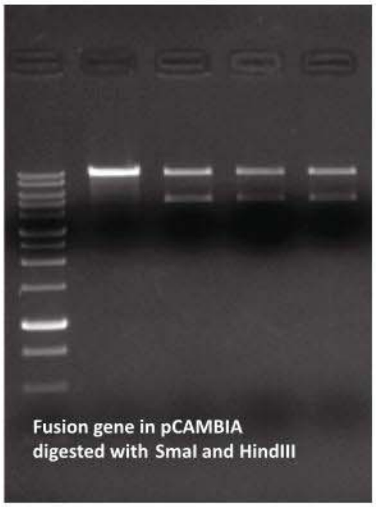
TMC MM I.1 : Development of multi-gene constructs and Bt cotton varieties for sustainable pest management.

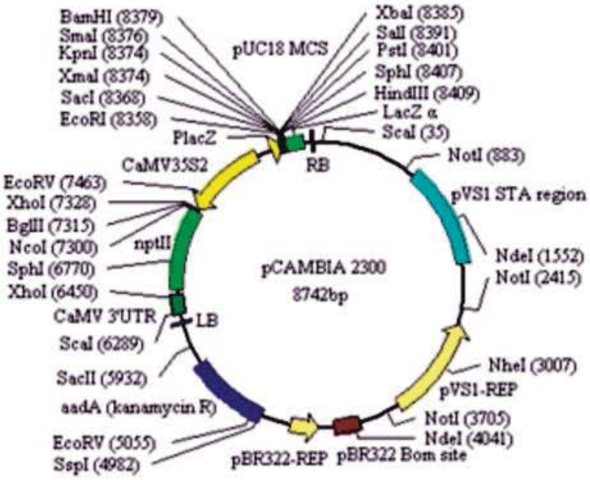
Lead Centre :	CICR, Nagpur
Cooperating centres	UAS, Dharwad PDKV, Alola

Introduction

Pests and diseases are major constraints in cotton production. Transgenic strategy using genes from bacteria Bacillus thuringiensis (Bt) has proved to be very successful in cotton crop protection against Lepidopteron insect pests. With the introduction of Bt gene in cotton (Bt-Cotton), cultivation has transfigured in India since 2002. However, resurgence/emergence of sucking pests has become serious cause of concern. Further no single cotton variety is available containing Bt gene which can be cultivated at reduced cost of seeds. As on today 1342 Bt hybrids belonging to eight events have been commercialized in India. The technology has helped to reduce 50-60% insecticide and doubling of yield. But yields are stagnating now at 460 to 560 kg/ha. Moreover Bt hybrids are more susceptible to sucking pest and diseases, require larger spacing for growth, are highly input intensive, require more fertilized causing long term soil nutrient deficiencies. Similarly, no viable strategy is available to combat leaf curl virus disease. Therefore, there is an urgent need to develop broad-based resistance against a wide spectrum of pests and diseases in a single cultivar. This can be achieved by expressing several genes in a single transgenic plant. CICR and most of the Agriculture Universities have developed excellent varieties and hybrids over the past 10 years, but these are not being preferred by farmers, because they do not have Bt gene in them.


Fusion gene in pCAMBIA digested with SmaI and HindIII

In this project we intend to design and express multiple genes in cotton for resistance to pests and diseases and in parallel, discover new genes and promoters that can be expressed in cotton. The genes targeted by us include the already existing arsenals like the "Cry" genes from Bacillus thuringiensis, the microbial chitinases for engineering enhanced susceptibility of insects to the Cry toxins and the herbicide resistance genes like "pat" with a dual purpose of transgene selection as well as herbicide resistance. In addition to making gene constructs with these available genes, we will attempt discovery of new genes that will enrich our arsenal for long-term plant protection. In this area, we will identify, clone and express



new genes (e.g., lectins) from plant/ microbial sources and validate by expression in E. coli/ yeast/ model plants for toxicity against insect pests. New promoters will be identified for selective expression under conditions of biotic stress, based on gene expression profiling. These promoters will in turn, be validated using "gus" or GFP as reporter.

The varieties having dwarf and compact stature from different agroclimatic zones will be targeted to transform with multigene construct for insect, diseases, leaf curl virus, herbicide and drought tolerant. Therefore the present studies involve transformation of multigene construct (combining different Cry genes with disease resistant gene / leaf curl virus resistance gene or herbicide resistance gene or drought tolerant gene in one cassette) in diverse cotton varieties. Four different multigene construct will be prepared and transferred into plant system. The transformed plants will be further subjected to event selection and trait evaluation. The transgenic plant carrying multiple "transgenes" ("Super-Bt") would be tested for biosafety and resistance to number of factors.

Objectives

1. Identification of novel candidate genes of microbes, plants and insect origin having potential for crop protection& production and identification of novel promoters based on transcriptome data.
2. Expression of novel insecticidal proteins in *E. coli*/ yeast/ model plant.
3. Development of four new multi-gene constructs for expression of useful genes in cotton varieties.
    a. Cryfusion : chitinase : *AroA* (insect-disease-glyphosate resistance)
    b. Cryfusion : ACPxSCP: *AroA* (insect:CLCV : glyphosate resistance)
    c. Cryfusion : VIP3A:chitinase :*AroA* (glyphosate resistance)
    d. Cryfusion:DREB1A:*AroA* (glyphosate resistance)
4. Transformation of cotton varieties by Agrobacterium tumefaciens containing multi-gene construct for insect, disease, CLCuV and herbicide resistance or drought tolerance.
5. Molecular characterization and gene expression studies in To and T1 plant progenies.
6. To identify homologous plants for seed multiplication and biosafety trials.

Technical programme

Activity / Programme	Centres
Development of four different Constructs for pest resistance	**CICR, Nagpur**
Transformation into elite varieties	**CICR, Nagpur** **Dr PDKV Akola** **UAS Dharwad**
Confirmation of gene integration, expression and molecular analyses	
Contained green house trial of putative transformed plants	
Field Evaluation, biosafety, Regulatory process & Commercialization	

Time frame

Sr. No.	Activity	Time frame									
		2012-13		2013-14		2014-15		2015-16		2016-17	
		I	II	I	II	I	II	I	II	I	II
1.	Development of four different Constructs for pest resistance	■	■	■	■						
2.	Transformation into elite varieties			■	■	■	■	■			
3.	Confirmation of gene integration, expression and molecular analyses				■	■	■	■			
4.	Contained green house trial of putative transformed plants						■	■	■		
5.	Field Evaluation, biosafety, Regulatory process & Commercialization							■	■	■	■

Output

1. Multiple gene construct
2. Development of GM cotton varieties resistance to multiple pests such as cotton bollworms, disease, leaf curl virus, herbicide and drought.