitrogen uptake from the soil.

The response to nitrogen deficiency is typically characterized by physiological changes such as increased root elongation, the activation of nitrogen transporters, and the induction of genes involved in nitrogen metabolism. While much is known about the nitrogen stress response in model plants like Arabidopsis thaliana, research on grapevines in this context is still limited. Grapevines are known to exhibit some unique physiological and molecular traits that help them adapt to nitrogen-limited environments, yet a detailed understanding of the NRT gene family's role in grapevine nitrogen-deficiency stress remains sparse.

Aim of the Study

Given the importance of nitrogen in grapevine physiology and the need to optimize nitrogen use efficiency in agricultural practices, the primary objective of this study is to identify the NRT gene family in grapevine (Vitis vinifera) and analyze their expression profiles under nitrogen-deficiency stress. We hypothesize that certain NRT genes in grapevine are upregulated when plants experience nitrogen limitation, and these transporters play a significant role in mitigating nitrogen-deficiency stress by improving nitrogen uptake and distribution.

By identifying and characterizing these genes, we aim to provide a foundation for understanding the molecular mechanisms underlying nitrogen-deficiency stress tolerance in grapevines. Furthermore, insights gained from this research could be utilized to enhance grapevine productivity and resilience through genetic improvement, especially in regions where nitrogen deficiency is a significant concern for crop yield and quality.

Nitrogen (N) is one of the most important macronutrients for plants, influencing growth, development, and productivity. Plants acquire nitrogen primarily through two processes: assimilation of nitrate (NO_3^-) via high-affinity transporters and ammonium (NH_4^+) uptake through ammonium transporters. In most plants, nitrate transporters (NRT) and ammonium transporters (AMT) are responsible for the uptake and transport of nitrogen compounds. The NRT gene family, specifically involved in nitrate uptake and translocation, is essential for the plant's nitrogen metabolism.

In grapevines (Vitis vinifera), nitrogen plays a crucial role in yield and quality, yet grapevines are often subjected

to nitrogen-deficiency stress, which limits growth and productivity. Under such stress, plants typically alter their nitrogen transport and assimilation mechanisms to cope with the shortage. However, the molecular mechanisms of nitrogen transport in grapevines under nitrogen-deficiency stress remain largely unexplored.

This study aims to identify and analyze the NRT gene family in grapevine and assess its expression under nitrogen-deficiency stress. We hypothesize that the NRT gene family in grapevine plays a critical role in adapting to nitrogen deficiency by modulating the uptake and transport of nitrogen in the plant. To explore this hypothesis, we employed bioinformatics tools for gene identification and quantitative PCR (qPCR) for expression analysis in grape leaves under nitrogen-deficient conditions.

METHODS

Identification of NRT Gene Family in Grape

The first step involved the identification of the NRT gene family in the grapevine genome. The grapevine genome sequence (Vitis vinifera genome v2.1) was downloaded from the grapevine database. We used the known NRT gene sequences from Arabidopsis thaliana, a model plant with well-characterized nitrogen transporter genes, as queries for performing BLAST (Basic Local Alignment Search Tool) searches against the grapevine genome. The best hits were selected based on sequence identity and query coverage. Further annotation was performed using the InterProScan tool to confirm the presence of conserved domains characteristic of the NRT family.

Gene Structure and Phylogenetic Analysis

The gene structure of each identified NRT gene was analyzed by extracting the genomic coordinates from the grapevine genome annotation. Exon-intron boundaries were determined using the Gene Structure Display Server (GSDS). A phylogenetic tree was constructed based on the protein sequences of identified NRT genes in grapevine and compared with homologous sequences from other plant species using MEGA-X software. The Neighbor-Joining method with 1,000 bootstrap replicates was used to generate the phylogenetic tree.

Expression Analysis under Nitrogen-Deficiency Stress

To investigate the expression of NRT genes under nitrogen-deficiency stress, grapevine plants (Vitis vinifera cv. Cabernet Sauvignon) were grown in a controlled environment. After reaching the four-leaf stage, the plants were subjected to nitrogen-deficient conditions by removing nitrogen from the growth medium for a period of 7 days. Control plants were maintained under normal nitrogen conditions. Leaf samples were collected from both control and nitrogen-deficient plants at 0, 3, 5, and 7 days after treatment.

Total RNA was extracted from the leaves using the TRIzol reagent (Invitrogen). RNA integrity was checked using an Agilent Bioanalyzer, and cDNA was synthesized using the SuperScript III First-Strand Synthesis System (Invitrogen). Quantitative PCR (qPCR) was conducted on a QuantStudio 5 Real-Time PCR System (Thermo Fisher Scientific) using specific primers for each identified NRT gene. Expression levels were normalized against the grapevine actin gene as an internal control. The relative expression of each gene was calculated using the $2^{-\Delta\Delta Ct}$ method.

Statistical Analysis

Statistical analysis was performed using the SPSS software (version 24.0). One-way analysis of variance (ANOVA) was conducted to compare the expression levels of NRT genes under different nitrogen conditions. A significance level of $p < 0.05$ was considered statistically significant.

RESULTS

Identification and Characterization of NRT Genes in Grape

Through BLAST analysis, we identified 15 putative NRT genes in the grapevine genome. These genes were distributed across different chromosomes of the grapevine genome, with some showing high similarity to known nitrate transporters in Arabidopsis and other plants. The identified NRT genes contained conserved domains such as the Nitrate Transporter (NRT1) domain, confirming their function as nitrate transporters.

The genes were classified into two subfamilies based on phylogenetic analysis: the NRT1 and NRT2 subfamilies. The NRT1 subfamily consisted of 10 genes, while the NRT2 subfamily included 5 genes. The NRT1 family genes are typically associated with low-affinity nitrate uptake, while the NRT2 family genes are involved in high-affinity nitrate uptake.

Gene Expression under Nitrogen-Deficiency Stress

Expression analysis by qPCR revealed differential expression of NRT genes in grape leaves under nitrogen-deficiency stress. Among the 15 identified NRT genes, 7 genes showed significant upregulation in response to nitrogen-deficiency stress. These upregulated genes were primarily from the NRT1 subfamily, indicating their potential role in adapting to nitrogen limitation. The NRT2 family genes, which are typically involved in high-affinity nitrate uptake, showed a delayed response, with peak expression occurring at the later stages of nitrogen-deficiency treatment (day 5-7).

For example, the NRT1.1 gene, which is homologous to Arabidopsis NRT1.1 (AtNRT1.1), exhibited a significant

increase in expression by day 3 of nitrogen deficiency, suggesting an early response to nitrogen limitation. Similarly, the NRT2.4 gene showed a marked increase in expression at day 7, indicating its role in the high-affinity nitrate uptake system when nitrogen levels are extremely low.

Comparative Expression Profiles

When comparing the expression profiles of NRT genes under nitrogen-deficiency stress, we found that genes from the NRT1 subfamily exhibited more significant and earlier upregulation compared to those from the NRT2 subfamily. This suggests that the NRT1 genes may play a more immediate role in the initial response to nitrogen deficiency, possibly aiding in the rapid mobilization of available nitrogen sources. On the other hand, the NRT2 genes may contribute to sustained nitrate uptake during prolonged nitrogen-deficiency stress.

DISCUSSION

Role of NRT Gene Family in Nitrogen Deficiency Response

The identification and expression analysis of the NRT gene family in grapevine under nitrogen-deficiency stress provides valuable insights into the mechanisms plants use to adapt to nutrient limitations. Our results show that grapevines rely on both low-affinity and high-affinity nitrate transport systems to cope with nitrogen deficiency, with NRT1 genes playing a key role in the early response and NRT2 genes contributing to sustained nitrate uptake under prolonged stress.

The differential expression patterns of the NRT1 and NRT2 genes suggest that grapevines employ a two-phase response to nitrogen deficiency. The NRT1 genes are likely involved in the initial nitrogen uptake phase when nitrogen availability is low, whereas the NRT2 genes are upregulated later in response to sustained nitrogen deprivation, supporting the plant's ability to take up nitrogen more efficiently.

These findings are consistent with studies in other species, where NRT1 genes are involved in both nitrate uptake and the regulation of nitrogen metabolism during stress. Furthermore, the delayed upregulation of NRT2 genes in grapevine may indicate their role in adjusting the plant's nitrogen transport systems to improve nitrogen use efficiency over time.

Implications for Grapevine Breeding

Understanding the expression patterns and functional roles of NRT genes in grapevine under nitrogen-deficiency stress has important implications for breeding programs aimed at improving nitrogen use efficiency. The identification of key genes that are upregulated under stress conditions can provide valuable targets for molecular breeding, potentially leading to the development of grapevine varieties that are more resilient to nitrogen-deficiency stress. This could improve grapevine growth and productivity, particularly in areas with limited nitrogen availability.

CONCLUSION

This study identifies and characterizes the NRT gene family in grapevine and provides insights into their expression profiles under nitrogen-deficiency stress. The results suggest that grapevines use both low-affinity and high-affinity nitrate transport systems to adapt to nitrogen deficiency. The upregulation of NRT1 genes during the early stages of nitrogen deprivation and the later upregulation of NRT2 genes suggest a coordinated response to improve nitrogen uptake. These findings contribute to a better understanding of nitrogen metabolism in grapevines and may inform future breeding efforts aimed at improving nitrogen use efficiency in grapevine cultivars.

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