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Topic: Generation of *cotton leaf curl virus*- resistant transgenic cotton plant

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ABSTRACT

Cotton is a valuable cash crop that has an important role in India's agrarian and industrial economy. Though, India is the second largest producer of cotton, the yields fall short even of domestic demands to allow for any export. Loss of vast crop hampered by cotton leaf curl disease is the major challenge staring Indian agriculture in the face. This necessitates immediate proactive approaches to be adopted to cater to the demands of the ever increasing population. With the development of advanced high throughput genomic methodologies new frontiers have been opened to develop plants tolerant to various biotic and abiotic stresses. Biotechnological approaches have long been in use to integrate new traits in crops to render them fit against various stress. The *in vitro* regeneration of plants, however, still remains the major bottleneck for realizing the goal of developing designer crops. Cotton in particular has long been one of the recalcitrant crops not easily amenable to propagate in tissue culture and genetic transformation procedures.

Being CLCuD susceptible, the present work was carried out with the objective of developing transgenic cotton (*Gossypium hirsutum*) plants resistant to CLCuD. This disease caused by *Cotton leaf curl virus* (CLCuV) species in association with betasatellites is the key biotic factor which limits the production of cotton in India. The symptoms include leaf curling, darkening of veins, vein swelling and cup-shaped

enations which are leaf-like structures on the undersides of the leaves. Several attempts were made to develop transgenic plants using genes or fragments of the genome of the pathogens (Beachy, 1993; Baulcombe, 1994; Lomonssof, 1995; Sanford and Jhonston, 1985) to suppress the virus in host plant. Some of these attempts have been successful which has led to the development of virus-resistant plants for commercial application.

We have applied RNAi-mediated approach for generating CLCuD resistant transgenic cotton plants. RNAi constructs derived from Intergenic region (IR) of CLCuV, and *βC*1 gene of betasatellites were successfully used for developing transgenic cotton resistant to CLCuD. Cotton (*G. hirsutum* var. Narshimha) plants were transformed with IR and *βC*1 based RNAi constructs. The gene products of CLCuV are transcribed via intergenic region which acts as a bidirectional promoter present between the first ORF of the virion sense DNA and the complementary sense DNA. The IR contains DNA elements that are required for replication of begomoviruses and their transcriptional regulation with presence of TATA boxes, Rep-binding site and stem-loop elements that contain a conserved nonanucleotide (TAATATTAC) sequence. The IR also contains *Ori* site i.e. replication origin of viral genome whereas *βC*1gene is a virulence determinant and is also a suppressor of plant PTGS.