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Virus Resistance and Gene Silencing in Plants Infected with Begomovirus

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Abstract: Gene silencing can occur either repression of transcription, termed Transcriptional Gene Silencing (TGS) or through mRNA degradation, termed post transcriptional gene silencing. Transcriptional gene silencing results from a marked decrease in tracription and hypermethylaton of genes affected. RNAi or PTGS discovered as a natural anti-viral system in plants. Studies revealed that geminiviruses which replicates in nucleus can induce PTGS and become the target for it. RNAi has a strong potential to reduce the infection of geminiviruses. Several studies are already conducted on different genes geminiviruses and that are used for generating virus resistance plants. Our main objective of present study is to develop resistance against geminivirus using a novel strategy based on RNAi. Here we summarise how the RNAi mechanism works against begomovirus infection in plants and how we can utilize it to reduce the losses.

Key words: Gene silencing, RNAi, PTGS, begomovirus, DNA-A, rolling circular amplification

INTRODUCTION

RNAi also known as RNA silencing and PTGS (Post transcriptional gene silencing), a natural mechanism works against viral infection in both plants and animals (Napoli *et al*., 1990). It is targeted by dsRNA which leads to the degradation of sequence specific viral RNA (Fig. 1) (Hamilton and Baulcombe, 1999). Double strandard RNAs generated by virus are recognized and cleaved into small interfering RNAs of approx 25nt by the RNAase III like enzymes (Bass, 2000) called as DICER. There are four dicer like enzymes identified in plants. First involved in miRNA biogenesis (Finnegan *et al*., 2003), second play role in viral siRNA production and third is responsible for transposon siRNA production (Xie *et al*., 2004). These siRNAs incorporate into the RISC (RNA induced silencing complex) and guide the degradation of viral RNA. Many viruses having ssRNA genome which released from the protein coat when they enter a cell. They replicate through virus encoded RNA-dependent RNA polymerase and generate sense and antisense RNA. These RNAs forms dsRNA and activate RNAi response (Hammond *et al*., 2000). Geminivirus like viruses which have ssDNA genome replicate through rolling circle mechanism and forms dsRNA structure by which they induce RNA silencing in plants.

Viral vector with specific genes can be use to induce RNA gene silencing or PTGS in many plants (Covey *et al*., 1997). This approach known as Virus induced gene silencing (Kumagai *et al*., 1995). In this approach hairpin transcripts are produce by inverted repeat as RNAi technology. This is useful in determination of gene function in transformation recalcitrant species. In the present study conducted in the Shekahawati region of

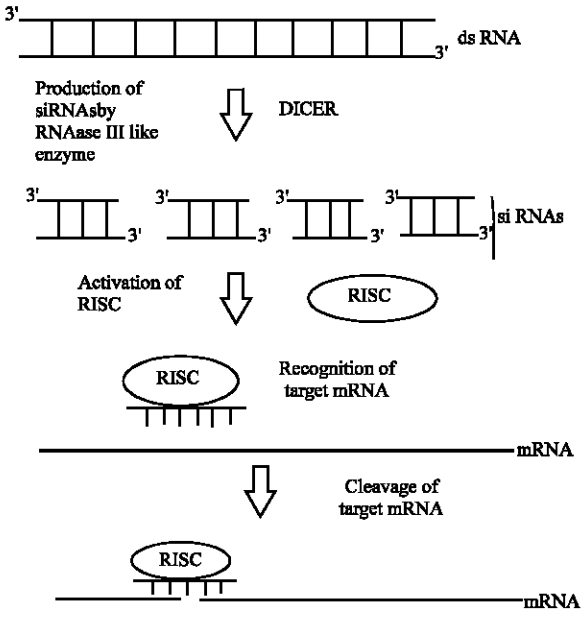

Fig. 1: General mechanism of iRNA

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India, are summarizes the different approach conducted for the RNAi gene silencing against the begomoviruses.

Begomovirus: Begomovirus is the largest genus of family *Geminiviridae*, which is responsible for the losses of crops worldwide. They are known to induce a diversity of symptoms in the plants they infect. However, leaf curling and other leaf distortions seem to be the most frequent symptoms associated with infections by begomovirus (Harrison and Robinson, 1999). Sever leaf curling symptoms were observed in recent years but no virus associated with such symptoms was reported (Tiendrebeogo *et al*., 2008). The high rate of symptoms severity on the plant in different region can be demonstrated by susceptibility of some cultivars in the proposed fields (Adjata *et al*., 2008). The virus is transmitted by white fly (*Bemisia tabaci*) and infects dicots. Begomoviruses contain genome either mono-partite or bipartite (Briddon *et al*., 1989). Bipartite begomoviruses contains two molecules of small single strandard circular DNA i.e., DNA-A and DNA-B which are same in size of 2.7 kb. These molecules are similar only in the Common Region (CR) of 200nt (Fauquet and Mayo, 2001). CR has significance in replication and transcription. CR is the site of both 1st and 2nd strand synthesis (Arguello-Astorga *et al*., 1994).

DNA-A has five ORFs (Rep, TrAp, REn, AC_4 and CP) which is responsible for replication and encapsidation (Elmer *et al*., 1988) whereas DNA-B has two ORFs (MP and NSP) which controls the movement in the host (Fig. 2) (Noueiry *et al*., 1994). Generally, monopartite begomoviruses is associated with a satellite DNA (β DNA) which is responsible for the symptom development (Saunders *et al*., 2000; Gaur and Rathore, 2009). All the geminiviruses replicate through Rolling Circle Replication (RCR). The organisations of genome and gene function are conserved in the begomoviruses. (Rojas *et al*., 1998).

In the initial stage, the ssDNA transported to the cell nucleus where it replicates through rolling circle mechanism. After replication, CP binds to replicated ssDNA in nucleus and makes a virion particle. Than it translocate to the cytoplasm for systemic movement. MP and NSP protein of DNA-B helps in the systemic infection of the virus (Pinner *et al*., 1993).

RNAi: Natural mechanism against viral infection: There are different studies which prove the RNAi mediated resistance against different begomoviruses. This strategy gives a high frequency of resistant plants. There are three ways identified for the gene silencing: cytoplasmic short interfering (siRNA) silencing, silencing of endogenous mRNAs by microRNAs (miRNAs) and DNA methylation and suppression of transcription (Baulcombe, 2004). Cytoplasmic siRNA silencing occurs in response to a viral infection in plants (Ahlquist, 2002). Genetic engineering of sense and antisense RNA in trangenics used effectively against Tomato golden mosaic virus (TGMV) (Day *et al*., 1991). It has been reported that Tomato yellow leaf curl Sardinia virus (TYLCSV), a monopartite begomovirus (Lucioli *et al*., 2003) and cassava infecting bipartite begomovirus namely Indian Cassava Mosaic Virus (ICMV) triggers the PTGS system in infected plants (Mendez-Lozano, *et al*., 2003) with the accumulation of virus specific siRNAs where TYLCSV related siRNAs are specific to viral rep and C4 genes (Lucioli *et al*., 2003). Another bipartite member of begomovirus genus Pepper golden mosaic virus (PepGMV) infects dicotyledonous crops like pepper, tomato, tomatillo and tobacco (Mendez-Lozano, *et al*., 2003). PepGMV-infected pepper plants show a recovery phenotype followed by the presence of virus-specific siRNAs (Carrillo-Tripp *et al*., 2007). In this way, its an interesting system to study the gene silencing in viral infected plants.

According to the previous studies there is a new upcoming group of monopartite begomovirus which causes infection in Okra (*Abelmoschus esculentus*) and leads to the economical losses. Bhendi Yellow Vein Mosaic Virus (BYVMV) is a complex monopartite begomovirus which requires satellite DNA component for the production of typical symptoms (Jose and Usha, 2003). Cotton leaf curl disease is very common in Indian sub-continent. The resistance development against this disease is challenging because of the diversity of the

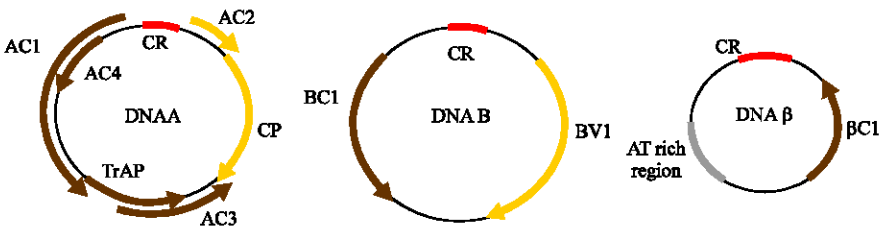

Fig. 2: Typical genome organization of a Begomovirus

viruses. There is requirement of such resistance system which can work against the broad range of viruses. In this case, the PTGS is most reliable strategy because its RNA mediated and induces resistance against invading virus. An approach to target Rep and AV_2 gene by antisense expression is more successful (Sanjaya *et al*., 2005). RNAi involved resistance against DNA virus also reported in Cassava. Many copies of AC_1 gene of ACMV found to be accumulated in plants. These siRNAs are effective against ACMV and two other geminiviruses (Chellappan *et al*., 2004). The AC_1 sequence is conserved (66-77%) among geminiviruses through which RNA silencing proved to be an effective strategy to produce stable and broad geminivirus resistance. Similarly, RNA induced gene silencing was studied with TYLCV. Non coding conserved region were taken from the different geminiviruses and used to make construct and targeted for the broad resistance against tomato leaf curl viral disease (Abhary *et al*., 2006). Golden mosaic disease of *Phaseolus vulgaris* is caused by BGMV (Bean golden mosaic virus). This is the largest constraints in bean production. Many strategies of genetic engineering are employed for the resistance of this virus. In which production of truncated defective gene (Antignus *et al*., 2004) and antisense RNA is included (Asad *et al*., 2003).

RNAi as a potent tool: The basic use of RNAi is to produce virus resistant crops. This natural process is interesting because it provides limits the viral infection (Voinnet and Baulcomb, 1997). Initially its importance was not recognized due to a misconception that it is limited to petunia with few other species (Cogoni and Macino, 1999). Nowadays it's the hot topic of molecular biology. RNAi is a favorable tool to knock down or silence a gene expression because it can target multiple gene family member by same RNAi inducing transgene. Another advantage of RNAi is related to its dominant aspect. It can knock down genes in polyploidy genome which contain four or more orthologs. Recent methods for RNAi silencing in plants are based upon an inverted repeat mRNA expression (Waterhouse *et al*., 1998). The best parts of these methods are that they are useful in both stable transformation and transient transfection. Few studies suggested an approach to improve the efficiency of silencing by the insertion of an intron between the inverted flanking target sequences (Smith and Gross, 2000). These RNA silencing strategies are suitable for crop improvement by reduce infection of RNA or DNA virus. Many plant viruse have ssRNA genome, which can deliver gene in PTGS system by inoculation of viral vector transcripts. In the case of DNA virus like geminivirus it become more easy because it need only the viral DNA. Except this, cDNA of viral genome can be introduced in plant cell with the binary vector. There are several traditional gene knock-out methods which require transformation and tissue culture but PTGS is rapid and more effective. RNAi is only six years old. Research on RNAi is increasing day by day yet this field is in its infancy. RNA gene silencing is the mechanism which is conserved across the kingdoms. Interesting feature of geminiviruses is that they induce gene silencing without having dsRNA phase in their replication. They activate host PTGS system by the siRNAs which are specific for viruses. Geminiviruses encode AC2 and AC4 PTGS suppressor proteins that indicate the different evolution of this virus in respect to interaction with the host. This has been clear with the previous studies that the geminiviruses and their statellite DNA are very useful for the gene silencing in various plants and also in post-genomic studies. Though a lot of studies has been done on RNAi mechanism related to geminiviruses but still many questions are there to be answered. As geminiviruse does not have any dsRNA structure in their replication cycle then how do they induce RNA silencing. There are some unproved theories which supports it, such as presence of overlapping transcripts and the presence of several mRNAs but no authenticated proof. How PTGS is controlled by suppressor proteins. Is there any inter-action of these suppressor proteins with miRNA pathway? The answers of these questions can help us to understand PTGS more precisely. As the new geminiviruses and their satellite DNAs are emerging rapidly, they can be devastating for the crops. To improve food production it must be controlled. For this we need to identify the common region of viral sequences to target a broad range of viruses and their PTGS suppressor system. RNAi or PTGS can be utilize as a potential tool against emerging geminiviruses to protect the crops. In respect to the present researches on RNAi can be considered as revolution in plant biotechnology and an effective tool in functional genomics. It's a broad application of biotechnology from molecular biology to gene therapy. Virus induced gene silencing has an enormous power to down regulate the endogenous genes that are virus- specific.

CONCLUSIONS

In this era of genomics, scientists are trying to evolve new methods to study gene function on genome. There are much advance techniques in this field like genome sequencing and micro-arrays but they require efficient procedures to study. RNAi is easy to approach and it can be use in broad spectrum. Tomato yellow leaf curl virus is

the worst virus considered in the world which causes 100% crop losses. There are approx. 60 forms of viruses which affect crops. They are highly prone to mutation and attacks phloem system. RNAi is the favorable tool to fight against this emerging threat of geminiviruses. RNAi gene silencing is helpful to identify gene function because many other different methods of gene knock out in plants generally do not produce phenotype under normal condition. RNAi is the pathway for silencing of whole gene families or unrelated genes through targeting of specific gene sequences or by using a single RNAi construct which contains several target sequencing. According to the present studies it has been proved that RNAi mediate the inhibition of replication of different viruses. Generally it is effective against RNA viruses which contain linear or fragmented genome and also DNA viruses. RNAi with different strategies has given new possibilities for control of many viruses.

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