

Considerations about cotton gene escape in Brazil: a review

Aluizio Borém^{*1}; Eleusio Curvêlo Freire²; Julio Cesar Viglioni Penna³ and Paulo Augusto Vianna Barroso⁴

¹ Universidade Federal de Viçosa, Departamento de Fitecnia, CEP 36570-000, Viçosa, MG, Brazil; ² Embrapa Algodão. E-mail: eleusio@cnpa.embrapa.br; ³ Universidade Federal de Uberlândia. E-mail: penna@umuarama.ufu.br; ⁴ Embrapa Algodão. E-mail: pbarroso@cnpa.embrapa.br. (* Corresponding Author. E-mail: borem@ufv.br)

ABSTRACT

The cotton crop has become recover its importance as a major crop in Brazil. Many growers use good management practices in their fields. One of the new technologies now available for cotton growers in many countries are the genetically modified varieties. The global area of genetically modified crop varieties has grown to 58,7 million of ha, planted by 16 countries. Although GM cotton could eventually be widely adopted, six countries present larger potential benefit of its use: China, India, US and Australia, which already are using these varieties and; Brazil and Pakistan, that still area evaluating the possibility to adopt this technology. The safety of GM varieties for the environment has been one of the main concerns addressed in the case adoption of GM cotton in Brazil. The main concern is the risk of gene flow between GM cotton and its native relatives in Brazil. Fifty species of cotton belonging to the *Gossypium* generum are distributed in the following continents: Asia, Africa, Oceania and America. In this paper we address the scientific basis of gene flow in cotton and some of the alternatives to make the GM technology available and safe to Brazilian growers and the environment.

KEY WORDS: Biosafety, gm cotton, bt cotton, transgenic varieties, gene flow.

The cotton crop in Brazil consolidated its recovery and has been gaining some international markets in the last years. After having been one of the largest world importers of cotton fiber, Brazil returned in 2001 to its self-sufficiency. The renaissance of the cotton crop in Brazil was due to several factors, but certainly the technological transformation of the farming system and of commercialization was one of the most important factors. The fast expansion of the cotton in the savannahs in the Center-west and Northeast of Brazil consolidated the migration to new agricultural frontiers. The favorable edafic and climatic conditions associated with total mechanization in these areas allowed larger productivities, recuperating the competitiveness of the cotton. The Brazilian production of cotton in 2001 reached 2.648.090 tons harvested in about 887.582 ha. The larger producers in Brazil are the states of Mato Grosso, Goiás, Mato Grosso do Sul, Bahia and Paraná.

Fifty species of cotton belonging to the *Gossypium* generum are distributed in the following continents: Asia, Africa, Oceania and America. The *Gossypium hirsutum* L. is one of the four cultivated species for the production of the cotton fiber (Lee, 1984). It is planted in a wide tropical area and in some subtropical regions. This species contributes to 90% of the world

cotton production and has great social and economical importance to Brazil. The harvested product has economical importance for its fiber and oil and protein, which are used in the textile industry and in the animal feeding and human (oil) industry, besides many other secondary products.

In 2002 the global area of genetically modified varieties was of 58,7 million of ha, grown by more than 13 countries and 5 million farmers. The total value of the GM crop was superior US\$4 billion. The resources invested in R&D (research and development) of GM varieties in the private and public sectors exceeded US\$4,5 billion. GM cotton was first commercially planted in 1996. In 2002 the GM cotton was planted at nine countries, seven of which are in developing nations: China, India, Indonesia, Argentina, Mexico, South Africa and Colombia, besides two developed countries: USA and Australia. Cotton is the third GM crop in area globally, after soybean and corn, respectively. Although GM cotton could eventually be widely adopted, six countries present a larger potential benefit for its use: China, India, the US and Australia, which are already using these varieties, and Brazil and Pakistan, which are still evaluating the possibility of adopting this technology. The safety of GM varieties for the environment has been one of the main concerns addressed in the case adoption of GM cotton in Brazil.

In 2001/2002, more than 20% of the area planted in the world was occupied by genetically modified cotton, representing about 6,8 million hectares (James, 2001). In spite of the benefits brought by GM varieties, the concern that these OGMs could produce adverse effects on the environment has been the goal of many studies and discussion among researchers around the world. Among the concerns, the escape of the transgenes through gene flow and its introgression into wild, feral and landrace relatives has received most of the attention. The gene flow from cultivated species to these relatives has been receiving larger attention, due to possible adverse effect on the biodiversity.

Gene flow is the exchange of alleles among individuals, populations or species. The effect of the alleles migration among populations of a species depends on the migrating proportion of individuals and on the difference in the allele frequencies of the two populations. Natural selection can increase the frequency of the introduced alleles, in case there is selective advantage of the individuals bearing the introduced allele. Gene flow can happen through seed dispersion or pollen movement. It can be classified as vertical when it involves individuals and/or populations of the same species; or horizontal when it involves the species that are filogenetically distant.

Factors Affecting the Pollen Dispersal

The subject species. The cultivated species differ in cross-pollination rate. They are therefore classified in self-pollinating - when there is predominance of self-pollination; and cross-pollination species that have predominance of cross-pollination. Typical self-pollinating species include soybean, wheat, common bean and lettuce, among others, whose frequencies of cross-pollination tend to be under 5%. Cross pollination species such as corn, sunflower, onion and eucalyptus, among others, usually possess more than 90% of cross-pollination. There is also an intermediate group that possess an intermediate rate of cross-pollination, that includes cotton and okra among other species.

Differences among cultivars of the species. There is a natural variability among cultivars as far as flower color and size, which may have various effects on pollinizing agents. Cultivars vary in pollen production, which also affect the rate of crossed pollination. The rate of crossed pollination among species and also among cultivars of a given species depends on pollen production and dispersal. Mathematical models have

been used to simulate the patterns of pollen dispersal in cotton and many other species.

THE COTTON CASE

Origin

The precise origin of the cultivated cotton is difficult to determine. The current knowledge on the genus *Gossypium* indicates that this is a very old genus, and that the tetraploid cotton species originated about 2,5 million years ago (Phillips, 1963; Fryxell, 1965). Archaeological evidences on remains of this species date to many centuries before the beginning of the Christian Era. In the site "Mohenjo-Daro", in Pakistan, Gulatti and Turner, mentioned by Lee (1984), found vestiges of fabrics and cotton strings of about 2,700 B.C., which were probably of the diploid species *G. arboreum*. Saunders (1961) tells that the center of origin of the genus is Central Africa. In America, the oldest discoveries are fibers of a primitive type of *G. barbadense*, found by Stephens and Moseley in the archaeological site of "Anchon-Chillon" in Peru, dating from 2500 to 1750 B.C. (Lee, 1984). As a species cultivated worldwide and used in manufacture, it is believed that India is the oldest center of domestication, remounting to the eighth century before the Christian Era.

Specialists also agree that the tetraploid cotton possesses genomic constitution AD (Beasley, 1940a, 1940b). They originated from the hybridization of two other species, followed by the duplication of the number of chromosomes, producing an amphydiploid (DD x AA \rightarrow AD \rightarrow AADD). There is no agreement, though, about the time of the polyploidization.

According to Hutchinson et al. (1947), tetraploid cottons have evolved under domestication after the introduction in South America of a cultivated species of the Old World having the A genome, *G. arboreum* probably, that hybridized with species related to the diploid *G. raimondii* (D genome). Wendel (1989) and Wendel and Albert (1992), based on the degree of divergence among tetraploids in relation to diploid species and also considering rates of evolutionary changes, have estimated the divergence of genomes A and D from a common ancestor as having occurred between 6 and 11 million years ago. They also estimated that the polyploidization is more recent, taking place in the New World between 1,1 and 1,9 million years ago. The oceanic migration of the genome A-bearing ancestor, in this case, should be

mandatory. Gerstel (1953) presented cytogenetic evidences which indicated that the genome A ancestor was probably *G. herbaceum*, a species of African distribution. The authors also concluded that the hybridization of the two genomes followed by polyploidization may have occurred in the beginnings of the Tertiary, circa 65 million years ago.

The theory of Skovsted (1934) and Saunders (1961) is based on the fact that hybridization of the two genomes has preceded the separation of the southern super-continent Gondwana, in the Cretaceous Period (from 136 to 65 million years ago), when the species bearing the genomes A and D would have had sympatric distribution.

Endrizzi et al. (1984) summarized the main events about the origin of allotetraploids at the light of studies related to DNA analyses: i) species bearing the genome A have occurred through macroevolution of a genitor of genome D; ii) hybridization followed by polyploidization has occurred in a short geological period, shortly after the down of the genome A species; iii) the amphidiploid probably appeared during the Cretaceous; and iv) the diploidization of the regulatory system of the hybrids has led to the formation of at least five amphidiploid species.

Taxonomy

Gossypium is one of the eight genus which comprises the tribe Gossypieae, family Malvaceae (Fryxell, 1979). The genus was previously placed in the tribe Hibisceae, but the author cited presented evidences for its classification into Gossypieae (Fryxell, 1968). Cottons under cultivation in the world belong to four distinct species of the genus: two allotetraploid species and two diploids, respectively *Gossypium hirsutum* L., *G. barbadense* L., *G. arboreum* L. and *G. herbaceum* L.

Segregation of the allotetraploid cottons corresponds to that of the diploid species, presenting predictable Mendelian proportions for simple inherited characters. *Gossypium hirsutum* L is the species of greater economic importance due to its great area of cultivation and its total lint production. This species is known in the Northern Hemisphere as *upland cotton*, and in Brazil as “annual” or “herbaceous” cotton. Its plants present sub-bushy growth and are not truly annual, because depending on favorable growing conditions after harvest, they may continue vegetating and therefore may produce for two or more years. In countries of temperate climate, frost and low

temperatures kill crops after harvest, whereas in Brazil and other countries of the Southern hemisphere they are destroyed by farmers after harvest.

The species *G. barbadense* L. assumes greater importance in the production of high-quality fibers, as in the cultivation of the “Pima” cottons in the Northern Hemisphere. In Brazil, this species encompasses a botanic race, the *brasiliense*, which occurs at Brazilian villages of natives and at backyards in the interior of the country. It is known as “kidney cotton”, due to the fact that they present linterless seeds clustered together in the shape of a kidney. The other two species, *G. arboreum* L., cultivated in Pakistan and India, and *G. herbaceum* L., cultivated in Africa, are utilized in small scale.

Other species occur in widely distant regions of the world, but mainly in arid zones in the tropics and subtropics. Cytogenetic studies have demonstrated the existence of genomic groups that gather species of high degree of homology in chromosome pairing amongst its components and therefore low pairing among species of different groups upon hybridization. Such groups were identified by letters, from A to G by Beasley (1942), with subscript numbers for genomes closely related. The diploid species ($2n = 2x = 26$) were classified into three geographic groups by Fryxell (1979): Australian, with 11 species (C genome); American, with 12 species (10 in Mexico e two in Peru and the Galapagos Archipelago - Ecuador) (D genome); and Afro-Arabic, with eight species (A, B, E and F genomes). Within this last group are the cultivated species *G. arboreum* and *G. herbaceum*. The allotetraploid species ($2n = 4x = 52$) are six, two of them are cultivated (*G. hirsutum* and *G. barbadense*) and the others are found in Hawaii (USA), Brazil, Ecuador (the Galapagos Archipelago) and Mexico (AD genome). Fryxell (1992) revised the genus and described 50 species, five of which are tetraploid considering the incorporation of *G. lanceolatum* to the species *G. hirsutum* (Table 1). There is no consensus, however, among specialists concerning the classification presented by Fryxell due mainly to the complexity of the variability found within the genus.

Domestication of Cottons

The domestication of the cultivated species in the Old World must have occurred in the present region of Saudi Arabia, according to Hutchinson (1959), from the perennial type *G. herbaceum*, race *africanum*.

Table 1. The species of *Gossypium*, their genomic constitution and geographic distribution

Species	Haploid Genome	Distribution
Tetraploids		
<i>G. hirsutum</i> Linnaeus (cultivated species)	(AD) ₁	Africa, America, Asia, Europe and Oceania
<i>G. barbadense</i> Linnaeus	(AD) ₂	Central and South America
<i>G. tomentosum</i> Nuttall ex Seemann	(AD) ₃	Hawaii (USA)
<i>G. mustelinum</i> Miers ex Watt	(AD) ₄	Northeast Brazil
<i>G. darwinii</i> Watt	(AD) ₅	The Galapagos (Ecuador)
Diploids		
<i>G. herbaceum</i> Linnaeus	A ₁	Cultivated species
<i>G. arboreum</i> Linnaeus	A ₂	Cultivated species
<i>G. anomalum</i> Wawra and Peyritsch	B ₁	Angola and Namibia
<i>G. anomalum</i> subsp. <i>Senarense</i> (Wawra and Peyrit.) Vollesen	B ₁	Niger, Chad, Sudan and Ethiopia
<i>G. triphyllum</i> (Harv. and Sond.) Hochreutiner	B ₂	Angola, Botswana and Namibia
<i>G. capitiviridis</i> Mauer	B ₃	Cabo Verde Isles
<i>G. sturtianum</i> J.H. Willis	C ₁	Australia
<i>G. nandewarense</i> Derera	C _{1-n}	Australia
<i>G. robinsonii</i> F. Mueller	C ₂	Australia
<i>G. londonderriense</i> Fryx., Crav. and Stew.	-	Australia
<i>G. rotundifolium</i> Fryx., Crav. and Stewart	-	Australia
<i>G. exiguum</i> Fryxell, Craven and Stewart	-	Australia
<i>G. pilosum</i> Fryxell	-	Australia
<i>G. australe</i> F. Mueller	-	Australia
<i>G. nelsonii</i> Fryxell	-	Australia
<i>G. thurberi</i> Todaro	D ₁	Mexico and USA
<i>G. armourianum</i> Kearney	D ₂₋₁	Mexico
<i>G. harknessii</i> Brandegee	D ₂₋₂	Mexico
<i>G. davidsonii</i> Kellog	D _{3-d}	Mexico
<i>G. klotzschianum</i> Andersson	D _{3-k}	The Galapagos (Ecuador)
<i>G. aridum</i> (Rose and Stan. ex Rose) Skovs.	D ₄	Mexico
<i>G. raimondii</i> Ulbrich	D ₅	Peru
<i>G. gossypoides</i> (Ulbr.) Standley	D ₆	Mexico
<i>G. lobatum</i> H. Gentry	D ₇	Mexico
<i>G. laxum</i> Phillips	D ₈	Mexico
<i>G. trilobum</i> (DC.) Skovsted	D ₉	Mexico
<i>G. schwendimanii</i> Fryxell and S. Koch	-	Mexico
<i>G. turneri</i> Fryxell	-	Mexico
<i>G. stocksii</i> Masters ex Hooker	E ₁	Somalia, Oman and Pakistan
<i>G. somalense</i> (Gurke) J.B. Hutchinson	E ₂	Eastern Africa
<i>G. areysianum</i> Deflers	E ₃	Iemen
<i>G. incanum</i> (Schwartz) Hillcoat	E ₄	Iemen
<i>G. bricchettii</i> (Ulbrich) Vollesen	-	Somalia
<i>G. benadirensis</i> Mattei	-	Ethiopia, Somalia and Kenya
<i>G. trifurcatum</i> Vollesen	-	Estern Somalia
<i>G. vollesenii</i> Fryxell	-	Somalia
<i>G. longicalyx</i> J.B.Hutchinson and Lee	F ₁	Sudan, Uganda and Tanzania
<i>G. bickii</i> Prokhanov	G ₁	Australia

^{1/} Genome not-determined; Source: Adapted from Fryxell (1969) and Endrizzi et al. (1984).

Hutchinson et al. (1947) believe that the cultivation and threading of cotton have developed in societies that already had proper technology for manufacturing products from line and wool.

In the New World, according to Fryxell (1979), there

are strong evidences that the events that made the establishment of the tetraploids possible preceded the presence of man and therefore were independent of his action. Such statement is based upon the following arguments: 1) the great diversity among tetraploids is

an indication of their origin as to what has happened prior to the existence of agriculture in the American Continent; 2) the great geographical distance existing among the species - *G. tomentosum* in Hawaii, *G. darwinii* in Ecuador (the Galapagos), *G. mustelinum* in Northeast Brazil, *G. hirsutum* and *G. lanceolatum* in Central America and *G. barbadense* in South America; 3) the morphological characters of the species and wild types are indeed primitive and can not be considered a simple return of cultivated types to wild forms; 4) the habitats of the wild tetraploids are shores and coastal plains, a fact that along with the presence of impermeable seed coats suggests aquatic dispersion. Therefore, man might have encountered the tetraploids and from them, he might have initiated the process of domestication through selection. Still according to the author, the effect of such selection was enormous for the four cultivated species, which made the development of an economic exploration of large scale - the cotton industry complex- possible.

DISPERSION

Archaic Dispersion

The geographic placement of the components of the tribe Gossypiae is worldwide, with the genera and species present in the tropical and subtropical regions with a pattern of distribution that might be related to oceanic dispersion. According to Fryxell (1979), the distribution of *Gossypium hirsutum* throughout the Pacific Ocean (Socorro Isles, Marquises, Samoa, Tahiti, Wake Island and North Australia), the Caribbean Sea and Mexican Gulf, suggests seed transport via oceanic currents towards far regions out of the center of origin (Central America). Stephens (1966) however, presented opposite evidences for the transoceanic transport of the majority of types throughout South Pacific, especially either the inability of seeds to float for long periods of time, or their low resistance to saline conditions. The author speculated the possibility of human dispersion by Polynesian travelers for the case of cotton species present in the South Pacific islands. With the exception of the genus *Hampea*, all genera of the tribe present hairy seeds. This is an extremely ancient adaptation that became very important for the use of the cultivated species of *Gossypium* as an economic important crop and industry (Fryxell, 1979). This is the only case of polyploids within the tribe and the origin of the phenomenon is intimately related to its invasion of new ecological niches. The diploid species

of the genus are present in the inner environments of the continents, generally arid habitats, even when they occur in islands. The types of tetraploid species (*G. hirsutum*, *G. barbadense*, *G. darwinii* and *G. tomentosum*) considered to be wild, typically appear in shores, occurring directly amidst coastal vegetation. *G. mustelinum*, however, distributes itself in the mountain regions of Northeast Brazil, especially in the state of Bahia (Freire et al., 1990). Such amphidiploids could have invaded the coastal environments when the sea levels were ascending and descending due to the alternate advance and retreat of glaciers. Shores might have been fertile environments for evolutionary changes and after man had recognized the utility of fibers, a new dispersal might have happened in a "second wave of evolutionary opportunism". Still according to Fryxell (1979), the characters that allowed such maritime dispersal were the impermeable seed coat, relatively long-living embryos and the hirsute seeds, together with the capacity of plants to grow in relatively saline substrates. This set of traits ("the tropical imperative") is used as arguments to reject Stebbins vision (Stebbins 1947, 1959), i.e., cottons related to present-days *G. arboreum* and *G. herbaceum* would have migrated from the Old to the New World through the strip of land at Bhering Strait between 65 and 54 million years ago. The low temperatures of such latitudes would be too extreme for the species even considering the warmer climate of the Eocen. The reproductive patterns of *Gossypium* would be ineffective at those latitudes.

Modern Dispersion

Alexander from Macedonia was probably the one who introduced the cotton plant and its manufactured products into the West (Scherer, 1916 cited by Lee, 1984). The Assyrian already cultivated cotton circa 700 b.C. The Arabs introduced cotton in Spain in 712 a. C. and in the Renaissance, the city-state of Venice represented an important dispersion center for manufactured cotton products coming from India to all Europe. Columbus found the New World natives already utilizing cotton cloths in their daily life.

Gossypium Species in Brazil

Three species of the genus *Gossypium* occur in Brazil, all of them allotetraploids: *G. hirsutum*, *G. barbadense* and *G. mustelinum*. None of these species are

considered weeds in cultivated fields or in natural areas. These occur as cultivated, landraces, dooryard, feral and wild species, as described in Table 2. The main regions where non-cultivated forms of *Gossypium* can be found in Brazil are in the Figure 1.

G. mustelinum is a wild species endemic to Brazil and was never improved or commercially grown (Freire et al., 1990). Its center of origin is the Northeast Region. A few natural populations are found in specific sites in Caicó (State of Rio Grande do Norte), and Macurerê and Caraíba (Bahia State) (Freire, 2000). The small number of individuals found might be an indication that the species is in eminent risk of extinction. According to the results obtained by Wendel et al. (1994) and Freire et al. (1998), gene flows from other species do not seem to be the key factor endangering the species. They report the main threat to the survival of *G. mustelinum* as habitat destruction caused by deforestation, unsustainable agricultural activities and cattle farming. This eliminates whole natural populations and could lead to the total extinction of this species in a short period of time.

Gossypium barbadense is a shrubby perennial species endemic to South America. It has a wide geographical distribution in Brazil, from the Amazon region, the low lands of Maranhão and Piauí States, in mining cities of Mato Grosso State, in areas around the Pantanal and in the Atlantic rain forest from Rio Grande do Norte to Espírito Santo. In the 16th century *G. barbadense* L. was cultivated in Maranhão, and at this time became one of the principal Brazilian export products. Two varieties are found in Brazil, basically differing only in their seed form: *G. barbadense* var. *barbadense*, known as Quebradinho or Maranhão and *G. barbadense* var. *brasiliense*, known as Rim-de-Boi (cattle kidney). Brubaker et al. (1999) consider that *G. barbadense* var. *brasiliense* is more adequately classified as a semi domesticated form that is geographically isolated from *G. barbadense* var. *barbadense*, rather than a taxonomically distinct

entity. The dispersion of *G. barbadense* occurred as a result of agricultural activities (Boulangier and Pinheiro, 1972). Small farmers have maintained them as landraces varieties through many generations, cultivated in small quantities to produce handicrafts, medicine and wicks for oil lamps.

Gossypium hirsutum originated in meso-America. Two biotypes are cultivated in Brazil. The biotype *latifolium*, (*G. hirsutum* L. race *latifolium* Hutch.) also known as herbaceous or upland cotton, is widely cultivated, particularly in areas where medium to high levels of technological inputs are used. It is an allotetraploid variety, originally from Central America, and only found in the cultivated form. The second biotype is *G. hirsutum* var. *Marie Galante*, known as mocó or arbóreo cotton. Its center of origin is large, ranging from the Colombian and Venezuelan coast to the Southeast of El Salvador and the Antilles (Stephens, 1973). Stephens (1967) has two hypotheses about the introduction of *Marie Galante* into Brazil: the Dutch may have introduced it between 1632 and 1654 from their Caribbean colonies to their colony on the northeast coast, or it may have been introduced from Africa. During the seventies, mocó cotton was grown on more than 2.5 million ha for its high fiber quality and drought resistance. Technical and economic constraints and the introduction of the boll weevil in the region resulted in a dramatic reduction, and today, only ca. 9 000 ha in the Seridó regions of Paraíba and Rio Grande do Norte are still cropped with this variety. Immigrants from the Northeast still plant mocó plants in some counties of the Amazon Region, mainly for medicine and domestic uses (Freire, 2000).

Verdão cotton is the only putative natural inter-specific hybrid of *G. hirsutum*, *G. barbadense* and *G. mustelinum* that was cultivated in the beginning of the 20th century, mainly semi-arid region in Northeast of Brazil. The cultivation of verdão was stopped some years after the introduction of mocó improved varieties.

Table 2. Populations' forms of *Gossypium* species and races in Brazil.

Specie	Wild	Feral	Dooryard	Landrace	Cultivated
<i>G. mustelinum</i>	X				
<i>G. barbadense</i> var. <i>brasiliensis</i>		X	X	X	
<i>G. barbadense</i> var. <i>barbadense</i>		X	X	X	
<i>G. hirsutum</i> var. <i>latifolium</i>					X
<i>G. hirsutum</i> var. <i>Marie galante</i>		X	X	X	X

The vanishing of landraces and non-cultivated relatives of the cultivated crops is a phenomenon documented from the beginning of the modernization of agriculture, which became especially evident after the Second World War and has been causing concern among the germplasm curators. It is important the preservation of the wild, feral and landraces as a variability reservoir.

The best alternatives for the conservation of the Brazilian wild cotton are:

i) Intensification of the collection of wild types, especially the Moco cotton- *G. hirsutum* Marie gallant and the *G. barbadense*, which may become extinct more rapidly due to the pressure by the improved cotton;

ii) Delimitation of the areas for preservation of the Brazilian wild cottons;

iii) Zoning of areas for planting of cotton transgenes.

Cotton Reproductive System

All the species of the genus *Gossypium* have complete flowers. Pollination occurs immediately after anthesis, with the likelihood of self-pollination and/or cross-pollination. Cotton plant pollen is relatively large, 81 to 143 microns, viscous (what makes the grains adhere to each other), spherically-shaped, covered with a great amount of spicules and practically not wind-pollinated (Kasiev, 1964 cited by McGregor, 1976; Oosterhuis and Jernstedt, 1999). Under field conditions, pollen remains viable until late afternoon but it may last for 24 hours if stored at 2 to 3°C (Calhoun and Bowman, 1999). Due to its size and the formation of small clusters, wind-pollinated pollen has not been reported for the genus *Gossypium* (Stewart, s.d.). Thus, for cross-pollination to occur, pollinating insects are needed, mainly from the Hymenoptera family, especially the *Bombus* spp (*Bombus* spp), the melliferous bees (*Apis mellifera*) and the wild bees of several genera (Silva et al., 1973). In the presence of pollinators, cross-pollination rate varies with the genotype, location, barriers, distance between fields, environmental factors, biotic factors and crop management. In the absence of pollinators, reproduction occurs exclusively by self-pollination.

The cotton pollinators most cited in the literature are *Apis mellifera*, *A. dorsata*, *A. florea*, *A. cecropia*, *Melissodes* spp., *Halictus* spp., *Bombus* spp., *Anthophora confusa*, *Elis thoracica* e *Scolia* spp. Pollen dispersal over long distances is associated to factors related to the foraging habits of its pollinators,

with flying capacity being a very important aspect. According to Malone (2002), most studies report that the maximum foraging distance of melliferous bees is 10 Km, and most of them are found at 6 Km from the hive, at an average foraging distance between 0.5 and 1.5 Km. Also, according to Malone (2002) distances greater than 13.7 Km have been reported, but this result was obtained in a desert without any other food source available. The flight distance of *Bombus* spp foraging is also high. Helnrich (1979) verified that at 5 Km foraging flights might be effective, provided that the likelihood of collection is greater than in nearby areas. Hedtke (1994) verified that *Bombus terrestris* and *B. lapidarius* can perform foraging at distant places up to 4 Km. Distance seems to vary with the species, with some hardly ever searching for food at distances over 500 m from their nest, while others tend to search for food in far away places. Sting-less bees have lower flight capacity as compared to the melliferous and *Bombus* spp bees. According to Kerr (1959), there is a certain level of correlation between the body size of these bees and flight distance. Small bees, such as those of the subgenus *Plebeia* (3-4 mm) display a flight range of around 300 meters; medium-sized bees, such as *Torigona* (5 mm), have a flight reach of approximately 600 meters; and large bees (10 mm) reach around 800 meters while very large bees (13-15 mm) as *Melipona fuliginosa*, could reach around 2.000 meters (Kerr, 1954; Wille, 1983). Some species can conduct extremely long flights, such as *Euplusia surinamensis* (Mc Gregor, 1976), but are not included among the species described as cotton pollinators.

Cotton flowers are large and complete, facilitating insect visitation. Pollen is dispersed through the anthers after the flower opens, remaining viable from approximately 12 to 24 hr (Cobley, 1956). The flowers open early morning and close late afternoon on the same day, without opening again. Pollen viability decreases gradually after 9:00 am although it has been verified that the pollen remains viable and over 80% of it can be shed after 5:00 pm. Only a small portion of the pollen can still perform pollination at 8:00a.m. the next day. Pollination occurs 30 hours after pollination (Free, 1993).

A factor that increases the capacity of attraction of cotton to the *Hymenopteros* is the presence of five sets of nectaries, a floral and four extra-floral ones. The floral nectary is formed by a ring of papilliform cells on the base of the inner face of the calyx.

The extra-floral nectaries include: a) three triangular

nectaries surrounding the calyx, next to the base; b) three nectaries in the pedicel of each flower, below each bract of the epicalyx; c) a nectary on the basal part of each flower; d) unipapilled nectaries on the floral peduncle and stem of young leaves. While the floral nectary secretes nectar only on the day the flower opens, the extra-floral nectaries do this for a few days. Although it has been observed that bees prefer to visit the floral nectaries, the extra-floral ones are important to maintain the bees in the culture before the flowers open (Moffett et al., 1979) and to attract part of the harmful insects, keeping them away from the flowers (McGregor, 1976).

Several methodologies have been applied to estimate the rate of cross-pollination in cotton. They can be classified into four types, according to the marker used: morphological, molecular, colorimetric and bioassays for transgenic characteristics.

The morphological methodologies are based on qualitative phenotype markers of simple inheritance. The absence of gossypol glands in the seeds, the color of the hypocotile and leaves, and leaf and bract shape have been utilized most frequently. Recent studies utilizing morphological markers employed the absence of gossypol in the seeds (Moresco et al., 1999; Xanthopoulos and Kechagia, 2000; Thitiprasert, 2001), mainly for presenting the xenia effect, i.e., the phenotype marker is expressed in the F_1 seed, without the need to germinate the seeds for evaluation. Although there are other loci controlling this characteristic, the most used ones are based on the locus $G1_2$ and $G1_3$, whose phenotype without gossypol is expressed only in homozygous plants for the recessive alleles, gl_2gl_2 , gl_3gl_3 . The remaining allelic combinations result in normal plants, with gossypol in the seeds. Under this system plants without gossypol are used as females, receptors of pollen of normal plants from a source plot. If the seed is originated from pollination from normal plants, it will display gossypol glands. Self-fertilized seeds will not have glands.

The colorimetric method uses a fluorescent pollination or a conventional dye to mark the flowers visited by pollinating insects. Queiroga et al. (1993) and Freire (2002b) used methylene blue to verify the potential rate for cross pollination mediated by insects, applying a simple method: immediately after opening, the flowers in the plot to be used as the source of pollen are pulverized with the powdered dye. At the end of the day, water is sprayed on the flowers of a pollen-receiving plot. If an insect that

visited a flower that had been marked with the dye also visited flowers from the receiving plot, it will shed grains of the substance, which will dissolve in contact with water, producing a bluish solution. The estimate generated is not direct, since the amount of cross-pollination is not measured, but rather the proportion of the flowers visited by insects. This method can provide estimates of the cross potential and help determine important parameters on the behavior of pollinating insects in the cultures and on the pollen dispersal conducted by them.

The third methodology is based on the analysis of a population to detect markers of DNA or protein originated from another population. This is frequently used to evaluate the crossing rate and the gene flow based on transgenic plants and may apply to markers specifically built to detect transgenic DNA (Umbeck et al., 1991; Shen et al., 2001 cited by Jia, 2002) or the proteins derived from it (Llewellyn e Fitt, 1996; Shen et al., 2001 cited by Jia, 2002). Molecular methodologies are also applied to estimate the Gene flow among natural populations of the genus *Gossypium*, with the analysis being conducted according to population genetic methodologies (Percy and Wendel, 1990; Wendel and Percy, 1990; Wendel et al., 1994; Wendel et al., 1992).

Bioassays have been used to evaluate the crossing rate between transgenic and conventional cotton populations. The expression of the transgenic characteristic in an individual descendant from a conventional plant (female parent) indicates that it was formed by cross pollination with transgenic pollen. Resistance to a particular selective marker is usually used in the transgenic plants, such as resistance to antibiotics (Umbeck et al., 1993; Llewellyn and Fitt, 1996), abnormal development or death of moths in assays involving type Bt transgenics (Llewellyn and Fitt, 1996) and the survival of plants submitted to herbicide application (Zhang et al., 1997 cited by Jia, 2002).

Several experiments to determine crossing rate have been developed in Brazil. Table 3 shows that the Moco cotton plant tends to cross more frequently and with greater variation than the herbaceous cotton plant. There is a great variation among the rates verified in different places, which may be related to a higher or lower presence of pollinating insects.

The degree of pollen dispersal from a given source has been measured by experiments specifically built for this purpose. The experimental designs applied

in these studies are highly variable. Some authors utilized a central pollen source plot surrounded by border receptors (Umbeck et al., 1991). Other studies presented an inverted plot arrangement with the source on the borders and the receptor at the center (Queiroga et al., 1993; Xanthopoulos and Kechagia, 2000). Other designs are a combination of the above-mentioned types with the receptor plots on the corners and at the center of a larger source-plot (Xanthopoulos and Kechagia, 2000). There is no evidence that an experimental design has a greater capacity than another in predicting what occurs inside a commercial plantation, whose size of the pollen source and receptor areas are much larger than the plots used in the studies.

Despite the differences in design, cultivars and locations where the experiments were conducted, the results obtained present a consensual aspect: the reduction of cross pollination rate as one goes inside the receptor plot (Green and Jones, 1953; Umbeck et al., 1991; Queiroga et al., 1993; Llewellyn and Fit, 1996; Zhang et al., 1997 cited by Jia, 2002; Shen, 2001 cited by Jia, 2002; Xanthopoulos and Kechagia, 2000; Freire, 2002b). Thus, the highest percentage of cross pollination-originated seeds has always been verified in the plants near the pollen source, drastically

reducing as distance increases, apparently in an exponential way. Not all the experiments conducted have shown an absence of cross-pollination in the greatest distances evaluated. However, in those in which this was verified, a correlation directly proportional to the frequency of cross-pollination at the shortest distance evaluated seems to occur. That is, the higher the cross-pollination rate of the individuals closer to the pollen source, the greater the distance necessary to nullify the cross-pollination rate.

This type of pollen dispersal was verified in experiments with the highest cross-pollination rates below 1% (Llewellyn and Fit, 1996), near 1% (Xanthopoulos and Kechagia, 2000), 5% (Umbeck et al., 1991), 10% (Zhang et al., 1997 cited by Jia, 2002; Shen, 2001 cited by Jia, 2002) 20% (Green and Jones, 1953), 70% (Freire 2002) and 100% (Queiroga et al., 1993). Such behavior occurred regardless of the type of cotton plant studied, with the same gene flow pattern being observed in experiments with transgenic herbaceous (Freire, 2002; Llewellyn and Fit, 1996; Umbeck et al., 1991), conventional herbaceous (Green and Jones, 1953; Xanthopoulos and Kechagia, 2000), Moco (Queiroga et al., 1993) and *G. barbadense* (Shen, 2001 cited by

Table 3. Cross pollination rates in different localities in Brazil.

Author	Cotton type	Location	Rate (%)
Cavaleri e Gridi-Papp (1963)	Herbaceous	São Paulo. SP	6 to 41
	Herbaceous	Campinas. SP	33
Mangueira (1971)	Moco	Serra Talhada. PE	1 to 100
Silva et al. (1973)	Moco	Ceará	55
Castro (1975)	Herbaceous	Sete Lagoas. MG	32
Crisóstomo (1988)	Herbaceous	Campinas. SP	30.5
	Herbaceous	Campinas. SP	40.8
Penna et al. (1991)	Herbaceous	Uberaba. MG	25
Queiroga et al. (1993)	Moco	Patos. PB	3 to 97
Resende e Fallieri (1995)	Herbaceous	Porteirinha. MG	10.11
Moresco (1999)	Herbaceous	Campo Verde. MT	6.54
	Herbaceous	Pedra Preta. MT	50.44
	Herbaceous	Pedra Preta. MT	68.83
	Herbaceous	Serra Petrovina. MT	46.85
	Herbaceous	Serra Petrovina. MT	44.98
	Herbaceous	Primavera do Leste. MT	29.26
Freire (2002)	Herbaceous	Capinópolis. MG	0 to 100
	Herbaceous	Santa Helena. GO	20 to 60
	Herbaceous	Acreuna. GO	0 to 12
	Herbaceous	Porteirão. GO	0 to 29

Jia, 2002). Llewellyn and Fit (1996) and Xanthopoulos et al. (2000) observed that the amount of cross-pollination varied, being higher in those in which pollination is presumably most intense. Thus, the direction towards which the source-plot and the receptor plot lie must be taken into account.

The only study aiming at the evaluation of the Gene flow from transgenic cotton plants in Brazil was carried out by Freire (2002a). All the analyses conducted in this study showed that a relatively small number of conventional cotton rows, such as borders, were sufficient to contain the pollen from the interior of the transgenic plant plot.

The highest cross pollination rates were verified in areas closer to places nearby native vegetation woods, from where the pollinators must originate. According to the data obtained, 10 conventional cotton rows would be sufficient to contain pollen from the transgenic cotton experimental areas, even in places where cross-pollination rates in the first border meters were quite high.

The separation of the pollen source and pollen receptor plots by barriers of other cultures taller than cotton or by non-cultivated areas, also reduce the amount of cross-pollination. Queiroga et al. (1996) verified that larger barriers between the source and the receptor plots increase the efficiency to avoid cross-pollination. The greater the barrier between the source and the receptor plots is, the greater the reduction of cross-pollination is. The species utilized in the barrier also influences the efficacy of the protection conferred (Castro et al., 1982), with those barriers constituted by the proper cotton plant apparently being the ones with greater capacity to contain pollen. (Carvalho, 2001; Freire 2002).

Hybridization capacity and Inter-specific Introgression

It is viable to conduct cross-pollination between *Gossypium* races and species occurring in Brazil and to obtain fertile descendants, including the transmission and expression of the transgene (Freire 2002a). The interracial hybrids are totally viable, with availability of commercial exploration of cultivars formed from the crossing between *G. hirsutum* races, such as the CNPA 7MH, one of the most cultivated in the northeastern semi arid region.

The majority of the *G. hirsutum* and *G. barbadense* hybrids are also fertile; however, a small percentage

displays a “corky” characteristic. The “corky” plants display short internodes and suberized stem. They flower and the pollen is viable, but female fertility is severely reduced. (Brubaker et al., 1999). This type of partial sterility is controlled by a locus with three codominant alleles: “0”, common to both species; “X”, *G. hirsutum* specific; and “Y”, *G. barbadense* specific. The combination of the X and Y alleles generates plants with a “corky” phenotype; the remaining combinations bear normal plants. The frequency of allele X, specific to *G. hirsutum*, is very low, while the frequency Y is high, being present in most *G. barbadense* plants.

The introgression studies in natural populations of *G. hirsutum* and *G. barbadense* have shown that cytoplasmic introgression is much smaller than nuclear introgression (Brubaker et al., 1993) and that *G. hirsutum* has a greater amount of *G. barbadense* alleles than conversely (Stephens, 1967; Percy and Wendel, 1990; Wendel et al., 1992; Brubaker et al., 1993; Rieseberg and Wendel, 1993). Rieseberg and Wendel (1993) consider that two factors may contribute to the low frequency of cytoplasmic introgression: lower female fertility in the “corky” type plants and the natural selection of specific cytoplasm-nucleus combinations. On the other hand, the higher allele frequency of *G. barbadense* in *G. hirsutum* may be partly understood by the analysis of the floral habits (Brubaker et al., 1993). As in all malvaceous species, the flowers of both species last only one day. *G. barbadense* corolla starts opening one hour after dawn, earlier than *G. hirsutum* flowers. Thus, the pollinators will be already carrying pollen from *G. barbadense* by the time *G. hirsutum* flowers open. The anti clockwise pollen flow may occur only a short time later, when many *G. barbadense* flowers have already been fertilized. This phenological difference favors a greater amount of *G. barbadense* pollen to be shed in *G. hirsutum* flowers.

The only instance in which a greater gene flow intensity occurred from *G. hirsutum* to *G. barbadense* was reported by Percy and Wendel (1990), in populations in Western Andes, Paraguay and Argentina. Since *G. hirsutum* does not occur naturally in these places and the plants displayed visible signs of improvement, the fact was attributed to recent introgressions, determined by selection for agronomic characteristics. A similar predominant sense of introgression was also verified in cultivated genotypes of these species (Brubaker et al., 1993).

Under the environmental conditions found in Brazil,

G. barbadense flowering apex occurs in July but, depending on water availability, one can find a small amount of flowers almost all year round. For the cultivated herbaceous cotton, flowering occurs from January to May in the Central West, which is presently the major cotton-producing region. Therefore, during cotton crop flowering, a reduced amount of *G. barbadense* flowers is available for cross-pollination, reducing the likelihood of Gene flow occurrence.

An interesting fact verified by Jiang et al. (2000) in descendants of *G. hirsutum* and *G. barbadense* cross was the favoring of specific allelic combinations, resulting in larger and widely distributed genomic regions, in which the frequency of *G. barbadense* sequences was much lower than the expected and in some regions in which *G. barbadense*'s chromatin frequency was above the expected. This makes evident that the introgression maintenance between the two species does not occur similarly throughout the genome. Such result implies that the importance of the Gene flow for the alteration of allelic frequencies must be a function of endogenous selection, besides the traditional effects considered (such as gene effect on the population, amount of immigrants, drift, etc), regardless of the environment, which will result in the unlikelihood of pollination of some gametic combinations and/or in the differential production of gametes. The epistatic interaction affecting gamete or fertility success is consistent with the theory of speciation, in which new alleles in divergent lines are the basis for reproductive isolation.

Inside *G. hirsutum*, morphological analyses have revealed that the botanical variety Marie Galante presents the highest amount of *G. barbadense* genes (Boulanger and Pinheiro, 1971; Stephens, 1967). The authors suggest that the cotton plant Marie Galante must have originated from *G. barbadense* introgressions in *G. hirsutum*. Another diversity analysis carried out on morphological characteristics verified that the exotic accesses of *G. hirsutum* var. Marie Galante, originated from the Antilles (probably the center of origin of this race), was more similar to the *G. barbadense* accesses found in Brazil than to the improved and earlier Moco genotypes (Freire and Moreira, 1998). The data suggest that after centuries of simpatria the Moco cotton plant and the two *G. barbadense* races remain rather distinct. Less morphological similarity with *G. barbadense* observed in the Moco variety than in Marie Galante from the Antilles may have been caused by the

selection of individuals that are more productive and adapted to the environmental conditions in Northeastern Brazil. The fact that the early Moco genotypes and genetically improved cultivars are very similar suggests that the reduced similarity to *G. barbadense* may have occurred before the large-scale Moco planting in the XXth Century.

Isoenzymatic studies using *G. mustelinum* showed that only 6 out of the 50 loci (2) sampled were polymorphic, without any heterozygous plant being verified (Wendel et al., 1994). This indicates that the populations are highly monomorphic and that self-pollination must be more important than cross-pollination. The authors compared data obtained on other allotetraploides species and verified that the level of introgression of *G. barbadense* and *G. hirsutum* in *G. mustelinum* is minimal, if any. In addition, the genetic diversity analysis based on morphological characters carried out by Freire et al. (1998) allowed to unequivocally separate Moco cotton from *G. mustelinum*. These results provide evidences that the genetic flow of the cotton plant cultivated for *G. mustelinum* is not significant. Although part of the *G. mustelinum* population is located in places that are difficult to reach - many kilometers away from plants of other *Gossypium* species - geographic isolation alone cannot explain the genetic isolation observed. In one of the populations studied by Wendel et al. (1994), *G. mustelinum* plants grew near a Moco plantation without any introgression signs being observed. Contrary to the authors' expectations, the only evidence for cross pollination was observed in some Moco plants, which presented colored fiber due to the transference of this characteristic by pollen from *G. mustelinum*.

Gene Flow in Cotton Plants

Gene flow can be an important evolutionary force (Slatkin, 1987). When the levels of hybridization are high and the rate of incorporation of external alleles surpasses the mutation rates, the gene flow becomes the main source of population (Ellstrand et al., 1999). Alleles which do not confer competitive advantage in the environment where these populations are found, i.e., neutral alleles, must not cause a great impact "per se" since there will be no selective pressure acting towards increasing or decreasing their frequencies. In this case, changes in the structures of the populations are dependent on genetic drift and are mainly important in small populations (Wright, 1969).

In the case of unfavorable alleles, if the frequency of introduction is inferior to the selection coefficient against these alleles, they must be eliminated within a few generations. But, if introduction is superior to selection, the population vigor will be reduced. (Slatkin, 1987).

If the introgressed alleles are favorable, natural selection must increase its frequencies in the population, with favorable genes being those conferring greater resistance against diseases and insect attack, whether from cultivars improved by classical methods or transgenically. This type of introgression is relatively common among cultivated and non-cultivated plants, causing an increased capacity for competition. Under agricultural environments, this process can result in a new weed species or in enhanced economic importance of a plant already considered a weed. In native populations, survival fitness in plants can result in a greater capacity to respond to unfavorable situations. The economic and environmental impact will depend on the extent of the increase of the competitive ability (Ellstrand et al., 1999).

In cotton, gene flow may occur in two distinct ways: by pollen or by seed. Dispersion by seeds rarely occurs directly in the field, since these are large, covered with an abundant amount of fibers and seldom transported by animals (Llewellyn and Fitt, 1996). A great amount of water is required for the seeds to germinate and when this occurs under non-agricultural environments, the plants formed have little chance of surviving due to their low colonizing capacity (Wozniack, 2002). Seed dispersion in Brazil generally occurs due to the inadequate use of kernels (normally used as food or to make edible oil) as propagating material, and during transportation of row cotton, pits and seeds, besides cotton mixtures from the Northeastern cotton plantations, which normally hull more than one cotton cultivar or species. Human immigrants are also an important source of gene flow for seeds in Brazil, mainly for *G. barbadense* and Moco cottonseeds.

Gene flow via pollen is likely to occur, since the *Gossypium* races and species occurring in Brazil are sexually compatible with each other and also with the transgenic cotton plants (Freire, 2002a). Under environmental conditions, it can occur in crops or isolated plants, with the help of pollinating insects. Theoretical mechanisms exist which may prevent transgene release by the pollen but there are problems to be solved before they can be applied. Male sterility,

if incorporated into transgenic plants, would not allow the production of viable grains, but the pollination rates obtained from the available systems result in low productivities, thus becoming economically unviable. Chloroplast and mitochondria transformation would make the transgenes maternally inherited, rather than transmitted to the progenies via pollen. However, no technology is available today to make this transformation. Other approaches aiming to solve this problem are available (Bock, 2002; Kwon et al., 2001), but need further experimentation to become viable.

Measures aiming to prevent transgene escape were implemented in the U.S.A and Australia, where large genetically modified cotton crops are cultivated and natural populations of the *Gossypium* genus are available, similarly to Brazil.

There are four *Gossypium* species in the USA, three allotetraploids – *G. hirsutum*, *G. barbadense* and *G. tomentosum* – and one diploid – *G. thurberi*. The Gene flow between the cultivated cotton plants and *G. thurberi* cannot occur due to ploid difference. *Gossypium tomentosum* originates from Hawaii and can be crossed with *G. hirsutum* and *G. barbadense*. In order to prevent the transfer of transgene to *G. tomentosum*, commercial cultivation of transgenic cotton plants was banned in Hawaii. Experimental plots and generation advancement fields located in Hawaii must be surrounded by 12 rows of non-transgenic cotton in plots smaller than 40,000 m² or by 24 lines, if larger. The plots must be located, at least, ¼ of a mile away from *G. tomentosum* plants. The distance of 3 miles (4,827 km) between *G. tomentosum* plants and transgenic plants surrounded by 24 lines (rows) of conventional cotton plants is sufficient to prevent cross pollination. Feral populations of *G. hirsutum* can be found in southwestern Florida. Cultivation south of Route 60 (EPA, 2001) was banned to prevent the release of the transgene of the varieties cultivated to these populations.

Seventeen diploid *Gossypium* species originate from Australia and are exclusively found there. Out of these seventeen, four occur in the same region where the herbaceous cotton plant is cultivated. Not all the species are sexually compatible with the tetraploid species cultivated, due to the difference in chromosome number (G.T.R., 2002). Two major measures were taken to avoid an unlikely gene flow: banning the cultivation of transgenic cotton plants north of latitude 22° S and banning their transport

and use as feed in this region (GMAC, 2001).

The measures implemented in the USA and Australia are complementary, both including exclusion zones. The ban on transportation and use of genetically modified cotton seeds and vegetative parts as cattle feed will substantially reduce the Gene flow via seeds. The adoption of non-transgenic borders in the plantations reduces the likelihood of transgenic pollen dispersal by pollinating insects. The combined contention strategies of the two countries seem to be a good alternative for countries such as Brazil, which have wild and feral populations and landraces varieties of *Gossypium*.

The adoption of exclusion zones was also proposed by Freire (2000) in order to preserve the natural populations and local varieties of *Gossypium* in Brazil. Such a zoning would be valid for conventional cotton and aims at the prevention of genetic mischaracterization of the materials via gene flow. The zones in which herbaceous cotton cultivation would be banned are comprised of 18 states, including the legal Amazon, Mata Atlantica from Rio Grande do Norte to Espírito Santo, Pantanal Matogrossense, southeastern Piauí and the municipalities Caico and

Acari (Rio Grande do Norte State), Macururé and Caraiba, (Bahia), where *G. mustelinum* and Moco cotton plants are found (Figure 1). This exclusion zone is not a definite proposal and its main objective is to prevent the loss of variability in the populations and landraces varieties. As information on the gene flow of the cultivated cotton to the other *Gossypium* races and species increases, adjustments must be made to expand the area where cotton plant cultivation would be allowed, without risking loss of diversity.

The transgenic plants currently available can present a modified survival capacity, conferring tolerance to the herbicides applied on the crops and a higher reproductive efficiency to the plants due to less damage caused by insects. Cotton is a low colonizing capacity plant, with only a few diploid species of the genus growing as weeds in agricultural environments. The tetraploids do not present characteristics commonly associated to weeds, such as seed dormancy, soil persistency in seed banks, germination under adverse environmental conditions, rapid vegetative growth, short cycle, production of a large amount of seeds, and high seed dispersal capacity over long distances (Keeler, 1985; Keeler, 1989). No

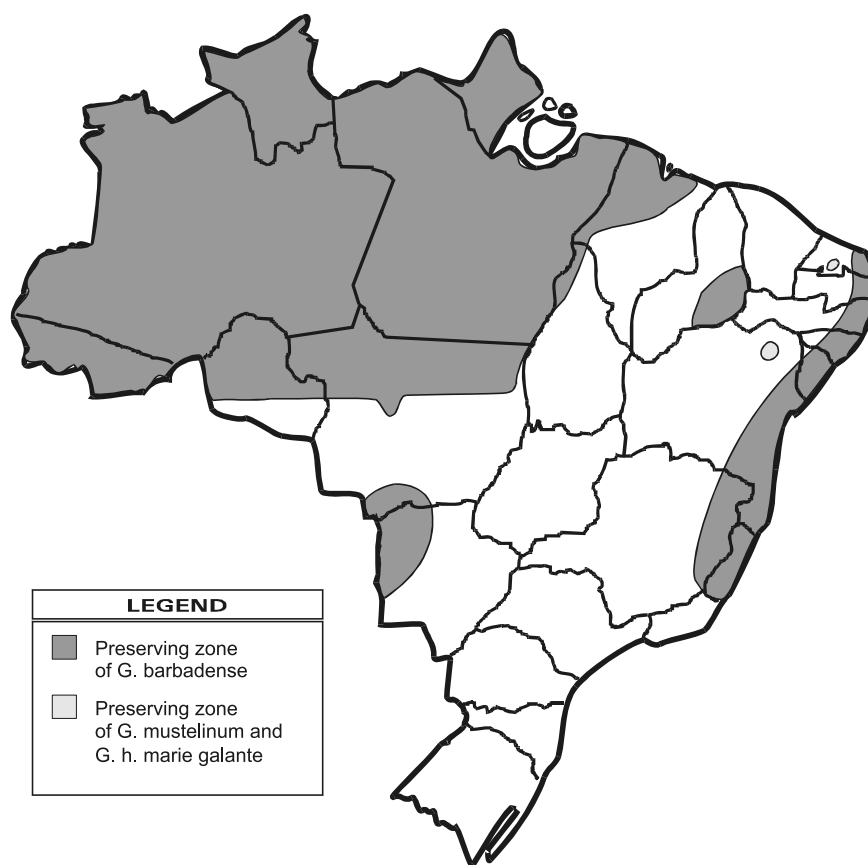


Figure 1. Zoning proposal for cotton plantation in Brazil, aiming at preserving *G. barbadense*, *G. mustelinum* and Moco cotton plants (*G. hirsutum* var Marie galante), according to Freire (2000).

reports exist on sexual compatibility among species of the genus *Gossypium* with other malvaceous species. If this lack of literature implies that it is not possible to obtain descendants between inter-generic crosses, the transgene present in cotton cultivars will be confined to the genus. Hence, greater care should be taken concerning the Gene flow in agricultural environments in Brazil during seed production to ensure the genetic purity of transgenic and conventional cultivars and certification of non-transgenic products since the presence of small amounts of transgenes in cotton products may cause commercialization problems.

Measures aiming to control pollen dispersion between cotton fields have been adopted in several countries. In Argentina, transgenic cotton plant experiments are isolated from experiments using other types of cotton by at least 500 m and if a transgenic cultivar is susceptible to the blue disease, isolation is increased by 800 m. Post-harvest monitoring is carried out for three years (CONABIA, 2002). In some North American states, special attention has been given to a likely genetic contamination of white fiber cotton, with fiber-color conferring genes. California has adopted the most rigorous procedures, by determining that the minimal distance between colored and white fiber cultivar seed production fields should be around 1.6 km, with a border of white fiber cotton rows between them. In case the border is not implanted, the minimal separating distance should be 4.8 km (EPA, 2001). The elimination of the undesired flow in Argentina and in the USA have the physical separation of transgenic cultivar fields in common.

In Brazil, the 607 Act of the Ministry of Agriculture, Cattle Production and Supply, published on 12/14/2001 in the Union Official Daily, determines that a field for the production of cotton seeds must be located 800 m from other *Gossypium* species, 250 m from other crops or 100 m when separated from another cotton plantation by a barrier of taller plants. Such distance must ensure the genetic quality of basic seeds, whose tolerance of plants of a different type is 0.1%. No indications exist in this report that the values have been sufficiently defined based on experiments conducted in fields that are large enough to allow a direct observation of genetic contamination via pollen. This determination must have been stipulated based on the analysis of historical data and extrapolation of the results obtained in experiments carried out in smaller areas than those determined by the act. If the transgene contamination level of

conventional seeds is below these values, greater distances must be obeyed, mainly for Bt plants, which should provide a more effective action of the pollinating agents in transporting their pollen, since they receive smaller amounts of insecticides.

The strategy recommended by the 607 Act is similar to that applied in California, combining physical separation and the use of barriers, but they differ in a basic aspect: the species to be utilized as a barrier. The literature results show that cotton barriers are more effective in reducing cross-pollination than those consisting of traditionally employed cultures, such as maize (Carvalho, 2001). According to this principle, cotton barriers must ensure a more efficient isolation by minimizing the necessary distance.

CONCLUSIONS

Brazil is the place of origin of *G. mustelinum* and an important diversity center of *G. barbadense* and *G. hirsutum* r. *marie galante*. The gene flow between these cotton plants and the commercial cultivars of *G. hirsutum* r. *latifolium*, especially the transgenic ones, may occur under Brazilian conditions. Measures to prevent the gene flow must cover both conventional and transgenic cultivars. Freire (2000) was the first to propose zoning to solve this problem. A more detailed proposal for the preservation of natural populations and landraces varieties should provide further information on the Gene flow between the cultivated cotton plant and other cotton plants in Brazil. Thus, research must be encouraged to generate information on gene flow between native populations and the cultivated cotton plant, floral visiting insect species in the boundary regions of wild and cultivated cotton plants, foraging behavior of the major pollinating insects and seed cotton routes for regions where natural populations and local varieties occur.

The following preventive measures may be taken for the preservation of variability in Brazil:

- i) Adoption of exclusion areas, based on the zoning proposed by Freire (2000) for cotton planting, extending the boundaries of the non-zoned locations to cotton planting to over 10 km, with GPS delimitation. This measure must prevent local populations and varieties located on the borders from receiving pollen originated from transgenic plants.
- ii) Banning the transport of seeds and vegetative parts of transgenic cotton plants to exclusion zones.

RESUMO

Considerações sobre escape gênico em algodão no Brasil

A cotonicultura no Brasil consolidou sua recuperação e hoje muitos cotonicultores do país estão usando elevada tecnologia em suas lavouras. Uma das tecnologias atualmente disponíveis em outros países são as variedades geneticamente modificadas. Em 2002 a área global plantada com variedades geneticamente modificadas (GM) de diferentes espécies foi de 58,7 milhões de ha. Variedades GM de algodão são atualmente cultivadas na China, Índia, USA e Austrália. A segurança dessas variedades para meio ambiente tem sido um dos principais aspectos em estudos no caso do algodão Bt no Brasil. Cinquenta espécies de silvestres parentes do algodoeiro cultivado são encontrados nos cinco continentes: Ásia, África, Oceania e América. Neste trabalho nos discutimos as bases científicas do fluxo gênico do algodão e algumas das alternativas para tornar esta tecnologia disponível e segura para os cotonicultores brasileiros e para o meio ambiente.

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