Cotton Biotechnology in Uzbekistan

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ABSTRACT

The Republic of Uzbekistan is a large producer and exporter of cotton but must overcome a number of limitations. Soil salinity has rendered about 20 % of the cotton producing area unsuitable for continued production. Increasing susceptibility to pathogens and pests reduces the yield by 10 – 12 %. Defoliants and harvest aid chemicals increase the cost of production and affect the environment adversely. Certain protein molecular markers are correlated with salt tolerance while others are receptors of abscisic acid, a key hormone in dehiscence of cotton. Key enzymes regulate the synthesis of ethylene that regulates boll opening. A fragment of DNA containing a copy of the gene for chitinase synthesis, the main enzyme in resistance to pathogens, has been cloned. New active Bt toxins have been generated from melon seeds. A vector has been constructed to provide for the transfer of foreign genes to cotton cells and a method has been developed for the transformation of single cotton cells.

Introduction

For many years Uzbekistan was the cotton base for the Soviet Union. During the last decade of the Soviet period, Uzbekistan produced more than six millions tons of raw cotton per year. An unwarranted amount of mineral fertilizers, pesticides and defoliants were used. This caused great harm to the environment. Hence, production of cotton in ecologically friendly manner is very urgent.

Material and Methods

There are two ways to solve this problem: 1) Find pathogen resistance genes or genes for self-defoliation among wild and cultivated forms and their hybrids; introduce them through hybridization and selection to create "ecologically friendly" cotton. This is a very lengthy process and could take 20-25 to create a new variety under normal conditions but it doesn't cost much, so many plant breeders and farmers can be involved. 2) Use achievements in plant biotechnology to create varieties by gene transfer for individual genes contributing to "ecological friendliness". This requires discovery and cloning of the beneficial genes, recombination of these genes with chromosomes of a single cotton cell, followed by regeneration of the cell to a fertile plant.

Single cells of some cotton varieties cannot regenerate to a normal fertile plant. This resulted in Plant Genetic Systems, Monsanto, Calgene, Agracetus and others using only the embryogenic variety "Coker-312" for transformation. Transgenic "Coker-312" plants that carried an insectotoxic gene (Bt-gene) or genes for resistance to herbicides were selected and crossed as donor with industrial varieties. After repeated backcrosses stable new transgenic cotton varieties were obtained. This way normally takes 5-6 years but utilization of Coker as donor can reflect on the recipient's properties.

This approach does not satisfy many needs so 15 years ago a task force was established that would directly transform industrial varieties of cotton and search for ways to regenerate the best Uzbeki cotton varieties. To induce somatic embryogenesis from callus tissue of industrial varieties at different steps of sub-cultivation, the ratios of different phytohormones such as 2,4-D, NUK, kinetin, and BAP were changed while leaving other components of the culture medium unchanged. Industrial varieties C-6524 and AN-409 have been successfully regenerated. Proliferation of embryogenic callus tissue of these varieties was observed in callus induced on media with minimum concentrations of 2,4-D and kinetin, then sub-cultured on media with kinetin replaced by BAP (also in minimum concentrations) followed by sub-culture on media with BAP again replaced by kinetin. Note that using BAP on the first passage was very successful. Somatic embryos began forming after transfer of embryogenic calli to media with concentrations of 2,4-D reduced to 0.01 mg/L and kinetin to 0.05 mg/L. It was important for embryo development to reduce the concentration of glucose but the mixture of hormones remains unchanged (Fig.1). Thus, embryogenesis from callus tissue of varieties C-6524 and AN-409 has been successfully induced. Other varieties require other regimes of phytohormones for callus induction and regeneration.

In parallel with this work, genes for insect toxicity and pathogen-resistance were sought and a collection of *Bacillus thuringiensis* isolates was amassed. At present there are about 250 strains in our collection, the majority having been identified by standard biochemical, physiological and morphological techniques and by plasmid profiles and bioassays (Fig.2). A fraction of these have been gene-typed by

PCR. A B.t. strain not giving PCR products with universal oligonucleotide primers for known Cry-type genes has been found (Fig.3). Identification of *B. thuringiensis* strains with novel Cry-type genes by PCR using mixtures of primers based on the conserved sequence blocks will be carried out.

Simultaneously we sought mycotoxic genes from microorganisms. Forty-five soil bacterial strains and *Trichoderma* fungi were tested for antagonism to the phytopathogenes *Verticillum dahliae* and *Fusarium oxysporum*. Fourteen of the bacterial isolates displayed active antagonism to both fungi, six of which comprised the growth-inhibiting activity in the protein fraction. Sources of antimycotic activity belong to *Bacillus* (5 strains), *Chromobacterium* and *Erwinia* genera. Using gel-filtration on TLC-gel HW55f, ion-exchange chromatography on DEAE or CM-sephadex, and ligand-exchange chromatography on chelates of copper and zinc, we have extracted from *Thrichoderma* a 38-42 kDa protein and a 2.5-4 kDa polypeptide fraction have antimycotic activity against *F. oxysporum* and *V. dahliae* (Table 1). According to ion exchange chromatography data the protein is anionic.

At present we are intensively seeking plant genes responsible for insect-resistance. In particular, we have extracted an insect-toxin that significantly exceeds Bt-toxin in spectral ranges and activity.

Conclusions

Widespread use of defoliants for machine harvesting is a major cause of ecological harm in cotton production. The solution directly depends on early-maturation and self-defoliation of cotton. With this aim, collaborators (A.Abdulaev) created an ultra-early-maturing, three-genomic hybrid that has the self-defoliating property. ABA binding is linked with the self-defoliation (Table 2).

It was established that a specific recipient molecule of 19 kDa was responsible for ABA binding and had high affinity to ABA (KD = $5x10^{-5}$-10^{-0}M) according to Scatchard's plot analysis. These data indicate that plants with a high content of the specific receptor molecule may be early-maturing and self-defoliating. Transfer of the corresponding gene into the genome of an existing variety is an alternative to conventional breeding for early-maturing and self-defoliating varieties. Accordingly, we are working on cloning of the ABA-receptor gene.

Conditions have been created in Uzbekistan for simultaneous cloning of genes for pathogen- and insect-resistance, early-maturity and self-defoliation to improve the ecological aspect of cotton.

Table 1. Properties of fungicidal proteins from soil microorganisms.

Microorganisms	Protein		Fungicidal Activity		Activity
	M_r, kDa	N-end	*V. dahliae*	*F. oxysporum*	of the enzyme
Bacillus sp. № 101	32-40	n.d*	+	+	chitinase
Bacillus sp. № 85	24-36	n.d	+	+	-
Bacillus sp. № 45	24-36	n.d	+	+	-
Bacillus sp. № 44	24-36	n.d	+	+	-
Bacillus sp. № 32	24-36	n.d	+	+	-
Chromobact. sp. № 5	32-40	n.d	+	+	cellulase
Erwina sp.	28	His	+	+	xylanase
Trichoderma sp.	38-42	n.d	+	+	cellulase

* no data

** chitinase, cellulase and xylanase activity is absent.

Table 2. Specific radioactivity of proteins prepared from seedlings of "Lystopadniy" variety and different hybrids of cotton (exposed to (3)H-ABA min-).

Variety and hybrids	Abscission	Specific radioactivity counts/min /mg protein	Percent
"Lystopadniy"	Early	39,200 (± 1,560)	100
"L-110"	Normal	17,300 (± 690)	44.13
"L-1579"	Normal	18,000 (± 720)	45.90
"L-27"	Late	1,000 (± 40)	2.55

Figure 1. Somatic embryogenesis of cotton variety AN-409.

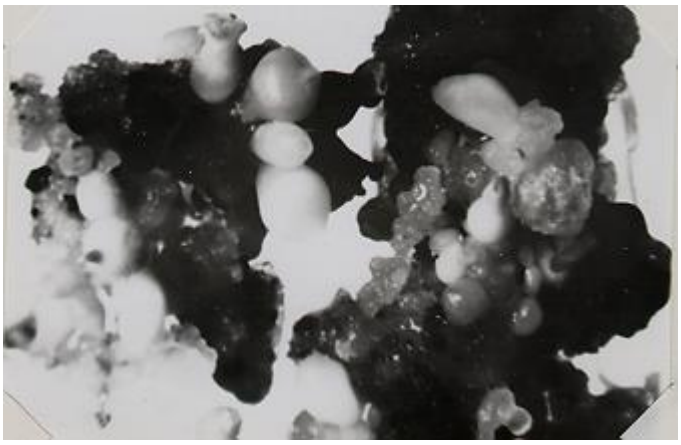

Figure 2. Comparative analysis of plasmid profiles of different B.t. strains by agarose gel electrophoresis: 1-B.t.galleria (type strain), 2-B.t.galleria 70-22, 3-B.t.galleria 38, 4-B.t.galleria 38/B, 5- B.t.sotto (type strain), 6-B.t.sotto 8-6P, 7-B.t.sotto 371, 8-B.t.sotto 375, 9-B.t.sotto 84-2, 10-B.t.alesti 121.

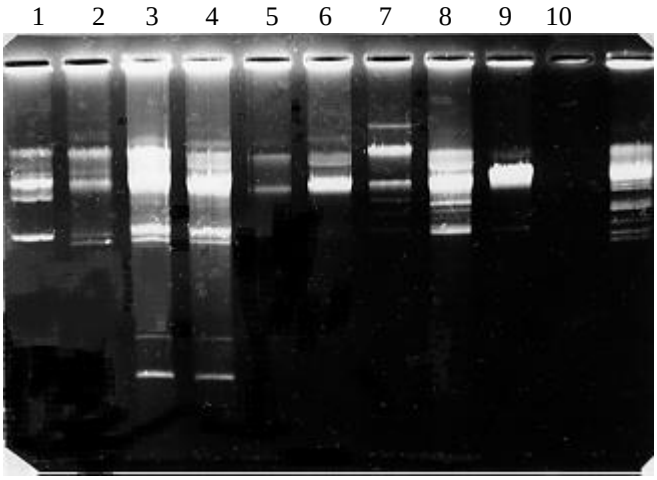

Figure 3. Blot-hybridization DNA from local B.t. strains with labelled fragments of Cry-I gene (A) and Cry- IV gene (B): 1-molecular weight marker, 2- B.t.galleria 70-22, 3-B.t.asia-media 78-71, 4-B.t.israelensis Fr, 5- fragment of cry-I gene (B.t.kurstaki HD-1) in M-13.