

CRISPR/Cas9 based virus resistance in cotton

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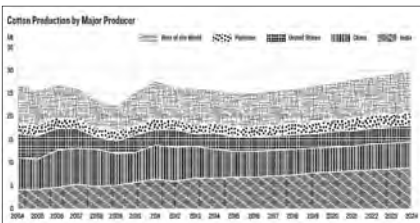
BACKGROUND: Pakistan is the fifth largest producer of cotton in the world, the third largest exporter of raw cotton, the fourth largest consumer of cotton and the largest exporter of cotton yarn. About one and half million farmers (out of a total of 5 million) cultivate cotton over 3 million hectares, covering 15% of the cultivable area in the country. Cotton and their products contribute about 10% to GDP and 55% to the foreign exchange earnings of the country. In recent years, yield of cotton has become static, rather it is declining due to the infestation of insects, pests and diseases. One of the major factors for low yield is the infestation of cotton leaf curl disease (CLCuD), as last epidemic of the disease resulted in a heavy loss of 7.4 million bales of cotton with an estimated value of 5 billion US\$. Engineering of resistance in cotton cultivars against CLCuD through RNA-directed Cas9 is a powerful tool to tackle the viral disease in cotton. In this study, we are using CRISPR/Cas system for genome editing technology in cotton to combat CLCuD. Development of transgenic cotton resistant to CLCuD using Cas9-gRNA technology is our main focus in this study. We are targeting *Rep* gene of cotton leaf curl viruses (CLCuVs) to specifically modulate its activity for engineering resistance in cotton. *Rep* protein binding iterons in promoter region of *Rep* gene are our potential target to inhibit the replication and ultimately suppression of CLCuVs in cotton plant. Conserved region of *Rep* gene in all available CLCuVs sequences in databases were used for construction of gRNAs. In plant expression vector containing Cas9, single and multiple gRNAs of *Rep* gene were cloned. *Nicotiana benthamiana* and cotton plants were transformed with Cas9-gRNA vector to evaluate the suppression of CLCuVs. Transgenic cotton, resistant to CLCuVs will be a major outcome of this proposed project. In addition, results of this study will be translated to other crop plants for the development of multigenic disease resistance. The expected results of this applied project will be of direct benefit to Pakistan agriculture sector and farmers.

Project Partners



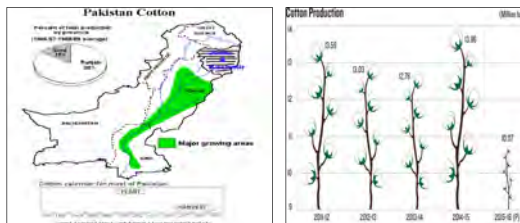
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Muhammad Salman Mubarik (CRISPR)

World Cotton Production



SOURCE: OECD-FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS (2015), OECD-FAO AGRICULTURAL OUTLOOK 2015

Cotton map of Pakistan

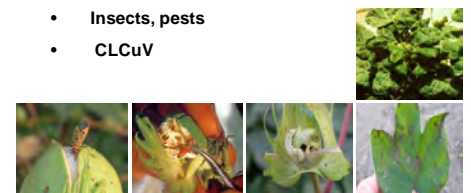


Agriculture Marketing Information Service: AMIS.PK

MIT Technology Review of Pakistan

Threats to Cotton Production in Pakistan

- Insects, pests
- CLCuV



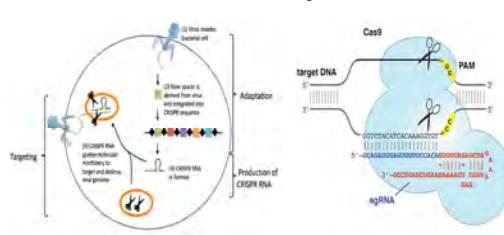
Cotton leaf curl virus (CLCuV)

- Family Geminiviridae
- Genus Begomovirus
- Vector Whitefly (*Bemisia tabaci*)
- ssDNA viruses
- Monopartite with alpha and beta satellites



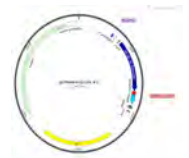
Gene Name	Protein	Function
AC1	Rep	Replication
C4	C4	Involved in symptom determinant
AC2	TRAP	Transcription activator for the virion sense genes
AC3	REn	Enhance viral DNA replication
AV1	CP	encapsidation and movement
AV2	AV2	Symptoms determination and expression of coat protein

CRISPR/Cas9 System

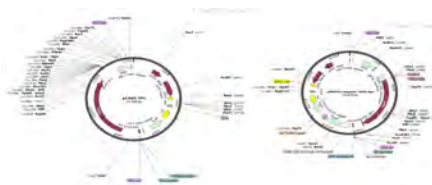


gRNA and cloning vectors

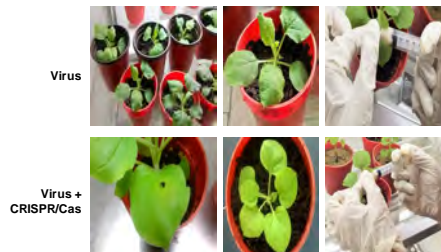
No.	Name	Sequence (5' -3')
1	CLCuV-K1-F	5'ATTGAGCCTGTGTCACCTTTGAT 3'
2	CLCuV-K1-R	5'AAACATCAAAGTCAGCACAGGCT 3'
3	CLCuV-K2-F	5'ATTGGCCATACCTCAAGTCTTC 3'
4	CLCuV-K2-R	5'AAACGAAGAACCTGAAGTGTGGC 3'
5	CLCuV-K3-F	5'ATTGAATAAATACGGTCCGGCT 3'
6	CLCuV-K3-R	5'AAACAAGCGGAACGGTATTATTTC 3'



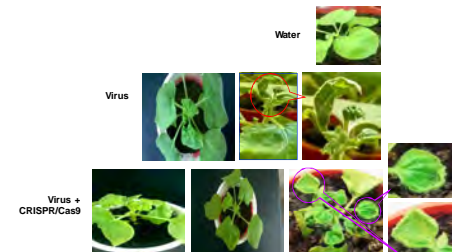
Cloning of gRNA Cassettes in Expression Vector



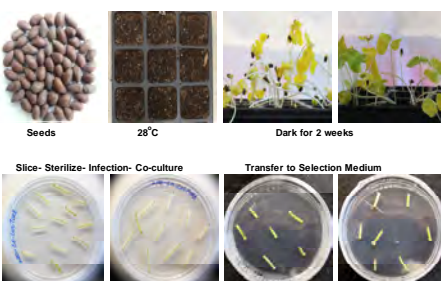
Infectivity assay in *benthamiana* plants



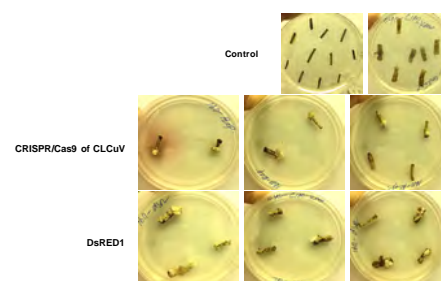
Infectivity Assay



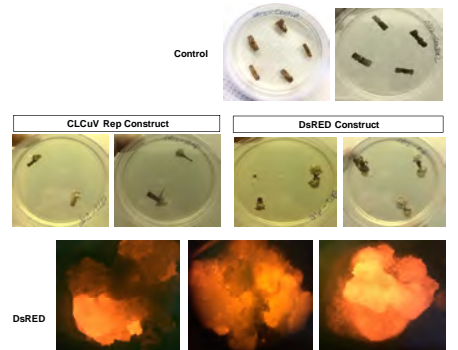
Cotton Transformation



Cotton Transformation



Cotton Transformation



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