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MICRO RNA RESPONSE TO CHRONIC AND SHORT-TERM NITROGEN DEPRIVATION IN DURUM WHEAT SEEDLINGS

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Nitrogen (N) is an important macronutrient for crop productivity; its availability is essential from the seedling stage since N is also a major component of chlorophyll. In durum wheat, phenotypic traits and yield are negatively influenced by N deficiency. MiRNAs are small non-coding RNA molecules that can regulate key genes in response to stress, thus contributing to environmental adaptation of plants.

In the present work, durum wheat cultivars Ciccio e Svevo were grown in standard and in N starvation conditions, and leaves and roots were collected at the seedling stage (Z14). Eight small RNA libraries were obtained and sequenced. After bioinformatics analyses, 294 miRNAs were detected, including 7 newly identified. Using quantitative qPCR, expression levels of selected miRNAs and corresponding target genes were evaluated in durum wheat seedlings subjected to chronic or short-term (6-48 hours) N stress conditions.

Some miRNAs showed an immediate response to N stress, others exhibited a gradual up- or downregulation during the short-term stress, which was either maintained or not during a longer (chronic) stress period. One of the newly identified miRNAs, ttu-novel-106, showed to be immediately downregulated after nitrogen starvation conditions, while MYB-A, its putative target gene, was straightaway upregulated. These results were validated using 5' RACE assay that allowed the identification of the ttu-novel-106 putative cleavage site in the MYB-A gene sequence. Additionally, the PHOSPHATE 2 (PHO2) gene showed an opposite expression trend compared to its regulatory miRNA ttu-miR399b. Identification of putative ttu-miR399 binding sites at the 5' UTR region of TtPHO2 and cleavage validation were carried out for this gene.

This work provides novel insights into the activation mode of durum wheat miRNAs in response to N deprivation, confirming the importance of miRNAs in the adaptation of seedlings to N stress, and opens new perspectives for the use of miRNAs in breeding programs.

ABSTRACT