

Identification and characterization of changes in soybean miRNA biosynthesis in response to low temperature stress

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Abstract

Soybean [*Glycine max* (L.) Merr.] is a significant oil crop and a highly valued food legume around the world. Soybeans are a rich source of oils and are one of the best plant-based sources of protein, as they contain all the essential amino acids necessary for human and animal nutrition. In Europe, soybean cultivation is limited because of the unfavorable climate. The demand for soybean in Europe, partly caused by the continual rise in population, is much greater than the actual amount produced, therefore Europe relies heavily on the imports of soybean mainly from Americas. Continuous improvement in the quality and yield of crop varieties is necessary for the advancement of agriculture, especially in the face of changing or difficult conditions. Recently, many miRNAs have been demonstrated to have important regulatory functions in plant growth, development, and stress responses. The emergence of high-throughput sequencing technology has enabled the discovery and analysis of many miRNAs in plants. Additionally, a great number of miRNA–mRNA target pairs have been successfully identified, enabling researchers to gain insight into the regulatory connections between miRNAs and their target genes. The aim of this study was to explain the molecular basis of the cold stress resistance in soybean, with the emphasis on miRNA. At the initial steps of work, the phenotyping of two soybean cultivars under chilling stress was conducted to investigate the impact on soybean productivity. The results of field trials and measurements taken during the growth of soybean in cold conditions showed that Augusta and Fiskeby V cultivars had different reactions to low temperatures. Despite the low temperatures having impact on the reproductive growth of both varieties, the number of seeds produced stayed the same. The differential expression of five candidate miRNAs under cold stress was detected using ddPCR. miRNAs that showed contrasting expression patterns in Augusta and Fiskeby V chilled in the reproductive stage have been identified. Based on the Gene Ontology analysis, a group of potential target genes was identified. A negative correlation between the expression of miR169, miR319 and miR398 and their targets was found in the roots of Augusta and Fiskeby V. In the next step the role of miRNAs and their target genes in the cold tolerance was analyzed in four different soybean varieties (Augusta, Fiskeby V, Toyomusume and *Glycine soja* [*G. soja*]) characterized by varying levels of stress resistance. High-throughput sequencing analysis of these four cultivars revealed the differential expression of 162 known miRNAs, as well as 18 novel miRNAs, under stress conditions. Analysis of the degradome enabled the assignment of differentially expressed miRNAs to their potential target genes. Based on the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) annotations, they were discovered to be

connected to plant abiotic stress response processes such as scavenging reactive oxygen species, synthesizing flavonoids, and controlling osmotic potential. The findings from this doctoral dissertation were published in two peer-reviewed experimental articles. Issues regarding high-throughput techniques used in the soybean stress response analysis were described and summarized in the review publication, which is also a part of this doctoral dissertation.