

## Mini review:

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

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elicitation changed around ten thousand transcripts in *Nicotiana attenuata* (Gulati et al., unpublished data).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



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.

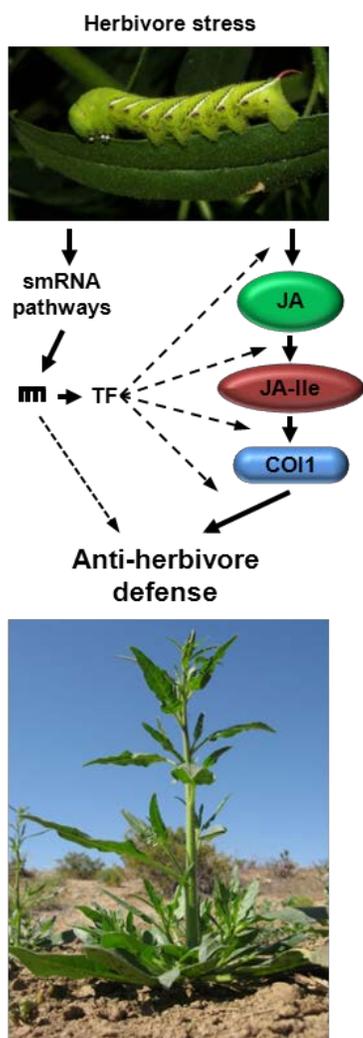


Figure 2: Role of small RNAs in *N. attenuata* anti-herbivore defense.

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