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Identification of cold stress and sulfate starvation induced microRNAs in maize roots

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The maize root system plays an essential role in mediating plant interaction with environmental stimuli. It has been shown that epigenetic mechanisms and small RNAs (sRNAs) are involved in mediating transcriptome changes induced by various environmental stresses. In particular, microRNAs (miRNAs) act in negatively regulating the mRNA level of target genes (Sunkar et al, Trends in Plant Science 12: 301-308, 2007; Zhang et al. PLoS Genetics 5: e716, 2009). To analyze the contribution of miRNAs regulation of gene transcription in maize roots and in response to abiotic stresses we used the microarray platforms previously described (Altana et al. a, MGC Newsletter, this issue). In particular, we analyzed changes in the miRNA root transcriptome induced by sulfate starvation and cold stress.

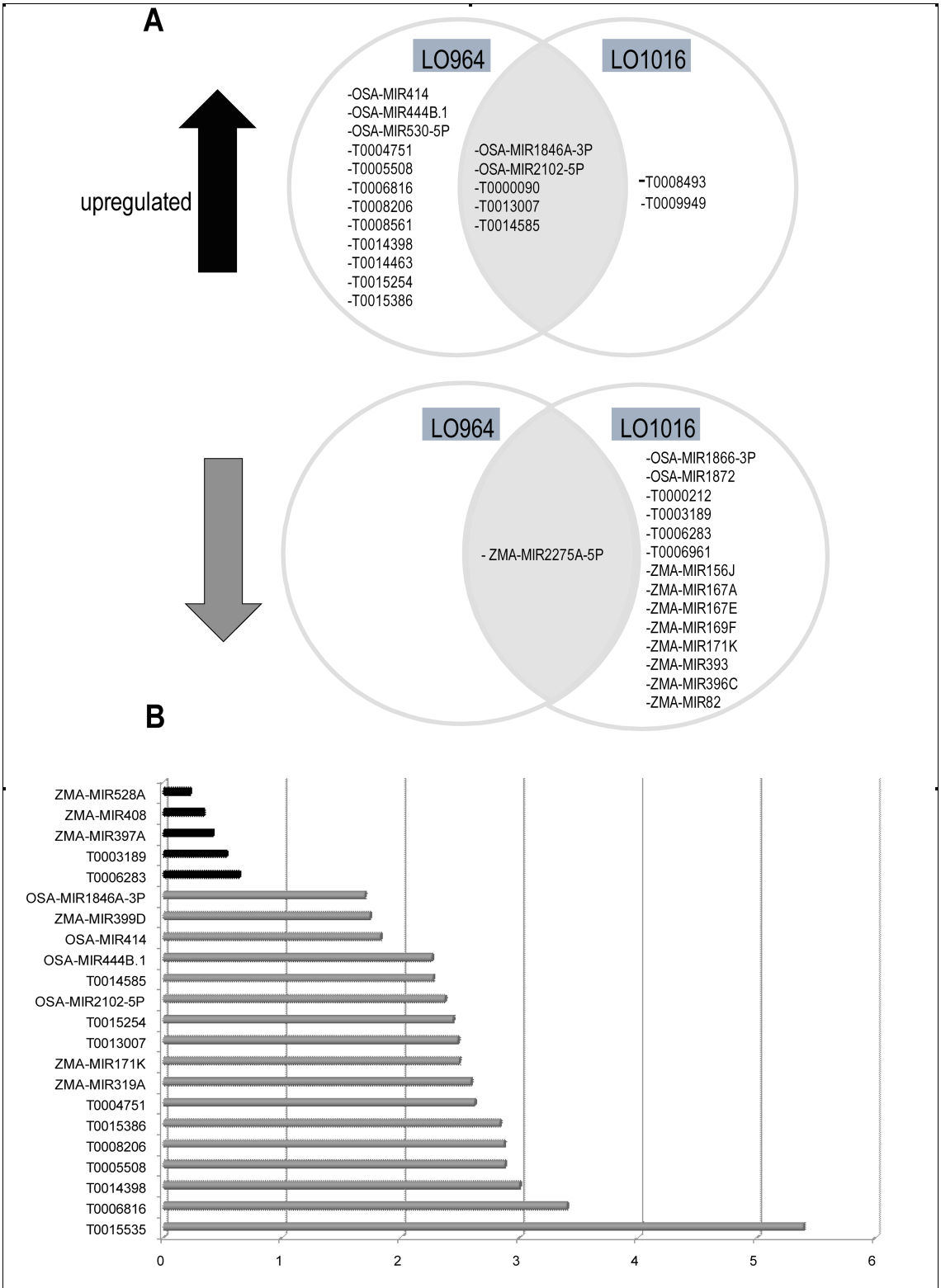
For the investigation regarding the sulfate starvation we employed two maize inbred lines: Lo964 and Lo1016, which are known to differ for root traits (Sanguineti et al., Maydica 43: 211-216, 1998) with the aim to analyze the genotype influence on the sulfate starvation mediated modification of miRNAs population. The protocol for sulfate starvation was established in hydroponically grown plants, by concomitantly monitoring sulfate uptake rate (in roots and in leaf) and root architectural parameters. The selected protocol (13 d-old plants grown 48 h without sulfate: hereinafter named: "-S" and with 500 µM sulfate: hereinafter named "+S) allowed to achieve sulfate starvation without significantly altering the growth rate of +S vs –S plants. The RNA was extracted by the primary root because the primary root diameter was one of the traits that significantly differ between Lo964 and Lo1016 lines. Three biological replicates were used for extraction of sRNA enriched fraction from each sample and Cy5 labeled RNA were employed for Combimatrix CustomArray 4X2K hybridization. Results showed that 18 and 22 miRNAs were differentially expressed in Lo964-S vs Lo964+S and in Lo1016-S vs Lo1016+S, respectively (a 1.5 fold change, between match probes in different sample types and between match/mismatch probes within the same sample type, was considered). Six and 18 miRNAs exhibited different abundance when the comparison was between Lo964+S vs Lo1016+S and Lo964-S vs Lo1016-S. Six miRNAs were commonly affected (5 up- and 1 down-regulated) by sulfate starvation in both lines (Figure 1A). These observations indicate that the major number of miRNAs changes in response to sulfate starvation than to genotype. However, because 17 of the

18 sulfate induced miRNAs are up-regulated in Lo964, whereas most of the miRNAs were down-regulated in Lo1016 (Figure 1A), a possible genotype-dependent miRNA transcriptome response to sulfate starvation may occurs. The 6 miRNAs differently expressed in both lines may be instead part of a genotype-independent conserved sRNA-related mechanism, activated by sulfate starvation to modulate gene expression.

To investigate miRNA response to cold stress, hydroponically grown plants were subjected to a cycle of six cold-pulses (13-d old plants were submitted to 6 days of growth at 25°C for 14 h followed by 10 h where the temperature dropped to 4 °C). Apical root tips were collected after that both cold stressed (CS) and not stressed (NS) samples were grown at 25°C for one day. Three biological replicates were used for each sample and the same approach described for sulfate starvation was used for miRNA microarray hybridization and data analysis. The results are illustrated in Figure 1B (22 miRNAs differentially expressed in CS vs NS). The mRNA transcriptome was also assessed for CS vs NS samples (false discovery rate < 0.05; only differences with a fold change > +/- 2 were considered). Because we know the predicted target genes of the differentially expressed miRNAs (Altana et al. a, MGC Newsletter, this issue) and because miRNAs are expected to negatively regulated expression of their targets, we searched for mRNA probes exhibiting a negative correlation with changes in the level of cold-induced miRNA expression. Seventeen miRNAs up-regulated and 5 miRNAs down-regulated by cold stress negatively correlated with the signal of 30 and 7 mRNA probes, respectively. The analysis of Gene ontology (GO) terms for genes represented by these mRNA probes indicated that specific GO categories were enriched within predicted miRNA targets (phosphate transmembrane transport, glycerol metabolism, and microtubule-based movement), thus providing information about possible specific functions for the miRNA-mediated gene regulation in response to cold stress.

Figure Legend

Figure 1. miRNAs differently expressed in maize roots in response to sulfate starvation and cold-stress. miRNAs up- and down-regulated (+/- 1.5 fold change) in primary root of two different inbred lines grown under sulfate starvation are reported in A, while in B the miRNAs with change in expression level after cold-stress treatment of apical root tips are indicated. The nomenclature adopted for miRNAs is the one from the miRBase, with addition of shRNA sequences (e.g. T0004751, see Altana et al. a MGC Newsletter, this issue) that are named according the nomenclature of reads from high-throughput sequencing reported by Wang et al. (Plant Cell 21:1053-1069, 2009).



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