

Identification of novel soybean microRNAs involved in abiotic and biotic stresses

Kulcheski, FR¹; de Oliveira, LFV^{1,2}; Molina, LG^{1,2}; Almerão, M¹; Rodrigues, FA³; Marcolino, J³; Barbosa, JF³; Stolf-Moreira, R³; Nepomuceno, AL³; Marcelino-Guimarães, FC³; Abdelnoor, RV³; Nascimento, LC⁴; Carazzolle, MF^{4,5}; Pereira, GAG⁴; Margis, R^{1,2,5}

¹Centre of Biotechnology, PPGBCM, Laboratory of Genomes and Plant Population, UFRGS, Porto Alegre, RS, 91501-970;

²PPGGBM, UFRGS, Porto Alegre, RS, 91501-970;

³EMBRAPA Soja, Londrina, PR, CEP 86001-970;

⁴Institute of Biology, Laboratory of Genomic and Expression, UNICAMP, Campinas, SP, 13083-970;

⁵CENAPAD, UNICAMP, Campinas, SP, 13083-970

*E-mail: frank@hotmail.com

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Small RNAs (19-24 nt) are key regulators of gene expression that guide both transcriptional and post-transcriptional silencing mechanisms in eukaryotes. Current studies have demonstrated that microRNAs (miRNAs) act in several plant pathways associated with tissue proliferation, differentiation, and development and in response to abiotic and biotic stresses. In order to identify new miRNAs in soybean and to verify those that are possibly drought and rust-stress regulated, eight libraries of small RNAs were constructed and submitted to Solexa sequencing. The libraries were developed from drought-sensitive and tolerant seedlings and rust-susceptible and resistant soybeans with or without stressors. Sequencing the library and subsequent analyses detected 269 miRNAs. From this total, we identified 37 families of novel miRNAs that had not been reported before, six families of conserved miRNAs that exist in other plants species, and 22 families previously reported in soybean. We also observed the presence of several isomiRNAs during our analyses, which were described for the first time in soybean. We examined miRNA expression profiles during biotic and abiotic stresses to soybean. The majority of miRNAs were up-regulated during drought stress in the sensitive plants. However, for the tolerant genotype, most of the miRNAs remained unchanged. The pattern of miRNAs expression was also different for the distinct genotypes submitted to the pathogen stress. Most miRNAs did not vary during the fungus infection in the susceptible genotype; however, in the resistant genotype, most miRNAs were up-regulated during rust attack. To validate a novel miRNA, we performed RT-qPCR across the eight different libraries. This showed it to be differently expressed and confirmed the profile obtained by sequencing. A prediction of the putative targets was carried out for a conserved and novel miRNA family. Validation of our results with quantitative RT-qPCR revealed that Solexa sequencing is a powerful tool for miRNA discovery. The identification of differentially expressed plant miRNAs provides molecular evidence for the possible involvement of miRNAs in the process of drought- and rust-stress responses.

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