Breeding for Insect Resistance in Cotton: Advances and Future Perspectives

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Abstract

Cotton crop was domesticated independently in separate parts of the world and comprises of at least four cultivated species and several geographical races. The crop is attacked by a wide variety of insect pests, which cause enormous losses in yield and lower the quality of fibre. Major efforts have been directed towards development of cultivars resistant to sucking pests (especially jassid and whitefly) and bollworms and budworms. Selection of hairy jassid resistant/tolerant genotypes in Africa and India are among the earliest examples of exploitation of host plant resistance in minimizing crop losses due to insect pests. While this trait helped in successfully managing the jassid problem, it led to increased susceptibility to whitefly and some bollworm species. Although several morphological and biochemical traits were found associated with resistance to one or more pests, the same traits resulted in increased susceptibility to other pests. A spectacular success in the development of bollworm- and budworm-resistant cotton was achieved with the development of Bt-transgenic cotton incorporating a gene encoding for delta-endotoxin from the soil-inhabiting bacterium, *B. thuringiensis*. A stacking of two or more resistance genes has helped to improve

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R. Arora, S. Sandhu (eds.), *Breeding Insect Resistant Crops for Sustainable Agriculture*, DOI 10.1007/978-981-10-6056-4_9

the activity spectrum of Bt cotton against several lepidopteran pests. Issues concerning biosafety of Bt cotton and management of resistance to Cry toxins in target pests are also discussed.

Keywords
Upland cotton • Genetic diversity • Insect resistance • Sucking insects • Bollworms and budworms • Transgenic cotton

9.1 Introduction

The cotton genus, *Gossypium*, contains around 50 species, grown for the fibre (cotton lint) obtained from the long seed hairs as well as for the oil obtained from the seeds. Cotton fibre has exercised a profound influence on humans from times immemorial. With a history going back to antiquity, the fibre has maintained its pristine purity and importance to this day. Currently, cultivated cotton is the single most important natural fibre crop in the world. Cotton fibre from *Gossypium* species has been a fibre component of textiles and other manufactured items for more than 5000 years in the New World (Damp and Pearsall 1994). Cotton cultivation in the Old World began from India, where it was grown for more than 6000 years since the pre-Harappan period. Indians used cotton for clothing, towels and sheets and sold these items as luxuries to the Chinese and Parthians (Dineen 1988). It even finds mention in the Rigveda, the oldest scripture of the Hindus in India (Sethi et al. 1960).

Gossypium includes species that originated in both the Old World and New World tropical and warm-temperature regions. It was domesticated independently in separate parts of the world. The four most widely cultivated species today are *G. arboreum* (tree cotton), *G. herbaceum* (Levant cotton) from the Old World, *G. barbadense* (Sea Island cotton or Pima cotton) and *G. hirsutum* (upland cotton, which accounts for the largest share of world production) from the New World (Wendel et al. 2009).

The world commercial production of cotton in 2016–2017 was 105.3 million bales from an area of 29.46 million hectares and a productivity of 756 kg/ha (National Cotton Council of America 2017). More than 100 countries are involved in the production of cotton and other related activities with China, India, USA, Pakistan, Uzbekistan and Brazil as the leading producers. The estimates of the United States Department of Agriculture for 2015–16 and 2016–17 indicate that India has displaced China to become the largest producer of cotton, while still maintaining the largest area under cotton. India has also sustained its position as the second largest consumer of cotton after China, as well as the second largest exporter of cotton next to the USA. However, the productivity of seed cotton in India (496 kg/ha) was still way below Australia (2038 kg/ha), China (1484 kg/ha), Brazil (1524 kg/ha), USA (870 kg/ha) and even Pakistan (552 kg/ha) during 2015 (National Cotton

Council of America 2016). Several biotic (insect pests, diseases, weeds) and abiotic (salinity, reduced moisture) stresses act as major constraints in attaining high productivity of cotton.

9.2 Insect Pests Damaging Cotton

Cotton ecosystems throughout the world harbour a wide variety of insects including pests, non-pest herbivores, natural enemies, pollinators and casual visitors. The number of insect species found in the crop may range from a few hundreds to more than a thousand. However, the number of pest-insect species ranges from around 20 to 60 with 5–10 key pests in most production systems (Luttrell et al. 1994). The important insect pests may be categorized into four groups: sucking pests (jassid, whiteflies, aphids, thrips, mirid bugs, stink bugs, mites); bollworms (pink, spotted, spiny and New World and Old World bollworms), budworms and boll weevil; defoliators (leaf rollers, leafworms, tobacco caterpillar, leaf perforator, cabbage looper, armyworms, cotton looper) and stem borers; and lint stainers (red cotton bug, dusky cotton bug) (Arora et al. 2006). Some of these pests like the heliothines, jassids, aphids, whiteflies and mites are polyphagous, and one or more species are recorded in each cotton-growing zone. Others, like the boll weevil and pink bollworm are specialized cotton feeders with a limited geographical distribution (Matthews 1999). For details on the distribution, nature of damage, life history and methods of control of all the important arthropod pests of cotton, the reader is referred to the excellent treatises on cotton pests edited by Matthews and Tunstall (1994) and King et al. (1996). The cotton plant, through its capacity for continued flower bud production and vegetative growth, can compensate for quite considerable damage, especially in varieties of an indeterminate growth habit, and so the damaging effect of an insect depends on the stage at which infestation occurs and other factors, such as availability of moisture, nutrients and temperature (Matthews 1999).

9.3 Genetic Diversity in Cotton

The genetic resources of cotton are extensively dispersed globally across five continents and consist of approximately 45 diploids (A–G and K genomes, $2n = 2 \times = 26$) and 5 tetraploid species (AD genomes, $2n = 4 \times = 52$) that belong to genus *Gossypium* in family Malvaceae (Lubbers and Chee 2009). To a large extent, the differences in cotton genomes are the result of geographical isolation: the ‘C’ genome is confined to Australia (10 species) and ‘D’ genome to America (12 species), while genomes ‘A’, ‘B’ and ‘E’ are found in Africa and Asia. Genomes ‘F’ and ‘G’ comprise one species each, which do not fit into the original five groups (Munro 1994). ‘A’ genome is the only one which produces cotton lint. It is found in the wild species *G. herbaceum* var. *africanum* in Southern Africa, which seems to be the nearest existing species to the wild ancestors of the cultivated cottons (Fryxell 1979).

9.3.1 Geographical Spread and Cotton Races

The cotton-growing areas of the world lie between 42°N and 33°S; outside these limits the summers are either not long enough or not hot enough for the cotton plant to complete its growth cycle (Munro 1994). Cotton is known to defy well-established Vavilovian principles as it acquired novel genetic variation during the course of its spread to new areas, which unlike other crops is much more than the variation found in their respective centre of origin (Gumber et al. 2014).

More than 90% of the world's cotton is supplied by modern cultivars of *G. hirsutum,* while *G. barbadense* provides long, strong and fine fibres and is cultivated in some areas of Central Asia, Egypt, Sudan, India, the USA and China. *G. arboreum* is a significant crop in India and Pakistan, while *G. herbaceum* is cultivated in some region of Africa and Asia (Wendel et al. 2009). The centre of origin of *G. hirsutum* is considered to be in Mexico, but diverse forms are spread throughout Central America and the Caribbean (Campbell et al. 2010).

Hutchinson et al. (1947) classified the diverse morphological forms of *G. hirsutum* into seven geographical races, viz. 'yucatanense', 'punctatum', 'palmeri', 'latifolium', 'marie-galante', 'morrilli' and 'richmondi'. Of these seven, punctatum, latifolium and marie-galante have dispersed the farthest with latifolium being considered the race from which modern cultivated 'upland' cotton was derived. During the botanical collection surveys, all races other than 'yucatanense' were strongly associated with humans and their activities (Lubbers and Chee 2009). The history of domestication of cotton and its impact on phenotypic and genetic traits has been discussed by Lubbers and Chee (2009).

The diversity of *G. hirsutum* germplasm base is currently narrow. However, there are many sources of diversity available from the primary, secondary and tertiary gene pools (Stewart 1995). The primary gene pool comprises all of five tetraploid AD species, viz. *G. hirsutum* L., *G. barbadense* L., *G. tomentosum* Nuttall ex Seemann, *G. mustelinum* Miers ex Watt and *G. darwinii* Watt. These species share the same genome chromosome constituency and can form fertile hybrids with cotton. The secondary gene pool includes A, B, D and F genome diploid cotton (20) species, which are relatives of the ancestral parents that gave rise to AD genome. The tertiary gene pool includes C, E, G and K genome diploid (25) species. The chromosomes of these species are quite divergent from the A to D genomes, and utilizing them for transferring tetraploids requires more extreme methods such as chromosome doubling and the use of bridge species (Stewart et al. 2010; Lubbers and Chee 2009).

9.3.2 Germplasm Collections

The exploitation of wide genetic diversity in *Gossypium* spp. necessitates establishment of germplasm collections for their utilization in crop improvement. Several cotton germplasm banks exist in the world, but the availability of the accessions are generally quite limited. To protect the worldwide economic value of cotton and cotton by-products, coordinated efforts to collect and maintain cotton genetic resources have been going on for more than 200 years. Campbell et al. (2010) presented an overview of the origin and expansion of cotton collections around the world. Currently, there are eight major dedicated cotton germplasm collections present in Australia, Brazil, China, France, India, Russia, the USA and Uzbekistan. The International Plant Genetic Resources Institute (IPGRI) has designated the *Gossypium* collections in the USA and India as the world cotton germplasm collections. In addition, some other international or national institutes also have limited germplasm collections as listed below. The following is a summary of these collections as described by Campbell et al. (2010).

9.3.2.1 USA

The US-sponsored cotton germplasm explorations date back to the early 1900s. Since 1960, these collections have been maintained by the National Centre for Genetic Resources Preservation (NCGRP). Currently, nearly 10,000 accessions covering 45 *Gossypium* species are maintained in the collection. The collection is subdivided into seven different parts: (i) variety collection, (ii) primitive landrace collection, (iii) *G. barbadense* collection, (iv) Asiatic (A genome species) collection, (v) wild species collection, (vi) genetic marker collection and (vii) a base collection (i.e. NCGRP) of all materials in Parts 1–6 and new plant introductions (Percival et al. 1999). Parts 1–5 constitute the working collection, which is routinely seed propagated and distributed by the USDA-ARS at College Station, Texas.

9.3.2.2 India

The Indian cotton germplasm collection is maintained as a working collection by the Central Institute of Cotton Research (CICR) at Nagpur and Coimbatore and as a permanent storage collection at the National Bureau of Plant Genetic Resources (NBPGR) in New Delhi. The collection consists of 10,227 accessions that represent almost entirely cultivated accessions of *G. hirsutum, G. barbadense*, *G. arboreum* and *G. herbaceum*. It also includes race stock accessions of each cultivated species, 26 wild species and 32 synthetic introgressed derivatives.

9.3.2.3 China

The Chinese cotton germplasm collection is housed by the Chinese Academy of Agricultural Sciences in Beijing, Anyang and Hainan Island. A working collection is housed at Anyang, a long-term collection at Beijing and an in vivo collection of wild species at Hainan Island. The total collection consists of 8868 accessions of all the 4 cultivated species and 41 wild species.

9.3.2.4 France

The French cotton germplasm collection is housed by the French Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD) in Montpellier, France. The collection contains 3070 accessions representing 5 tetraploid species and 27 diploid species.

9.3.2.5 Brazil

The Brazilian collection is maintained by the Brazilian Agricultural Research Corporation (Embrapa) at the National Centre for Genetic Resources and Biotechnology. There are about 4361 accessions representing mainly *G. hirsutum* and *G. barbadense* along with 26 diploid species and the other 3 tetraploid species.

9.3.2.6 Australia

Cotton collections in Australia reside in two places: the Commonwealth Scientific and Industrial Research Organization (CSIRO Plant Industry), Narrabri, NSW, and the Australian Tropical Grains Germplasm Centre (ATGGC), Biloela, QLD. The CSIRO collection consists of 542 *G. hirsutum* accessions, 63 *G. barbadense* accessions and 30 races and wild diploid species. The ATGGC collection consists of 1080 accessions mainly of *G. hirsutum* and a small number of accessions of 27 other *Gossypium* species.

9.3.2.7 Russia

The current Russian cotton germplasm collection better known as the VIR collection is housed at Vavilov Institute of Plant Industry (VIR) in St. Petersburg. It consists of 6322 accessions comprised of 24 diploid species, 3 tetraploid species and several diploid and tetraploid hybrids. Seventy percent of the VIR collection is represented by *G. hirsutum* cultivars, landraces and germplasm lines.

9.3.2.8 Uzbekistan

Cotton germplasm collections in Uzbekistan reside in three locations: the Cotton Breeding Institute of Agriculture Ministry, the Institute of Genetics and Plant Experimental Biology at the Academy of Sciences of Uzbekistan and the National University of Uzbekistan at Tashkent. In total, there are >20,000 accessions including isogenic, inbred lines, recombinant inbred lines and elite AD allotetraploid lines, along with wild primitive and extant representatives of the A to G and K genome species.

In spite of these extensive collections, there are species which are not conserved or are under-represented in these collections. Species that are not conserved include the E genome species *G. benadirense* Mattei, *G. bricchettii* (Ulbrich) Vollesen and *G. vollesenii* Fryxell and the K genome species *G. anapoides* Stewart, Wendel and Craven. Most of the K genome species are under-represented in the collections. Habitat loss and potential species loss are lending an urgency to collecting efforts that has not previously existed (Campbell et al. 2010).

9.4 Breeding for Resistance to Insect Pests

In the absence of protection provided by pesticides, the mean losses inflicted by insect pests in cotton were predicted to be a whopping 84% (O'erke et al. 1994). In India, actual mean yield losses of 50–60% were attributed to insect pests even with the adoption of crop protection measures before the advent of Bt cotton (Arora and Dhaliwal 1996). Due to the enormous losses caused by a multitude of insect pests in cotton, major efforts have been directed at developing insect-resistant cultivars with emphasis on leafhoppers, whitefly, thrips and bollworms and budworms.

9.4.1 Leafhoppers

Several species of leafhoppers or jassids are serious pests of cotton crop around the world. The important ones are *Amrasca biguttula* (Ishida) in India, *Jacobiella facialis* (Jacobi) and *J. lybica* (Bergevin and Zanon) in Africa, *Amrasca terraereginae* (Paoli) in Australia, *Empoasca decipiens* Paoli in Egypt, *Empoasca distinguenda* Paoli in Zaire and South Africa and *Empoasca dolichi* Paoli in Somalia (Matthews 1999).

The Indian cotton jassid, *A. biguttula*, is widely distributed in India, and in addition to cotton, it also feeds on okra, potato, brinjal and some wild plants. Adults are about 3 mm long and greenish yellow during the summer, acquiring a reddish tinge in the winter. Injury to plants is caused both by the adults and nymphs which suck sap from the foliage and due to the injection of toxins into the plant tissues. The attacked leaves turn pale and then rust red. With change in appearance, the leaves also turn downwards, dry up and fall to the ground. Owing to the loss of plant vitality, the cotton bolls may also drop off resulting in loss of yield (Atwal 1986).

Some of the earliest work on host plant resistance was done for the selection of jassid (*J. facialis*)-resistant/jassid (*J. facialis*)-tolerant genotypes in Africa (Painter 1951). Hairiness was found to be associated with resistance to jassid. An extensive screening of cotton germplasm revealed that without exception, the resistant types were hairier than the susceptible ones. Zululand hybrid was the most resistant, although all hairy plants were not necessarily resistant (Parnell 1925). The selection 44 from a variety 'Uganda' had considerable tolerance as well as generally lower populations of leafhoppers. Later, strain A 2106 was found highly resistant to leafhoppers (Parnall et al. 1949).

In India also, hairy genotypes were found tolerant to *A. biguttula*. The selected genotypes included 4F, LSS and 289F/43 (Afzal and Abbas 1944). But U4 from Africa was susceptible to jassid in India. Likewise, in Australia, hairy variety Miller 41J was found to be resistant to *A. terraereginae* as were crosses of Miller x U4 (Marriott 1943).

Although pubescence has been widely exploited as a resistance characteristic against jassid, it imparts susceptibility to several other major pests especially whitefly and some bollworm species. There is thus a need to look for other traits imparting jassid resistance. Sikka et al. (1966) observed that hair density on the midrib was not important, but the density and hair length on the leaf lamina were involved in jassid resistance. Batra and Gupta (1970) indicated that in addition to the hair length and density of hairs on midrib or leaf lamina, the thickness of palisade tissues was also important in imparting resistance to cotton cultivars against jassid. Khan and Agarwal (1984) observed that varieties of cotton with hair length on the midveins of the ventral surface of leaves longer than the ovipositor of females were not preferred for egg laying. Murugesan and Kavitha (2010) conducted a detailed analysis of physico-chemical characteristics associated with jassid resistance in cotton and observed that plant height, internodal length, trichome density on the ventral surface of the leaves, hair length and hair density on midrib had negative association with leafhopper damage as well as oviposition. Among the biochemicals, free gossypol in cotton leaves has been reported to be negatively correlated with number of eggs of cotton jassid (Singh and Agarwal 1988), while protein content had no significant effect (Murugesan and Kavitha 2010).

9.4.2 Whitefly

Several whitefly species infest cotton, the most important of which is the sweet potato whitefly *Bemisia tabaci* (Gennadius), which is nearly cosmopolitan in distribution. The banded-wing whitefly, *Trialeurodes abutilonea* (Haldeman), has been recorded in the USA, Mexico and the West Indies. The greenhouse whitefly, *Trialeurodes vaporariorum* Westwood, is also a secondary pest of cotton in California (Leigh et al. 1996). The silverleaf whitefly *B. argentifolii* Perring and Bellows is considered biotype B of *B. tabaci* by many (Commonwealth Agricultural Bureaux International 2017a).

The sweet potato whitefly is a cosmopolitan phloem-feeding insect that lives on a diverse range of herbaceous host plants, numbering over 900. *B. tabaci* possibly originated in India and spread to different parts of the world through transport of infested plant products (Global Invasive Species Database 2015). Besides cotton, *B. tabaci* is a major pest of ornamentals, vegetables and grain legumes, causing damage directly through feeding on phloem and deposition of honeydew on leaves or indirectly through the transmission of plant pathogenic viruses in the genera *Begomovirus* (*Geminiviridae*), *Crinivirus* (*Closteroviridae*) and *Carlavirus* or *Ipomovirus* (*Potyviridae*) (Jones 2003).

It has been observed since the 1950s that morphologically indistinguishable populations of *B. tabaci* differ with respect to host range, host plant adaptability and plant virus transmission capabilities (Brown et al. 1995). Recent molecular and phylogenetic studies have revealed that *B. tabaci* is a complex of 11 well-defined genetic groups containing at least 34 morphologically indistinguishable species, which are merely separated by a minimum of 3.5% mtCOI nucleotide divergence (Dinsdale et al. 2010; De Barro et al. 2011; Commonwealth Agricultural Bureaux International 2017a).

In contrast to leafhoppers, several studies have demonstrated that smooth-leaf trait conferred lowered whitefly susceptibility (Pollard and Saunders 1956; Mound 1965; Bindra 1985; Venugopal Rao et al. 1990; Chu et al. 1998; Walker and Natwick 2006). Another plant morphological trait contributing to lowered whitefly susceptibility was okra-leaf trait (Jones et al. 1974; Chu et al. 1999; Walker and Natwick 2006). Among the wild cottons, *G. thurberi* was found to possess high level of resistance to whitefly by Walker and Natwick (2006). Based on their studies, these authors concluded that the high level of resistance in *G. thurberi* seemed to be due to unknown factors above and beyond smooth- and okra-leaf traits. Khalil et al. (2015) studied the impact of leaf hairiness and other physicomorphic plant characters on whitefly susceptibility and reported that whitefly population correlated positively with hair density on leaf lamina and vein and length of hairs on leaf midrib, but it correlated positively with hairy density on midrib and veins as well as length of hairs on leaf midrib. Among the other factors, whitefly population exhibited negative response with gossypol glands on leaf lamina, midrib and veins and with plant height. Jindal (2004) reported that cotton genotypes 'Supriya' and 'NHH 44' were resistant to whitefly. Trichome length and distance from lower leaf surface to nearest vascular bundles were negatively correlated, while compactness of vascular bundles and leaf lamina thickness were positively correlated with development duration of whitefly. Egg laying by the pest was negatively correlated with compactness of vascular bundles but positively correlated with leaf lamina thickness. Epicuticular waxes were positively correlated with number of eggs laid. However, none of these characteristics has been found to impart sufficient level of resistance to whitefly in commercial American cotton cultivars.

Since the development of bollworm-resistant Bt-transgenic cotton, efforts have been made to incorporate resistance to whitefly and other sucking pests in transgenic cotton. Recently, Shukla et al. (2016) reported identification of a protein (Tma 12) from an edible fern, *Tectaria macrodonta* (Fee), that is insecticidal to whitefly. Transgenic cotton lines expressing Tma 12 at about 0.01% of total soluble protein were resistant to whitefly infestation in contained field trials. In view of its proven safety, Tma 12 is a promising candidate gene that could be pyramided with Bt toxin genes to develop transgenic cotton resistant to bollworms as well as whitefly.

9.4.3 Thrips

The thrips are among the important insect pests damaging young cotton plants. Several researchers have observed decreases in yield from thrips or increases in yield when seedling thrips were controlled (Cook et al. 2011). Among these, the onion thrips, *Thrips tabaci* (Lindeman), western flower thrips, *Frankliniella occidentalis* (Pergande), and flower thrips, *F. tritici* (Fitch), are the most important. Both adults and larvae of thrips feed on the contents of plant epidermal cells. Damaged areas of leaves do not develop in a normal manner causing leaves to twist. Distortion, malformation and tearing of leaves occur at the site of injury as leaf size increases. Seedling damage by thrips may result in reduction in plant height and leaf area and may even delay crop maturity due to its impact on growth parameters (Cook et al. 2011).

Genetic variation in thrips resistance exists among cotton species and genotypes within cultivated species (Ballard 1951; Hawkins et al. 1966; Zhang et al. 2014). Many lines in *G. barbadense* and *G. arboreum* are more resistant than *G. hirsutum* genotypes (Stanton et al. 1992; Zhang et al. 2013). The plant characteristics contributing to thrips resistance included pilosity (Quisenberry and Rummel 1979) and being glandless (Zhang et al. 2014), while okra-leaf shape was more susceptible than normal-leaf cotton (Syed et al. 1996; Chen et al. 2006). Based on extensive evaluation, Zhang et al. (2011, 2013, 2014) observed Acala 1517-08, Acala 1517-99 and Pima as more resistant to thrips than other commercial cultivars. The glandless cotton may, however, not impart resistance to all thrips species as the same has been reported to be more susceptible to onion thrips *T. tabaci* in China (Fang et al. 1995), India (Bhatnagar and Sharma 1991) and Pakistan (Arif et al. 2004). As per Arif et al. (2004), hair density on midrib had a positive correlation, while length of hairs on veins and gossypol glands on veins and midrib showed a negative correlation with *T. tabaci* population.

9.4.4 Bollworms and Budworms

Several species of bollworms and budworms attack the fruiting bodies of the cotton plants, the most important being the heliothines, pink bollworm and spotted and spiny bollworms. In India Kranthi and Russel (2009) reported that for nearly 2 decades before the advent of bollworm-resistant transgenic cotton, these pests caused yield losses to the extent of 70–80% even after the adoption of plant protection measures. Among these, *Helicoverpa/Heliothis* species are the major pests. These four major species are found on a wide range of wild and cultivated host plants, with the later larval instars preferentially feeding upon the fruiting bodies.

Helicoverpa armigera Hubner popularly known as the Old World bollworm or African cotton bollworm is a cosmopolitan, polyphagous pest of cotton, which also attacks a wide range of legumes, vegetables, cereals, oilseeds and ornamentals. The larvae bore into the flower buds; the attacked buds show bracteoles spread out and curled downwards. Larger larvae bore into maturing green bolls, and young bolls fall after larval damage. Leaves and shoots may also be attacked, especially at high pest population densities (Commonwealth Agricultural Bureau International 2017b).

Helicoverpa punctigera (Wallengren) known as the Australian bollworm and endemic to Australia shares the damage with the more notorious species, *H. armigera*. The two species combined represent the most significant agricultural insect pests in Australia (Matthews 1999).

The tobacco budworm, *Heliothis virescens* (Fabricius), is a native of North America found throughout the eastern and southwestern USA. It disperses northward annually and can be found in New England, New York and southern Canada during late summer. It also occurs widely in the Caribbean and sporadically in Central and South America. Tobacco budworm attacks several field crops including tobacco, cotton, alfalfa, clover, flax and soybean and is sometimes also recorded feeding on vegetable and ornamental plants (University of Florida Entomology & Nematology 2017). Budworm larvae damage bolls and squares by chewing holes into the base of bolls.

American cotton bollworm, *Helicoverpa zea* (Boddie), commonly known as cotton bollworm, corn earworm and tomato fruitworm, is confined to the New World and occurs throughout the Americas from Canada to Argentina. It is a polyphagous pest damaging a wide range of crops including cotton, corn, sorghum, tomato, legumes and vegetable crops. In cotton, squares, flowers and young bolls are attacked. Young shoots and leaves can also be damaged, especially in the absence of fruiting structures (Commonwealth Agricultural Bureau International 2017c).

The pink bollworm *Pectinophora gossypiella* Saunders is a worldwide pest of cotton and is the key cotton pest in North and South America and Asia (O'erke et al. 1994). The pink bollworm larvae enter the cotton buds, flowers and bolls shortly after emergence from eggs and feed internally on the fruiting bodies. The pink bollworm causes failure of buds to open properly, fruit shedding, lint damage and seed loss (Commonwealth Agricultural Bureau International 2017a).

The *Earias* species attacking cotton include the spotted bollworm, *Earias vittella* (Fabricius), and spiny bollworm, *E. insulana* (Boisduval), in India, *E. biplaga* Walker in Africa, *E. huegeliana* Gaede in Australia and *E. cupreoviridis* Walker in China (Pearson and Maxwell-Darling 1958). The larvae of both *E. insulana* and *E. vittella* cause damage by boring into growing shoots, buds, flowers and bolls. As soon as the terminal shoot of young cotton is bored, the growing tip loses its turgidity and droops. The larvae can cause excessive shedding of fruiting bodies, and the circular holes produced by larvae in the fruiting bodies remain filled with excreta.

Before the advent of DDT and other synthetic organic insecticides in 1940s, several varietal traits especially earliness and short duration were incorporated into commercial cotton cultivars to minimize damage by late season boll weevils, bollworms and other pests (Bottrell and Adkisson 1977). When organic insecticides became available, breeders began to develop longer duration cultivars which produced more lint and were more profitable than the short-season cultivars (Adkisson et al. 1982). Several morphological and biochemical traits were found associated with lower bollworm damage and incorporated into commercial cultivars for lowering bollworm damage (Smith 1992; Jenkins and Wilson 1996).

Among the morphological traits conferring resistance, nectariless improved resistance to *H. zea* and *H. virescens*. Glabrous or smooth-leaf lacking pubescence reduced oviposition by the pests (Lukefahr et al. 1971; Robinson et al. 1980). Pubescence also adversely affected the mobility and survival of young *H. virescens* larvae (Ramalho et al. 1984). The characteristics imparting resistance to pink bollworm include nectariless, okra leaf, super-okra leaf and earliness (Ingram 1994). Stiffness of shoot tips contributed to resistance against spotted bollworm (Singh 1989).

An important biochemical conferring resistance to bollworms and tobacco budworms is gossypol, which adversely affects development of lepidopteran larvae (Jones et al. 1988). Several studies have demonstrated the effectiveness of gossypol and other allelochemicals like catchin, quercitin and condensed tannins in retarding growth of bollworm and tobacco budworm (Bell and Stipanovic 1977; Chan et al. 1978; Waiss et al. 1981; Jenkins et al. 1983; Narayanan et al. 1990; Taneja et al. 1994). Stipanovic et al. (1988) reported that hemigossypolone and the heliocides H_1, H_2, H_3 and H_4 were also associated with resistance to *Heliothis*/*Helicoverpa* spp.

However, none of these characteristics provided stable and high level of resistance for incorporation in commercial cultivars. Moreover, the requirements for resistance to one or a group of pests often resulted in increased susceptibility to some other pests. A spectacular success in the development of bollworm (Lepidoptera)-resistant cotton has been achieved with genetically engineered transgenic cotton developed during the 1990s (Peferoen 1997; Helider and Boulter 1999). Genetic engineering of crops enables introduction of one or more useful genes from microorganisms or plants into commercial cultivars and reduces the time needed to introgress introduced character into an elite genetic background (Helider and Boulter 1999). The insect-resistant transgenic cotton, also known as Bt cotton, incorporates a gene from the soil-inhabiting entomopathogenic bacterium *Bacillus thuringiensis* Berliner in the cotton plant (Peferoen 1997). In addition to endospores, *B. thuringiensis* produces a parasporal crystal in the sporangium at the time of sporulation. The insecticidal toxicity of *B. thuringiensis* in susceptible insects is largely due to the crystal protein (abbreviated as Cry protein), and different Bt strains produce one or more distinct Cry proteins. Numerous Bt Cry toxins have been isolated and characterized (Hofte and Whiteley 1989; Crickmore et al. 1998). The updated Bt toxin lists, their dendrograms and further details are available at the Bt toxin nomenclature website (Crickmore et al. 2016).

The Cry protein is produced in the form of a protoxin, which is degraded by proteolytic enzymes in the alkaline midgut of the susceptible insects into an activated toxin which then attaches with the specific receptor on the brush border of midgut epithelial cells to cause pathological effects ultimately leading to insect mortality (Sanahuja et al. 2011). Each Cry protein has a specific and rather narrow spectrum of activity against some insect species (Jurat-Fuentes and Jackson 2012), while being safe to all other organisms (Siegel 2001; Naranjo et al. 2008).

The natural enemies including parasitoids and predators are not adversely affected by the Cry toxin (Romeis et al. 2006; Naranjo 2011). Rather, recent studies have indicated strengthening of biological control in transgenic cotton due to reduced usage of insecticides (Lu et al. 2012). The only major problem encountered in large-scale adoption of foliar *B. thuringiensis* applications was its rapid degradation on the plant surface (Arora 2015). Therefore, successful efforts were made to clone Cry protein genes and incorporate these in crop plants (Nester et al. 2002; Sanahuja et al. 2011). Initially, the expression levels of Cry proteins in experimental plants were not sufficient for insecticidal activity (Peferoen 1997). Substantial increases in expression levels have since been achieved using strong promoters and enhancers and by engineering the codon usage to bring it more in line with the plant-preferred codon usage (Helider and Boulter 1999). Consequently, the expression levels of Cry proteins in transgenic plants have increased to over 100 times those obtained using native Bt genes (Wong et al. 1992). More than 20 Bt genes have so far been incorporated into cotton, corn, soybean and other important crops for lepidopteran and/or coleopteran activity (Shera and Arora 2015).

The genetically engineered cotton called Bollgard incorporates a *cry1Ac* gene from *B. thuringiensis*. The technology developed by Monsanto was used to transfer the Bt gene into the Delta and Pine Land varieties (Coker type) using DP5415 and

DP5690 as recurrent parents. The D&PL brand Bt varieties were designated as NuCOTN 33^B and NuCOTN35^B, respectively, and were the first Bt cotton varieties released for commercial cultivation in the USA in 1996 (International Cotton Advisory Committee 1995, 1997). It was released as 'Ingard' in Australia by Deltapine and Cotton Seed Distributors, both subsidiaries of Monsanto (Fitt 2003). In China, the Chinese Academy of Agricultural Sciences developed Bt cotton by using modified Bt fusion gene (*cry1ab*, *cry1Ac*) inserted in local varieties, which was commercially released in 1997 (Pray et al. 2001). The primary target pests successfully managed by these varieties included various species of bollworms and budworms including *Helicoverpa* spp., *Heliothis* spp., *P. gossypiella* and *Earias* spp. (Naranjo 2011). Bt cotton also reduced survival of other lepidopteran such as armyworms, cabbage loopers, leaf perforators and soybean loopers (Hardee et al. 2001). The adoption of Bt cotton in USA, Australia, China, India and other countries resulted in a sharp decline in insecticide application to cotton crop, increased the yield of seed cotton and benefited cotton growers through improved income (International Cotton Advisory Committee 2001a; Brookes and Barfoot 2015). The reduced insecticide usage also helped to increase the abundance of natural enemies in Bt cotton fields (Lu et al. 2012).

In spite of its widespread adoption, the control of some of the bollworm and defoliator pests of cotton with the *cry1Ac* Bt cotton was not achieved up to the desired level (Fitt et al. 1988; Forrester et al. 1998; Hardee et al. 2001). Therefore, stacked Bt cotton (Bollgard II or BG II) with two cry genes (*cry1Ac*, *cry2Ab*) was developed (International Cotton Advisory Committee 2001a, b). This two-toxin cotton was first planted in 2003 in the USA and in 2006 in India (International Cotton Advisory Committee 2003a; Fabrick et al. 2015). The BG II cotton genotypes provided for a broader spectrum of activity against the lepidopteran pests (International Cotton Advisory Committee 2003a; Naranjo 2011). The *cry2Ab* gene in Bollgard II ensured good control of the fall armyworm *Spodoptera frugiperda*, beet armyworm *S. exigua*, cabbage looper *Trichoplusia ni* and soybean looper *Pseudoplusia includens*, in addition to bollworms and budworms already controlled by Bollgard (International Cotton Advisory Committee 2003a). It has also been observed to provide better protection from the tobacco caterpillar *Spodoptera litura*, a sporadic pest of cotton in India (Mann et al. 2010), and red bollworm, *Diparopsis watersi*, in Burkina Faso in Africa (International Cotton Advisory Committee 2004b). Bollgard II also produces the β-D-glucoronidase (GUS) marker protein to facilitate detection of plants capable of producing *cry2Ab* (International Cotton Advisory Committee 2008). Further, in view of large-scale adoption of Bt cotton, reports of field-evolved resistance to *cry1Ac* containing Bollgard cotton by the pink bollworm from Gujarat, India, were received by 2008 (Dhurua and Gujar 2011). Double-stacked cotton is believed to help in managing resistance to Cry toxins (Ferre et al. 2008; Tabashnik et al. 2009).

In addition to endotoxins (Cry toxins), some strains of *B. thuringiensis* also produce exotoxins during the vegetative phase. These toxins are known as vegetative insecticidal proteins (VIPs) and a large number of such proteins have been isolated from different *B. thuringiensis* strains (Crickmore et al. 2016). While Monsanto produced Bollgard and Bollgard II cottons, Syngenta came up with transgenic cotton containing VIP 3A, which was selectively toxic to a number of lepidopteran insects (Mascarnhas et al. 2003; International Cotton Advisory Committee 2003b). Similarly Dow Agrosciences came up with its own version of Bt cotton called 'Widestrike' containing *cry1Ac* and *cry1F* genes from *B. thuringiensis*. It provided season-long protection from a broad spectrum of lepidopteran pests (International Cotton Advisory Committee 2004a) and was released for commercial cultivation during 2005 in USA. Investigations into the comparative efficacy of Bollgard II and Widestrike cottons against different lepidopteran pests by a number of researchers revealed that both the stacked genotypes were effective against all the important lepidopteran pests. However, the Widestrike cotton with Cry1Ac and Cry1F was highly effective and provided better control of *S. frugiperda* as compared to Bollgard II cotton with Cry1Ac and Cry2Ab toxins (International Cotton Advisory Committee 2008). The Widestrike 2 cotton was released for commercial cultivation in Australia, Brazil, Costa Rica and Mexico besides the United States. In addition to these countries, the Bollgard II was adopted in Columbia, India and South Africa (International Cotton Advisory Committee 2014).

Recently Bollgard III and Widestrike 3, with three-stacked insect resistance genes have been developed. Both Bollgard III and Widestrike 3 contain *vip 3A* in addition to *cry 1Ac* plus *cry 2Ab* genes in the former and *cry 1Ac* plus *cry 1F* genes in the latter genotype (International Cotton Advisory Committee 2014; Whitehouse et al. 2014). Bollgard III was granted regulatory approval for commercial cultivation in Australia in 2014 and Brazil in 2016 with the hope that the additional toxin (VIP 3A) will reduce the selection pressure for resistance to Bt toxins and extend the life-span of Bt cotton.

9.5 Concerns About Bt Cotton

Any new technology comes with its own set of advantages and limitations, and transgenics are no exception. The major areas of concern in case of Bt cotton include impact on non-target organisms, development of resistance to Bt toxins and broader socio-economic impacts on the adopting farmers (Naranjo et al. 2008).

9.5.1 Toxicity to Non-Target Organisms

The season-long expression of Bt toxins in cotton plants has aroused concerns about their safety to nontarget organisms, especially the natural enemies encountered in the cotton ecosystem. Several studies have revealed that there are no meaningful impacts of Bt cotton on predator populations (Naranjo et al. 2005; Romeis et al. 2006; Gatehouse et al. 2011).

Lu et al. (2012) carried out an extensive analysis of predator populations in Bt cotton at 36 locations across China over a 20-year period (1990–2010). The authors reported a marked increase in abundance of three types of generalist arthropod predators (ladybirds, lacewings, spiders). These predators helped to provide natural control of the aphid pests reducing the need for pesticide sprays. The biocontrol services by the conserved predators even extended to the neighbouring crops of maize, peanut and soybean.

In contrast, specialist parasitoid population was adversely affected by reduced host abundance and/or reducing individual fitness through indirect host-mediated effects within Bt-susceptible hosts (Romeis et al. 2006). However, a meta-analysis of several of these studies revealed the overall impacts on arthropod communities were significantly less than those encountered in pesticide-treated conventional cotton (Marvier et al. 2007; Gatehouse et al. 2011). Based on analysis of 360 published studies and scores of meta-analyses on the subject, Naranjo (2011) concluded that unlike conventional bred insect-resistant plants that may sometimes be detrimental to natural enemies, Bt crops have been documented to be essentially benign to a wide range of nontarget invertebrates.

9.5.2 Pest Resistance to Bt Toxins

The selection pressure exerted by the application of highly toxic insecticides to manage nefarious pests has resulted in the development of insecticide resistance in hundreds of species of insect pests. In case of transgenic crops including Bt cotton, the insect pests are continuously exposed to minute amounts of Cry toxins throughout their lifespan. Therefore, probability of development of resistance to these toxins is quite high (Kaur and Arora 2015). Such resistance has been termed as field-evolved resistance and defined as a genetically based decrease in susceptibility of a population to a toxin caused by exposure of the population to the toxin in the field (Tabashnik et al. 2014).

The Bt cotton was first released in 1996, and within a couple of years, Gould (1998) expressed fears about the long-term sustainability of Bt crops due to the ability of insect pests to adapt to these toxins. However, the first report of increasing tolerance of cotton bollworm, *H. armigera* to Cry1Ac cotton in China appeared a decade later (Li et al. 2007). Since then, another two cotton pests, pink bollworm for India (Dhurua and Gujar 2011) and *H. zea* from the USA (Tabashnik et al. 2013), have been suspected to have developed resistance to Bt cotton containing Cry1Ac toxin. In addition, Downes et al. (2010) have reported incipient resistance to cry2Ab (Bollgard II) toxin in *H. punctigera* from Australia.

The refuge (non-Bt cotton or other hosts) coupled with high dose has been the major strategy for delaying pest resistance to Bt crops including cotton (Tabashnik et al. 2008; Tabashnik et al. 2013). The strategy has worked well to the extent that no major cotton crop failures due to pest outbreaks on Bt cotton have been reported from anywhere in spite of some reports of development of pest resistance to one or more Bt toxins (Kaur and Arora 2015). The high-dose refuge strategy works by diluting the frequency of resistant allele and delaying the production of a resistant pest population (Gould 1998; Ives et al. 2011). Non-Bt cotton plants have been used as refugia in the USA, Australia, India and elsewhere, while China has relied on natural refugia of non-Bt alternate host plants of *H. armigera*, the primary target of Bt cotton in China (Wan et al. 2012; Lu et al. 2013). The natural refugia of alternate non-Bt host plants have also worked to an extent in Australia (Sequeira and Playfield 2001) and India (Ravi et al. 2005). But the alternate host strategy cannot be applied for pink bollworm, which is a rather specific pest of cotton.

The second major strategy for delaying development of resistance has been the pyramiding of Bt genes in cotton (Naranjo 2011). The *cry1Ac* gene (Bollgard) was pyramided with *cry2Ab* (Bollgard II) or *cry1F* (WideStrike) to produce double-stacked insect-resistant Bt cotton (International Cotton Advisory Committee 2008). With the advent of these genotypes, the refuge requirements have also generally become less stringent (US Environmental Protection Agency 2007; Carriere et al. 2015).

Carriere et al. (2015) conducted a meta-analysis of 38 studies that reported the effects of 10 Bt toxins used in transgenic corn and cotton against 15 species of insect pests. Surprisingly, they found that compared with optimal low level of insect survival, survival on currently used pyramids was often higher for both susceptible insects and insects resistant to one of the toxins in the pyramid. The researchers concluded that cross-resistance and antagonism between toxins used in pyramid was common. Further, the authors suggested directed pyramid design based on their own and similar studies in future.

Several alternate strategies including the use of seed mixtures, mosaics and tissue-specific and stage-specific toxin expression, combining Bt toxins with biological control and deploying additional microbial or plant genes along with Bt genes have been proposed and tried on a limited scale (Gould 1998; Tabashnik et al. 2013; Kaur and Arora 2015; Carriere et al. 2016).

9.5.3 Socio-economic Impact

Transgenic cotton is now grown in 22 countries and the European union, spanning six continents: Africa (Burkina Faso, South Africa, Sudan), Asia (China, India, Japan, Myanmar, Philippines, Pakistan, Singapore, South Korea, Taiwan), Europe (European union), Oceania (Australia, New Zealand), South America (Argentina, Brazil, Colombia, Paraguay) and North America (Canada, Costa Rica, Mexico, USA) (International Service for the Acquisition of Agri-biotech Applications 2017). Numerous studies conducted across major cotton-growing countries during the last three decades have revealed substantial economic, environmental and social benefits from the cultivation of Bt cotton (International Cotton Advisory Committee, 2000; Shelton et al. 2002; Smale et al. 2006; Mayee and Choudhary 2013; Brookes and Barfoot 2015; Choudhary and Gaur 2015).

Beginning with an area of 0.8 million hectares in the USA, Mexico and Australia, transgenic cotton (insect resistant plus herbicide tolerant) was grown over 75% of the more than 31 million hectares in 2016–2017 (James 2015). With an adoption rate of more than 95%, India has emerged as the largest producer of cotton in the world. In the 13-year period, 2002–2014, India tripled its cotton production from

13 million bales to 39 million bales. The increase in Bt cotton hectares from 50,000 in 2002 to 11.6 million in 2014 represents an unprecedented 230-fold increase during the same period. India more than doubled its share of global cotton production from 12% in 2002 to 25% in 2014. The yield of seed cotton increased from 308 kg/ha in 2001–2002 to 570 kg/ha in 2013–2014. This achievement was combined with a sharp decline in insecticide use on cotton from 46% of total insecticide use in agriculture in 2001 to 20% in 2011. Additionally, cotton seed oil production rose from 0.46 million tonnes in 2002–2003 to 1.5 million tonnes in 2013–2014 (Choudhary and Gaur 2015). In spite of such spectacular performance, increase in suicide rate of cotton farmers since the 1990s has been cited as evidence for failure of Bt cotton in India. One of the environmentalists even called it genocide. Suicides are a complex issue dependant on many factors. A recent analysis of factors contributing to farmer's suicide concluded that implicating Bt cotton in such cases was not based on facts and there has been no increase in farmer's suicide rate since the introduction of Bt cotton (Gilbert 2013). The near total adoption of Bt cotton by more than 7 million predominantly small and marginal cotton growers is itself biggest proof of the profitability and utility of transgenic technology for the farmers.

9.6 Outlook

Transgenic insect-resistant cotton has helped to minimize losses caused by bollworms/budworms and other lepidopteran pests. But whitefly and other sucking pests continue to cause serious damage to cotton crop. In addition, there are reports of bollworms becoming resistant to *cry1Ac* and *cry2Ab* genes. Therefore, there is a need to locate new resistance genes, which can be incorporated into commercial cotton cultivars. Induced defences (Zarate et al. 2007) and RNAi-based gene silencing (Chen et al. 2015) appear promising for developing future insect-resistant cultivars. The recent characterization of Tma 12 protein from a fern is another promising step towards developing whitefly-resistant cultivars (Shukla et al. 2016). In addition, application of *Isaria fumosorosea* (a fungal pathogen of whitefly) expressing dsRNA of whitefly immunity-related gene may aid in developing RNAi technology for whitefly management (Chen et al. 2015). The efforts of the Institute of Cotton Research (ICR) of the Chinese Academy of Agricultural Sciences (CAAS) have resulted in the sequencing and assembling of the genome of *G. arboreum* (Li et al. 2015). This may lead to identification of insect resistance genes in the crop and ultimately lead to development of specifically targeted insect-resistant cultivars. There is an urgency to integrate transgenic insecticidal cultivars with other components of pest management to minimize pest damage as well as to extend the useful life of insecticidal proteins (Naranjo 2011). The combined efforts of agronomists, breeders, biotechnologists and crop protection scientists may lead towards a sustainable cotton production and protection system in future.

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