Chapter 6

Noncoding RNA-Based Genetically Modified Crops: Concepts and Challenges

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INTRODUCTION

Genetically modified (GM) crops originated in 1983 with the concept of introducing a trait by expressing protein in the plant, which does not occur naturally in species. From 1996 onward the success story of genetically modified plants by expressing foreign protein revolutionized the concept of genetic engineering, for example, Bt crops, which are genetically engineered to produce a toxin with the goal of protecting the crop from insects dominate. Many more traits were addressed by expressing a foreign protein. The first genetically engineered crop product approved for sale (in 1994) was the Flavr-Savr tomato. It was developed based on the concept of noncoding RNA and the need for the introduction of noncoding RNAs (ncRNAs) for viral protection and to fine-tune host gene expression, was felt to incorporate the desired trait more effectively.

Noncoding RNAs are indispensable effector molecules of many biological processes like gene expression, defense against invading nucleic acids (viruses, transposons), chromatin maintenance, etc. in eukaryotic organisms (Lee et al., 1993; Baulcombe et al., 1996; Reinhart et al., 2002). The significance of ncRNAs in gene regulatory mechanisms came from the inadvertent discovery of the dsRNA-induced gene silencing phenomenon called RNA interference (RNAi) in *Caenorhabditis elegans* (Fire et al., 1998) and in plants (Waterhouse et al., 1998). Prior to dsRNA-mediated gene silencing effects in plants, occurrences of antisense RNA- and sense RNA (co-suppression)-induced gene repression were known in plants as post-transcriptional gene silencing (PTGS) (Napoli et al., 1990) and in fungi as quelling (Romano and Macino, 1992).

The discovery of the RNAi phenomenon coupled with research in the fields of transgenic plants and plant molecular virology has led to a greater understanding of small ncRNAs (sncRNA)-based gene silencing (Baulcombe et al., 1996; Hamilton and Baulcombe, 1999). Studies have proven that RNAi-derived ncRNAs are evolutionarily conserved in plants and play diverse gene regulatory roles (Vaucheret, 2006). This led to the development of ncRNA-based genetically engineered crops. ncRNAs are deployed as potential effectors of gene regulation whenever expression of certain proteins or associated traits is undesired. Major achievements of ncRNA-based crop genetic engineering are in the field of pathogen resistance wherein down-regulation of viral, fungal, or viroid gene expression is imperative in developing resistant crop phenotype. Besides the microbial pathogens, the phenomenon of ncRNA genetically modified crops has played a greater role in expressing dsRNA as plant incorporated protectants (PIPs) against insects, nematodes, and insect vectors that spread deadly viral diseases. In addition, expression of small ncRNAs has resulted in the elimination of toxic allergenic compounds in plants, adding to the nutritive value of many crops, and in secondary metabolite engineering. The role of ncRNA-based genetic modification in developing male sterile genetic lines is another salient application of ncRNAs in accelerating the process of crop breeding (Table 1).

VARIOUS ncRNA-BASED SILENCING PLATFORMS

The development of various ncRNA-based gene silencing platforms owes much to the scientific approaches associated with the development of virus-resistant transgenics (Figure 1). For developing virus resistance, as early as in 1986 the concept of coat protein-mediated resistance appeared (Powell-Abel et al., 1986). It involves expression of complete coat protein gene derived from viral genome to confer resistance against a particular virus. Genetically engineered papaya plants were developed using this concept for conferring Papaya ringspot virus resistance. Besides coat protein, other viral-derived nonstructural enzymatic proteins, like RNA-dependent RNA polymerase (RdRP), showed a similar response (Golemboski et al., 1990; Fitchen and Beachy, 1993). During the same period the role of RNA in conferring resistance has gained

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