

Micro RNA Interference: A New Platform for Crop Protection

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Introduction

Micro RNAs (miRNAs) are a class of small non-coding RNAs with 21-22 nucleotides (nt) size, which play important role in the regulation of gene expression, development, stress reactions, diseases and cell death in different organisms. They are generated from their own genes present in intergenic regions and also from their host protein coding genes (from intragenic regions, particularly introns) [1]. The miRNA genes are transcribed mostly by pol. II as long distinct transcriptional units as well as in clusters of polycistronic units, known as primary miRNAs (pri-miRNAs [2,3]. Later, pri-miRNAs are processed to ~70 nt precursor miRNAs (pre-miRNAs) by RNase III enzyme, Drosha [4], which is then transported to the cytoplasm by exportin 5 and Ran-GTP [5], where they are further processed to generate 19-25 nt mature miRNA duplexes by RNase III protein, Dicer [6]. However, in plants these miRNA duplexes are formed in nucleus itself from pre-miRNAs by dicer-like enzyme, Dcl1, which is also involved in the processing of pri-miRNAs to pre-miRNA [7,8]. These miRNA duplexes are exported out of the nucleus by Hasty and are recruited later by multi-protein nuclease complex known as RNA-induced silencing complex (RISC), where only the guide strand of miRNA remains with the RISC and the passenger strand are selectively eliminated. This RISC (mi-RISC) then binds to the motifs usually present in 3' UTRs of the targeted genes by exact or near-exact complementary base pairing, thereby silencing the target gene(s) through cleavage of target mRNA or translational repression, respectively [1,7,8].

A large number of miRNAs have been reported in different organisms, which are evolutionally conserved in the kingdom-specific (animal or plant) manner. These miRNAs are differentially expressed during cell differentiation and development as well as stress responses. In fact, tremendous progress has been made during the last few years in identifying the novel miRNAs that are intimately associated with various important cellular functions, including stress responses and disease development in plants [9].

miRNAs During Biotic Stresses in Plants

The expression profile of miRNA genes has been studied in plants in response to biotic stresses. These investigations revealed over or under-expression of certain miRNAs or synthesis of new miRNAs to withstand the stress conditions [10,11]. For instance, the miRNA, miR393 was demonstrated to play a role in antibacterial PTI (Pattern Triggered Immunity) in *Arabidopsis* by regulating the auxin signaling pathway [12]. Fahlgren *et al.* (2007) [13] reported three miRNAs, miR160, miR167 and miR393 which were highly induced and one miRNA, miR825 that was down-regulated in *Arabidopsis* leaves collected after 1 and 3 h of infection with *Pseudomonas syringae*. Two miRNAs - miR156 and miR164 were induced in *Arabidopsis* following infection with the Turnip Mosaic Virus (TuMV), and these were also induced in transgenic *Arabidopsis* expressing the viral silencing suppressor P1/HC-Pro [14]. Two new miRNAs, miR158 and miR1885 were also identified in *Brassica* following infection with ToMV, but not by other viruses - cucumber mosaic virus (CTV) and tobacco mosaic virus (TMV) [15]. The expression of several host miRNAs was shown to be affected in response to viral infection in tomato [16]. Likewise, it has been shown that a common set of developmental miRNAs are

up-regulated in *Nicotiana benthamiana* by infection with diverse begomoviruses [17].

There are also studies on the expression profile of host plant miRNAs following fungal infections. Lu *et al.* [18] reported that miRNAs were differentially expressed in response to infection by the rust fungus, *Cronartium quecuum f. sp. fusiforme*, which cause fusiform rust disease in pines. They have shown that 10 out of 11 miRNA families were down-regulated, including 7 that were pine specific. For example, miR156 and miR160 were down-regulated in pine stems infected with the rust fungus. The differential expression of some of the conserved miRNAs was observed in wheat infected with the powdery mildew fungus [19]. They have shown that miR156, miR159, miR164, miR171 and miR396 were repressed and miR393, miR444 and miR827 were up-regulated. More recently, two miRNAs - miR160 and miR171a were found to be down-regulated and one miRNA - miR169 was up-regulated in tomato infected with *Botrytis cinerea* [20]. Similarly, twelve miRNAs, including miR156, miR159, miR160, miR164 and miR168 were found to be up-regulated in the stem bark of *Populus trichocarpa* following stem canker disease, caused by *Botryosphaeria dothidea* [21]. However, there is scanty literature on host plant miRNAs that are associated with insect attack and nematode parasites. A majority of the mentioned miRs is believed to play a role in biotic stress adaptation.

Micro RNA Interference Technology for Crop Protection

The identification of novel miRNAs in various pathogens and pests which are vital for their growth and development, and the discovery of novel miRNAs in the host plant that respond to pathogen and pest attack can be utilized for crop protection by two different strategies. First one, where we can over-express the host plant miRNA genes which responds to pathogen or pest attack for protection against these invaders. Secondly, we can also target (suppress) the miRNA genes of the target pathogens or pests which are vital for their growth and development by *in plant* expression of an antisense RNA specific to their miRNA sequences. Alternately, other miRNA gene silencing methods [1], for example, artificial microRNA technology [22] can also be adopted to target important miRNA genes of pathogens and pests for their control. These novel strategies may prove to be highly potent in protecting our crop plants against important pathogens and pests. However, these novel strategies have not been explored in plants for resistance against biotic stresses. But, this miRNA-based therapeutics appears to be quite promising in treating the most important human diseases, including cancers and heart diseases [23].

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Received September 21, 2012; Accepted September 21, 2012; Published September 23, 2012

Citation: Venkat Rajam M (2012) Micro RNA Interference: A New Platform for Crop Protection. Cell Dev Biol 1:e115. doi:10.4172/2168-9296.1000e115

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Acknowledgements

My research programmes on RNA silencing are generously supported by the Department of Biotechnology and Department of Science and Technology, New Delhi. I thank my graduate students, Ms. Sneha Yogindran and Mr. Manish Pareek for their help in literature collection.

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