

Mini review:

Small RNAs and transcriptional regulation of plant defense responses against insect herbivory

Tohir A. Bozorov and Ian T. Baldwin*

Department of Molecular Ecology, Max Planck Institute for Chemical Ecology, Hans-Knöll-Str. 8, 07745 Jena, Germany;

*Correspondence to: baldwin@ice.mpg.de

Journal of Endocytobiosis and Cell Research (2012) 82-85

Category: mini review

Keywords: small RNAs, microRNAs, insect herbivory, jasmonate, plant defense, *Nicotiana attenuata*

Received: 10 November 2012; Accepted: 21 December 2012

Introduction

Plants attract insects as pollinators but at the same time suffer damage from the same insect species, especially during their larval stages. To defend against herbivore attack, plants have evolved efficient defense and tolerance strategies, such as producing toxic chemicals, emitting volatiles to attract predators, and transferring nutrients to roots so as to better tolerate herbivore attack (Pare and Tumlinson 1999; Schwachtje and Baldwin 2008; Schuman et al. 2012). Changes in gene regulation play essential roles in all of these defense responses.

Large-scale reconfigurations of a plant's transcriptome occur during abiotic and biotic stresses, including herbivore attack (Hermsmeier et al. 2001; Schittko et al. 2001; Hui et al. 2003; De Vos et al. 2005; Giri et al. 2006; Phillips et al. 2007; Gaquerel et al. 2009). *Arapidopsis* exposed to pathogenic leaf bacteria (*Pseudomonas syringae* pv. tomato) or fungi (*Alternaria brassicicola*), tissue-chewing caterpillars (*Pieris rapae*), cell-content-feeding thrips (*Frankliniella occidentalis*), or phloem-feeding aphids (*Myzus persicae*) exhibit complex transcriptional alterations in which, in all cases, the representation of transcripts of stress-related genes in the transcriptome are increased (De Vos et al. 2005). Notably, although these four attackers all stimulated jasmonate (JA) biosynthesis, the majority of the changes in JA-responsive gene expression was attacker-specific (De Vos et al. 2005). Applying oral secretions (OS) from insect herbivores to damaged leaves generally changes a plant's transcriptome by up- and down-regulation of transcripts of genes involved in the regulations and biosynthesis of secondary metabolites (Halitschke et al. 2001; Hermsmeier et al. 2001; Winz and Baldwin 2001; Schmidt et al. 2005; Schwachtje and Baldwin 2008; Gaquerel et al. 2009). A recent study by our group revealed that OS

elicitation changed around ten thousand transcripts in *Nicotiana attenuata* (Gulati et al., unpublished data).

Transcriptional changes require rapidly elicited and transported regulators, and small RNAs (smRNAs) functioning as "fine tuners" are likely candidates for this role. Several studies have shown that smRNAs are involved in abiotic and biotic stress responses (Choi and Sano 2007; Phillips et al. 2007; Ruiz-Ferrer and Voinnet 2009; Zhang et al. 2010; Kulcheski et al. 2011; Yan et al. 2011; Khraiweh et al. 2012). SmRNAs have recently been intensively investigated because of their important regulatory role in gene expression. The 18-24 nt-size class of smRNAs such as microRNAs (miRNAs) and small interfering RNAs (siRNAs) control gene expression at the transcriptional and post-transcriptional levels (Ruiz-Ferrer and Voinnet 2009; Chellappan et al. 2010; Chen et al. 2011). Aside from their roles in developmental patterning and maintaining genome integrity by modifying chromatin and DNA methylation (Selvi et al. 2010; van Wolfswinkel and Ketting 2010), they also play key roles in plant responses to environmental stresses (Reinhart et al. 2002; Ruiz-Ferrer and Voinnet 2009). Plants benefit from transcriptional control which allows them to quickly and plastically adapt their physiologies to stresses (Schmidt et al. 2005).

SmRNAs are classified into miRNAs and endogenous siRNAs, which are in turn classified as chromatin-associated siRNAs (hcsiRNAs), natural antisense siRNAs (natsiRNAs), and transacting siRNAs (tasiRNAs) (Reinhart et al. 2002; Bartel 2004). Biogenesis of all of these smRNAs requires specific components of the RNAi pathway (Figure 1) (Bartel 2004; Vaucheret 2006). Different proteins of the RNA interference (RNAi) pathways such as Dicer-like (DCL), RNA-dependent RNA polymerases (RDR), double-strand RNA binding (DRB), Argonaut (AGO), and other protein families are involved in the biogenesis of each class of smRNAs (Figure 1) (Vaucheret 2006; Chapman and Carrington 2007; Ruiz-Ferrer and Voinnet 2009). Primary miRNA genes (*MIR*) are transcribed in the nucleus as primary transcripts and subsequently processed into precursor stem and loop structures (pre-miRNA). These pre-miRNAs are processed by DCL1 into mature miRNA/miRNA duplexes which are transported into the cytoplasm (Bartel 2004; Chen 2005; Bologna et al. 2012). In contrast to miRNAs, the biogenesis of siRNAs begins from primary non-protein-coding transcripts processed into double-stranded RNAs (dsRNAs) which are synthesized by RDRs. These dsRNAs are "diced" by dicer-like proteins (DCL2, 3 and 4) into 21-24 nt pieces depending on the particular DCL involved (Gascioli et al. 2005). Both smRNAs bind to mRNA targets in a perfect or imperfect comple-

mentary manner, resulting in inhibition or degradation of the target mRNA implicated in developmental and stress responses.

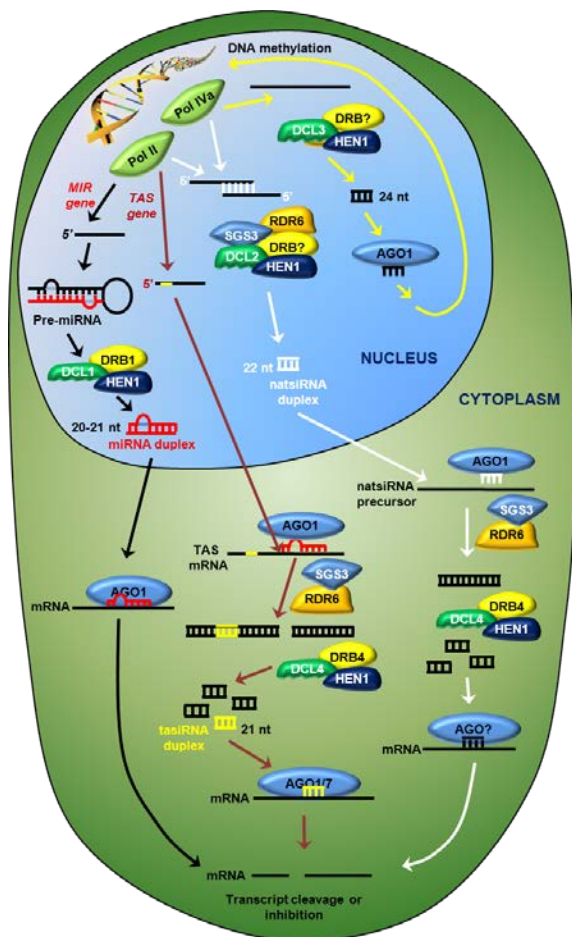


Figure 1: Overview of smRNA processing in plants.

Abiotic and biotic stresses change smRNA abundance in plants as well as in animals (Bhattacharyya et al. 2006; Mallory and Vaucheret 2006; Zhao et al. 2007; Zhou et al. 2007; Zhou et al. 2010; Bozorov et al. 2012a; Tang et al. 2012). When plants are exposed to stresses, they must switch off or reduce the expression of genes involved in growth and developmental processes, and activate stress-responsive genes that adapt the plant's physiology to better cope with the stress (Sunkar 2010). It would be costly for plants to maintain the expression of transcriptional responses under non-stressful conditions. This could be costly in two ways: maintaining synthesis of proteins which cannot be used, and the potential loss of photosynthesis and primary metabolites to attackers. A recent study by our group identified miRNAs and trans-acting siRNAs (tasiRNAs) in *N. attenuata* and profiled their abundance during herbivory (Bozorov et al. 2012a). The result revealed that smRNAs fine-tune developmental and defense responses in jasmonate-dependent and independent manners (Bozorov et al. 2012a). However, siR-

NAs have also been implicated in DNA methylation of gene regulatory elements, resulting in transcriptional inhibition of their targets (Pontes et al. 2009; Baev et al. 2010; Chellappan et al. 2010; Chen et al. 2011).

Regulation of the transcriptome during abiotic and biotic stress responses often depends on histone and DNA modifications (Chinnusamy and Zhu 2009; Verhoeven et al. 2010; Karan et al. 2012) which transcriptionally regulate gene expression. Several studies have reported that genome rearrangements play a key role in gene expression and plant development under stress (Chinnusamy and Zhu 2009). Under continuous stress responses, such genome rearrangements may become transgenerationally inherited, increasing the fitness of subsequent generations also exposed to stressful environments (Boyko et al. 2010; Boyko and Kovalchuk 2010; Verhoeven and van Gurp 2012). It was shown that herbivore stress and pathogen defenses triggered considerable methylation throughout the genome (Verhoeven et al. 2010; Rasmann et al. 2012). Genome modifications such as methylation and acetylation can result in altered regulation of gene activity. These changes are associated with the silencing of transposons, imprinting, and silencing transgenes and endogenous genes (Kooter et al. 1999; Zilberman et al. 2007). The transmission of such genome modifications to offspring (Johannes et al. 2009; Verhoeven et al. 2010; Rasmann et al. 2012) is a mechanism of plant plasticity. Recent studies demonstrated that transgenerational inheritance of DNA modifications mediates phenotypic plasticity in *Arabidopsis* and tomato plants during herbivory (Rasmann et al. 2012). *Arabidopsis* mutants deficient in jasmonate perception (*coronatine insensitive 1*) or in the biogenesis of small interfering RNAs (*dicer-like2, 3, 4* and *nuclear RNA polymerase d2a and d2b*) do not exhibit this type of inherited resistance (Rasmann et al. 2012).

Moreover, interruption of proteins functioning in RNAi pathways demonstrates their involvement in responses to stress (Blevins et al. 2009; Liu et al. 2009; Liu et al. 2009; Ziebell and Carr 2009; Boyko et al. 2010; Yang et al. 2011). Applying the reverse genetics approach to individually characterized functions of components of the RNAi machinery demonstrated a key role in the regulation of stress-responsive genes, including genes responsive to herbivory (Liu et al. 2005; Pandey and Baldwin 2007, 2008; Pandey et al. 2008a, 2008b; Liu et al. 2009; Yang et al. 2011; Bozorov et al. 2012b). Silencing *RDR1* in *N. attenuata* impairs the ability of plants to activate JA-mediated responses and makes them highly susceptible to insects (Pandey and Baldwin 2007). Moreover, RDR-silenced plants display large differences in their smRNA transcriptome during herbivory in comparison to wild-type (WT) plants (Pandey et al. 2008b).

Another important piece of the RNAi machinery, DCL proteins play a central role in processing smRNAs from double-stranded RNA precursors and are involved in smRNAs regulating defense responses to herbivory (Bozorov et al. 2012b). Silencing of the four individual *DCL* genes differently impaired JA-mediated direct defense accumulation during her-

bivory in *N. attenuata*. Both NaDCL3 and NaDCL4 were found to regulate the levels of nicotine accumulation, but trypsin proteinase inhibitors were regulated specifically by NaDCL3, which was not compensated by the function of other NaDCLs. However, DCLs interact with each other to regulate defense responses to herbivory. Defense responses were not affected in NaDCL2-silenced plants, but double-silencing with NaDCL3 or NaDCL4 restored nicotine level (Bozorov et al. 2012b). These results demonstrate that smRNA pathways interact in a complex ways to regulate anti-herbivory defense (Figure 2), and it will be an exciting challenge to unravel all the ways in which this clearly fine-tuned regulation comes about.

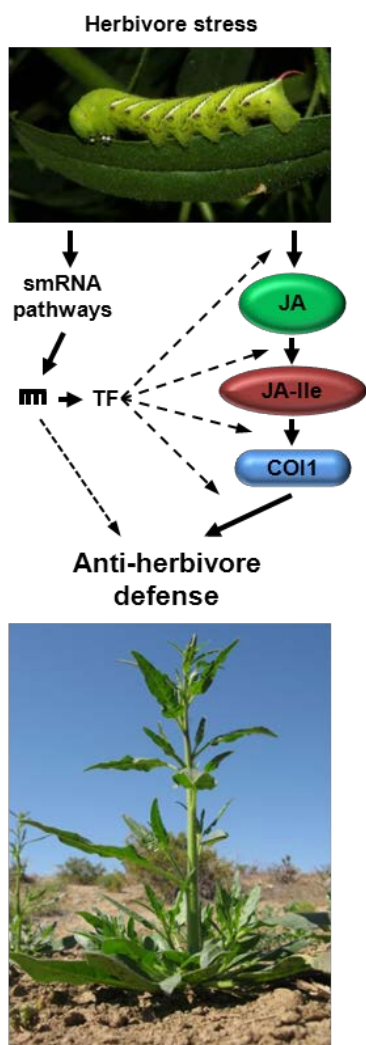


Figure 2: Role of small RNAs in *N. attenuata* anti-herbivore defense.

References

Baev V, Naydenov M, Apostolova E, Ivanova D, Doncheva S, Minkov I, Yahubyan G. (2010) Identification of RNA-dependent DNA-

- methylation regulated promoters in *Arabidopsis*. *Plant Physiol Biochem*. 48:393-400.
- Bartel DP. (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell*. 116:281-297.
- Bhattacharyya SN, Habermacher R, Martine U, Closs EI, Filipowicz W. (2006) Stress-induced reversal of microRNA repression and mRNA P-body localization in human cells. *Cold Spring Harb Symp Quant Biol*. 71:513-521.
- Blevins T, Pontes O, Pikaard CS, Meins F Jr. (2009) Heterochromatic siRNAs and DDM1 independently silence aberrant 5S rDNA transcripts in *Arabidopsis*. *PLoS One*. 4:e5932.
- Bologna NG, Schapire AL, Palatnik JF. (2012) Processing of plant microRNA precursors. *Brief Funct Genomic*. Doi:10.1093/bfgp/els050.
- Boyko A, Blevins T, Yao Y, Golubov A, Bilichak A, Ilnytsky Y, Hollunder J, Meins F, Jr., Kovalchuk I. (2010) Transgenerational adaptation of *Arabidopsis* to stress requires DNA methylation and the function of Dicer-like proteins. *PLoS One*. 5:e9514.
- Boyko A, Kovalchuk I. (2010) Transgenerational response to stress in *Arabidopsis thaliana*. *Plant Signal Behav*. 5:995-998.
- Bozorov TA, Baldwin IT, Kim SG. (2012a) Identification and profiling of miRNAs during herbivory reveals jasmonate-dependent and -independent patterns of accumulation in *Nicotiana attenuata*. *BMC Plant Biol*. 12:209.
- Bozorov TA, Prakash Pandey S, Dinh ST, Kim SG, Heinrich M, Gase K, Baldwin IT. (2012b) DICER-like proteins and their role in plant-herbivore interactions in *Nicotiana attenuata*. *J Integr Plant Biol*. 54:189-206.
- Chapman EJ, Carrington JC. (2007) Specialization and evolution of endogenous small RNA pathways. *Nat Rev Genet*. 8:884-896.
- Chellappan P, Xia J, Zhou X, Gao S, Zhang X, Coutino G, Vazquez F, Zhang W, Jin H. (2010) siRNAs from miRNA sites mediate DNA methylation of target genes. *Nucleic Acids Res*. 38:6883-6894.
- Chen D, Meng Y, Yuan C, Bai L, Huang D, Lv S, Wu P, Chen LL, Chen M. (2011) Plant siRNAs from introns mediate DNA methylation of host genes. *RNA*. 17:1012-1024.
- Chen X. (2005) MicroRNA biogenesis and function in plants. *FEBS Lett*. 579:5923-5931
- Chinnusamy V, Zhu JK. (2009) Epigenetic regulation of stress responses in plants. *Curr Opin Plant Biol*. 12:133-139.
- Choi CS, Sano H. (2007) Abiotic-stress induces demethylation and transcriptional activation of a gene encoding a glycerophosphodiesterase-like protein in tobacco plants. *Mol Genet Genomics*. 277:589-600.
- De Vos M, Van Oosten VR, Van Poecke RM, Van Pelt JA, Pozo MJ, Mueller MJ, Buchala AJ, Mettraux JP, Van Loon LC, Dicke M, Pieterse CM. (2005) Signal signature and transcriptome changes of *Arabidopsis* during pathogen and insect attack. *Mol Plant Microbe Interact*. 18:923-937.
- Gaquerel E, Weinhold A, Baldwin IT. (2009) Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata*. VIII. An unbiased GCxGC-ToFMS analysis of the plant's elicited volatile emissions. *Plant Physiol*. 149:1408-1423.
- Gascioli V, Mallory AC, Bartel DP, Vaucheret H. (2005) Partially redundant functions of *Arabidopsis* DICER-like enzymes and a role for DCL4 in producing trans-acting siRNAs. *Curr Biol*. 15:1494-1500.
- Giri AP, Wunsche H, Mitra S, Zavala JA, Muck A, Svatos A, Baldwin IT. (2006) Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata*. VII. Changes in the plant's proteome. *Plant Physiol*. 142:1621-1641.
- Halitschke R, Schittko U, Pohnert G, Boland W, Baldwin IT. (2001) Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata*. III. Fatty acid-amino acid conjugates in herbivore oral secretions are necessary and sufficient for herbivore-specific plant responses. *Plant Physiol*. 125:711-717.

- Hermesmeier D, Schittko U, Baldwin IT. (2001) Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata*. I. Large-scale changes in the accumulation of growth- and defense-related plant mRNAs. *Plant Physiol.* 125:683-700.
- Hui D, Iqbal J, Lehmann K, Gase K, Saluz HP, Baldwin IT. (2003) Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, sphingidae) and its natural host *Nicotiana attenuata*: V. microarray analysis and further characterization of large-scale changes in herbivore-induced mRNAs. *Plant Physiol.* 131:1877-1893.
- Johannes F, Porcher E, Teixeira FK, Saliba-Colombani V, Simon M, Agier N, Bulski A, Albuissou J, Heredia F, Audigier P, Bouchez D, Dillmann C, Guerche P, Hospital F, Colot V. (2009) Assessing the impact of transgenerational epigenetic variation on complex traits. *PLoS Genet.* 5:e1000530.
- Karan R, Deleon T, Biradar H, Subudhi PK. (2012) Salt stress induced variation in DNA methylation pattern and its influence on gene expression in contrasting rice genotypes. *PLoS One.* 7:e40203.
- Khraiwesh B, Zhu JK, Zhu J. (2012) Role of miRNAs and siRNAs in biotic and abiotic stress responses of plants. *Biochim Biophys Acta.* 1819:137-148.
- Kooter JM, Matzke MA, Meyer P. (1999) Listening to the silent genes: transgene silencing, gene regulation and pathogen control. *Trends Plant Sci.* 4:340-347.
- Kulcheski FR, de Oliveira LF, Molina LG, Almerao MP, Rodrigues FA, Marcolino J, Barbosa JF, Stolf-Moreira R, Nepomuceno AL, Marcelino-Guimaraes FC, Abdelnoor RV, Nascimento LC, Carazzolle MF, Pereira GA, Margis R. (2011) Identification of novel soybean microRNAs involved in abiotic and biotic stresses. *BMC Genomics.* 12:307.
- Liu B, Li P, Li X, Liu C, Cao S, Chu C, Cao X. (2005) Loss of function of OsDCL1 affects microRNA accumulation and causes developmental defects in rice. *Plant Physiol.* 139:296-305.
- Liu Q, Feng Y, Zhu Z. (2009) Dicer-like (DCL) proteins in plants. *Funct Integr Genomics.* 9:277-286.
- Liu Y, Gao Q, Wu B, Ai T, Guo X. (2009) NgRDR1, an RNA-dependent RNA polymerase isolated from *Nicotiana glutinosa*, was involved in biotic and abiotic stresses. *Plant Physiol Biochem.* 47:359-368.
- Mallory AC, Vaucheret H. (2006) Functions of microRNAs and related small RNAs in plants. *Nat Genet.* 38:S31-36.
- Pandey SP, Baldwin IT. (2007) RNA-directed RNA polymerase 1 (RdR1) mediates the resistance of *Nicotiana attenuata* to herbivore attack in nature. *Plant J.* 50:40-53.
- Pandey SP, Baldwin IT. (2008) Silencing RNA-directed RNA polymerase 2 increases the susceptibility of *Nicotiana attenuata* to UV in the field and in the glasshouse. *Plant J.* 54:845-862.
- Pandey SP, Gaquerel E, Gase K, Baldwin IT. (2008a) RNA-directed RNA polymerase 3 from *Nicotiana attenuata* is required for competitive growth in natural environments. *Plant Physiol.* 147:1212-1224.
- Pandey SP, Shahi P, Gase K, Baldwin IT. (2008b) Herbivory-induced changes in the small-RNA transcriptome and phytohormone signaling in *Nicotiana attenuata*. *Proc Natl Acad Sci USA.* 105:4559-4564.
- Pare PW, Tumlinson JH. (1999) Plant volatiles as a defense against insect herbivores. *Plant Physiol.* 121:325-331.
- Phillips JR, Dalmay T, Bartels D. (2007) The role of small RNAs in abiotic stress. *FEBS Lett.* 581:3592-3597.
- Pontes O, Costa-Nunes P, Vithayathil P, Pikaard CS. (2009) RNA polymerase V functions in *Arabidopsis* interphase heterochromatin organization independently of the 24-nt siRNA-directed DNA methylation pathway. *Mol Plant.* 2:700-710.
- Rasmann S, De Vos M, Casteel CL, Tian D, Halitschke R, Sun JY, Agrawal AA, Felton GW, Jander G. (2012) Herbivory in the previous generation primes plants for enhanced insect resistance. *Plant Physiol.* 158:854-863.
- Reinhart BJ, Weinstein EG, Rhoades MW, Bartel B, Bartel DP. (2002) MicroRNAs in plants. *Genes Dev.* 16:1616-1626.
- Ruiz-Ferrer V, Voinnet O. (2009) Roles of plant small RNAs in biotic stress responses. *Annu Rev Plant Biol.* 60:485-510.
- Schittko U, Hermesmeier D, Baldwin IT. (2001) Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata*. II. Accumulation of plant mRNAs in response to insect-derived cues. *Plant Physiol.* 125:701-710.
- Schmidt DD, Voelckel C, Hartl M, Schmidt S, Baldwin IT. (2005) Specificity in ecological interactions: attack from the same lepidopteran herbivore results in species-specific transcriptional responses in two solanaceous host plants. *Plant Physiol.* 138:1763-1773.
- Schwachtje J, Baldwin IT. (2008) Why does herbivore attack reconfigure primary metabolism? *Plant Physiol.* 146:845-851.
- Schuman MC, Barthel K, Baldwin IT. (2012) Herbivory-induced volatiles function as defenses increasing fitness of the native plant *Nicotiana attenuata* in nature. *Elife.* 1:e00007.
- Selvi BR, Mohankrishna DV, Ostwal YB, Kundu TK (2010) Small molecule modulators of histone acetylation and methylation: a disease perspective. *Biochim Biophys Acta.* 1799:810-828.
- Sunkar R. (2010) MicroRNAs with macro-effects on plant stress responses. *Semin Cell Dev Biol.* 21:805-811.
- Tang S, Wang Y, Li Z, Gui Y, Xiao B, Xie J, Zhu QH, Fan L. (2012) Identification of wounding and topping responsive small RNAs in tobacco (*Nicotiana tabacum*). *BMC Plant Biol.* 12:28.
- van Wolfswinkel JC, Ketting RF. (2010) The role of small non-coding RNAs in genome stability and chromatin organization. *J Cell Sci.* 123:1825-1839.
- Vaucheret H. (2006) Post-transcriptional small RNA pathways in plants: mechanisms and regulations. *Genes Dev.* 20:759-771.
- Verhoeven KJ, Jansen JJ, van Dijk PJ, Biere A. (2010) Stress-induced DNA methylation changes and their heritability in a sexual dandelion. *New Phytol.* 185:1108-1118.
- Verhoeven KJ, van Gorp TP. (2012) Transgenerational effects of stress exposure on offspring phenotypes in apomictic dandelion. *PLoS One.* 7:e38605.
- Winz RA, Baldwin IT. (2001) Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata*. IV. Insect-induced ethylene reduces jasmonate-induced nicotine accumulation by regulating putrescine N-methyltransferase transcripts. *Plant Physiol.* 125:2189-2202.
- Yan H, Deng X, Cao Y, Huang J, Ma L, Zhao B. (2011) A novel approach for the construction of plant amiRNA expression vectors. *J Biotechnol.* 151:9-14.
- Yang H, Wang M, Gao Z, Zhu C, Guo X. (2011) Isolation of a novel RNA-dependent RNA polymerase 6 from *Nicotiana glutinosa*, NgRDR6, and analysis of its response to biotic and abiotic stresses. *Mol Biol Rep.* 38:929-937.
- Zhang S, Zhou J, Han S, Yang W, Li W, Wei H, Li X, Qi L. (2010) Four abiotic stress-induced miRNA families differentially regulated in the embryogenic and non-embryogenic callus tissues of *Larix leptolepis*. *Biochem Biophys Res Commun.* 398:355-360.
- Zhao B, Liang R, Ge L, Li W, Xiao H, Lin H, Ruan K, Jin Y. (2007) Identification of drought-induced microRNAs in rice. *Biochem Biophys Res Commun.* 354:585-590.
- Zhou L, Liu Y, Liu Z, Kong D, Duan M, Luo L. (2010) Genome-wide identification and analysis of drought-responsive microRNAs in *Oryza sativa*. *J Exp Bot.* 61:4157-4168.
- Zhou X, Wang G, Zhang W. (2007) UV-B responsive microRNA genes in *Arabidopsis thaliana*. *Mol Syst Biol.* 3:103.
- Ziebell H, Carr JP. (2009) Effects of dicer-like endoribonucleases 2 and 4 on infection of *Arabidopsis thaliana* by cucumber mosaic virus and a mutant virus lacking the 2b counter-defence protein gene. *J Gen Virol.* 90:2288-2292.
- Zilberman D, Gehring M, Tran RK, Ballinger T, Henikoff S. (2007) Genome-wide analysis of *Arabidopsis thaliana* DNA methylation uncovers an interdependence between methylation and transcription. *Nat Genet.* 39:61-69.