Name: Sadia Akhtar

Supervisor: Prof. Jawaid A. Khan

Department: Biosciences

Topic: Designing and development of RNAi constructs for Cotton leaf curl disease

Abstract

Cotton leaf curl disease (CLCuD) has been a serious threat to successful cotton production throughout the Indian subcontinent. CLCuD is mainly associated with *Cotton leaf curl virus* (CLCuV) species, a whitefly mediated begomovirus, and its associated satellite (betasatellite DNA). Majority ofthe plant viruses have evolved silencing suppressor proteins to evade the Post-transcriptional gene silencing (PTGS) defense mechanism of the plant initiated by the plants against the viruses. Begomoviruses produce three different suppressor proteins encoded by ORFs viz., C4,C2 and βC1 (in betasatellite molecules).

The major objective of this study was to develop RNAi-mediated resistance against CLCuV by targeting the viral suppressor genes, *C4* and *βC1* produced by the virus. Source of *C4* gene was from *Cotton leaf curl Rajasthan virus* while the *βC1* gene was derived from Cotton leaf curl virus betasatellite obtained from leaf samples from Sirsa, Haryana. The *C4* gene was characterized- cloned in pDrive cloning vector and sequenced. The *C4* gene shared 98% sequence homology with *Cotton leaf curl Rajasthan virus* - India [India:Sri-Ganganagar:2008] (GQ220850.1). The βC1 gene was cloned in pDrive cloning vector and sequenced. The *βC1* gene shared 99% homology with Cotton leaf curl Multan betasatellite-[In:Abohar:2003] (JF509752). In addition to the maximum homology with CLCuV, these cloned genes also revealed high nucleotide sequence homology varying from 88-94% withC4 and 96-98%with*βC*1 genes of other geminiviruses that affect the crop production in economically important crops like tomato, etc. With this level of high homology ,RNAi constructs derived from the *C4* and *βC1* genes could effectively be utilized against a wide range of important geminiviruses, and thus confer a broad spectrum resistance for the development of viral resistant transgenic crops.

In this study, two RNAigene constructs containing *C*4 and *βC*1 genes for silencing of CLCuV and betasatellite, respectively were developed. C4 is a pathogenicity determinant which is involved in PTGS suppression. βC1 is a pathogenicity determinant and has an important role in symptom induction- which it accomplishes by suppressing the silencing mechanism of plants.

In order to confirm the efficacy of the cloned genes in imparting tolerance against CLCuD in target plants, the *C*4 and *βC*1 genes were cloned into the RNA silencing vector pFGC1008. *Agrobacterium tumefaciens*GV3101 containing the C4RNAi and βC1RNAi constructs were used to develop transgenic tobacco which showed resistance to CLCuD. The regeneration of transformed leaf discs showed good results in regeneration under hygromycin selection pressure (25mg/l).About 70% of the transgenic plants from C4RNAi constructs and 70% βC1RNAi constructs showed positive results.

The shoots with positive hybridization showed multiple copy insertion ranging from 1 to 4 in the transgenic tobacco transformed with the *C4* and *βC1* genes. A possible reason for the transgenic lines showing resistance could be owed to the presence of multiple copy insertion of the transgene in plant genome. These multiple copy insertions may lead to a higher transcript production and thus high amount of siRNA generated due to the degradation of transcript. The T0-generation plants containing C4RNAi and βC1RNAi constructs displayed variable degrees of resistance towards the CLCuV when challenged with viruliferous whiteflies. The majority of inoculated transgenic plants remained symptomless with two lines of C4RNAi, lines C4RNAi-6 and -8, showing 100% resistance (no plants with symptoms) after inoculation with CLCuV and 2 lines of βC1RNAi, lines βC1RNAi-5 and -7 showed 100% resistance after virus inoculation in both T0 and T1 generations.

From this study, it can be concluded that C4hpRNA and βC1hpRNA have the ability to inhibit CLCuV DNA accumulation which makes these genes an attractive candidate gene for engineering CLCUV resistance.

My results thus indicate that ORFs C4 and βC1 can be used as potent targets to develop a specific RNAi-mediated resistance in cotton plants against *Cotton leaf curl virus.*