

22 Nucleotide siRNAs: Emerging Players in Plant Stress Adaptation

Deepti Tiwari^{1*} and Manoj Kumar Patel²

¹Division of Plant Physiology, ²Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi (110 012), India



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Corresponding Author

Deepti Tiwari

✉: deeptit12@gmail.com

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Abstract

The RNA interference (RNAi) is process of modulating gene expression and holds significant promise for crop improvement. The intricate mechanisms underlying RNA silencing, particularly the significance of 22-nucleotide small interfering RNAs (siRNAs), remain elusive. In this article, we explore the biogenesis and biological significance of 22 nucleotide siRNAs in plants. These siRNAs, emerging as crucial regulators of gene expression, mediate translational repression and facilitate stress mitigation. The biogenesis of 22 nucleotide siRNAs is dependent on Dicer like 2 (DCL2), Argonaute 1 (AGO1), RNA dependent RNA polymerase 6 (RDR6) and HUA ENHANCER 1 (HEN1). The induction of 22 nucleotide siRNAs, particularly from genes encoding nitrate reductases (*NIA1/2*), under stress conditions, leads to severe growth disorders, emphasizing their significance in plant stress responses. This article offers valuable insights into the diverse roles of 22 nucleotide siRNAs in plants, highlighting their significance in agriculture and biotechnology for enhancing crop resilience and productivity.

Keywords: 22 nucleotide siRNAs, Plant stress adaptation, RNA interference, RNA silencing

Introduction

RNA interference (RNAi) is employed to regulate internal gene expression, suppress transposons and heterochromatic areas, and to combat viral infections by plants and other eukaryotes. Its application in plants has led to the development of valuable traits, with considerable potential for further enhancements in crop improvement.

RNA silencing is initiated by double stranded RNA (dsRNA) or hairpin structured RNA (hpRNA). It involves crucial components like Dicer or Dicer like (DCL) and Argonaute (AGO) family proteins. These dsRNA originates from diverse sources, illustrated in figure 1(A).

Generally, Dicer family proteins cleave dsRNA into 20 to 24 nucleotide siRNA duplexes. A single strand of the siRNA duplex integrates in an AGO, facilitating the creation of an RNA induced silencing complex (RISC). This complex then governs target regulation either post-transcriptionally or through RNA directed DNA methylation (RdDM). Arabidopsis has four DCL proteins (DCL1 to DCL4), each fulfilling distinct roles, as depicted in figure 1(B). Different types of RNA Silencing Pathways that occur in plants are shown in figure 2(A).

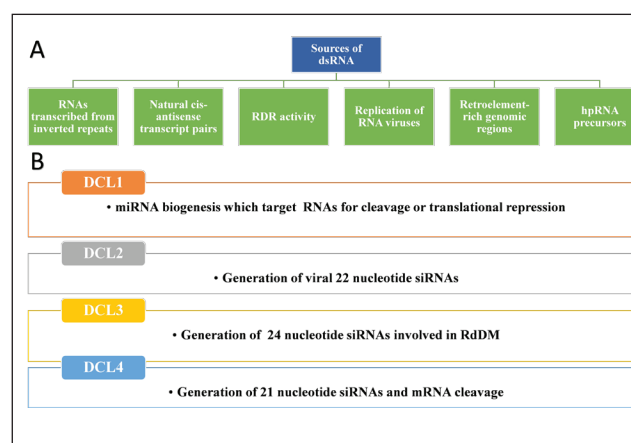


Figure 1: (A) Various sources for the origin of dsRNA; (B) Types of DCL proteins (DCL1 to DCL4) present in Arabidopsis and their distinct roles [Here, hpRNA - hairpin structured RNA, RdDM - RNA directed DNA methylation, RDR - RNA dependent RNA polymerase and siRNAs - small interfering RNAs]

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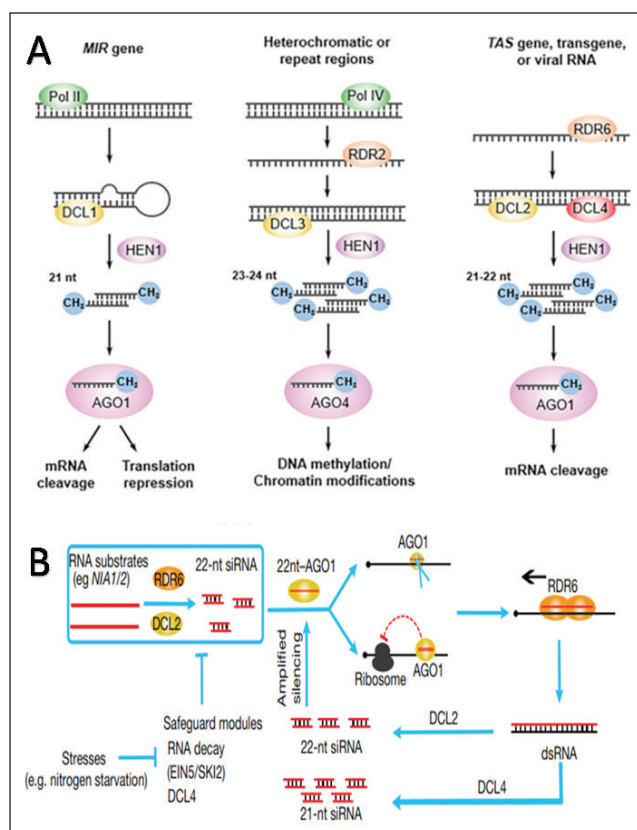


Figure 2: (A) RNA silencing pathways in plants (Source: Liu and Chen, 2016); (B) A proposed mechanism for 22 nucleotide siRNAs mediated translational repression (Source: Wu *et al.*, 2020) [Here, AGO - Argonaute, DCL - Dicer like, HEN 1 - HUA ENHANCER 1, MIR - microRNAs, RDR - RNA dependent RNA polymerase, siRNAs - small interfering RNAs and TAS - Trans-Acting siRNA]

Biogenesis of 22 Nucleotide siRNAs

siRNAs are pivotal in gene expression regulation, facilitating the degradation of specific mRNA molecules. Their significance spans across eukaryotic growth and defence. Plants generate siRNAs of diverse lengths, such as 21, 22, and 24 nucleotides. While the roles of 21 and 24 nucleotide siRNAs in DNA methylation and mRNA cleavage are widely recognized, the limited presence of endogenic 22 nucleotide siRNAs have hindered their thorough examination.

Wu *et al.* (2020) observed that genes under adverse environments, *NIA1* and *NIA2*, responsible for encoding nitrate reductases, generate 22 nucleotide siRNAs. The production is particularly in plants lacking cytoplasmic RNA decay and DCL4 activity, which results in severe growth abnormalities, attributed to translational repression and heightened stress responses. DCL2 and RDR6 are crucial for the production of these 22 nucleotide siRNAs, with *NIA1* and *NIA2* contributing significantly to their total pool. Moreover, genes essential for phloem development like *SMAX1 LIKE* (*SMXL4* and *SMXL5*), also exhibit copious production of these siRNAs. AGO1 governs the biogenesis and functionality of these siRNAs, while *HUA ENHANCER 1* (*HEN1*) facilitates their methylation.

Recent findings by Fei *et al.* (2018) elucidate the process by which a 21 nucleotide miRNA undergoes monouridylation to form a 22 nucleotide miRNA, which in turn plays a crucial role in the generation of secondary siRNAs. This research highlights the importance of miRNA length in these processes. Furthermore, the study reveals that the production of 22 nucleotide siRNAs is notably enhanced under conditions of environmental stress, emphasizing their adaptive significance.

Functional Significance of 22 Nucleotide siRNAs in Plants

Wu *et al.* (2020) provided evidence proving that translational repression is facilitated by 22 nucleotide siRNAs and hence, inhibition of growth. The 22 nucleotide siRNAs demonstrate proficiency in translational repression but display reduced efficiency in target cleavage compared to their counterparts 21 and 24 nucleotide siRNAs. Additionally, they prompt transitive RNA interference and silencing amplification processes. However, the precise mechanisms governing translational repression and transitive RNA interference by these siRNAs remain unclear. It is plausible that AGO1, laden with 22 nucleotide siRNAs, might selectively impede ribosomes. The association between 22 nucleotide siRNAs and AGO1, in conjunction with cleaved mRNA fragments or ribosomes, could possibly engage RDR6 to begin synthesizing dsRNA [Figure 2(B)]. Consequently, it would amplify siRNA production. In stressful situations like nitrogen deficiency, the generation of 22 nucleotide siRNAs triggers the initiation of multiple plant stress response pathways while simultaneously suppressing pathways that promote growth. These findings highlight the crucial part of 22 nucleotide siRNAs in finely regulating both plant responses to stress and growth.

Secondary or transitive siRNAs are created from miRNAs or siRNAs targeted transcripts. These secondary siRNAs have the potential to amplify the silencing effect on the primary target or contribute to the trans-silencing of members within the gene family. The importance of 22 nucleotide miRNAs was seen in the experiment by McHale *et al.* (2020) conducted in transgenic Arabidopsis plants utilizing artificial miRNAs (amiRNAs). It was found that knockdown of a targeted chalcone synthase (CHS) gene occurred in case of using a RDR6 dependent, 22 nucleotide amiRNA, not its corresponding 21 nucleotide counterpart.

Systemic silencing refers to a phenomenon in which RNAi signals initiated in one part of a plant spread throughout the organism, leading to the silencing of target genes in distant tissues. While the signal for systemic silencing remains inconclusive, it is generally believed to be an RNA molecule. sRNAs have the ability to travel within plants *via* plasmodesmata or through vascular tissues (Liu and Chen, 2016). Another mechanism outlined for the extended distance transport of sRNA requires template dependent signal amplification coupled with cell-to-cell movement. Recent research has emphasized that in systemic RNAi, there is a crucial involvement of DCL2 and consequently, 22 nucleotide siRNAs (Chen *et al.*, 2018).

Conclusion

siRNAs are pivotal in gene expression regulation, facilitating the degradation of specific mRNA molecules. Translational repression and stress adaption are mediated by plant 22 nucleotide siRNAs. Large-scale endogenous 22 nucleotide siRNA synthesis has been seen in plants by *NIA1/2* genes when DCL4 activity and cytoplasmic RNA degradation is lacking. For the enhanced synthesis of 22 nucleotide siRNAs, RDR6 and DCL2 activity are necessary. Additionally, their functioning and biosynthesis both depend on AGO1. 22 nucleotide siRNAs stand apart from other siRNAs by initiating silencing amplification, transitive RNA interference and translational repression, while showing reduced efficacy in target cleavage. Under stress conditions, induction of 22 nucleotide siRNAs causes growth inhibition due to translational repression. 22 nucleotide siRNAs are crucial in fine-tuning plant responses through the activation of stress response pathways and the inhibition of growth related pathways under stress. Secondary siRNAs further contribute to gene silencing amplification or trans-silencing of gene family members. Systemic silencing, facilitated by 22 nucleotide siRNAs movement, particularly through DCL2-mediated pathways, ensures widespread RNA interference throughout the plant. Overall, these findings elucidate that 22 nucleotide siRNAs have the multifaceted roles in orchestrating plant stress responses and growth, offering valuable insights into plant biology and potential applications in agriculture and biotechnology.

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