

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

Maria Barciszewska-Pacak, Katarzyna Knop, Artur Jarmołowski and Zofia Szwejkowska-Kulińska✉

Department of Gene Expression, Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University in Poznań, Poznań, Poland

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* *MIR162a* 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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INTRODUCTION

Plant miRNAs are important general negative regulators of gene expression. They are short, single stranded, noncoding RNAs that act at the posttranscriptional level and guide target mRNA cleavage or translation inhibition (Bartel 2004; Brodersen *et al.*, 2008; Beauclair *et al.*, 2010). As the products of action of RNA Pol II, the miRNA gene (*MIR*) primary transcripts (pri-miRNAs) are 5' capped and 3' polyadenylated (Xie *et al.* 2005). They contain a characteristic stem and loop structure in which microRNA and its cognate, the so called microRNA star (*), are embedded. The plant pri-miRNAs' processing occurs in the nucleus where an RNase III-type enzyme, DICER LIKE 1 (DCL1), is responsible for all their endonucleolytic cleavages leading to the formation of pre-miRNAs, further cleaved by the same enzyme to produce the microRNA and microRNA* duplexes (Park *et al.*, 2002). However, an

efficient biogenesis of plant miRNAs requires the involvement of plenty of proteins (Vazquez *et al.*, 2004; Kurihara *et al.*, 2006; Dong *et al.*, 2008; Laubinger *et al.*, 2008; Manavella *et al.*, 2012; Ren *et al.*, 2012; Zhan *et al.*, 2012; Kruszka *et al.*, 2013; Rogers & Chen, 2013; Wang *et al.*, 2013), from which DCL1, HYPONASTIC LEAVES 1 (HYL1) (Han *et al.*, 2004; Vazquez *et al.*, 2004), and SERRATE (SE) (Lobbes *et al.*, 2006; Yang *et al.*, 2006; Dong *et al.*, 2008) appear to be the most crucial. The complex plant miRNAs' biogenesis is mainly due to the structure of *MIR* genes. The promoter regions of many plant microRNA genes contain numerous abiotic response elements (RE) found using bioinformatic tools (Higo *et al.*, 1999; Megraw *et al.*, 2006; Zhao *et al.*, 2013). Half of the plant miRNA gene bodies of independent transcription units possess introns (Szarczynska *et al.* 2009; Rogers and Chen, 2013; Szwejkowska-Kulinska *et al.*, 2013; Kruszka *et al.*, 2013; Kruszka *et al.*, 2014; Zielezinski *et al.*, 2015) and the *Arabidopsis* pri-miRNAs were found to contain many alternative polyadenylation signals (Szarczynska *et al.*, 2009; Bielewicz *et al.*, 2012; Bielewicz *et al.*, 2013; Schwab *et al.*, 2013; Zielezinski *et al.*, 2015). Additionally, 29 *Arabidopsis* miRNAs characterized so far are embedded within the introns of other genes encoding proteins or noncoding RNAs (Brown *et al.*, 2008; Yan *et al.*, 2012; Zielezinski *et al.*, 2015). Therefore, not only transcriptional regulation of at least some microRNA gene expression must exist but also the constitutive and alternative splicing and polyadenylation processes can indeed posttranscriptionally regulate the miRNA level (Bielewicz *et al.*, 2012; Bielewicz *et al.*, 2013; Yan *et al.*, 2012; Jia *et al.*, 2013).

Among the first identified plant miRNAs from *Arabidopsis* (Reinhart *et al.*, 2002; Jones-Rhodes *et al.*, 2004), miRNA162 was found to be encoded by two genes: *MIR162a* and *MIR162b*, respectively. *MIR162b* is intronless (*At5g23065*) while the *MIR162a* gene contains multiple introns and its transcript undergoes a complex constitutive and alternative splicing (Reinhart *et al.*, 2002; Hirsch *et al.*, 2006; Brown *et al.*, 2008). The pre-miRNA162a is located in the intron 2 of the protein coding *At5g08185* gene. Hirsch *et al.* in 2006 reported the identification of four *MIR162a* splice isoforms of which only one is functional and can give rise to miR162 production (Hirsch *et al.*, 2006), while Brown and coworkers in 2008 characterised five splice iso-

✉ e-mail: zofszwey@amu.edu.pl

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

forms of which two are potentially functional and can give rise to mature miRNA (Brown *et al.*, 2008). Both ath-miRNA162a and ath-miRNA162b have identical nucleotide sequences and it is known that ath-miR162 targets the *DCL1* mRNA (Xie *et al.*, 2003). Arabidopsis miR162 can be detected in a *mir162a* mutant which suggests that the *MIR162b* gene can compensate for the loss-of-function of *MIR162a* (Hirsch *et al.*, 2006). Arabidopsis *MIR162b* transcript appeared to have at least two alternative 3' ends, while *MIR162a* was not reported to have alternative polyA sites (Hirsch *et al.*, 2006).

A relationship between splicing and processing of an intronic pri-miRNA in Arabidopsis plants has been studied in the case of miR162a and its host gene (Hirsch *et al.*, 2006; Brown *et al.*, 2008). Here, we show the post-transcriptional regulation of miR162 abundance in *Arabidopsis thaliana* in control and two abiotic stress conditions: 20% soil water content (SWC) drought and salinity. The data presented point to the role of splicing and polyadenylation already at the pri-miRNA level during the intronic miRNA162a biogenesis. They confirm the posttranscriptional regulation of ath-miRNA162 under both abiotic stresses applied. This regulation affects the miRNA stress responsiveness and therefore the ability of plants to resist the stress.

MATERIALS AND METHODS

Plant material and growth conditions. *Arabidopsis thaliana* (L.) Heynh, Col-0 wild type plants were grown and stressed as previously described (Barciszewska-Pacak *et al.*, 2015). The control and both stress experiments were done in three biological replicates. The stress application was confirmed by the amplification of stress marker mRNAs (Barciszewska-Pacak *et al.*, 2015).

RNA isolation. For semi-quantitative RT-PCR and quantitative real-time PCR analyses, including TaqMan® miRNA assays (ABI, Life Technologies, USA), total RNA was isolated from 100 mg of 15-day old seedlings and 1.13 growth stage (Boyes *et al.*, 2001) plant leaves, and assessed with regard to the RNA concentration, RNA integrity determination, and DNA contamination removal, as previously described (Szarzynska *et al.*, 2009; Barciszewska-Pacak *et al.*, 2015).

Quantitative real-time PCR profiling of miRNAs and the pri-miRNA splicing and polyA isoforms. Three µg and 10 ng of DNA-free RNA were used for real-time PCR and TaqMan® miRNA assay (ABI, Life Technologies, USA) analyses, respectively, carried out as described in (Kruszka *et al.*, 2013; Barciszewska-Pacak *et al.*, 2015). All measurements were performed in three biological replicates. The list of qPCR primers for splicing and polyA transcript isoforms, as well as the TaqMan® miRNA assay probes can be found in the supplementary data Table S1 (at www.actabp.pl). The amplification efficiency of each primer pair (200 nM each, final concentration) was calculated by making a 2-fold dilution series of the mix of templates, calculating a linear regression based on the data, and estimating the efficiency from the line slope. Only primer pairs with the highest and almost equal efficiency (max. difference of 1% was approved) were used for analyses.

pri-miRNA 5' and 3' RLM-RACE experiments. The 5' and 3' RLM-RACE experiments were performed using a GeneRacer™ Kit (Invitrogen™ Life Technologies, Carlsbad, CA, USA), according to the manufacturer's protocol. The 5' and 3' RLM-RACE PCRs were done using an Advantage® 2 PCR Enzyme Sys-

tem (Clontech, Mountain View, CA, USA), according to the manufacturer's protocol. All primers used in the experiments are listed in Table S1 (Supplementary data at www.actabp.pl). Cloning and sequencing of 5' and 3' RLM-RACE products were done as previously reported in Kruszka *et al.*, 2013.

RESULTS

Drought and salt stress upregulation of *Arabidopsis thaliana* miRNA162

We have previously shown by using a high throughput real-time PCR platform, mirEX (modified mirEX platform, Bielewicz *et al.*, 2012, http://comgen.pl/mirex2/; Zielezinski *et al.*, 2015) that *Arabidopsis thaliana* pri-miRNA162a and b were both down-regulated under 20% SWC drought stress and their expression was not changed under a salinity stress (Barciszewska-Pacak *et al.*, 2015). Small RNA next generation sequencing (NGS) results had shown a slight elevation of ath-miRNA162 level that was almost statistically significant (*p*-values = 0.055 and 0.06) under the drought and salinity stress conditions (Barciszewska-Pacak *et al.*, 2015). We thus decided to verify the NGS data by applying a sensitive TaqMan® miRNA assay (ABI, Life Technologies, USA), and show that indeed the level of *A. thaliana* miRNA162 is 2.1- and 2.9-fold increased under drought and salinity stresses, respectively (*p*-value < 0.01) (Fig. 1). These results confirm the *Arabidopsis thaliana* miRNA162 responsiveness to drought and salt stresses, which has not been shown so far.

Transcriptional regulation of *Arabidopsis thaliana* MIR162a and b expression

Drought and salinity responsiveness of Arabidopsis miR162 prompted us to investigate the potential transcriptional regulation of the miRNA genes under the stress conditions. For this purpose, we examined the promoter regions of both, *A. thaliana* MIR162a and MIR162b. The Database of Plant Cis-acting Regulatory DNA Elements (Higo *et al.*, 1999) has revealed the presence of six MYB motifs (MYBST1, MYBCORE, two MYB1AT, MYBGAHV, MYBPLANT) and five MYC motifs (MYCCONSENSUSAT type) in the two

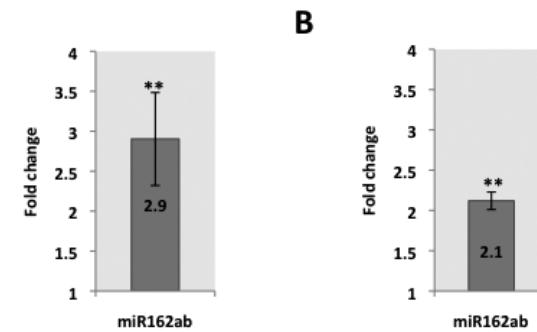


Figure 1. Quantification of *A. thaliana* miRNA162a, b levels under salinity and drought stresses.

(A) MiRNA162a, b level detection under salinity stress revealed by TaqMan® miRNA assay. (B) MiRNA162a, b level detection under 20%SWC drought stress revealed by TaqMan® miRNA assay. Grey bars depict the fold change of miRNA levels under the stresses when compared to the control conditions. Values on the charts are shown as the mean ± S.D. fold change from three independent experiments. Double stars depict a two-tailed Student's *t*-test (*p* ≤ 0.01) suggested statistically significant results.

kilobase (kb) *MIR162a* potential promoter region. In the case of 2 kb *MIR162b* promoter region, the same database indicated seven MYB motifs (MYBCORE, two MYBGAHV, two MYBPLANT, MYB26PS, MYBCOREATCYCB1), and three MYC (MYCCONSENSUS type) motifs. Additionally, the *MIR162a* promoter region contains four ABRE (ACGT-containing abscisic acid response element)-like sequences (three ACGTATERD1 type, one ABRELATERD1 type) required for etiolation-induced expression of *Arabidopsis erd1* (*early responsive to dehydration1*). The *MIR162b* promoter region also possesses one ABRE (ACGTATERD1) and one DRE (DRE2COREZMRAB17) motif. All of the above motifs refer to regulation of expression of genes that are responsive to the water stress in *Arabidopsis* (Higo *et al.*, 1999), suggesting a potential of transcriptional regulation of *A. thaliana MIR162a* and *b* expression under drought stress. Based on the fact that salinity, drought, and also cold, elicit many common and interactive downstream effects (Liu *et al.*, 2007), we examined the *MIR162a* and *b* promoter regions with regard to salinity responsiveness searching for different responsive elements that were reported to play important roles in the plant responses to salinity, drought, osmotic stress, and reactive oxygen species (ROS) stress. We have found the already mentioned DRE element in the *MIR162b* promoter that is a cis-acting promoter element known to regulate gene expression in response not only to drought but also salt and cold stresses in *Arabidopsis* (Cheng *et al.*, 2013). The *MIR162a* promoter also contains a single GCCCORE motif that is the ethylene-responsive element, and *Ethylene Response Factor1* (ERF1) is often considered as the one related to the salt stress response through ethylene signalling in *Arabidopsis* (Cheng *et al.*, 2013). Within the same promoter, a GT1GNSCAM4 motif was found, as GT1 is known to be the RE present in pathogen and salt-induced genes of *Glycine max* (Higo *et al.*, 1999). As

drought and salt stresses activate the dehydration response element binding factor 2 (DREB2) and elevate abscisic acid (ABA) levels (Liu *et al.*, 2007), the DRE response element, and the above ABRE and MYC/MYB responsive elements can be potentially recognized in plants under the salt and drought stresses, suggesting a potential transcriptional regulation of both *ath-miR162* genes under stress conditions studied.

Posttranscriptional regulation of *Arabidopsis thaliana MIR162a* and *b* expression

The discrepancy between the levels of *Arabidopsis* pri-miRNAs 162a and b (Barciszewska-Pacak *et al.*, 2015), and the mature miRNAs 162a and b in the course of the plant's response to both, drought and salt stresses, encouraged us to investigate the possible post-transcriptional ways of regulation of the miRNAs abundance under the stress conditions. Firstly, we confirmed an already described for wild type (*wt*) *Arabidopsis* plants *MIR162a* splicing pattern (Hirsch *et al.*, 2006) in 15 day-old seedlings under control and salt stress conditions, as well as in 30 day-old adult plants under control and 20% SWC drought conditions (Figs. 2A, 3). With regard to the miRNA162a production, the differentially spliced *MIR162a* transcripts were called: a, b, c, d (Hirsch *et al.*, 2006), as functional (a) and non-functional: b, c, d, respectively. The functional splicing isoform is the splicing variant retaining all introns, where the pre-miRNA162a hairpin's left arm is formed by an alternative exon 3 containing miRNA162a-5p, and the right arm – by a retained intron 3 containing miRNA162a-3p. All non-functional splicing variants of pri-miRNA162a represent transcripts lacking either exon 3 or intron 3, or both of them (Fig. 3). For the *A. thaliana MIR162b* gene, we also confirmed the lack of any introns in the gene body (Figs. 2B, 3), as it was shown previously (Hirsch *et al.*, 2006). However, 5'RLM-RACE experiments for

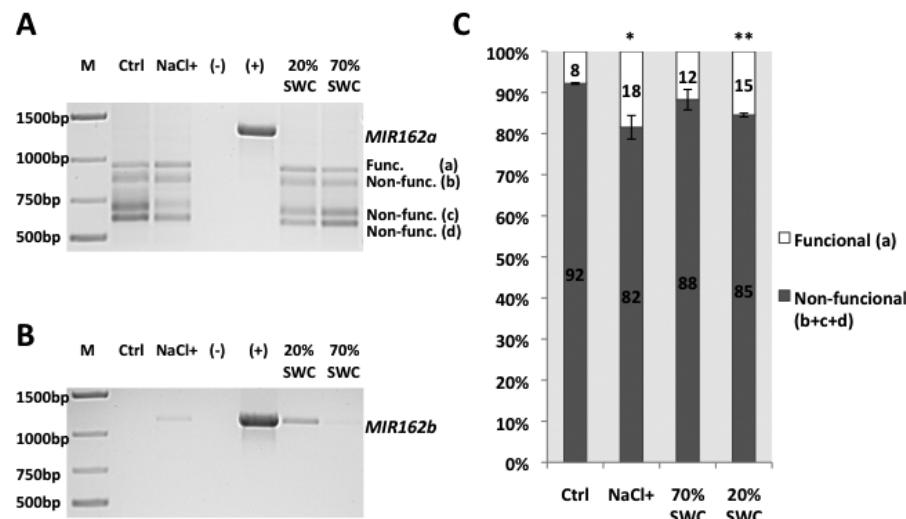


Figure 2. *A. thaliana MIR162a* and *MIR162b* transcript analyses and quantification of pri-miRNA162a splicing isoforms under salinity and drought stresses.

(A) RT-PCR detection of Functional (a) and Non-functional (b, c, d) *MIR162a* transcripts in control (Ctrl) and salinity (NaCl+) stressed seedlings, and 70% SWC (control) and 20% SWC (drought) treated adult plants. (–) and (+) depict negative and positive (genomic DNA) controls, respectively. M depicts 1 Kb Plus DNA Ladder (Thermo Fisher Scientific). **(B)** RT-PCR detection of *MIR162b* transcript in control (Ctrl) and salinity (NaCl+) stressed seedlings, and 70% SWC (control) and 20% SWC (drought) treated adult plants. (–) and (+) depict negative and positive (genomic DNA) controls, respectively. M depicts 1 Kb Plus DNA Ladder (Thermo Fisher Scientific). **(C)** Pri-miRNA162a splicing functional (a) (white bars) and non-functional (b+c+d) (grey bars) isoform level detections under salinity and drought stresses revealed by RT-qPCR. The bars depict the percentage of particular splicing isoforms. Values on the charts are shown as the mean ± S.D. percentage from three independent experiments. Single and double stars depict a two-tailed Student's t-test ($p \leq 0.05$) and ($p \leq 0.01$) suggested statistically significant results, respectively.

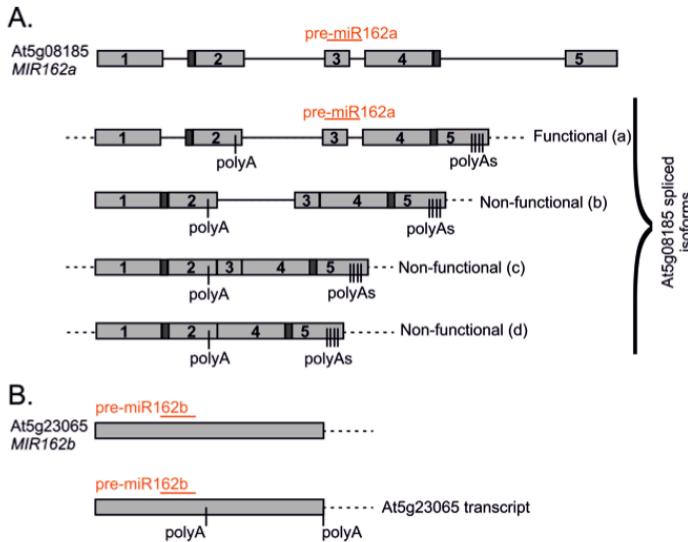


Figure 3. Schematic representation of *MIR162a* (*At5g08185*), *MIR162b* (*At5g23065*) genes, and the differentially spliced: functional (a) and non-functional (b, c, d) transcripts of *MIR162a*.

Grey boxes represent the numbered exons, black horizontal lines between exons represent introns, dark grey boxes depict 5' and 3' untranslated regions (UTRs). The polyadenylation site selection events are depicted by short, vertical lines named polyA or polyAs and pre-miR162a location is visualised by red, short horizontal lines above a particular gene and mRNA region.

control and salt-stressed seedlings enabled us to determine 134 nucleotides (nt) longer 5'end of the *MIR162b* transcript (KX228747) when compared to the 5'end described earlier by Hirsch and coworkers (2006). Under the same conditions, 3'RACE experiments performed on the 5'RLM-RACE derived cDNA led us to define a 298 nt longer 3'end of the *MIR162b* transcript (KX228747), when compared to the 3'end defined by the already mentioned research of Hirsch and coworkers (2006).

We then quantitatively evaluated the level of the *MIR162a* functional and non-functional splicing isoforms in control, salt, and drought stress treated *Arabidopsis* plants by RT-qPCR (Fig. 2C). The functional pri-miRNA162a splicing isoform level increased more than two fold under the salt stress and slightly (1.25 fold) under the 20% SWC drought stress. These changes were accompanied by detection of decreased levels of non-functional pri-miRNA162a splicing isoforms (Fig. 2C). These results pinpointed a significant splicing pattern impact of *MIR162a* transcript on mature ath-miRNA162a production.

The 3'RACE *MIR162a* and *MIR162b* experiments performed on the control and stressed seedlings revealed numerous polyadenylation site selection events in these *MIR* transcripts. We have grouped and named them as proximal (located in the *MIR162a* exon 2 and the middle part of *MIR162b*) and distal (located in the last *MIR162a* exon 5 and at the end of *MIR162b*) polyadenylations (polyAs) (Fig. 3). We quantified the *MIR162a* and *MIR162b* polyA isoforms under control and both stress conditions by RT-qPCR (Fig. 4). Distal *MIR162a* polyAs selection increased under drought when compared to the control, while the polyA site selection was not affected under the salinity stress (Fig. 4). It is important to

realize that only distal polyA site selection leads to the production of a functional pri-miR162a splice isoform (see Fig. 3). In the case of the *MIR162b* transcripts, proximal polyA site selection increased under drought when compared to the control conditions (Fig. 4), while under control and salinity conditions the distal polyA isoform was predominant, and the proximal polyA site selection was barely detectable (data not shown). The

polyadenylation analyses of *MIRs: 162a* and *b* transcripts revealed how important it is to consider the process of polyadenylation with regard to the plant miRNA gene expression regulation under both, the control and stress conditions.

DISCUSSION

MicroRNA162 is known to be stress responsive in many plant species, although it is not reported as such in *Arabidopsis*. Its level was reported to be elevated in salt stressed maize roots (Ding *et al.*, 2009) and it also belongs to hypoxia responsive maize miRNA families (Zhang *et al.*, 2008). It is responsive to mechanical stress in poplar (Lu *et al.*, 2005). It is drought and salt responsive in cellulosic biofuel crop switchgrass (Sun *et al.*, 2012). It also responds to drought in rice (Zhou *et al.*, 2010) and to non-essential metals, like cadmium in rice and rapeseed, aluminum in *Nicotiana tabacum* and mercury in *Medicago truncatula* (Gielen *et al.*, 2012). Here we present data showing *Arabidopsis thaliana* mature miRNA162 responding to drought and salinity stresses that severely threaten plant growth and crop productivity worldwide. As miRNA162 regulates *DCL1*

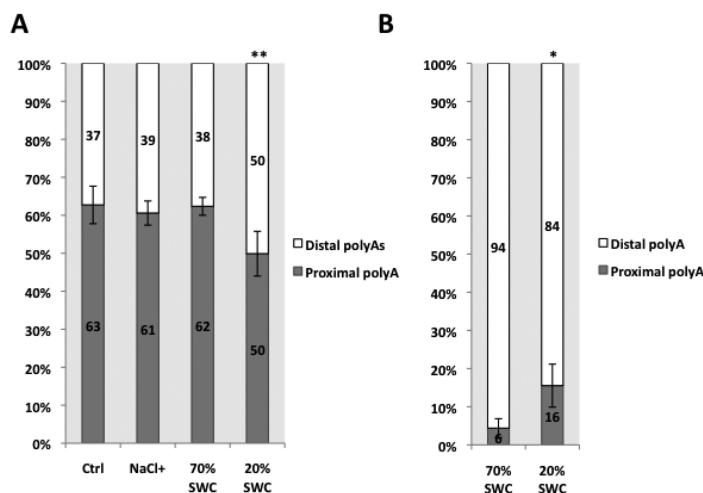


Figure 4. Quantification of *A. thaliana* pri-miRNA162a and pri-miRNA162b polyadenylation isoforms under salinity and drought stresses.

(A) Pri-miRNA162a polyadenylation isoform level detections under salinity and drought stresses revealed by RT-qPCR. (B) Pri-miRNA162b polyadenylation isoform level detections under drought stress revealed by RT-qPCR. The white and grey bars depict the percentage of distal and proximal polyA isoforms, respectively. Values on the charts are shown as the mean \pm S.D. percentage from three independent experiments. Single and double stars depict a two-tailed Student's *t*-test ($p \leq 0.05$) and ($p \leq 0.01$) suggested statistically significant results, respectively.

expression in plants, its particular stress responsiveness may potentially have a significant impact on the plant miRNA biogenesis within different species under various conditions. As under stress conditions the miRNAs and their targets' expression changes certainly reflect plants adaptations to stresses, *MIR162a* and *b* and other miRNA genes are good candidates for crops' improvement engineering experiments.

The promoter regions of both, ath-miR162a and b genes, contain plenty of drought stress related response elements, as well as REs indirectly connected to salt stress via other signalling pathways. This suggests that transcriptional regulation of these genes must be already very complex. However, our previous results have shown that *Arabidopsis thaliana* pri-miRNA162a and pri-miRNA162b were down-regulated under drought stress, and their levels were not affected under a salinity stress (Barciszewska-Pacak *et al.*, 2015). It suggests that transcriptional induction does not play a main role in increasing the level of the mature ath-miR162 under the stresses applied. The discrepancies between the levels of pri-miR162a, b and the mature ath-miR162 can be explained by a more efficient processing of pri-miRNA162a and b under drought and salinity stresses. Certainly, based on bioinformatically predicted REs in their promoter regions, this still does not exclude the transcriptional regulation of *MIR162a* and *b*. The latter could be verified by determination of pri-miRNA expression and mature miRNA levels in *Arabidopsis* miRNA biogenesis mutants subjected to stress conditions. The pri-miRNAs' accumulation and the lowered levels of mature miRNAs could be indicative of their genes' transcriptional regulation. Furthermore, chromatin immunoprecipitation (ChIP) experiments with antibodies against RNA polymerase II could be used to show its distribution within the *MIR* promoter and the gene body, and to help to make conclusions about the gene transcription rate the run-on transcription experiments showing *in vivo* transcription rates of a gene of interest could be performed.

RNA regulatory mechanisms, including RNA synthesis, processing, transport, translation, storage, stability and degradation are emerging as key processes in modulation of stress responses (Ambrosone *et al.*, 2012). In this context, miRNA studies in response to different stresses fit perfectly. Additionally, from the previous studies on *Arabidopsis* plants we concluded the pri-miRNA expression was not predictive for the level of mature miRNA (Barciszewska-Pacak *et al.*, 2015). Thus thorough analyses of posttranscriptional miRNA gene regulation are often necessary and advisable under various conditions the plants encounter. Here, we have shown not only the existence of a complicated splicing pattern of the pri-miRNA162a under stresses applied to *Arabidopsis* *wt* plants, but also changes in the level of particular functional and non-functional splicing variants occurring under the stresses. The only functional intron retaining splicing variant appeared to be crucial for the ath-miR162a up-regulation under salinity and drought stresses, suggesting that splicing inhibition had a decisive effect on the ath-miRNA162a production under the stresses. The results proved a possible competition between splicing efficiency and miRNA production (Brown *et al.*, 2008). So far, reports concerning the stimulatory effect of splicing on miRNAs encoded within the first exons of intron-containing miRNA genes have been published (Bielewicz *et al.*, 2013; Schwab *et al.*, 2013). Here, we show stimulatory effect of splicing inhibition on the intron embedded ath-miRNA162a production.

Generally, in the case of plant intronic miRNAs, the knowledge about communication between spliceosome and the plant microprocessor is limited. In 2008, the cooperation or competition between the complexes has been proposed (Brown *et al.*, 2008). Then, for intronic miR400, a heat stress was found to activate an alternative 5' splice site (5'ss) downstream of its hairpin structure, which lowered the miR400 levels under the stress and suggested miRNA production from spliced-out introns (Yan *et al.*, 2012). However, in the case of pri-miRNA162a the presence of abundant non-functional splicing variants next to the functional intron retaining variant suggests that splicing and miRNA production compete with each other.

In animals, non-canonical splicing events, alternative transcription start site, and polyadenylation site selection affect the biogenesis of miR25, miR93, miR106b (Ramalingam *et al.*, 2014; Agranat-Tamir *et al.*, 2014). In the case of *Arabidopsis MIRs: 162a* and *b*, numerous polyadenylation site selections were found. This may indicate that polyadenylation, next to the splicing process, is another important level of regulation in the plant miRNA biogenesis. In the case of *MIR162a*, only the distal polyA isoforms are functional and were more abundant under drought but not salinity stress, when compared to the non-functional proximal polyA transcripts. Altogether, our results show that the level of ath-miR162 is regulated by posttranscriptional events like splicing and alternative polyA site selection. These results are important because of the miR162 function: miR162 controls the level of the *DCL1* mRNA that is crucial for the production of almost all miRNAs in plants.

Conflict of interest statement

The authors declare no conflict of interests.

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