More Genetically Engineered Cottons

Genetic engineering of crop plants involves induction of a foreign gene or genes into the seed or plant to perform a specialized task for achieving specific objectives. The objective could be protection against insects, quality improvement, or any other aspect of crop production, preservation and marketing. It is easier to insert non-related species genes into some crop plants than into others. Unfortunately, unlike some other field crops, cotton is not very receptive to foreign genes. Varietal responses also exist. Of the 2,500 field tests conducted in the USA to September 1996 only 8% of trials involved cotton. The field trials on all transgenic plants were for herbicide tolerance (57%), insect control (43%), product quality (7%) and agronomic performance (2%). Thus, in all crop species, including cotton, most of the work done so far has been to control insect pests.

Very few efforts have been devoted to quality improvement. Only a small number of trials involved more than one feature.

Since the mid 1980s, research efforts to develop transgenic Bt cotton varieties resistant to bollworms have been intense. For almost ten years, researchers have been testing the usefulness of bioproduction of a toxin within the cotton plant and its impact on other morphological and quality characteristics in cotton. In October 1995, the first insect resistant Bt cotton was formally cleared for commercial cultivation in the USA. Later, Australian authorities also permitted commercial scale cultivation of Bt cotton in 1996/97. The insect resistant Bt gene has been licensed by Monsanto as Bollgard™ in the USA and Ingard™ in Australia. The Bt gene produces the protein toxin CryIA(c), found in most biological insecticides, throughout the life of the plant, without regard to growing conditions. Many other countries will ultimately start growing Bt cotton but how it will reach developing countries is not clear at this stage. One of the possible options could be joint ventures with owners of resistant genes and technologies. Such an agreement has recently been signed by Delta and Pine Land Company of the USA with Chinese companies. It is expected that transgenic Bt cotton with the Bollgard™ gene will be grown in China on about 200,000 hectares in 1998. Herbicide tolerant cotton was also grown on a commercial scale in the USA in 1996/97.

Insect control costs form the main component of the total cost of cotton production. In the face of enormous insect pressure, unusual in 1996, Bollgard cotton proved the worth of Bt gene in cotton. Further success of Bt cotton in the USA, Australia and, in two years, in China (Mainland) could redirect the money currently spent on chemical pesticides to biotech research.

It is reported from China and India that, in addition to a gene from soil bacteria, a cowpea gene has been inducted into cotton to protect it against tobacco budworm, *Heliothis virescens*. Two other areas where significant progress has been made are colored cotton and cotton with changed thermal properties.

Recently, a pigment alteration patent has been granted to the American company Calgene, Inc. by the US Patent Office. A gene construct for the expression of the pigmentation gene, melanin, in cotton fiber has been identified and inducted into cotton. The company owns Stoneville Pedigree Seed Company and claims that their scientists have developed blue and red fibers and now are focussing on enhancing the shades. Pigment alteration of genetically-modified cotton plants has improved the prospects of producing naturally-colored blue jeans. The fiber quality of naturally-colored green and brown fibers is not equivalent to that of white cotton. Development of pigment alteration in genetically-modified cottons will not require additional work to improve fiber quality. Pigment alteration will be an addition to the existing qualities of white cottons with no other effects on the plant. The foreign genes inducted into the cotton plant are present in all cells and have a specific task to perform at particular stages of plant development. This specific task in the future could be pigment production for black, yellow and all other colors in cotton.

Work to identify genes which could be assigned the task of doubling the size of bolls without affecting boll number, or doubling the number of bolls without affecting boll size, has not been reported as yet. Perhaps there is a need for improved understanding of the genetic control of boll numbers and size. Similarly, fiber quality characters like length, strength and micronaire could be improved significantly but no such genes have been identified yet which could bring improvements which have not been achieved through conventional breeding. However, with the latest achievements in genetic manipulation of non-species genes for accomplishing particular and well defined objectives, such developments do not seem to be impossible.

A report was published in the Proceedings of the National Academy of Sciences of the USA where the above mentioned approach has been adopted. According to the paper, a group of researchers at Agracetus (Monsanto) have changed the thermal characteristics of the cotton fiber through insertion of bacterial genes. The cotton fiber, which is an unicellular outgrowth from the seed coat, is comprised of up to 90% cellulose. It has three important developmental stages: Elongation and formation of a primary cell wall, which is completed in about twenty days after anthesis; formation of a secondary wall and deposition of cellulose, which starts about sixteen days after anthesis and is usually completed at forty-five days after anthesis; and chemical changes. Chemical changes, usually related to mineral contents and protein levels, take place after the formation of a secondary wall has been completed. Cellulose deposition and chemical composition of the cotton fiber not only affect the commonly measured fiber parameters but also affect properties like shrinkage, chemical reactivity, heat retention, water absorption, etc., which are very important in the manufacturing of textile products. The researchers at the Agracetus have been able to produce a new biopolymer inside the cellulosic walls which is cable of producing thermoplastic properties of the compound poly–D–(—)–3–hydroxy butyrate (PHB). PHB, produced by many genera of bacteria, is a naturally biodegradable thermoplastic with physical and chemical properties similar to polypropylene.

Using the particle bombardment method for transformation of cotton, researchers inducted two bacterial genes capable of producing enzymes responsible for the production of PHB in the cotton fiber. As expected, the percentage of successfully transformed plants was very low, but plants with epidermal and germ-line transformations were achieved. In total, 14,000 seeds were attempted and thirty seeds after germination showed epidermal (twenty-one seeds) and germ-line (nine seeds) inductions. Because cotton fibers are an epidermal growth, even epidermal transformations could be used for evaluating modifications in fiber properties. Laboratory analysis by more than one method confirmed the presence of PHB in transgenic fibers. Quantitative measurement of PHB contents in the developing fibers was also studied at an interval of ten days until fifty days after anthesis. The data showed that the quantity of PHB abruptly increased for 5-6 days after ten days of anthesis. The fiber weight continued increasing after fifteen days but the amount of PHB did not increase, thus reducing the quantity of PHB in proportion to fiber weight. The actual amount of PHB did not increase after the primary wall was formed (fifteen days after anthesis), and no degradation of PHB was noted with the formation of secondary walls and maturation of fibers.

Thermal properties of transgenic fibers vs. normal fibers were tested by measuring the heat flow rate through the cotton samples. The total heat uptake was 11.6% higher in transgenic fibers than in normal fibers. The analysis of samples from other plants with a varying amount of PHB showed that the heat uptake was proportional to the quantity of PHB in transgenic fibers.

Results showed that fibers from transgenic plants had 6.7% lower thermal conductivity compared to normal fibers, indicating slower cooling down of the fibers with PHB. The heat retention capacity of both cottons was tested at 36^{o}C and 60^{o}C. The data showed 8.6% higher heat retention at 36^{o}C in the case of cotton having PHB. The heat retention capacity of cotton with PHB increased to 44.5% higher at 60^{o}C.

Cotton from transgenic plants and normal plants was spun into yarn and unbleached and undyed fabrics were subjected to the heat uptake test again. Fabric made from the cotton from transgenic plants once again confirmed a higher uptake of heat by the same margin as in the case of lint.

Like Bt cotton resistant to lepidopteran insects, transgenic cotton having the bacterial genes *phaB* and *phaC* (capable of producing PHB) showed normal growth and morphology. Fiber quality in terms of length, strength and micronaire also remained unaffected. The PHB cotton was stored for several months at room temperature and no change was found in PHB contents. However, similar stability effects in finished textile products have not been studied.

The authors consider the insulation properties of the experimental cotton not sufficient to satisfy customer needs. Agracetus is working to enhance the effect by increasing the amount of PHB produced in the fibers. PHB contents could be increased either through induction of more genes or different genes with stronger effects. However, identification of measurable quantities of PHB in transgenic plants has demonstrated that it is possible to change the thermal properties of cotton.

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