A STUDY ON siRNA MEDIATED RESISTANCE AGAINST BEGOMOVIRUS(ES) CAUSING PAPAYA LEAF CURL DISEASE

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Abstract

Background

The phytoparasites infect garden and crop plants, causing economic losses costing millions of dollars every year. These phytoparasites also hamper global crop production by disturbing host plant's metabolic and developmental pathways that results into decrease in fruit and grain yield. Thus, the infection due to these parasites is a universal threat to sustainable food security and increases global hunger rate.

The viruses are phytoendoparasites, which make an entry into a plant tissue and take over the host plants molecular machinery to replicate and spread across cells. This phenomenon leads to production of developmental and physiological anomalies resulting into a collection of symptoms, collectively called disease. Major crop diseases are identified by mosaic, leaf curl, vein yellowing, leaf roll and a combination of any or all of the mentioned symptoms. These symptoms affect floral and vegetative organs with varying severity leading to abnormal/deformed fruit production and reduced/degraded grain yield. Therefore, prevention strategies need to be developed against these viral diseases. Geminiviruses are one of the largest group of plant infecting virus, which infect all dicot plants and crops of the world. Begomoviruses belong to Geminivirus, are omnipresent sub-group causing huge losses to farmers across globe, especially in tropical and sub-tropical regions of the world. Recently, a begomovirus epidemic in India infected cotton crops, accounting to more than Rupees 150 million in crop damage. This economic loss is reflective of the magnitude of damage due to begomovirus and its vector i.e. *Bemisia tabaci* Gennadius, also known as Whitefly.

Several strategies were applied in past to develop begomovirus resistant transgenic crops such as anti-sense RNA and RNA interference techniques. These techniques involve plant and pathogen derived factors as the potential candidates providing antiviral resistance in plants. The molecular components of pathogen i.e. nucleic acids and proteins or the host immune machinery i.e. siRNA components such as proteins and non-coding RNAs can act as a source of resistance against

these viruses. Mechanistically, the begomovirus group is a single stranded DNA virus, which replicates by forming a double stranded DNA intermediate, followed by transcription and translation of viral proteins. The multifunctional proteins encoded by viral open reading frames are capable of supporting viral machinery as well as helping in its survival by suppressing host immune machinery. These host immune suppressors encoded by viral ORF's are major contributors to the high rate of viral infection prevalent in a variety of crops worldwide.

Origin of problem

Papaya leaf curl disease (PLCD) is caused by a group of begomoviruses, which has wiped out papaya cultivation in many regions of northern India. It is reported to be identified by leaf curl, vein swelling, lamina crumpling, stem looping and deformed fruit formation, eventually leading to plant death. It is mainly caused by begomovirus such as *Papaya leaf curl virus*, *Chili leaf curl virus*, *Tomato leaf curl virus*, *Papaya leaf crumple virus* and their associated betasatellite molecules; resulting into mild to severely damaged crops. Since, this disease is caused by many genetically distant species of begomoviruses; therefore, it is difficult to plan intervention strategy against this disease group.

The diversity of infecting begomoviruses in this disease group demands a generic approach to develop a disease resistant crop variety. For this purpose, a deep insight into the genomic components is necessary i.e. to understand implication of high frequency of recombination and mutation induced changes affecting nucleic acid composition and patterns. The conserved patterns across genera of begomovirus in the PLCD complex can be used to design effective, broad range and physiologically feasible strategy for developing papaya leaf curl disease resistant crops. Therefore, a comprehensive study was conducted using *in-silico* and molecular approach to understand the molecular complexity of PLCD complex at the genomic level.

Proposed hypothesis

The ‘proof of concept’ is an essential part of a good strategy, therefore, a field isolate of one of the begomovirus species causing papaya leaf curl disease is isolated, identified and used to demonstrate the efficacy of the resistance strategy proposed in this study. Severe leaf curl causing monopartite begomoviruses identified were found to be associated with a betasatellite viral component. Both viral components are essential for infection and thus, cause PLCD in papaya plants.

The begomovirus genome was introduced into a plant binary expression vector as a dimer, to produce viral particles inside host plants. The viral multi-functional proteins initiate virus mRNA transcription using host machinery and establish a begomovirus infection. Plant’s innate immunity induces a molecular response against virus genomes by producing 21-24-nts size dsRNA fragments called small interfering RNAs (siRNAs). These molecules act in homology dependent manner and suppress viral infection by cleaving target viral mRNAs into small fragments.

Our strategy focused on the same principle i.e. utilizing siRNA producing long hairpin fragments incorporated into a cassette containing target viral mRNA region in sense and anti-sense orientation. This is how a conserved Upper half fragment of DNA-A was introduced into a long hairpin like siRNA construct. This siRNA construct is mobilized into a *Nicotiana benthamiana* plant with the help of an *Agrobacterium* strain, bearing capability to transfer any foreign DNA present between its T-DNA borders. These plants were challenged with virus inoculum (infectious clones of DNA-A and betasatellite components) to investigate siRNA constructs efficacy in imparting resistance/tolerance against the papaya leaf curl disease complex virus component. Decline in symptom severity and loss of viral population in host plant is a clear indicator of action of siRNA-mediated resistance in host plants. Therefore, the viral population was monitored at different stages of plant growth using various molecular techniques.

Proposed outcome of this study

The study conducted in this report has potential to provide a cue for a generic resistance strategy against the PLCD causing begomoviruses. The begomovirus, especially those having associated betasatellites have propensity of spreading at extreme rate in fields. The strategy presented in this study has flexibility to incorporate number of viral fragments in a single siRNA hairpin construct for developing resistance against these begomoviruses too. In past, a long hairpin siRNA construct has shown ability to carry fragments up to 500kb in length with high efficiency, hence multiple fragments from different genomic components can be incorporated to provide resistance against a group of begomovirus. Through this study, we would like to show if larger fragments containing functionally important ORFs could be used to impart resistance against PLCD causing begomoviruses. Therefore, this study significantly contributes to the area of plant virus resistance. It can be helpful in development of resistant crops against begomoviruses and associated viruses. Thus, farming with virus resistant varieties will result in high grain and fruit yield, which in turn can significantly contribute in reduction in global hunger rate and enhanced food security and sustainability.