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GENETIC VARIABILITY AND POTENTIAL RECOMBINATION EVENTS IN THE P0 GENE OF SUGARCANE YELLOW LEAF VIRUS CAUSING YELLOW LEAF DISEASE IN SUGARCANE

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Sugarcane is an important commercial crop and has served as a source of sugar for hundreds of years; recently it is being used to produce bioethanol, a renewable bio-fuel energy source. The crop productivity is seriously affected by many fungal diseases like red rot, smut and wilt. Recently, we found epidemic occurrence of yellow leaf disease (YLD) caused by Sugarcane yellow leaf virus (SCYLV) in different parts of the country. The virus infection causes up to 43% reduction in plant growth and ~34% in juice yield in susceptible varieties of sugarcane (Viswanathan et al. 2014). The disease is also responsible for the poor performance of ruling varieties that is referred as 'varietal degeneration' (Viswanathan and Rao, 2011). Since the disease causes serious economic damages to crop productivity, disease management through meristem culture and developing disease free nurseries in the factory areas has been recommended. To manage the disease in a long term basis, studies are emphasized on the host resistance and/or developing transgenic lines resistant to SCYLV. Earlier, variation in SCYLV isolates occurring in the country was studied in detail and this revealed occurrence of three virus genotypes (Viswanathan et al. 2008). Further studies characterized the complete genome of the virus and established the genotype SCYLV-IND and its close relation with the genotypes reported from China (Chinnaraja et al. 2013).

The diversity and changes in the genetic structure of plant virus populations are important aspects in plant virology and may be highly relevant for developing strategies to control viral diseases of crop plants. SCYLV possesses a positive-sense single-stranded RNA of ~5900 nucleotides (nt) in length, which contains six recognized open reading frames (ORFs, 0-5) and three untranslated regions (UTRs), the 5′ UTR, the 3′ UTR, and the intergenic UTR located between ORF2 and ORF3. The P0 protein encoded by ORF0 is a suppressor of post-transcriptional gene silencing (PTGS). ORF1 overlaps ORF0 and ORF2 in the 5′ and 3′ termini, respectively, encoding a serine protease (P1). ORF2 encodes the RNA-dependent RNA polymerase (RdRp), ORF3 encodes the viral coat protein (CP). ORF4 encodes a movement protein (MP) and ORF5 is responsible for aphid transmission of the virus.

Recent studies of Lin et al. (2014) indicates that the P0 gene would be a good candidate to assess the phylogenetic relatedness based on amino acid sequence identities among different genotypes if complete SCYLV genome sequences were not available for new isolates. The P0 protein plays a critical role in virus infectivity, as P0 is an RNA silencing suppressor associated with virus pathogenicity (Mangwende et al. 2009). Since P0 shows high genetic variation it was previously proposed as a sensitive alternative diagnostic segment to detect different SCYLV genotypes (El-Sayed and Komor, 2012). The purpose of the present study was to investigate and characterize putative recombination events and selective constraints in the P0 gene of SCYLV worldwide and finally to determine the predicted evolutionary relationships among their respective genotypes. This was achieved by analyzing the P0 sequences of 55 accessions retrieved from GenBank including the 19 SCYLV sequences determined in this study.

Recombination analysis was performed with the Recombination Detection Program v4.16 (RDP4) with input of ClustalW aligned sequences. Recombination events, likely arising parental isolates of recombinants, and recombination breakpoints were identified using the seven algorithms available in the RDP4 package (RDP, GENECONV, CHIMAERA, MAXIMUM CHISQUARE, BOOTSCAN, SISTER SCAN and 3Seq). To determine the site-specific selection pressures acting on the P0 gene, the ML codon-substitution model implemented in the CODEML program of the PAML4 package (Yang. 2007) and four different codon-based maximum-likelihood methods implemented on the Datamonkey server (http://www.datamonkey.org) Delport et al. (2010) were used to estimate the rate of non-synonymous (dN) and synonymous (dS) substitutions at each codon site. DnaSP version 5.10 (Librado et al. 2009) was used to analyzed the data by Tajima's D test, and Fuand Li's D and F tests.

The 19 P0 sequences of SCYLV isolates originating from India were determined, analyzed and compared with sequences of 55 other isolates of SCYLV from different genotypes (BRA, PER, HAW, REU, CUB, CHN1 and CHN3). Comparative sequence analysis of 19 Indian P0 isolates revealed 95-100% nt sequence identity among themselves and 69-98% identity to the other SCYLV isolates compared in this study. Among the different genotypes, Indian isolates were closely related with CHN1 and CUB genotypes and showed 95-98% nt sequence identities with them. In the deduced aa sequences, Indian isolates shared 93-100% identity among themselves and 93-97% and 95-98% with the isolates from CHN1 and CUB genotypes, respectively. The amino acid distances for all 74 of the available P0 sequences, including 19 Indian isolates, were used to generate the Phylogenetic trees. The neighbor-joining tree for the aligned P0 sequences at the amino acid level showed that there are seven major groups. All the Indian isolates (IND) clustered together and showed a close relationship with CHN1 and CUB genotypes.

In the present study, the recombination sites were detected throughout the P0

sequences of different SCYLV isolates. In total, 74 of the 54 SCYLV-P0 isolates analyzed have showed the evidence for recombination under the six recombination detection methods. Of the 54 putative recombination sites, observed maximum number of (36) recombination sites were observed in IND isolates. It is noteworthy that the Indian isolate CBD1135 and BRA isolate SP811763 appear as a major parent of the different P0 genotypes reported worldwide. Results of the selection pressure analysis of 74 SCYLV isolates revealed that most of the codons in the P0 gene were under negative selection or neutral evolution except for codons 225, 249 and 255, which were under positive selection.

To date, SCYLV has been divided into nine different genotypes and of these genotypes, BRA is widespread worldwide. The HAW and PER genotypes tend to cluster together as HAW-PER due to close genetic relatedness. In China, SCYLV is mostly distributed in sugarcane-growing areas, and at least six different SCYLV genotypes (CHN1, CHN2, CHN3, BRA, PER and CUB) have been identified. In the present study more number of P0 sequences used for analysis confirmed with the earlier studies (Chinnaraja et al. 2013; Lin et al. 2014) that Indian isolates belong to IND genotype and they showed close relationship with CHN1 and CUB genotypes.

Recombination observed in 74 P0 sequences of SCYLV supports earlier observations that recombination is a dominant feature of SCYLV evolution. The greater number of P0 sequences available in GenBank, particularly from India, allowed us to identify the more robust occurrence of recombination sites and hotspots, a major constraint in previous studies (Lin et al. 2014, Chinnaraja et al. 2013). According to the mutational deterministic hypothesis of Kondrashov, (1998), mutations are mainly deleterious, causing mutational loads and creating isolates that contain many slightly deleterious mutations. On the other hand, recombination creates genotypes with different levels of deleterious mutations, and selection forces remove genotypes with a larger number of deleterious mutations. This hypothesis may explain the 54 recombination events discovered in the present study, and it is possible that the recombination, along with strong negative selection, enhances the speed of elimination of deleterious mutations in the P0 gene of SCYLV. Similar results have been described for the HC-Pro genes of Sugarcane streak mosaic virus (SCSMV) (Bagyalakshmi et al 2012) and Potato virus Y (Tian et al. 2011).

Conclusion

Plant viruses are subjected to purifying selection, which is the major selection force shaping their evolution and P0 of SCYLV is no exception. Using more number of methods and P0 sequences to detect the recombination, IND isolates from India were shown as major contributor in genome architecture of all other genotypes of the virus. The maximum sequence variability observed in the P0 genes of Indian SCYLV isolates and involvement of Indian isolates in genetic recombination with all other genotypes

suggest an ancestral Indian origin of this virus.

Future line of work

P0 of most SCYLV isolates exhibits RNA silencing suppressor (RSS) activity, although at varying efficiencies. Conversely, P0 of SCYLV isolates may display no RSS activity under the experimental conditions used. P0 proteins of two closely related SCYLV isolates may show strain-specific differences in their effects on RNA silencing. Further studies are required on whether P0 of the closely related SCYLV genotypes display a similar function and whether this ability vary among the isolates. Further studies are in progress to demonstrate the difference in the suppression induced by P0 of SCYLV is long-lasting in agro-infiltration experiments.

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