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Review

A comparative study of transgenic cotton development, impacts, challenges and prospects with respect to China and Africa

Nnaemeka E. Vitalis* and Yuqiang Sun

Plant Genomics and Molecular Improvement of Coloured Fiber Laboratory, College of Life Science and Medicine, Zhejiang Sci-Tech University, Hangzhou 310016, Zhejiang, China.

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Cotton is grown in about 101 countries with about 10 countries including China contributing highest quantity. Africa contributes less than 5% to the global demand for cotton. Processing of cotton generate so many businesses for the rural people of China and Africa. Like other cotton producing continents, the majorly cultivated species of cotton in China and Africa is *Gossypium hirsutisms* Gh (Upland Cotton) and is mostly grown by the smallholder farmers using crop rotation with few large plantations. China and Africa climate condition encourage pest growth which brings about pest attack on cotton followed by yield losses. Effort towards solving this problem was based on integration of transgenic cotton into cotton farming. It has been found that *Bacillus thuringiensis* strains produce crystal (Cry) and cytolytic (Cyt) toxins at the beginning of sporulation and during the stationary growth phase. These crystals are aggregate of proteins encoded by Cry genes and they have insecticidal properties. The Bt Cry genes have been isolated and used to transform cotton seed thus, the term Bt cotton/transgenic cotton which now have in built Cry gene to resist insect attack. In cotton, Bt gene is mostly expressed in the green parts of plant compared to the non-green parts and in the young plants compared to the older plants. This work therefore focused on cotton farming, the Bt crystal, Bt gene, methods of transformation of cotton with Bt gene, Bt gene expression level, resistances, mode of action, limitations and possible recommendations with respect to its use in China and Africa.

Key words: Cotton, cotton farming, genetic engineering, *Bacillus thuringiensis* (Bt), Bt cotton, Bt gene, Bt crystal, China, Africa.

INTRODUCTION

Cotton is grown in about 101 countries with about 10 countries contributing highest quantity. One of the majorly cotton producing nations on earth is China. Africa contributes less than 5% to the global demand for cotton (OECD-FAO Agricultural outlook, 2020). Cotton is an important rare economic story in sub Saharan Africa, a major source of foreign exchange earning in more than 15 countries of the continent and a crucial source of

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*Corresponding author. E-mail: vitalisekene@gmail.com.

income for millions of rural people (Nnaemeka and Sun, 2021). Cotton as one of the most important economic crops provides more than 50% of the fiber source in the textile industry (Gao et al., 2019). Cotton stands as both a valuable fiber crop and an oil- producing crop on a global scale (Hongli et al., 2021) (Table 1).

In China and Africa, cotton farming has faced lot of setbacks, depriving them huge economy benefit from cotton and these has resulted to integration of genetic engineering approaches towards salvaging the challenges. Among other cotton species, the most abundant, largely cultivated and modified in China and Africa is *Gossypium hirsutum* Gh. Like other cotton producing continents, the majorly cultivated species of cotton is *Gossypium hirsutum* (Gh). Gh is also known as upland cotton or Mexican cotton considering its historical trace to Mexico. About 90% of all cotton production globally is of cultivars derived from this species. In tropical Africa, Gh is grown from sea-level up to 200 m altitude. Gh can also be grown on medium to deep, light to heavy well drained soils with a moderate fertility and a pH of 5-6 and 6-7.5 (-9.5) (Ikitoo, 2011). The lint of Gh is about 20 to 30 mm long in international market. Stem fiber cell of Gh investigated in Greece were about 0.8 mm long with a diameter of 18-20 um, a cell wall thickness of 3 to 4 um and a linen width of 12-13 um. The stems contained 40 to 44% a- cellulose and 13 to 18% lignin (Ikitoo, 2011). Also, the oil of Gh has shown antibacterial activity against Gram-positive and Gram-negative bacterial and the antibacterial activity was not affected by fermentation of the oil. Gh importance and demand contributed to it wide cultivation and interest.

COTTON FARMING IN CHINA AND AFRICA

In China and Africa, cotton is almost exclusively grown by smallholder farmers, and there are very few large plantations. The cotton plant loves warmth: It needs about 200 days of sunshine in the season to flourish and bear fruit. For that reason alone, it does well in the dry or humid Savannahs. China and Africa climate with its high average temperatures and alternation between dry and wet seasons favour the cultivation of this natural fiber crop. In many parts of the world, cotton is grown in large plantations, but in Africa the number of smallholder farmers cultivates cotton more than smallholder farmers in China. It is almost exclusively cultivated in Africa using crop rotation (Nkechi, 2020). That is, cotton is grown alternately with other crops such as basic food crops like maize, soybeans or groundnuts unlike China. The cultivation method imparted by cotton made in Africa also support smallholder farmers in growing food and this make an important contribution to food security. Artificial irrigation often used in large planting in China is practically unknown in Africa. Smallholder farmers in Africa practice rain fed cultivation, in other words they rely on natural rainfall being sufficient to water the crops. The wet and dry phases in agricultural part of China and Africa suit the cotton plant. In China, machine is use to harvest cotton but in Africa, harvesting is done mainly by hand picking. Hand pick cotton is also cleaner. However, in China and Africa cotton farming faced severe challenges due to pest attack. China and Africa have a climate favorable for pest growth and this result in sever attack of pests on cotton and subsequent yield losses.

Weather is one of the critical factors that affect insect breeding and movement. Positive physiological responses to increasing temperature allow for a faster insect population growth and facilitate movement. Most analysis by Weizhang et al. (2018) shows that in a warmer climate, pests may become more abundant and may expand their geographical range. Precipitation also affects crop-pest interactions. Both direct and indirect effects of moisture stress on crops make them more vulnerable to damage by pests especially in the early stages of growth. China and Africa climate also have warmed with stronger warming in the north and increased rainfall contrast between northeastern and Southern China. Weather effects on agricultural pests need to be assessed in the light of climate change which is projected to bring significant warming to large parts of China and Africa over changing decades.

INSECTICIDAL APPROACH TO COMBATING COTTON PEST ATTACK IN CHINA AND AFRICA

High population of insects attacking crops in China and Africa have been witnessed with insecticide application the only combating alternative as at that time. Despite growing evidence that *Bacillus thurigiensis* (Bt) cotton reduces use of insecticides, cuts farmers production costs and increase yields in the United States (Perlak et al., 2001), key countries that criticized biotechnology continue to doubt its usefulness, particularly for small farmers in developing countries. Examples of such countries as at that time include China (Pray et al., 2001) and South Africa (Ismael et al., 2001). This implies that China and Africa initially did not welcome Bt maybe for the argument that arose during that time that Bt cotton does not have any positive impact on yields and that bollworms are becoming a problem in China. Before the Bt invention, Chinese farmers have learned to combat this pest using pesticides. Initially, they used chlorinated hydrocarbons (DDT) until in the early 1980 (Stone, 1988).

In the mid - 1980s, farmers began to use organophosphates; however, in the case of cotton, pests developed resistance in the early 1990s, farmers began to use Pyrethroids, which were more effective and safer than organophosphates. Just like the case of other pesticides, China's bollworms began to rapidly develop

Table 1. Summary of production statistics for *Bt* cotton adopting country, 2009.

Country	Yield (M Kg)	Total ha (1000s)	% Bt	First Bt production
Argentina	181	430	70	1998
Australia	384	200	86	1996
Brazil	1252	836	14	2005
Burkina Faso	152	420	29	2008
China	7076	5300	68	1997
Colombia	30	38	64	2002
Costa Rica	0.2	1		2009
India	5117	10260	87	2002
Mexico	92	70	58	1996
South Africa	8	10	88	1997
United States	2654	3047	63	1996

Source: Adopted from Steven (2010).

resistance to Pyrethroids in the mid- 1990s. At this time farmers resorted to chemical cocktails or organophosphates, Pyrethroids and other chemicals (including DDT, although use of chlorinated hydrocarbons is illegal) with less and less impact on pests). With the rising pest population and increasing ineffective pesticides the volume of pesticides used by Chinese cotton farmers rose sharply. China became one of the largest pesticide consumers worldwide. An estimated 30 to 40% of all pesticides applied in China are used on cotton (Weizhang et al., 2018).

LIMITATIONS OF THE INSECTICIDAL APPROACH

Nearly 40% of the pesticides used by the Chinese cotton farmers contain active ingredients that are classified as extremely or highly hazardous by the World Health Organization contributing to around 400 to 500 cotton farmers death every year from pesticide poisoning. During the period of 1992-1996, the last five years for which aggregate data are available there was an average of 54,000 poisoning of farmers annually (Ferdaus et al., 2004). In addition, pesticides make their way to consumers as residues in fruits, vegetables and grains and through contaminated water supplies. It is clear in many countries that the use of pesticides which was the alternative carries many immediate as well as long term risks to human health. Farmers use more pesticides per hectare on cotton than on any other field crop in China (Huang Hu et al., 2002) and in the aggregate Chinese cotton farmers use more pesticides than farmers of any other crop with the exception of rice. Over all Chinese cotton production expands nearly US$500million on pesticides annually (Huang et al., 2002). Africa during this period was facing similar severe pests attack on cotton and was using only pesticides to combat this war with pests and most of the pesticides were imported from China. Majority of the imported goods in Africa are from China including pesticides. Thus, similar pesticide poisoning danger was posed to African cotton farmers. The use of pesticides against pest attack and its corresponding side effects in China and Africa have been in place for the past years. This adverse effect contributed to the choice made for alternative pest control approach which paved way to the integration of genetic engineering.

GENETIC ENGINEERING APPROACH

China pest problems led the nation scientists to pursue a variety of strategies including development of new pesticides, breeding of new pest-resistant cotton varieties and development of integrated pest management (IPM) programs for pest control with Africa sharply dependent on the best recommended approach after all. Consequently, when the possibility of incorporating genes for pest resistance came closer to reality, China's scientists became actively involved with funding primarily from the government research sources, a group of public research institutes led by the Chinese Academy of Agriculture Science (CAAS). China has devoted considerable resources into developing GM cotton expressing endotoxins from *Bacillus thurigiensis* (Bt) to control insect pests. The gene was transformed into major Chinese cotton varieties using China's own methods (Pollen tube pathways). The researchers tested the varieties for their impact on the environment and then released them for commercial use in 1997 (Pray et al., 2001). Monsanto in collaboration with the cotton seed company Delta and Pineland developed Bt cotton varieties that were approved for US commercial use in 1996. They began to collaborate with the Chinese National Cotton Research Institute of the CAAS at Anyang, Henan in the mid-1990s. In 1997, several varieties were tested and approved by the Chinese Biosafety Committee for commercialization. Concurrently, scientists in the Cotton Research Institute were working on their own varieties. The research team began to release their varieties in the late 1990s (AgBioForum, 2002). As the adoption of Bt cotton spread, China's government research institutes at the province and prefecture levels produced new Bt varieties by backcrossing the Monsanto and CAAS varieties into their own local varieties. These varieties are now being adopted in Henan, Shandong and elsewhere. In the wake of commercialization of these approved and non-approved varieties, the spread of Bt cotton has been rapid. It has been estimated that farmers planted more than 2 million hectares of Bt cotton from 1996 to 2001 that is 45% of China's cotton growing area was planted with Bt cotton in 2001. In 1998 commercial production of Bt cotton by the Chinese farmers started in the Yellow River cotton producing region of Hebei, Shandong and Henan. Production rapidly expanded to 97% of the respective cotton growing areas in Hebei by 2000 and in Shandong by 2001. In Henan the adoption rate reached nearly 70% in 2001 (AgBioForum, 2002). The adoption rate was less in Jiangsu may be the cause of the observations during the field survey; the red spider mite problem was more serious than bollworm in their cotton production. In Hebei, Monsanto varieties were first approved. Genetically modified *Bacillus thurigiensis* Bt insect- resistant cotton and adoption progressed at different rates in different regions depending on the timing of Bt cotton varieties approved for commercialization and the availability of Bt cotton seed in local markets.

COMMERCIALIZATION OF BT COTTON

In China commercialization of genetically modified cotton (GMC) started in 1997; thereafter, a comparison of Bt cotton and non Bt was surveyed in terms of yield impacts, cost of production, farmer's income impacts, farmers health and environmental impacts. In all, Bt cotton has better positive result than the non-Bt. Although the spread of Bt cotton in China has relied on the varieties introduced by the public research system and seeds sold (at least initially) by the state-run seed network; the adoption of Bt varieties has been the result of decisions by millions of Chinese small farmers and is regulated by the government with less involvement of the private companies. The sustainability of GM crops has been subjected to heated debate in China but Bt cotton has proven less controversial as production, ecological and human health benefits have been realized and cotton is a non-food produce. The empirical evidence that GM technology offers long- term economic benefits than ecological benefits are realized, the economic benefits for Bt cotton exceeded US$5.3 billion over the years after it was commercialized (Yunhe et al., 2017). In China, Bt cotton had pervasive effects on the whole pest complex in cotton and its management adoption resulted in major reduction in insecticide use for bollworms control (Weizhang et al., 2018). Also, the 1999 and 2000 production survey (Pray et al., 2001) showed that Bt cotton continued to do well and increase yield in the northeast China (Yellow River) and central (Yantze River) cotton zone after the introduction and spread of Bt cotton using the nationally representative long panel data for 1997 to 2012. Fangbian Qiao showed that the economic benefit in China continues many years after the commercialization of Bt cotton. In West Africa precisely, approximately 25 to 35% of cotton yield is lost because of pest. The most important group of insects in terms of economic costs is the bollworm which causes discoloration of the cotton lint and automatically represents a serious decline in quality and substantial reduction in price. Aphids and bacteria blight (*Xanthomonas malvacearum*) are also examples of insects that affects cotton yield. These problems posed severe effect on the cotton industries in Africa and majority of them shutdown (Nnaemeka and Sun, 2021). Unfortunately, this has remained since that time and the total production remained far below the requirements of the textile and the oil mills. Although there are other challenges that contributed to the severe cotton production decline in Africa but pest infestation among others have greater significance damage. Restoration of the cotton glory in Africa has however become a subject of serious concern to cotton concerned Africans. There have been many improvements in the management of insect pests in cotton that have contributed to a reduction of insecticide use in this crop in the past two decades with perhaps the most notable being advances in biotechnology that have allowed engineering of plants to provide highly effective and selective control of caterpillar (order Lepidoptera) pests, the most significant pest group of cotton globally (Steven, 2010). Therefore, effort towards solving this problem was based on integration of transgenic cotton into cotton farming in Africa as was done in China etc. After the Bt discovery and application in China, China sister counterpart (Africa) and precisely South Africa as at that time followed through the processes and methodologies of the Chinese to develop and adopt the Bt cotton in same 1997. South Africa therefore became the first African country to adopt this technology after careful study of the Chinese success thus, bringing Africa to the map of Bt cotton farmers.

Therefore, *G. hirsutum* which is the most cultivated in Africa become genetically modified to resist the insects attack thus, the transgenic cotton currently in Africa consist mainly the Bt cotton. In Africa majority of the Bt cotton in use are produced by the Monsanto Company. The success of Bt cotton in Africa has been recorded for instance Kenya use to produce 20,000 bales of cotton every year against a demand of 140,000 meaning they have to import the deficit. As the National Performance Trials for Bt cotton was completed with Bt they can produce up to “260,000 bales”. Like other countries, pests attack was the main reasons that caused cotton growing and textile industry shutdown, but Bt cotton which was adopted in 2012 after the constitution of the National Biosafety Council revitalized cotton farming in the country and only one variety named Seini (a Chinese variety) was released for commercialization (Nagala, 2013). In Sudan, Bt cotton out yielded the non Bt varieties more than 5 to 6 times evaluated in open field trials in six environments. Bt cotton contributed to a reduction in the damages caused by sucking insects and in the improvement of cotton quality by limiting stickiness. The cost of cotton insecticides and application for non Bt cotton cost $892 per hectare, reducing to $586 for Bt cotton, a saving of about 35%. The net profit for farmers for cultivating Bt cotton was estimated to reach $405 per hectare. South Africa began planting Bt cotton in 1998. The adoption rate continued to increase and the Bt cotton coverage reached 95% in 2007 (James, 2004). Bt cotton was adopted by large scale farmers and smallholders in South Africa. Besides the economic benefits, the number of insecticide sprayings related to the Bt cotton has decreased with a beneficial impact on the environment (Morse et al., 2006). In Burkina Faso, two regional BolgardII varieties were generated and commercialized in 2008, in collaboration with Monsanto (Vitale and Greenplate, 2014). In Nigeria, an economy rich country in Africa, her textile manufacturers association said in 2016 that “Genetically modified insect protected (Bt) cotton can play an immense role in restoring attraction to cotton farming as well as reviving and repositioning the textile sector in the country (Nnaemeka and Sun, 2021). The lack of confidence by participants across the cotton value chain over the years restricted the much-needed investment so it became the most important input industry “the cotton crop”, genetically modified insect protected (Bt) cotton” could improve cotton lint quality, farmers benefit and yields increase due to reduced insect pest damage; the release and commercialization of the Bt cotton could be related to successes recorded about the Bt cotton in other countries. Thus, in Nigeria the transgenic cotton was commercialized as the first genetically modified crop to boost the textile industry in 2018. Two home grown cotton varieties: MRC7377BGII and MRC7361BGII were developed by Mayhco Nigeria Private Ltd. in collaboration with the Nigeria Institute for Agricultural Research (IAR). Nigeria’s new Bt cotton was suitable for cultivation in all of Nigeria cotton growing zones and produced 4.1 to 4.4 tons per hectare compared to the local variety which yield just 600 to 900 kg ha^{-1}. In addition to the pest resistance traits, they offer early maturity, fiber length of 30.0 to 30.5 mm, fiber strength of 26.5 to 27. 0 g/tex (tenacity) and micronair (strength) of 3.9 to 4.1. The new varieties have saved farmers the trouble of contending with the local conventional variety, which is no longer accepted at the international market. Some other African countries have adopted Bt cotton as the transgenic cotton in use to combat pest infestation. Africa Bt cotton though developed in collaboration with foreign companies has proven safe, no negative impact on the environment and consumers. The Bollgard cotton reduced cotton production costs and insecticide use for the control of tobacco budworm (*H. virescens*) cotton bollworm (*H. zea*) and Pink bollworm (*P. gossypiella*) (Perlak et al., 1990, 2001). Agronomics trait, fiber quality and seed composition remain unchanged in the transgenic cotton. “Bt cotton after several food safety and risk assessment studies”, it is confirmable that Bt cotton hybrids pose no obvious toxic effects on non- target species. The analysis of Bt protein in the soil indicated that CryIAc protein is degradable without negative environmental impact. Further evaluation of the impact of GM protein leached by roots of GM cotton on the soil microflora showed that there was no significant difference in the population of microbes and soil invertebrates between both samples. Food safety assessment have shown that nutrient composition analysis of protein, carbohydrates, oil and calories also disclose no obvious difference in the Bt and non Bt cotton seed this was observed after comparison of animals fed with Bt and non Bt cotton seed thus, supporting the campaign that Bt cotton is as safe as the conventional cotton in almost every ramification (Nnaemeka and Sun, 2021). Nigeria Bt cotton success and Nigeria decisions to commercialize Bt cotton has revived hopes for the novel variety in Ghana (Gakpo, 2018) as well as other African countries. Nigeria’s green lightening of Bt insect resistant cotton spurred Africa’s increase interest acceptance of GMO’s (Steven, 2010).

The reduction in pesticides use due to the adoption of Bt cotton in China and Africa has been substantial. However, Africa cannot currently be equally compared to China as Africa depends on China for so many things including Agriculture. It cannot be stated however that Africa has learned useful lessons from China which they are integrating in their economy and agricultural policy. Advances in the Bt cotton commercialization was made and currently it is estimated that Bt cotton is covering about 60% of the total Chinese cotton area but one can consider that the coverage is close to 100% wherever the target pests of the Bt cotton are a real threat (Michael and Naiyin, 2007).

LIMITATIONS OF THE GENETIC ENGINEERING APPROACH

Despite tremendous improvement in breeding and other technologies for robust yield, increase crop pests remain an important cause of considerable yield loss triggering the use of insecticides that once affected farm profit and the health of human and their environment (Weizhang et al., 2018). Cornell University researchers at the American Agricultural Economics Association (AAEA) annual meeting in Long Beach, Calif, July 25 reported that other pests are now attacking the GM cotton in China. Although Chinese cotton growers were among the first farmers worldwide to plant genetically modified (GM) cotton to resist bollworms, the substantial profits they have reaped for several years by saving on pesticides is now been eroded because population of other insects such as Mirids have increased so much that farmers are now having to spray their crops up to 20 times a growing season to control them according to the study of 481 Chinese farmers in five major cotton producing provinces. The problem in China therefore is not due to the bollworm developing resistance to the Bt cotton as some researchers feared but is due to secondary pests that are not targeted by the Bt cotton which previously have been controlled by the broad -spectrum pesticides used to control bollworms. Furthermore, the practice of applying excessive amount of highly toxic pesticides has continued even after the adoption of Bt cotton in China and Africa, perhaps as a result of lack of knowledge and some behavioral factors by the farmers following reoccurrence of secondary pest in China and pest development of resistance to Bt cotton in Africa as seen in Burkina Faso. As late comers in the Bt technology, this reoccurrence may also be viewed as lack of clear understanding of the pests by the farmers because when US farmers plant Bt crops they unlike farmers in Africa required by contract with seed producers to plant a refuge, a field of non- Bt crops to maintain a bollworm population nearby to help prevent the pest from developing resistance to the Bt cotton. The pesticides used in these refuge field help control secondary pest populations on the nearby Bt cotton field (Pinstrup-Anderson, AAEA, 2007). This observation from US practices has inspired further efforts in China and Africa. Wang et al. (2016) said that one of the solutions to eradicating secondary pests attack is also by introducing natural predators to kill the secondary pests or enforcing the planting of refuge areas where broad- spectrum pesticides are used.

THE *BACILLUS THURINGIENSIS* CRYSTAL STRUCTURE AND BIOCHEMISTRY

Bt stands for the naturally occurring bacterium (*Bacillus thuringiensis*). Bt is a ubiquitous Gram- positive rod –shaped and sporulating bacterium that has been isolated worldwide from a great diversity of ecosystems including soil, water, dead insects, dust from silos, leaves from deciduous trees, diverse Conifers and insectivorous mammals as well as from human tissues with severe necrosis (Koller et al., 1992,). Bt strain synthesizes crystal (Cry) and cytolytic (Cyt) toxins at the onset of sporulation and during the stationary growth phase as parasporal crystalline inclusions (Du et al., 1994). In the past decades, more than 700 cry genes sequence that codes for crystal (Cry) proteins have been identified and the large plasmids appear to be the usual location of these genes. The crystal proteins are aggregates of proteins that builds up to form crystal. Cry protein are encoded by Cry genes. Apart from the crystal, other Bt isolates have various functions such as attack on human cancer cells apart from the known insecticidal properties (Ekino et al., 2014). Since the identification and cloning of the first Bt insecticidal crystal protein gene in 1981, the number of genes coding for novel insecticidal proteins has continuously increase namely: CryI for proteins toxic for Lepidopterans, CryII for proteins with toxins against Lepidoptera and Dipterans, CryIII for proteins toxic for Coleopterans, CryIV for proteins exclusively toxic for dipterans. About 73 different types of Cry are known (Cry1-Cry73) (Ekino et al., 2014). In addition, other criteria can be used to identify Cry apart from base on the target insect. Currently the Cry proteins constitute the largest group of insecticidal proteins produced by species of *Bacillus.* Within the decade, Cry genes with specificity for different groups of insects have been cloned and sequenced. Regarding the Cry toxins, at least 5 different groups not related in their sequence has been characterized (Soberon et al., 2018). In addition to the Cry toxins, Bt contains transposomes (transposable genetic element that flanked genres and that can be excised from one part of the genome and inserted elsewhere). All these properties increase the variety of toxins produced naturally by Bt strains and provide the basis for commercial companies to create genetically engineered strains with novel toxin combination (Jim Deacon, 2001). The different strains available is because there can be up to 5 to 6 different plasmids in single Bt strain and these plasmids can encode different toxin genes and the plasmids can be exchanged between Bt strains by a conjugation-like process, thus, paving way for potential wide variety of strains. By the mid-1970s, about 13 Bt strains effective against Lepidopterans had been identified and classified according to their Cry genes. With different combinations of Cry toxins (Jim Deacon, 2001) the crystalline parasporal inclusions (CPI) produced by the Bt is usually composed of one or several polypeptide subunits which are toxic when ingested by susceptible insects. These CPI contain proteins that exhibit a variety of biological actions including cytolytic, hemolytic and entomocidal activities (Aronson et al., 1986, Hofte and Whiteley, 1989). Numerous natural variations in the primary structure of the crystal proteins exist and are responsible for differences in susceptible

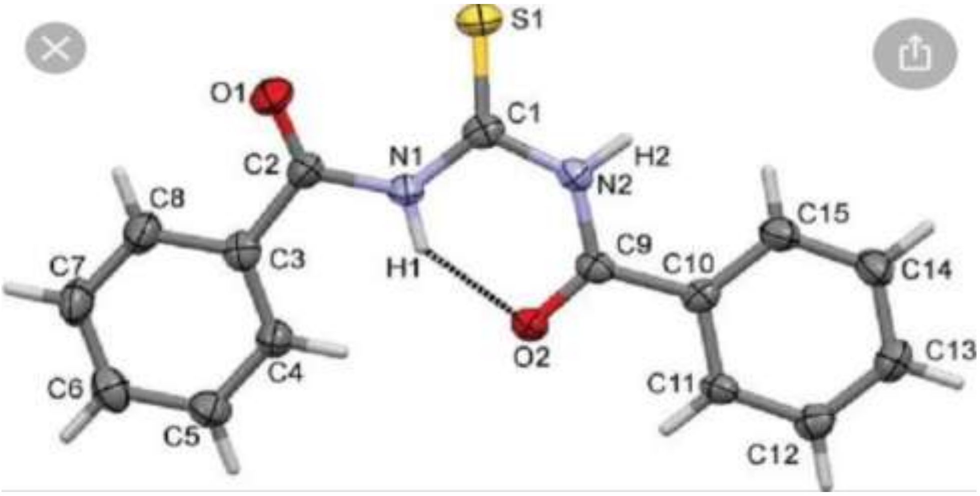

Figure 1. Crystal structure of the Bt compound with selected atoms labeled (Ekino et al., 2014).

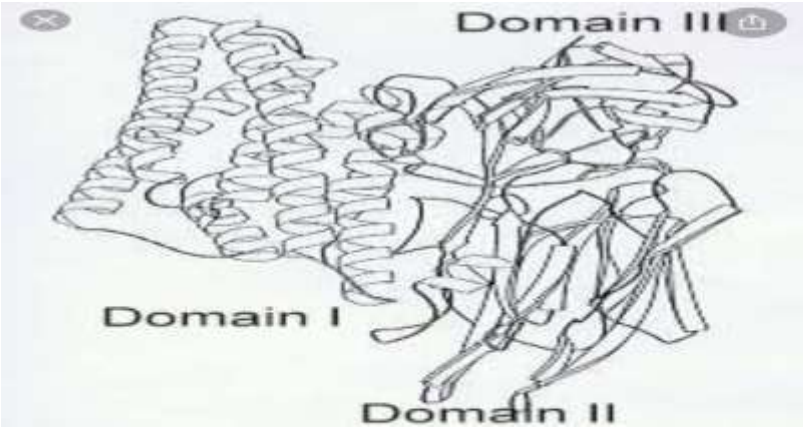

Figure 2. *Bacillus thuringiensis* toxin (Schnepf et al., 1998).

host range of each toxin (Hofte and Whiteley, 1989). Many physiochemical properties of the crystal inclusions have been reviewed (Huber and Luthy, 1981, Koller et al., 1992, Du et al., 1994) including the pH required for solubilization of the crystal an important parameter, since it is an essential step for toxicity in susceptible insects. The Bt Cry proteins comprise at least 50 subgroups with more than 200 members (Figure 1). The members belong to a three-domain family and the larger group of Cry protein is globular molecules with three structural domains connected by single linkers (Ekino et al., 2014).

A common characteristic of Cry genes is that they are expressed during the stationary phase growth. Cry proteins, the end-products of Cry gene expression constitute 20 to 30% of the cell dry weight and generally accumulate in the mother cell (Bravo, 2007). The highest level of Cry gene synthesis appears to be coordinately controlled by a variety of mechanisms occurring at the transcriptional, posttranscriptional and post translational levels. Several Cry gene promoters have been identified and their sequence determined (Figure 2).

BT GENE MODIFICATION HISTORY AND METHODS

This new technology for managing insect pests in China and Africa was approved for commercialization in the United States by the US Environmental Protection Agency in 1995 and is currently available in many other countries. The Bt cotton variety presently used against tobacco budworms, bollworms and certain other caterpillars attacking cotton produce the CryIAc protein. Therefore, a cotton plant is modified to produce Cry protein within the plant tissues from which the insects can eat it that is the Cry protein traits is carried in plants genes as is traditional plant resistance to insects. During the development process, biotechnology created Bt cotton by inserting selected exotic DNA from a Bt bacterium into the cotton plants own DNA. Following the insertion of modified Bt DNA into the cotton plant DNA, seed companies moved the Cry proteins trait into high performance cotton varieties by traditional plant breeding methods meanwhile agronomics qualities for yield, harvestability, fiber quality and other important characteristics were preserved at the same time the Cry proteins gene was added to commercial varieties. The three primary components of the genetic package inserted into cotton DNA include protein gene, promoter, and genetic marker.

This genetic package can be inserted into cotton plant DNA through a variety of plant transformation techniques. Transformed plants may be affected by the genetic package as well as the location of the new genes in the plant DNA. The insertion site may affect the Bt protein production and other plant functions as well. Therefore, biotechnology companies carefully scrutinized each transformation to ensure adequate production of Bt protein and to limit possible negative effects on agronomic traits. Following a successful transformation, plants are entered into traditional backcross breeding program with the variety chosen to receive the foreign Bt gene package. The final product of a Bt cotton variety is developed after four or five backcross generations. Although the new transgenic Bt cotton variety, agronomic qualities can be considerably different. This process of making Bt cotton was adopted from the United States Department of Agriculture, Agricultural Research Service ARS-154, January 2001 (Hardee et al., 2001). Several companies can now make Bt cotton.

However, out of a number of strategies for insect resistance management, three are key: (i) Achieving high toxin dosage either by the use of strong promoters or by targeting the protein to organelles or by tissue specific expression of the protein (ii) use of multiple genes preferably, those that work through different mechanisms and (iii) use of a refuge along with (i) and (ii) (Singh et al., 2016).

BT GENE EXPRESSION LEVELS

In Singh et al. (2016) experiment, the Cry protein expression resulting from this experiment was observed only in the green plant parts. No transgenic protein expression was observed in the non-green parts including roots, seeds and non-green floral tissues. The result of this experiment also showed that a transgenic protein having the transit peptide of a protein that accumulated in green plastids does not get targeted to the leucoplasts. Thus, applying such a transit peptide could be an effective method of expressing a transgene- encoded protein only in green aerial tissues. These features also allay public concern about the safety of Bt cotton since it expression level in the seed is low.

Also, Allah et al. (2012) stated that the production of transgenic plants with stable high- level transgenic expression is important for the success of crop improvement programs based on genetic engineering. In their study they evaluated genomic integration and spatio temporal expression of an insecticidal gene (Cry2A) in pre-existing transgenic lines of cotton. Genomic integration of Cry2A was evaluated using various molecular approaches. The expression levels of Cry2A were determined at vegetative and reproductive stages of cotton at regular intervals. Gene expression was found variable at various growth stages as well as in different plant parts throughout the season: The leaves of transgenic were found to have maximum expression of Cry2A gene followed by squares, bolls, anthers and petals. The protein level in fruiting part was less as compared to other parts showing inconsistency in gene expression. Expression level also varies with age of the plant. Spatio temporal study by Greenplate et al. (1998) reveals that expression level of Cry2A declined during the crop growth with toxin level falling to 15 to 20 nanogram per gram of fresh tissue weight. That is young plants tend to show higher expression than the older plants. The reduction in Bt protein contents in late-season cotton tissues could be attributed to the over expression of the Bt gene at earlier stages which leads to gene regulation at post-transcription levels and consequently results in gene silencing at a later stage (Allah et al., 2012). Although, the mechanisms of variation in endotoxin protein content in plant tissues are rather complicated, the level and efficiency to which genes are expressed are mainly regulated by their cis-regulatory elements such as the promoters.

BT GENE EXPRESSION RESISTANCE MAINTAIN AND LOSSES

A major weakness of the products (Bollgard I and II) currently used in the field is a drop in the Cry1Ac protein’s expression level as the plant matures and sets bolls. Furthermore, there is a high expression level in the roots that provides no resistance against *H. armigera* and other lepidopteran pests, as they do not feed on roots. Another weakness is that a secondary lepidopteran pest on cotton can survive the low Cry1Ac protein dose present in the developing bolls and leave progeny (Singh et al., 2016). As is already known, Tobacco budworms and bollworms are not the only insect pests that attack cotton and unfortunately, the CryIAc protein has essentially no effect on many of the secondary pest such as *Pectinophora gossypiella*, boll weevils, cotton aphids, cotton fleahoppers, cutworms, spider mites, Stink bugs, tarnished plant bugs, and white flies. In some caterpillar species Bt may provide only 10 to 50% control. Research has suggested that Bt cotton insecticidal protein is not expressed steadily (Knox et al., 2006) due to a number of factors: Soil water deficit significantly affects insecticidal protein expression in the leaves of Bt cotton (Rochester, 2006; Parimala and Muthuchelian, 2010). Increased damage to Bt cotton by cotton worm in Shandong and Hebei provinces of China in 2005 and 2006 may have been due to lack of rain and a resulting soil water deficit from June to July (Liu et al., 2008). Likewise, Carter et al. (1997) and Benedict et al. 1996) found that lack of rain resulted in soil water deficit and associated water stress reduced the content of total soluble protein and insecticidal protein in June and July. Drought stress could lead to DNA degradation in cotton seedling tissues, producing many residual DNA fragments that could inhibit the synthesis of functional proteins and structural proteins (Yang et al., 2016). Thus, several lines of independent evidence implicate drought stress in the failure of insect resistance of Bt cotton. In most of the world, drought is an important problem during the cotton whole growing period (Li et al., 2010). Environment is also an important factor that contributes to influence insect resistance of Bt cotton in a number of ways. One hypothesis suggested that under an adverse environment, DNA methylation of the promoter regions of the Bt gene switches off gene expression (Stam et al., 1997). Another hypothesis suggested that tannin, generated by cotton plants exposed to adverse environments, was binding to Bt insecticidal protein and inactivating it (Holt, 1998). A third hypothesis suggested that the protein synthesis decreased, resulted in decreased Bt insecticidal protein content (Chen et al., 2005). However, how soil water deficit affects the expression of Bt insecticidal protein in bolls, and what mechanism is responsible for these effects has not been efficiently reported. Methylation of the promoters may also play a role in the declined expression of endotoxin proteins (Singh et al., 2016).

Fitness is also one of the key parameters to evaluate the effects of transformed plants on the ecological environment (Liu et al., 2020). Bt cotton growing in different habitats (farmland, grassland and shrubs) were assessed for fitness level. It was found that the expression of Bt protein in the farmland was significantly higher than that in the other habitats (Liu et al., 2020).

Theoretically, Bt cotton may also indirectly lower the general abundance of some beneficial insects. It is commonly known that more than 500 species of insects and mites have developed at least some degree of resistance to insecticides (Georghion and Wirth, 1997). Most scientists therefore, agree that the tobacco budworm and the bollworm will eventually become resistant to the CryIAc protein used in current Bt cotton varieties someday due to a number of predicted factors. Hardee et al. (2001) also stated that before exposure to Cry toxins by planting Bt cotton the very few tobacco budworms and bollworms (Perhaps 1 in 100,000 or 1 in 1 million) carry two copies of a resistance allele (RR) meaning they are fully resistant to Bt cotton while some have a single copy of a resistant allele and a susceptible allele (RS), meanwhile the overwhelming majority have two copies of a susceptible allele (SS). Most of the (SS) are killed after feeding on Bt cotton depending on the dose of Cry toxin in the plant. The (RS) usually are more difficult to kill than the (SS) still the (RS) are not considered Bt resistant in most cases but (RR) are not killed. This perhaps is why Bt cotton cannot achieve 100% eradication of the insect pest. A clear evidence of pests developing resistance to Bt cotton have been seen in China and Africa (Burkina Faso precisely) where there was reoccurrence of the insect pest in the cotton farm after a period of time and this nearly demoralized the use of Bt transgenic cotton in China and in some part of Africa if not for the precaution measures taken by Nigerians during their Bt cotton development, the future of Bt cotton in Africa may have been impossible to predict as a result of the pest resistance observed in Burkina Faso. The resistance has been traced to the (RR) species because after the introduction of Bt cotton in China and Burkina Faso; non-target pests became more abundant due to less pesticide that were sprayed followed by the gradual pests resistant to the toxin. Regardless, improvements in the Bt cotton technology continue towards completely eradicating any insect attacking cotton (Figures 3 and 4).

CONCLUSION

Cotton farming in China and Africa has shown fluctuation in quantity over the years: Less quantity before Bt technology, greater quantity with Bt technology and lesser quantity again after secondary pest and target pests developed resistance. The Cry gene use in making Bt cotton in China and Africa have shown variable expression with the nucleotide sequence of the gene, promoters and the insertion point of the gene in the DNA of the transgenic variety, transgene copy number, the internal cell environment as well as several external factors in the environment (Guo et al., 2001) being responsible. Prompting investigation at molecular, genetic as well as physiological levels with the aim of understanding the differential expression of transgenes and the quantitative changes in insecticidal proteins in insect resistant cotton plant which has been known to have beneficial impact on global cotton farming due to the reduction in the number of pests and hence the total application of chemical insecticides used for it control as well as the final production especially in China and Africa

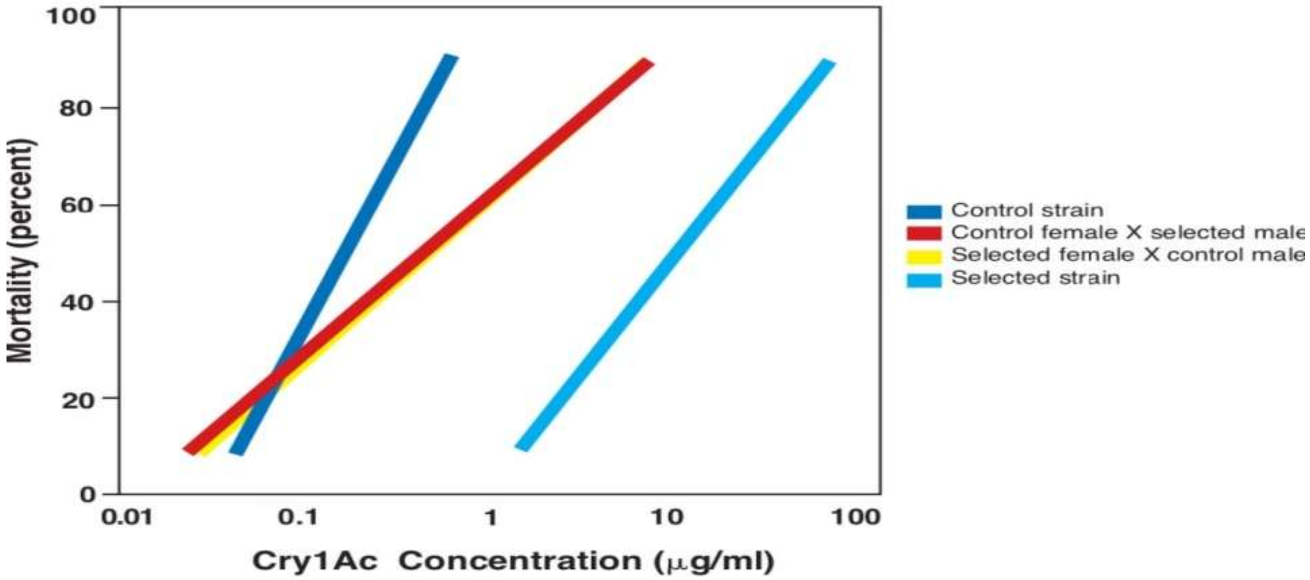

Figure 3. Development of resistance to Bt cotton in tobacco budworm in laboratory experiment has been found (Gould et al., 1992).

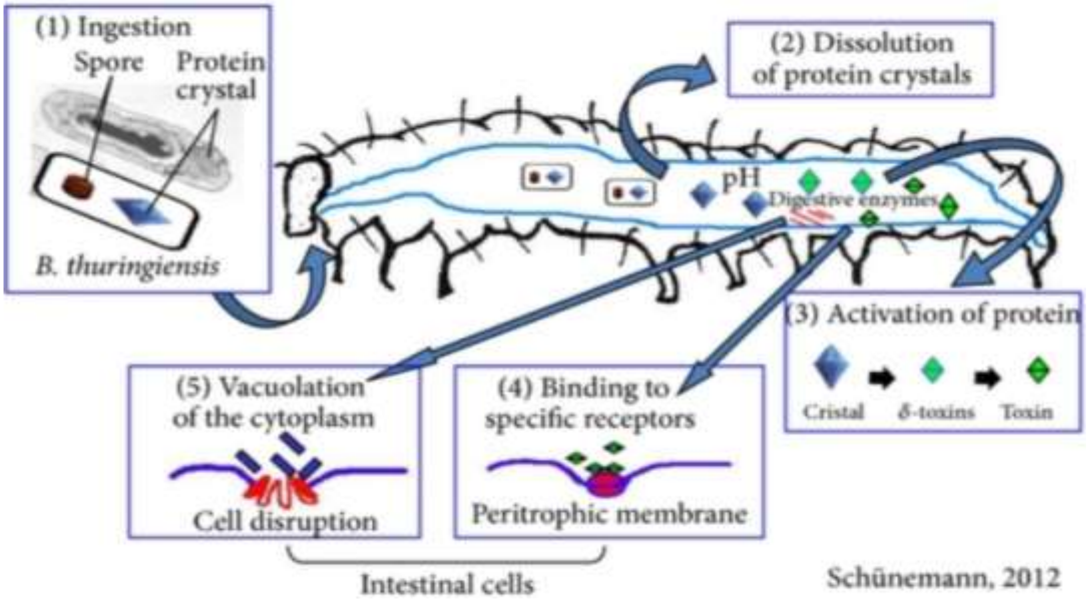

Figure 4. Mode of action of *Bacillus thuringiensis* in Lepidoptera (Rogerio et al., 2014).

where insects infestation is high but low pest management practices mainly in Africa. Following this investigation, it is vital to ensure efficient expression of the insecticidal protein in Bt cotton through genetic engineering and the utilized mechanisms should be well understood in other to plan rational resistance management strategies to slow the rate at which insects develop resistance and to control target pests effectively by enhancing endotoxin expression through genetic or agronomic management. Because this technology has faced serious challenges especially in the quality of the cotton fibers, abundant growth of non-target pests and the insects developing resistance to it, China and Africa growers should therefore know that production costs can increase as insects develop resistance to the Bt toxin and the Cry toxins in Bt plants are not easily replaced when insect develop resistance, developing new transgenic insecticidal crop is also difficult, time consuming and cost intensive. Thus, preserving the effectiveness of Bt cotton is one way to keep pest management costs at the lowest level and this can be further achieved by producing a high dose of Bt plant Cry toxins throughout the season; effective IRM refuges must be maintained, using vegetable insecticidal proteins (VIPs) and using genes from plants or animals which encode immunosuppressive proteins are recommended to enable continuation of transgenic plant and precisely transgenic cotton farming in China and Africa without future setbacks. Finally, developing new cotton varieties with more powerful resistance, applying certain plant growth regulators and maintaining general health of the transgenic crop are substantial in realizing the full transgenic potential in transgenic Bt cotton in China and Africa. Development of new promoter that will induce more consistent production of insecticidal genes throughout the life of the cotton plant and also throughout the plant part especially the fruiting parts that are also susceptible to attack should not also be excluded in the advancement for effective Bt cotton. Integration of these recommendations can go a long way in sustaining Bt technology in China and Africa

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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