Chapter 15
Cotton

Keerti S. Rathore

15.1 Introduction

The cotton plant is the most important source of natural fiber and has met the clothing needs of mankind for several millennia. This plant plays a major role in the economy and social structure of many countries, and in fact, has helped shape the history of some parts of the world. Although many other natural and synthetic fibers have been available, advantages related to the cost of production, many unique features offered by cotton lint, and the growing world population will ensure a continued increase in the demand for cotton. Not only will the tools offered by biotechnology help us to achieve the needed increase in the production of this crop, this technology will also be used to confer properties to the fiber that are difficult or impossible to achieve by traditional breeding. The increasing demand for food, feed, and energy will also help the cottonseed to achieve a better status than merely a byproduct of lint production. Again, there is tremendous scope for improving the quality of cottonseed through biotechnology.

Cotton is grown in over 80, mostly developing countries where it is a cash crop for many poor farmers. With its share of more than 90% of the worldwide acreage, the tetraploid *Gossypium hirsutum* or upland cotton is the predominant cultivated species. The other tetraploid species, *G. barbadense* or Egyptian cotton, is also grown in some parts of the world for its prized extra-long staple. Because of their low productivity and poor quality fiber, the two Old World, diploid species, *G. arboreum* and *G. herbaceum*, are cultivated only on limited acreage in some parts of Africa and Asia. China is the largest producer of cotton, followed by India and the United States. In 2006, the global area under cotton cultivation was estimated to be approximately 34.3 million hectares (Mha) leading to ~25 million

K.S. Rathore
Institute for Plant Genomics & Biotechnology and Department of Soil and Crop Science, Texas A&M University, College Station, TX 77843-2123, USA
e-mail: rathore@tamu.edu

F. Kempken and C. Jung (eds.), *Genetic Modification of Plants*,
Biotechnology in Agriculture and Forestry 64,
DOI 10.1007/978-3-642-02391-0_15,

metric tonnes (MMT) of fiber production (FAO 2008). Cotton is grown primarily for its fiber, however, the plant also produces large quantities of seeds. Worldwide, ~44 MMT of cottonseed was produced in the year 2006 (FAO 2008). A portion of this abundant agricultural byproduct is used to obtain edible oil; however, a large share of the cottonseed/cottonseed meal is simply used as a feed for the ruminant animals. The presence of a toxic terpenoid, gossypol, renders the protein-rich seed unfit for consumption by monogastric animals and prevents its direct use as food. Elimination of this toxin from the seed will ensure a more efficient utilization of this resource and help fulfill the growing, worldwide demand for food and feed.

15.2 Importance and Potential Impact of Genetic Modification in Cotton

Cotton plants are particularly susceptible to a wide variety of insect pests and nematodes, and their cultivation has traditionally relied on the use of large amounts of highly toxic pesticides. Some estimates suggest that, prior to the widespread adoption of Bt cotton, nearly 25% of all insecticides used worldwide were needed for the production of cotton (Pannetier et al. 1997). Genetically modified cotton produced by incorporating the Bt gene was therefore a huge success in the United States following its introduction in 1996 (see also Chap. 11). Amongst the cotton-producing countries, India has the largest area under cotton cultivation and yet it has ranked third in terms of production until very recently. This was because the average yield of cotton in India was one of the lowest in the world. Several factors accounted for this low productivity including insect pests. The yield of lint+cotton-seed in this country averaged 561 kg ha^{-1} in 2001; however, it increased to 1019 kg ha^{-1} in 2007 (FAO 2008). In a recent publication from the International Food Policy Research Institute (IFPRI), Gruere et al. (2008) report that in the year 2007/08, India's cotton production exceeded that of the United States. Most of this rise in the production is attributed to an increasing use of Bt cotton varieties following their introduction in the year 2002 (Qaim and Zilberman 2003; James 2007; Gruere et al. 2008). It is not surprising then that, once approved by the respective regulatory agencies, cotton growers in many other countries have readily adopted GM cotton. The example of India illustrates the potential impact of biotechnology in enhancing global cotton production. Overall improvements in the production of cotton will be considerable once this technology is adopted by the rest of the cotton producing countries. Currently, Bt-mediated insect resistance and herbicide resistance are the only two transgenic traits available in cotton. When the traits that confer resistance to various other biotic and abiotic stresses become available, the combined impact of various transgenic traits on the total output will be much more substantial than what has been achieved thus far. In addition to its impact on the production, genetic engineering is likely to play a very important role in improving the quality of fiber as well as the seed.

15.3 Transformation of Cotton and its Improvement via Genetic Modification

There are some excellent reviews available on transgenic cotton (Murray et al. 1993; John 1997; Chlan et al. 2000; Wilkins et al. 2000; Rajasekaran et al. 2001; Kumria et al. 2003; Rathore et al. 2008). Table 15.1 provides a list of selected papers describing the key transformation methods and the introduction of certain useful traits via genetic engineering. General aspects of genetic transformation are discussed in Chap. 1.

15.3.1 Methods Used to Transform Cotton

The first two reports on successful cotton transformation were published by scientists at Agracetus (Umbeck et al. 1987) and Agrigenetics (Firoozabady et al. 1987). In both cases, tissue explants obtained from a young seedling were transformed via *Agrobacterium tumefaciens*. The transformed tissues growing on selection medium were cultured for several months before recovering the transgenic plants via somatic embryogenesis. This procedure is rather long and laborious, and is limited for use in only a few genotypes that are able to regenerate via somatic embryogenesis. However, it is a robust protocol and with some modifications, is widely used to obtain transgenic cotton plants in both academic and industrial laboratories (Table 15.1). A comprehensive investigation was undertaken in author's laboratory to understand both the transformation and regeneration processes (Sunilkumar and Rathore 2001; Rathore et al. 2006). This study made use of green fluorescent protein (GFP) gene as a reporter and showed clearly that the transfer of T-DNA per se, from *Agrobacterium* to the cotton cells at the wound site in a cotyledon, hypocotyl and cotyledonary petiole, is an efficient process. In addition, its integration into the cotton genome is also quite effective. It is the culture of transformed cells to obtain a friable, embryogenic callus capable of plant regeneration, that is a highly genotype-dependent process (Trolinder and Xhixian 1989). Even with the regenerable genotypes, a high degree of tissue culture skills are required to obtain transformed cotton plants. Bearing in mind the difficulties faced by many researchers in producing transgenic cotton, a simplified protocol describing various steps in detail has been published (Rathore et al. 2006).

Thus, genotype-dependence, in terms of regeneration via somatic embryogenesis, does remain a limitation in introducing a transgenic trait directly into commercial varieties. The same constraints also apply to methods that utilize particle bombardment-mediated transformation of cultured cells. These limitations have served as an impetus to find alternative methods to produce transgenic cotton. Since regeneration of plants from shoot apical meristem is genotype-independent, relatively rapid, and a rather straightforward process, many laboratories have targeted the cells within this explant for transformation. The research involving

Table 15.1 Summary of selected studies on transgenic cotton. *C* Cotyledon, *ESC* embryogenic cell suspension, *H* hypocotyls, *P* cotyledonary petiole, *SAM* shoot apical meristem

Transformation method	Cultivar	Target tissue/mode of transformant recovery	Transgenes	Analysis and Comments	Reference
Agrobacterium	Coker 310, 312, 5110	H/somatic embryogenesis	*cat* and *nptII*	Enzyme assays and Southern for confirmation	Umbeck et al. (1987)
Agrobacterium	Coker 201	C/somatic embryogenesis	*nptII* and OCS	Immunoblot and Southern for confirmation	Firoozabady et al. (1987)
Gene gun	Coker 310	ESC/somatic embryogenesis	*hpt*	Southern for confirmation	Finer and McMullen (1990)
Agrobacterium	Coker 312	H/somatic embryogenesis	*CryIAc*, *CryIAb* and *nptII*	Western and bioassay for confirmation	Perlak et al. (1990)
Agrobacterium	Coker 312	H/somatic embryogenesis	*nptII* and *tfd*A	2,4-D monooxygenase activity, PCR, and 2,4-D resistance for confirmation	Bayley et al. (1992)
Agrobacterium	Coker 315	C/somatic embryogenesis	*nptII*, *gusA* and *tfd*A	Southern, GUS enzyme assay and 2,4-D resistance for confirmation	Lyon et al. (1993)
Gene gun	Delta Pine 50, Delta Pine 90, Sea Island, Pima S-6	SAM from mature seed/shoot regeneration in culture	*gusA*	GUS histochemical analysis and Southern for confirmation	McCabe and Martinell (1993)
Gene gun	Coker 312, Delta Pine 50, Sea Island	SAM from mature seed/shoot regeneration in culture	Fiber-specific, FbL2A promoter driving *phaB* and *phaC*, and *gusA*	GUS assay, Southern, Western, and biochemical analyses for confirmation	Rinehart et al. (1996)
Gene gun	Delta Pine 50	SAM from mature seed/ Shoot regeneration in culture	Fiber-specific, E6 or FbL2A promoter driving *phaB* and *phaC*, and *gusA*	GUS assay, Southern, Northern, microscopic, and biochemical analyses for confirmation; fiber's thermal properties were altered	John and Keller (1996), Chowdhury and John (1998)

Agrobacterium and gene gun	Coker 315 and Acala varieties	C, H, ESC/somatic embryogenesis	*nptII*, mutant native AHAS genes	Southern and resistance to herbicides, imidazolinone and sulfonylurea, for confirmation	Rajasekaran et al. (1996)
Agrobacterium	Coker 312	H/somatic embryogenesis	*nptII*, FMV 35S promoter diving *CP4-EPSPS*	Southern, ELISA, and resistance to herbicide, glyphosate for confirmation	Nida et al. (1996), Chen et al. (2006)
Agrobacterium	CUBQHRPIS	SAM from seedling/ shoot regeneration in culture	*nptII*, *gusA*	Resistance to kanamycin and Southern for confirmation	Zapata et al. (1999)
Agrobacterium	Coker 315	H/somatic embryogenesis	Tobacco basic chitinase, glucose oxidase, *nptII*	Some protection against verticillium wilt with each gene	McFadden et al. (2000)
Agrobacterium	Coker 312	H/somatic embryogenesis	*nptII*, *gusA*	PCR and Southern for confirmation; a step-wise and comprehensive account of transgenic cotton production	Sunilkumar and Rathore (2001), Rathore et al. (2006)
Agrobacterium	Coker 312	H/somatic embryogenesis	Mn-SOD, APX, GR, *nptII*	Enzymatic assays confirmed overexpression; chilling-induced photoinhibition of photosystem II reduced	Kornyeyev et al. (2001, 2003a, b)
Agrobacterium	Coker 312	H/somatic embryogenesis	Cotton α-globulin promoter driving *gusA*, *nptII*	Histochemical and biochemical analyses; seed-specificity of promoter demonstrated	Sunilkumar et al. (2002a)
Agrobacterium	Coker 312	H/somatic embryogenesis	CaMV 35S promoter driving GFP gene, *nptII*	Fluorescence microscopy analysis; developmental- and tissue-specific activity of promoter demonstrated	Sunilkumar et al. (2002b)
Agrobacterium	Coker 315	C/somatic embryogenesis	Seed-specific RNAi of Cotton SAD-1	Southern, Northern and biochemical analyses; seed oil	Liu et al. (2002)

(*continued*)

Table 15.1 (continued)

Transformation method	Cultivar	Target tissue/mode of transformant recovery	Transgenes	Analysis and Comments	Reference
			and Cotton FAD2-1, *nptII*	with substantially higher stearic acid or oleic acid levels	
Agrobacterium	MCU5, DCH32, Coker 310FR	Shoot tip from seedling/shoot regeneration in culture	*gusA, nptII*	Histochemical, PCR and Southern analyses and resistance of progeny to kanamycin for confirmation	Satyavathi et al. (2002)
Agrobacterium	Coker 312	C, H/ somatic embryogenesis	Cotton β-tubulin promoter driving *gusA, nptII*	Histochemical analyses; preferential activity in fiber and root tip observed	Li et al. (2002)
Agrobacterium	Coker 312	H/somatic embryogenesis	Endochitinase gene from *Trichoderma virens*, *nptII*	Southern, Northern and biochemical analyses; protection against *Rhizoctonia solani* and *Alternaria alternata* observed	Emani et al. (2003)
Agrobacterium	Coker 315	C/somatic embryogenesis	Sense and antisense suppression of sucrose synthase, *nptII*	Southern, immunolocalization, electron microscopy and biochemical analyses; fiber development inhibited	Ruan et al. (2003)
Gene gun	Coker 310FR	H-derived friable callus/somatic embryogenesis	Chloroplast-specific expression of *aph*A-6 and *nptII*	PCR and Southern to confirm plastid genome transformation; strict maternal inheritance of Kanamycin-resistance	Kumar et al. (2004)
Agrobacterium	Coker 312	H/somatic embryogenesis	Cotton *ghCTL2* promoter driving *gusA, nptII*	Preferential activity in different cell types during secondary wall deposition including lint fibers	Zhang et al. (2004)
Agrobacterium	Coker 312	H/somatic embryogenesis	Seed-specific antisense of cotton FAD-2, *nptII*	Biochemical analysis; seed oil with higher oleic acid level	Sunilkumar et al. (2005)

Agrobacterium	Coker 315	C/somatic embryogenesis	Soybean lectin promoter or CaMV 35S promoter driving antisense *cdn*1-C4, *nptII*	Southern, Northern, and Western analyses; no reduction in gossypol levels; induction of the target gene by bacterial blight was blocked	Townsend et al. (2005)
Agrobacterium	Coker 312	C, H/ somatic embryogenesis	Synthetic antimicrobial peptide *D4E1*, *nptII*	Southern, PCR and RT-PCR analyses; transgenic plants resistant to several fungal pathogens	Rajasekaran et al. (2005)
Agrobacterium	Coker 312	H/somatic embryogenesis	*Arabidopsis NHX1*, *nptII*	PCR, Northern, and Western analyses; more biomass and more fiber produced under salt stress conditions	He et al. (2005)
Agrobacterium	Coker 312	C, H/somatic embryogenesis	Cotton ACTIN1 promoter driving *gusA*, RNAi of *ghACT1*, *nptII*	Histochemical analyses; preferential activity observed in the fiber; RNAi-inhibition of fiber elongation	Li et al. (2005)
Agrobacterium	Zhongmiansuo 35	H/somatic embryogenesis	Phloem-specific promoter driving *ACA* gene, *nptII*	Southern and Western for confirmation; resistance to cotton aphid observed	Wu et al. (2006)
Agrobacterium	Coker 312	H, P/somatic embryogenesis	Seed-specific RNAi of cotton δ-cadinene synthase, *nptII*	Southern, RT-PCR, Northern and biochemical analyses for confirmation; over 98% reduction in the seed gossypol level obtained	Sunilkumar et al. (2006)
Agrobacterium	Coker 312	H/somatic embryogenesis	CaMV 35S promoter driving spinach SPS gene, *nptII*	Southern, RT-PCR, Northern, Western and biochemical analyses for confirmation; improved fiber quality	Haigler et al. (2007)

particle bombardment of isolated shoot apical meristems followed by the recovery of plants has provided unambiguous evidence for the transgenic status of the regenerants and proved the feasibility of this approach (McCabe and Martinell 1993; McCabe et al. 1998). The gene gun-based microprojectile bombardment is a direct, physical method that can deliver the genes into the epidermal cells of the L1 layer or the germline progenitor cells of L2/L3 layer within the apical meristem. As expected, the progeny plants from L1 transformants did not inherit the transgene. In contrast, the germline transformants resulting from the transformation of L2/L3 cells passed on the transgenic trait to subsequent generations. However, the primary transformants recovered from these shoot apices are chimeric and the efficiencies of recovering germline transformation events are very low. As this method is highly labor- and resource-intensive, it has not been used by others. There are reports from three laboratories on *Agrobacterium*-mediated transformation of shoot apical meristem to obtain transgenic cotton plants (Zapata et al. 1999; Satyavathi et al. 2002; Uceer and Koc 2006). The ability to tolerate kanamycin was used as a major criterion to identify the putative transformants and each report provided some molecular evidence. However, these reports did not provide any information on the type of cells that were transformed within the shoot apical meristem. The transformation efficiencies reported in these papers differ drastically, thus raising questions about the criteria used to assign transgenic status to the regenerated plants. Additional, convincing evidence that includes phenotypic analysis, molecular proof that discounts the possibility of *Agrobacterium* contamination of the plant tissue, and genetic analysis in several generations will be needed to confirm the reliability, efficiency, and robustness of this method. If an unambiguous proof of *Agrobacterium*-mediated transformation of shoot apical meristem is provided, it will ensure a widespread adoption of this technique by other researchers who are interested in a genotype-independent method to transform cotton.

15.3.2 Selectable Markers and Reporter Genes used for Cotton Transformation

Chap. 3 generally discusses the use of marker genes in transgenic plants. Neomycin phosphotransferase II (*nptII*) gene in combination with kanamycin as the selection agent was used in the first two investigations reporting successful cotton transformation (Firoozabady et al. 1987; Umbeck et al. 1987). The papers listed in Table 15.1 suggest that this gene continues to be used widely to obtain transgenic cotton. Its wide popularity stems from the fact that kanamycin is relatively inexpensive and does not adversely affect regeneration from cultured cotton tissues. Hygromycin phosphotransferase (*hpt*) gene is also suitable for producing transgenic cotton and has been used as a selectable marker in some studies (Finer and McMullen 1990). Cotton has been transformed with the bialaphos resistance (*bar*) gene; however, the initial selection of transgenic tissue was based on the expression of a linked *nptII*

gene in these studies (Keller et al. 1997). Bialaphos-tolerant cotton has been also developed by Bayer CropScience and is marketed by FiberMax under the name LibertyLink (Perkins 2004). The list provided in Table 15.1 shows that *β*-glucuronidase (*gusA*) remains the gene of choice to evaluate different transformation methods as well as for the characterization of promoter activities in various tissues in cotton. This is because GUS activity assays are relatively simple and the enzyme activity can be quantitated (Jefferson et al. 1987). The utility and versatility of GFP reporter gene (allowing non-invasive monitoring of its expression) was demonstrated by observing the tissue- and development-specific activity of CaMV 35S promoter in cotton (Sunilkumar et al. 2002b).

15.3.3 Genetically Engineered Traits in Cotton

Bt was the first commercially useful gene introduced into cotton (Perlak et al. 1990). This cotton was later developed and marketed under the trade name Bollgard by Monsanto/Delta & Pine Land (Jones et al. 1996; Jenkins et al. 1997). These plants expressed a truncated, codon-modified *CryIAc* gene from *Bacillus thuringiensis* (Bt) encoding a δ-endotoxin that is toxic to tobacco budworm and American bollworm (Jenkins et al. 1997). These Bt cottons were readily accepted by farmers in the United States and other countries that had allowed their cultivation. Bollgard II, introduced in 2003, contains *Cry2Ab* in addition to *CryIAc* (Micinski et al. 2006; Robinson 2006). This second *Bt* gene broadens the resistance to include fall armyworm, beet armyworm, cabbage looper, and soybean looper (Perlak et al. 2001). Syngenta has developed VIP-Cotton containing a different gene from *B. thuringiensis* that encodes a vegetative insecticidal protein (VIP; Estruch et al. 1996). The VIP is structurally, biochemically, and functionally different from the Bt δ-endotoxins and exhibits insecticidal activity against a variety of lepidopterans (McCaffery et al. 2006). Another type of insect-resistant cotton has been developed by Dow AgroSciences by combining *CryIF* and *CryIAc* genes. This product, WideStrike cotton, also confers resistance to several Lepidopteran pests (Bacheler et al. 2006; Micinski et al. 2006). Thus, a choice of more than one insect resistance genes with different modes of action, especially if they are stacked, will help broaden the spectrum of insects that can be controlled by the genetically modified plants and also help counter the development of resistance in the target insects.

Roundup Ready cotton that is resistant to glyphosate-based herbicide (see also Chap. 10 for references) was introduced in 1997 by Monsanto (Nida et al. 1996). This trait was engineered by expressing a gene encoding 5-enolpyruvylshikimate-3-phosphate synthase (derived from *Agrobacterium* sp. strain CP4) under the control of FMV 35S promoter. In 2006, Roundup Ready Flex cotton became available that allows safe application of the herbicide well beyond the five-leaf stage (Chen et al. 2006). Glyphosate-tolerant cottons help in the effective management of weeds and were also readily adopted by the United States cotton growers. Glufosinate- or bialaphos-tolerant cotton, developed by Bayer CropScience and

marketed by FiberMax under the name LibertyLink, is also available commercially (Perkins 2004).

As is the case with most other crop plants, no commercial, transgenic products are yet available in cotton that address the problems of biotic or abiotic stresses. However, there are some published reports describing transgene-mediated resistance to various fungal diseases in cotton (Murray et al. 1999; McFadden et al. 2000; Emani et al. 2003; Wang et al. 2004b; Rajasekaran et al. 2005). Although some of these studies appear promising, in each case, the transgene conferred protection to only a limited spectrum of pathogens. Similarly, there are a number of reports describing attempts to engineer cotton to tolerate abiotic stresses, including freezing (Kornyeyev et al. 2001, 2003a, b; Payton et al. 2001), water-logging (Ellis et al. 2000), salt stress (He et al. 2005) and drought (Yan et al. 2004).

Since cotton is grown mainly for its fiber, it is an obvious target for improvement via genetic engineering. In addition to the usual desirable properties that include strength, fineness, length, and uniformity, cotton fiber can benefit from characteristics such as better dye binding, wrinkle resistance, and shrinkage resistance. Improvements in these last three categories will help cotton fiber compete more effectively against synthetic fibers. The number of genes involved in controlling some of these traits is likely to be large and the mechanism controlling these characteristics is expected to be complex. Several laboratories are involved in identifying and isolating genes that are involved in fiber initiation, elongation, and development. As these genes become available and are characterized, their coding and regulatory sequences will be used to engineer the cotton plant to address issues related to fiber quality improvement. Nevertheless, some interesting work to modify cotton fiber has been already conducted by scientists at Agracetus and elsewhere. An early example of such research involved the synthesis of novel biological materials in the fiber. Expression of some genes derived from *Alcaligenes eutrophus* in the developing cotton fibers resulted in the deposition of poly-D-(–)-3-hydroxybutyrate (PHB) in their lumens (John and Keller 1996; Rinehart et al. 1996). The modified fiber exhibited altered thermal properties resulting in improved insulating characteristics (Chowdhury and John 1998). Although this product was not developed further, the results demonstrated the feasibility of improving cotton fiber in a manner that is impossible to achieve by traditional breeding methods. Two recent studies have examined the effects of manipulating endogenous gene expression in cotton fiber cells (Ruan et al. 2003; Li et al. 2005). Although each of these studies involved suppression of a cotton gene that adversely affected fiber growth/development, the results indicate the feasibility of altering fiber properties. In a more recent study, Haigler et al. (2007) showed that constitutive overexpression of spinach sucrose phosphate synthase gene in cotton resulted in the improvements in fiber quality when the cotton plants were grown under controlled environmental conditions. Chapman et al. (2008) reported an interesting and unexpected outcome of manipulating the oil composition by overexpressing a non-functional rapeseed FAD-2 gene in cottonseed. They showed that, while the seeds from transformed lines were smaller, of poor quality and had lower oil content, the lint produced was significantly increased, suggesting a redirection of carbon reserves. These reports on

transgenic manipulation of cotton fiber are promising. However, considering the importance of this agricultural product, the progress in improving its characteristics and yield through biotechnology has been rather slow. As more fiber-specific genes and their regulatory sequences become available, transgenic technology is expected to make a significant impact on the quality and yield of this most important product of the cotton plant (Li et al. 2002; Wang et al. 2004a).

Cotton plant produces about 1.6 times more seed than fiber. Cottonseed contains ~21% oil and a substantial portion of the global production is used to obtain edible oil. Since cottonseed oil is rather low in monounsaturated fatty acid, gene-suppression technologies have been used to improve its fatty acid composition in favor of higher oleic acid. Use of antisense technology to suppress Δ-12 desaturase gene resulted in doubling of oleic acid from a wild-type level of ~15% to ~30% and a reduction in linoleic acid level from ~55% to ~35% (Sunilkumar et al. 2005). Interestingly, RNAi-mediated suppression of the same target gene resulted in a fivefold increase in oleic acid level and a concomitant reduction in the linoleic acid (Liu et al. 2002). In a separate set of transformants, RNAi-mediated downregulation of the SAD-1 gene resulted in a >10-fold increase in stearic acid level in cottonseed oil. Importantly, it was possible to stack the two traits by intercrossing (Liu et al. 2002). These results demonstrate that transgenic technology can be used to modify fatty acid biosynthetic pathway in a tissue-specific manner to improve storage and cooking properties of the cottonseed oil. In addition to the oil, cottonseed also contains ~23% protein that is of relatively high quality. Global cottonseed output of ~44 MMT year^{-1} can potentially meet the basic protein requirements of 500 million people. However, the ability to utilize this abundant, protein-rich resource for food is hampered by the presence of toxic gossypol. This cardio- and hepatotoxic terpenoid, present in cottonseed glands, renders the seed unsafe for human and monogastric animal consumption. Glands containing gossypol and related terpenoids are present in most parts of a cotton plant. The terpenoids are believed to play a protective role in defending the cotton plant against various insect pests and diseases (Hedin et al. 1992; Townsend et al. 2005). To avoid the weakening of defensive capability of the cotton plant, the elimination of gossypol must be strictly limited to the seed. Since traditional breeding methods have failed to achieve this goal, biotechnological approaches were tested in many laboratories around the world to solve the problem of cottonseed toxicity. Most of these attempts over the past decade have been unsuccessful (see Townsend et al. 2005 and references therein). However, in a relatively recent breakthrough, the feat of selective and significant reduction of gossypol in cottonseed was achieved by disrupting its biosynthesis through RNAi-mediated suppression of δ-cadinene synthase activity in the developing seed (Sunilkumar et al. 2006). Some of the RNAi lines obtained showed a 98% reduction in the concentrations of gossypol in the seed. Importantly, these transformants maintained normal levels of gossypol and related terpenoids in all other parts of the plant. These studies involving alteration of oil composition and gossypol reduction suggest that a genetically modified cotton plant, in addition to meeting the clothing requirements, can also play an important role in fulfilling the nutritional needs of the growing human

population. More details on different engineered traits may be found in Sect. C of this volume.

15.3.4 The Role of New Technological Advances in Cotton Improvement

Successful transformation of the plastid genome in cotton has been achieved by Kumar et al. (2004). Although chloroplast transformation is more difficult and less efficient compared to the nuclear transformation, it does offers some advantages, including transgene containment because of maternal inheritance of the trait and a high level of consistent transgene expression. Lower efficiency and the complexity of the plastid transformation system have prevented widespread adoption of this technology. However, it may be useful for some specific applications.

The two examples provided earlier of transgene-encoded RNAi to improve cottonseed quality demonstrate the power of this gene silencing technology. Undoubtedly, it will be used to improve other properties of this important resource in the future. As the genes involved in controlling various aspects of fiber growth and development are identified, RNAi will serve as a valuable tool in the engineering of desired characteristics in the fiber. In addition to the use of RNAi to improve the quality of seed and fiber, some recent reports suggest exciting new possibilities in harnessing the power of this technology to control nematodes and insect pests of cotton. Yadav et al. (2006) transformed tobacco plants to express dsRNA against important genes of a root-knot nematode (RKN) which resulted in a virtual elimination of the target mRNA in the parasite and significant resistance in the host plant. In another report, Huang et al. (2006) describe results of a transgene-encoded expression of ingestible dsRNA in *Arabidopsis* targeting an RKN gene that encodes *16D10*, a secretory peptide essential for the nematode parasitism of the plant. The transformants showed significant resistance to four major RKN species. Corn plants expressing dsRNA against Western corn root-worm V-ATPase were effectively protected against the insect (Baum et al. 2007) and this protection was comparable to that provided by Bt. The results obtained by Mao et al. (2007) illustrate an interesting possibility to confer protection against cotton bollworm by expressing dsRNA in the plant that targets an insect cytochrome P450 monooxygenase, believed to be involved in detoxifying the natural insecticide gossypol in cotton plants. These examples illustrate that RNAi is a versatile and highly effective tool that can be used to engineer cotton plants to confer resistance to various pests.

15.4 Future Perspectives

It has been two decades since the first reports on cotton transformation were published. Bt cotton was introduced in the marketplace in the United States in 1996, with herbicide-tolerant cotton a year later. In 2005, GM cotton (Bt cotton,

herbicide-resistant cotton) garnered 79% of the cotton acreage in the United States (Brookes and Barfoot 2006). Especially, Bt cotton has enjoyed the same enthusiastic acceptance by the farmers in many other countries where its use was permitted by their respective regulatory agencies. In 2006, of the 11 million small farmers who grew GM crops, most were Bt cotton farmers, including 7.1 million in China and 3.8 million in India (James 2007). However, the current GM cotton varieties offer only insect- and herbicide-resistance traits that benefit largely the growers. Availability and choice of these and other input traits is likely to increase in future. The published reports described in this chapter show the efforts underway to engineer a number of useful output traits into cotton. As new genes and their regulatory sequences become available from cotton and other species, and as the genetic modification technologies are further refined and improved, we can expect cotton plants with novel input and output traits for the benefit of growers, consumers, and the environment. The current product-line is available from just a few large companies and the farmers have to pay a premium to grow their GM cotton varieties. However, some of the basic patents on various GM-related technologies will start to expire soon. This will open up opportunities for the scientists to engineer cotton to meet the more specific, local needs of the poor farmers in the developing countries.

Acknowledgements Research in the author's laboratory has been supported by funds from Cotton Incorporated, Texas Cotton Biotechnology Initiative (TxCOT), Texas Higher Education Coordinating Board (Advanced Research Program), Texas Food & Fibers Commission, and Texas Agricultural Experiment Station.

References

Bacheler J, Mott D, Bowman DT (2006) The relative efficacy of Bollgard, Bollgard II and WideStrike lines against bollworms in North Carolina in 2003 and 2005: implications for producer choices. Proc Beltwide Cotton Conf 2006:1536–1540

Baum JA, Bogaert T, Clinton W, Heck GR, Feldmann P, Ilagan O, Johnson S, Plaetinck G, Munyikwa T, Pleau M, Vaughn T, Roberts J (2007) Control of coleopteran insect pests through RNA interference. Nat Biotechnol 25:1322–1326

Bayley C, Trolinder N, Ray C, Morgan M, Quisenberry JE, Ow DW (1992) Engineering 2,4-D resistance into cotton. Theor Appl Genet 83:645–649

Brookes G, Barfoot P (2006) GM crops: the first ten years – global socio-economic and environmental impacts. ISAAA Briefs 36. ISAAA, Ithaca, N.Y.

Chapman KD, Neogi PB, Hake KD, Stawska AA, Speed TR, Cotter MQ, Garrett DC, Kerby T, Richardson CD, Ayre BG, Ghosh S, Kinney AJ (2008) Reduced oil accumulation in cottonseeds transformed with a *Brassica* non-functional allele of a delta-12 fatty acid desaturase (FAD2). Crop Sci 48:1470–1481

Chen YS, Hubmeier C, Tran M, Martens A, Cerny RE, Sammons RD, CaJacob C (2006) Expression of CP4 EPSPS in microspores and tapetum cells of cotton (*Gossypium hirsutum*) is critical for male reproductive development in response to late-stage glyphosate applications. Plant Biotechnol J 4:477–487

Chlan CA, Rajasekaran K, Cleveland TE (2000) Transgenic cotton (*Gossypium hirsutum*) In: Bajaj YPS (ed) Transgenic crops I. Biotechnology in agriculture and forestry, vol 46. Springer, Heidelberg, pp 283–301

Chowdhury B, John ME (1998) Thermal evaluation of transgenic cotton containing polyhydroxybutyrate. Thermochim Acta 313:43–53

Ellis MH, Millar AA, Llewellyn DJ, Peacock WJ, Dennis ES (2000) Transgenic cotton (*Gossypium hirsutum*) over-expressing alcohol dehydrogenase shows increased ethanol fermentation but no increase in tolerance to oxygen deficiency. Aust J Plant Physiol 27:1041–1050

Emani C, Garcia JM, Lopata-Finch E, Pozo MJ, Uribe P, Kim D-J, Sunilkumar G, Cook DR, Kenerley CM, Rathore KS (2003) Enhanced fungal resistance in transgenic cotton expressing an endochitinase gene from *Trichoderma virens*. Plant Biotechnol J 1:321–336

Estruch JJ, Warren GW, Mullins MA, Nye GJ, Craig JA, Koziel MG (1996) Vip3A, a novel *Bacillus thuringiensis* vegetative insecticidal protein with a wide spectrum of activities against lepidopteran insects. Proc Natl Acad Sci USA 93:5389–5394

FAO (2008) FAO statistics. http://www.fao.org. Accessed 7 Jan 2008

Finer JJ, McMullen MD (1990) Transformation of cotton (*Gossypium hirsutum* L.) via particle bombardment. Plant Cell Rep 8:586–589

Firoozabady E, DeBoer DL, Merlo DJ, Halk EL, Amerson LN, Rashka KE, Murray EE (1987) Transformation of cotton (*Gossypium hirsutum* L.) by *Agrobacterium tumefaciens* and regeneration of transgenic plants. Plant Mol Biol 10:105–116

Gruere GP, Mehta-Bhatt P, Sengupta D (2008) Bt cotton and farmer suicides in India: reviewing the evidence. IFPRI discussion paper 808. International Food Policy Research Institute, Delhi. http://www.ifpri.org/pubs/dp/ifpridp00808.asp

Haigler CH, Singh B, Zhang D, Hwang S, Wu C, Cai WX, Hozain M, Kang W, Kiedaisch B, Strauss RE, Hequet EF, Wyatt BG, Jividen GM, Haladay AS (2007) Transgenic cotton overproducing spinach sucrose phosphate synthase showed enhanced leaf sucrose synthesis and improved fiber quality under controlled environmental conditions. Plant Mol Biol 63:815–832

He C, Yan J, Shen G, Fu L, Holaday AS, Auld D, Blumwald E, Zhang H (2005) Expression of an Arabidopsis vacuolar sodium/proton antiporter gene in cotton improves photosynthetic performance under salt conditions and increases fiber yield in the field. Plant Cell Physiol 46:1848–1854

Hedin PA, Parrott WL, Jenkins JN (1992) Relationships of glands, cotton square terpenoid aldehydes, and other allelochemicals to larval growth of *Heliothis virescens* (Lepidoptera, Noctuidae). J Econ Entomol 85:359–364

Huang G, Allen R, Davis EL, Baum TJ, Hussey RS (2006) Engineering broad root-knot resistance in transgenic plants by RNAi silencing of a conserved and essential root-knot nematode parasitism gene. Proc Natl Acad Sci USA 103:14302–14306

James C (2007) Global status of commercialized biotech/GM crops: 2007. ISAAA Briefs 37. ISAAA, Ithaca, N.Y.

Jefferson RA, Kavanagh TA, Bevan MW (1987) GUS fusions: β-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO J 6:3901–3907

Jenkins JA, McCarty JC, Buehler RE, Kiser J, Williams C, Wofford T (1997) Resistance of cotton with endotoxin genes from *Bacillus thuringiensis* var. *kurstaki* on selected lepidoptaran insects. Agron J 89:768–780

John ME (1997) Cotton crop improvement through genetic engineering. Crit Rev Biotechnol 17:185–208

John ME, Keller G (1996) Metabolic pathway engineering in cotton: biosynthesis of polyhydroxybutyrate in fiber cells. Proc Natl Acad Sci USA 93:12768–12773

Jones K, Kerby T, Collins H, Wofford T, Bates M, Presley J, Burgess J, Beuhler B, Deaton R (1996) Performance of NuCotn with Bollgard. Proc Beltwide Cotton Conf 1996:46–47

Keller G, Spatola L, McCabe D, Martinell B, Swain W, John ME (1997) Transgenic cotton resistant to herbicide bialaphos. Transgenic Res 6:385–392

Kornyeyev D, Logan BA, Payton P, Allen RD, Holaday AS (2001) Enhanced photochemical light utilization and decreased chilling-induced photoinhibition of photosystem II in cotton overexpressing genes encoding chloroplast-targeted antioxidant enzymes. Physiol Plant 113:323-331

Kornyeyev D, Logan BA, Payton P, Allen RD, Holaday AS (2003a) Elevated chloroplastic glutathione reductase activities decrease chilling-induced photoinhibition by increasing rates of photochemistry, but not thermal energy dissipation, in transgenic cotton. Funct Plant Biol 30:101–110

Kornyeyev D, Logan BA, Allen RD, Holaday AS (2003b) Effect of chloroplastic overproduction of ascorbate peroxidase on photosynthesis and photoprotection in cotton leaves subjected to low temperature photoinhibition. Plant Sci 165:1033–1041

Kumar S, Dhingra A, Daniell H (2004) Stable transformation of the cotton plastid genome and maternal inheritance of transgenes. Plant Mol Biol 56:203–216

Kumria R, Leelavathi S, Bhatnagar RJ, Reddy VS (2003) Regeneration and genetic transformation of cotton: present status and future perspectives. Plant Tissue Cult 13:211–225

Li X-B, Cai L, Cheng N-H, Liu J-W (2002) Molecular characterization of the cotton *ghTUB1* gene that is preferentially expressed in fiber. Plant Physiol 130:666–674

Li X-B, Fan X-P, Wang X-L, Cai L, Yang WC (2005) The cotton *ACTIN1* gene is functionally expressed in fibers and participates in fiber elongation. Plant Cell 17:859–875

Liu Q, Singh SP, Green AG (2002) High-stearic and high-oleic cottonseed oils produced by hairpin RNA-mediated post-transcriptional gene silencing. Plant Physiol 129:1732–1743

Lyon BR, Cousins YL, Llewellyn DJ, Dennis ES (1993) Cotton plants transformed with a bacterial degradation gene are protected from accidental spray drift damage by the herbicide 2,4-dichlorophenoxyacetic acid. Transgenic Res 2:162–169

Mao Y-B, Cai WJ, Wang JW, Hong G-J, Tao X-Y, Wang L-J, Huang Y-P, Chen X-Y (2007) Silencing a cotton bollworm P450 monooxygenase gene by plant-mediated RNAi impairs larval tolerance of gossypol. Nat Biotechnol 25:1307–1313

McCabe DE, Martinell BJ (1993) Transformation of elite cotton cultivars via particle bombardment of meristems. Bio/Technol 11:596–598

McCabe DE, Martinell BJ, John ME (1998) Genetic transformation of cotton through particle bombardment. In: Bajaj YPS (ed) Cotton. Biotechnology in agriculture and forestry, vol 42. Springer, Heidelberg, pp 263–273

McCaffery A, Caprio M, Jackson R, Marcus M, Martin T, Dickerson D, Negrotto D, O'Reilly D, Chen E, Lee M (2006) Effective IRM with the novel insecticidal protein Vip3A. Proc Beltwide Cotton Conf 2006:1229–1235

McFadden H, de Feyter R, Murray F, Grover A., Llewellyn D, Dennis E, Peacock WJ (2000) Genetic engineering approaches to the improvement of cotton's tolerance to *Verticillium* wilt. In: Tjamos EC, Rowe RC, Heale JB, Fravel DR (eds) Advances in *Verticillium* research and disease management. APS, St Paul, pp 187–191

Micinski S, Waltman WF, Spaulding HL (2006) Efficacy of WideStrike for control of the bollworm/tobacco budworm complex in northwest Lousiana. Proc Beltwide Cotton Conf 2006:1090–1094

Murray EE, DeBoer DL, Firoozabady E (1993) Transgenic cotton. In: Kung S-D, Wu R (eds) Transgenic plants, present status and social and economic impacts, vol 2. Academic, San Diego, pp 153–168

Murray F, Llewellyn D, McFadden H, Last D, Dennis ES, Peacock WJ (1999) Expression of the *Talaromyces flavus* glucose oxidase gene in cotton and tobacco reduces fungal infection, but is also phytotoxic. Mol Breed 5:219–232

Nida DL, Kolacz KH, Buehler RE, Deaton WR, Schuler WR, Armstrong TA, Taylor ML, Ebert CC, Rogan GJ, Padgette SR, Fuchs RL (1996) Glyphosate-tolerant cotton: genetic characterization and protein expression. J Agric Food Chem 44:1960–1966

Pannetier C, Giband M, Couzi P, Tan VL, Mazier M, Tourneur J, Hau B (1997) Introduction of new traits into cotton through genetic engineering: insect resistance as example. Euphytica 96:163–166

Payton P, Webb R, Kornyeyev D, Allen RD, Holaday AS (2001) Protecting cotton photosynthesis during moderate chilling at high light intensity by increasing chloroplastic antioxidant enzyme activity. J Exp Bot 52:2345–2354

Perlak FJ, Deaton RW, Armstrong TA, Fuchs RL, Sims SR, Greenplate JT, Fischhoff DA (1990) Insect resistant cotton plants. Bio/Technology 8:939–943

Perlak FJ, Oppenhuizen M, Gustafson K, Voth R, Sivasupramaniam S, Heering D, Carey B, Ihrig RA, Roberts JK (2001) Development and commercial use of Bollgard cotton in the USA – early promises versus today's reality. Plant J 27:489–501

Perkins R (2004) A new herbicide from Bayer CropScience for use in LibertyLink cotton. Proc Beltwide Cotton Conf 2004:114

Qaim M, Zilberman D (2003) Yield effects of genetically modified crops in developing countries. Science 299:900–902

Rajasekaran K, Grula JW, Hudspeth RL, Pofelis S, Anderson DM (1996) Herbicide-resistant Acala and Coker cottons transformed with a native gene encoding mutant forms of acetohydroxyacid synthase. Mol Breed 2:307–319

Rajasekaran K, Chlan CA, Cleveland TE (2001) Tissue culture and genetic transformation of cotton. In: Jenkins JN, Saha S (eds) Genetic improvement of cotton: emerging technologies. Science Publishers, Enfield, pp 269–290

Rajasekaran K, Cary JW, Jaynes JM, Cleveland TE (2005) Disease resistance conferred by the expression of a gene encoding a synthetic peptide in transgenic cotton (*Gossypium hirsutum* L.) plants. Plant Biotechnol J 3:545–554

Rathore KS, Sunilkumar G, Campbell LM (2006) Cotton (*Gossypium hirsutum* L.). In: Wang K (ed) Agrobacterium protocols, 2nd edn, vol 1. Methods in molecular biology, Vol 343. Humana, Totowa, N.J., pp 267–279

Rathore KS, Sunilkumar G, Cantrell RG, Hague S, Reding HK (2008) Cotton. In: Kole C, Hall TC (eds) Transgenic sugar, tuber and fiber crops. Compendium of transgenic crop plants, vol 7. Wiley–Blackwell, Chichester, pp 199–238

Rinehart JA, Petersen MW, John ME (1996) Tissue-specific and developmental regulation of cotton gene FbL2A. Plant Physiol 112:1331–1341

Robinson E (2006) Second generation technology: what's advantage of next Bt cotton? Delta Farm, London. http://deltafarmpress.com/mag/farming_second_generation_technology/

Ruan Y-L, Llewellyn DJ, Furbank RT (2003) Suppression of sucrose synthase gene expression represses cotton fiber cell initiation, elongation, and seed development. Plant Cell 15:952–964

Satyavathi VV, Prasad V, Gita Lakshmi B, Lakshmi Sita G (2002) High efficiency transformation protocol for three Indian cotton varieties via *Agrobactrium tumefaciens*. Plant Sci 162:215–223

Sunilkumar G, Rathore KS (2001) Transgenic cotton: factors influencing *Agrobacterium*Agrobacterium-mediated transformation and regeneration. Mol Breed 8:37–52

Sunilkumar G, Connell JP, Smith CW, Reddy AS, Rathore KS (2002a) Cotton α-globulin promoter: isolation and functional characterization in transgenic cotton, *Arabidopsis*, and tobacco. Transgenic Res 11:347–359

Sunilkumar G, Mohr L, Lopata-Finch E, Emani C, Rathore KS (2002b) Developmental and tissue-specific expression of CaMV 35S promoter in cotton as revealed by GFP. Plant Mol Biol 50:463–474

Sunilkumar G, Campbell LM, Hossen M, Connell JP, Hernandez E, Reddy AS, Smith CW, Rathore KS (2005) A comprehensive study of the use of a homologous promoter in antisense cotton lines exhibiting a high seed oleic acid phenotype. Plant Biotechnol J 3:319–330

Sunilkumar G, Campbell LM, Puckhaber L, Stipanovic RD, Rathore KS (2006) Engineering cottonseed for use in human nutrition by tissue-specific reduction of toxic gossypol. Proc Natl Acad Sci USA 103:18054–18059

Townsend BJ, Poole A, Blake CJ, Llewellyn DJ (2005) Antisense suppression of a (+)-δ-cadinene synthase gene in cotton prevents the induction of this defense response gene during bacterial blight infection but not its constitutive expression. Plant Physiol 138:516–528

Trolinder NL, Xhixian C (1989) Genotype specificity of the somatic embryogenesis response in cotton. Plant Cell Rep 8:133–136

Umbeck P, Johnson G, Barton K, Swain W (1987) Genetically transformed cotton (*Gossypium hirsutum* L.) plants. Bio/Technology 5:263–266

Wang S, Wang J-W, Yu N, Li C-H, Luo B, Gou J-Y, Wang L-J, Chen X-Y (2004a) Control of plant trichome development by a cotton fiber MYB gene. Plant Cell 16:2323–2334

Wang YQ, Chen DJ, Wang DM, Huang QS, Yao ZP, Liu FJ, Wei XW, Li RJ, Zhang ZN, Sun YR (2004b) Over-expression of *Gastrodia* anti-fungal protein enhances *Verticillium* wilt resistance in coloured cotton. Plant Breed 123:454–459

Wilkins TA, Rajasekaran K, Anderson DM (2000) Cotton biotechnology. CRC Cr Rev Plant Sci 19:511–550

Wu J, Luo X, Guo H, Xiao J, Tian Y (2006) Transgenic cotton expressing *Amaranthus caudatus* agglutinin, confers enhanced resistance to aphids. Plant Breed 125:390–394

Yadav BC, Veluthambi K, Subramaniam K (2006) Host-generated double stranded RNA induces RNAi in plant-parasitic nematodes and protects the host from infection. Mol Biochem Parasit 148:219–222

Yan J, He C, Wang J, Mao Z, Holaday SA, Allen RD, Zhang H (2004) Overexpression of the *Arabidopsis* 14-3-3 protein GF14l in cotton leads to a "Stay-Green" phenotype and improves stress tolerance under moderate drought conditions. Plant Cell Physiol 45:1007–1014

Yuceer SU, Koc NK (2006) *Agrobacterium*-mediated transformation and regeneration of cotton plants. Russ J Plant Physiol 53:413–417

Zapata C, Park SH, El-Zik KM, Smith RH (1999) Transformation of a Texas cotton cultivar by using *Agrobacterium* and the shoot apex. Theor Appl Genet 98:252–256

Zhang D, Hrmova M, Wan C-H, Wu C, Balzen J, Cai W, Wang J, Densmore LD, Fincher GB, Zhang H, Haigler CH (2004) Members of a new group of chitinase-like genes are expressed preferentially in cotton cells with secondary walls. Plant Mol Biol 54:353–372