DEVELOPMENT OF GENERIC RESISTANCE STRATEGY AGAINST PaLCuV INFECTION BASED ON VIRAL GENOMIC VARIABILITY

ABSTRACT
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Background

Papaya is a popular horticultural crop throughout the world grown in tropical and subtropical countries. Unfortunately, severe papaya leaf curl infection caused by begomoviruses hampers the crop cultivation, marketability and economy at large. Although India is the largest papaya producing country but ever expanding presence of begomoviruses throughout India is a major concern for papaya growers. To understand the effect of geographic locations, climatic conditions and host ecology on leaf curl disease, it is necessary to perform surveys at different locations at specific time period to collect infected samples. Study of infected plant materials provide the information about virus distribution, introduction of new begomoviruses, alternate hosts for the viruses and long term biological interactions of viruses with their plant hosts, which may provide necessary information to design any effective management strategy against the disease. Symptomatology of leaf curl disease is thickening of leaf midrib and veins, downward, upward leaf curling, mottling of leaves and stunted plant growth. Infection of several begomoviral species, betasatellite molecules and mixed viral infection produce diverse symptoms in addition to above mentioned general disease symptoms on plants which makes the diagnosis of specific viral isolates difficult.

Detection of these phloem inhabiting begomovirus infections in agricultural crops is mandatory for the earlier disease recognition. Scanning electron microscopy (SEM) is a basic technique to observe internal structure of leaf tissues to examine the changes in leaf structure due to viral infection and can be successfully used in preliminary detection of disease symptoms. Begomoviruses are a group of plant pathogen that emerged as a potential threat for agriculture worldwide causing significant crop losses and their expanding host range is also an attentive measure for global papaya production. Although these viruses were observed long time back but information available about them is still very less. Recombination and genetic variability among different viral genes play a very important role during evolution which is a reason for emergence of different begomovirus species, betasatellite molecules and their complex distribution. While developing any disease resistance strategy it is necessary to emphasize on generic resistance against these begomoviruses (PaLCuV isolates) infecting papaya in India to overcome the problem of complex distribution of begomoviruses.

Common management practices used to control viral diseases include appropriate cultural practices by using resistant cultivars and virus free propagating material, use of insecticides to control vector population etc. Nearly all the control practices including physical, chemical as well as biological, which are being followed now a days have several drawbacks and therefore not much effective against papaya leaf curl virus (PaLCuV) for combating leaf curl disease in papaya. Development of resistant papaya crop against geminiviruses to control viral infection is one of the major challenges for plant virologist till date. We are proposing a novel siRNA based strategy for the development of geminivirus resistant papaya crop in this study. This strategy utilizes RNA interference (RNAi) mechanism to disrupt virus survival by silencing the expression of target viral gene. Short interfering RNAs (siRNAs) of 21-23 nucleotide long worked in a highly homology dependent manner that selectively knock down the expression of the target gene during RNAi mechanism. Since siRNA is a homology based sequence specific strategy, information on the genomic variability/ conserved regions of begomovirus isolates infecting papaya will be of great importance to design generic resistance strategy against several begomoviruses and their strains infecting papaya. This is of particular importance as the siRNA mediated strategy developed against one virus isolate may not work against another hence, targeting of conserved regions of viral genes among all the possible viral isolates taken into account in this study was considered to develop a broad resistance. Investigation of sequence homology/levels of variability of papaya infecting begomoviruses (PaLCuV isolates) are necessary mainly to select the most appropriate conserved sequence(s) to use as siRNA targets and also to assist in the prediction of stability of resistance. Hence, present work includes analysis of genetic variability among existing begomovirus isolates to design *insilico* siRNA to develop generic resistance strategy against papaya leaf curl disease.

Results

In present study, the diversity and distribution of begomoviruses infecting papaya in India was studied to develop a generic resistance strategy against papaya leaf curl disease. For this study we have surveyed different geographical locations to collect leaf tissues affected with severe leaf curl symptoms. Changes in leaf tissues after viral infection were analyzed through SEM to reveal disease symptoms at cellular level that

clearly differentiate between the morphology of healthy and infected papaya leaves. Further, all the symptomatic leaves were screened for the presence of begomoviruses using universal degenerate primers specific for DNA-A, DNA-B component and betasatellites to check their association with leaf curl disease of papaya. Complete DNA-A genome of begomoviruses were amplified through RCA and degenerate abutting primer pairs (PSBP-F & PSBP-R) were designed during this study. However, reports on distribution of different begomoviruses and satellite molecules on papaya leaf curl disease occurring in India are limited. So, we have studied the diversity of begomovirus complexes infecting papaya and their diversity to know their distribution across India and reveal the association of unusual begomovirus-betasatellite complex.

Total 82 samples were collected from various locations all over India. The whitefly transmitted begomoviruses were detected by PCR with several universal degenerate primers pairs specifically designed for begomoviruses. Total 16 isolates were preceded for the complete characterization of DNA-A component of begomoviruses and their associated betasatellite molecules during study. Complete DNA-A component of 16 isolates and 10 associated betasatellite sequences were characterized and the presence of 10 distinct begomovirus species and 8 different betasatellite molecules infecting papaya were identified. Among all identified begomoviral species one novel species i.e. papaya severe leaf curl virus (PaSLCuV) showing less than 91% identity with the previously available begomoviruses was identified which has not been previously reported. Subsequently, sequences of two PaSLCuV isolates namely PSB8 and PSB 14 with accession no. MH988457 and MH988458 have been submitted to GenBank from this study.

Muscle algorithm implemented in mega v6.0 software was applied to infer multiple sequence alignment and maximum likelihood method for the evolutionary background of begomovirus isolates identified during the work. Alignment of all genes of DNA-A component of papaya infecting begomoviruses revealed AC1, AC2, AV1 and AV2 genes as highly conserved among all begomoviruses while AC3 and AC4 genes were highly variable throughout the genera. Recombination analysis using RDP4 and phylogenetic analysis reconstruct the plausible history of papaya infecting begomoviruses and associated betasatellites that showed their diversification and migration from different

places and plant hosts. Phylogenetic studies of begomoviruses and betasatellites identified during this study showed relationship with their closely related species thus illustrating their origin from their closely related begomovirus species. Recombination analysis also provided the evidence for their evolution and explained several recombination events between viruses co-existing in phylogeographic analyses suggesting that expanding range of begomoviruses on papaya in India is probably the result of genomic variability and recombination events occurred during evolution and adaptation. During *insilico* siRNA search AC1, AC2, AV1 and AV2 genes are found as good candidate for siRNA based approach as they have conserved regions across begomovirus genomes, infecting papaya plants. Hence, RNAi using siRNA can be considered as a preferred approach to develop broad spectrum resistance against begomoviral complexes infecting papaya.

Conclusion

Rapid and extensive virus spread in India was demonstrated through identification of various begomoviruses causing leaf curl disease in India. SEM has been successfully employed to investigate the begomoviral infection in the crop through morphological and anatomical differences between healthy and infected plant leaves. To study the impact of insect and human transmission extensive sampling in papaya growing regions was performed. The isolation and analysis of full length DNA-A and betasatellite sequences demonstrated that papaya leaf curl disease is caused by complex of different begomovirus species. Results of our study indicate that various begomovirus species and betasatellites are infecting papaya plants naturally. Begomoviruses and betasatellite molecules associated with papaya leaf curl disease were found associated in recombination events during evolution that explains the influence of recombinations in begomovirus diversity. These begomoviruses may infect the papaya crop while having weeds as secondary host during off-season. Our results indicate that different begomovirus species and their strains associated with the disease have a complex history of recombination with a relevant contribution of interspecific recombination and various hosts.

The data reported here may be useful in the development of control measures against begomoviruses. Our approach has based on *insilico* siRNA designing from conserved

regions among different begomoviral genes to develop a stable and sustainable disease management. Current efforts are focused on developing a sustainable disease management strategy based on genomic variability among begomoviral isolates identified on papaya. Recent progress in determining the variability in the viral genome/ betasatellites are discussed in the context of designing of siRNAs against all viral genes showing its potential for genetically engineered disease-resistant plants.