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Regular paper

Arabidopsis thaliana microRNA162 level is posttranscriptionally regulated *via* splicing and polyadenylation site selection

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Arabidopsis microRNA162 (miRNA162) level regulation was studied under abiotic stresses, such as drought and salinity. The TaqMan® microRNA assay proved that *A. thaliana* miRNA162 level was elevated under these stresses, confirming its salt and drought responsiveness. The promoter region analyses of *A. thaliana miRNA162a* and *b* genes *(MIR162a* and *MIR162b)* identified numerous salinity and drought responsive elements. However, our results indicated that Arabidopsis *MIR162a* was presumably the main locus responsible for the mature ath-miRNA162 accumulation under the stresses tested, and the *MIR162b* was generally rather weakly expressed, both in control and under the stress conditions. The *MIR162a* structure was confirmed to be complex and the pri-miRNA162a hairpin structure was shown to span an alternative exon and an intron. The *MIR162a* transcription generated a few pri-miRNA162a splicing isoforms that could be functional and non-functional. Upon drought and salinity stresses, the regulation of the pri-miRNA162a alternative splicing pattern revealed an increase of a functional pri-miR162a isoform and a preferential distal polyA site selection under the stress conditions. Apart from the potential transcriptional regulation of the miRNA genes (*MIRs)* expression, the data obtained point to an essential role of posttranscriptional regulation of Arabidopsis microRNA162 level.

Key words: miRNA, pri-miRNA, abiotic stress, gene expression

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Abbreviations: ath-miRNA, *Arabidopsis thaliana* miRNA; *MIR*, miRNA gene

Table S1. Oligonucleotide sequences used as primers and TaqMan® MiRNA and Gene Expression Assays (Life Technologies).

Oligonucleotide	**Sequence**
***MIR162a* and *MIR162b* splicing pattern**	
F_RT_162a_whole	5'AGAAAAAACCAGATCTATAAAGTTTGT3'
R_RT_162a_whole	5'ATCACAAAACAAAACAGTGGATAA3'
F_RT_162b_whole	5'TAAAACGGTGAGTCATCAGATTC3'
R_RT_162b_whole	5'TATACTATTCTACGTGGATTCCTTATCA3'
***MIR162a* splicing isoforms qPCR**	
162a.6MBPFor (Func. a)	5'GAATCTTTTTGTTTATTGGTTTTTGA3'
162a.5MBPRev (Func. a)	5'CAGAAACAACAGTCACCTCTTCA3'
162aform4FwdI (Non-func. b)	5'TTTGGAGTTTAGTTGGAAGAAGA3'
162aformRevI (Non-func. b)	5'TCCCTCACTTTTTTATTTTTAATGTGTTC3'
F_162a.8 (Non-func. c)	5'CCAGCTATTTACTACTTGTGTTGGAA3'
R_162a.5 (Non-func. c,d)	5'CAACAGTCACCTCTTCATCTGC3'
F_162a.7 (Non-func. d)	5'GATCCAGCTATTTACTACTTGTGAAGAAA3'
***MIR162a* polyA isoforms qPCR**	
162aIDF (proximal polyA)	5'TGCATGTGTGTAATCTAGGGTATATG3'
162aIDR (proximal polyA)	5'AAATAGCTGGATCTTTATTGCCTTA3'
162aIVa4 (distal polyA)	5'GGTGACTGTTGTTTCTGGTGAG3'
162aIVa8 (distal polyA)	5'GTCATCCTCGCTTCACCACT3'
***MIR162b* polyA isoforms qPCR**	
162bICnew (proximal polyA)	5'TGCATCTATCCACCTCTCTCTG3'
162bICnew (proximal polyA)	5'TCGGTTGATGAACAAACACAA3'
162bIVbAF (distal polyA)	5'TGTTGAGCCACTGAATCCAA3'
162bIVb6 (distal polyA)	5'GCGTTGAGGGTCTGTAGTGA3'
***MIR162b* 5'RLM-RACE qPCR**	
RN_5RACE_162b	5'CGCTGCCTCCAGCGACTTCACTC3'
New5RN_5RACE_162b	5'TGAGAAAATCGGTTGATGAACAAACACA3'
***MIR162b* 3'RACE qPCR**	
F_3RACE_162b	5'GCGGTTCATCGATCAATTCCTGTG3'
FN_3RACE_162b	5'AAACCTCTGCATCCAGCGCTGCTT3'
TaqMan® MiRNA Assay	**Assay ID**
ath-miR162a	TM 000342
TaqMan® Gene Expression Assay	**Assay ID**
Actin 8 (ACT8)	At02270958_gH