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Bt cotton: A boon against insect resistance

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Abstract
The cultivation of transgenic cotton is increased in a large scale since from its introduction globally. *Bacillus thuringiensis* is produced crystalline proteins, these proteins were used in transgenic cotton to control insect pest. Resistance in insects is a major problem in transgenic cotton growing regions in all over the world especially, pink boll worm developed resistance in all major cotton regions in India. It was noticed in our studies that the expression levels of Cry1Ac in different plant parts such as upper leaves, lower leaves, sepals and boll bracts was increased while increasing refuge percentage. Planting refuge with alternatively with *Bt* cotton also increase the Cry1Ac expression levels compare with border refuge. An optimized unique combination of refuge crop *viz*., 75% *Bt* with 25% *nBt* showed higher expression level of Cry1Ac and highest yield was also achieved. This combination is useful to counter insect resistance and the staking of two toxin Cry proteins in same transgenic cotton.

Keywords: *Bacillus thuringiensis*, refuge strategy, pyramid strategy and resistance

Introduction
Bacillus thuringiensis is produced crystalline proteins, these proteins was used in transgenic cotton to control insect pest in cotton growing regions (Bravo, 2011; Sanahuja, 2011) [1, 2]. World wide, transgenic cotton growing regions increased from 1million hectares to 75 million hectares in 2013 (James, 2013) [3]. Transgenic cotton increases the yield and produces more income to farmers and reduces the pesticide application in cotton crops (Tabashnik, 2013) [4].
Resistance in insects is a major problem in transgenic cotton growing regions in all over the world. Especially, pink boll worm developed resistance in all major cotton regions in India. (Storer *et al.*, 2010) [5]**.** In India, pink boll worm developed resistance to transgenic cotton. Transgenic cotton major growing states are Gujarat and Maharashtra tough it is found in recent years that in Amerali district of Gujrat, pink bollworm increased 75% pest surveillance resistance was identify with diet bioassay. Monsanto also noticed that pink boll worm developed resistance in Gujarat (Dhurua, 2011) [6].
Survey reports from 2010 to 2017 also states that increased resistance in pink bollworm population in all transgenic cotton growing regions in India (Fabrick *et al.* 2015) [7]. Pyramid and refuge strategies are major resistance management tactics. In pyramid strategy, two are more Cry proteins were used at a time in transgenic crops. If insects developed resistance one toxin and it is difficult to develop resistance to second toxin (Fabrick *et al.* 2014) [8].
Refuge is a plant that does not have insecticidal proteins and provide host to susceptible insects. These susceptible insects mate with resistance insects and produce the heterozygous population of insects. These leads to produce less population insects and refuge strategy mainly delay the resistance in insects. Resistance development in insects may depend up on the rate of resistance allele present in the resistance insects (Tabashnik *et al*. 2013) [4].

Bacillus thuringiensis
Bacillus thuringiensis (*Bt*) is widely adopted insecticidal protein to control insect pests in agriculture crops. *Bacillus thuringiensis* is a gram positive soil dwelling bacteria which is present in soil. *Bt* shares 2% of insecticidal market globally, these *Bt* toxins contain crystalline toxins which kills the insect larvae (Raymond *et al.* 2010) [9].
Bt produce different types of crystalline proteins, these proteins kill different kinds of insects. These Cry toxins come under bacterial class pore forming toxins. These toxins have water soluble nature and kill different kind of insects (Bravo *et al*., 2011) [1].

Mode of action of Cry toxin in insect midgut
The *Bacillus thuringiensis* produce crystalline toxins, these toxins produce 70 to 130 kDa size protoxins. The protoxins have three domains 1. Domain I having seven α - helix bundle, these α - helix bundle was used in formation off oligomers. 2. Domain II having β – prism three anti-

parallel β - sheets. 3. Domain III having sandwich type of β – two anti parallel sheets. Domain II and Domain III play major role in insect midget to form pores (Zavla *et al*., 2011) [10].

The Crystalline toxin was engulfing by the insect larvae. These crystalline toxin is activated by the gut proteases and form monomeric Cry toxins, these monomeric cry toxins bind with abundant receptors such as GPI, ALP and APN with low affinity at specific regions loop3 of Domain I and β - 16 regions of Domain III because of single regions binding of Domain II and Domain III with crystalline toxins (Ali *et al.*, 2006) [11].

After the first interactions, the monomeric toxin binds with a high affinity to low abundant CAD receptor. These interaction of monomeric toxin is a complex interaction because, of loop 2, loop 3 and α – 8 regions was bind with the Domain II region of Cry protein. Domain II binding with CAD receptor promotes the photolytic cleavage of N - terminal end of the toxin to eliminate helix α – 1 of Domain I (Bravo *et al*., 2011) [1].

Cleavage of N - terminal end of the toxin to eliminate helix α – 1 of Domain I initiates assembling oligomeric forms of the cry toxins. These oligomers gone through conformational changes and 100 fold increased binding affinity of toxin to bind with GPI, ALP and APN receptors through the loop 2 region of Domain II. These oligomers bind with receptors and forms pores in the apical membrane of mid gut causing osmotic shock leads to death of the larvae.

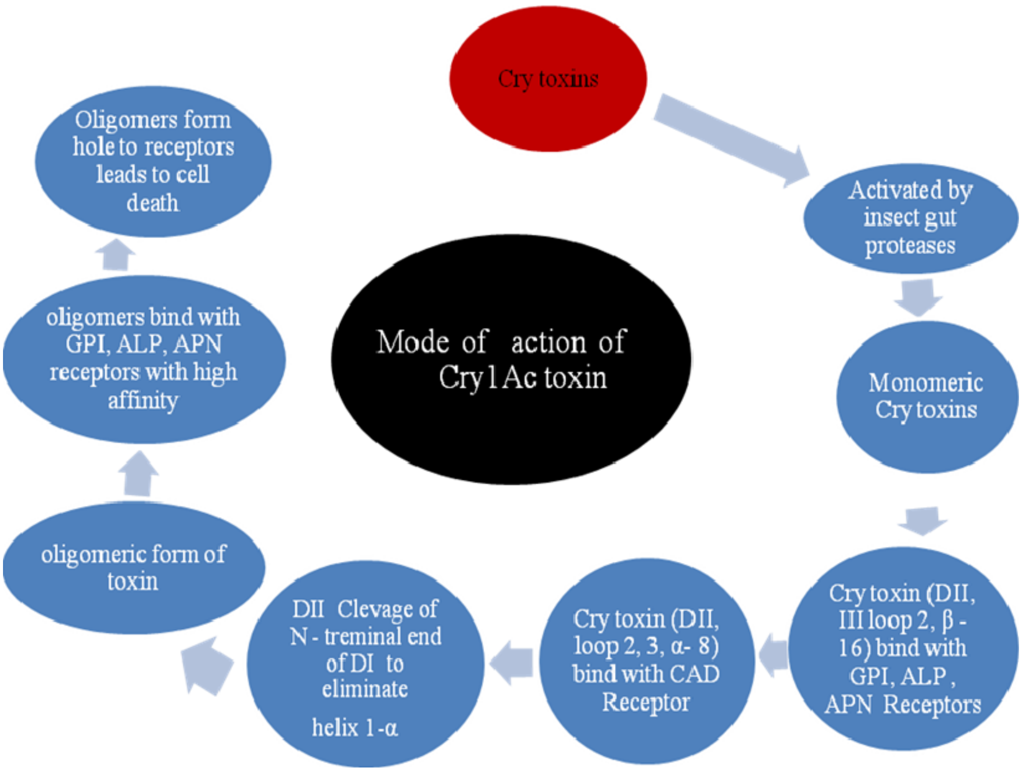

Fig 1: Mode of action of Cry toxin in insect midgut

Bt cotton

The cultivation of transgenic cotton is increased in a large scale since from its introduction from 1996 globally (Tian *et al*., 2018) [12]. The transgenic cotton cultivation to insect pest control is widely increased cultivated area 22 million hectares and 64% increased its adaptation by the farmers globally (Tian, 2015) [13].

In India, Bollgard I was introduced in 2002 consisting of Cry1Ac protein and Bollgard II was introduced in 2008 consisting of Cry1Ac and Cry2Ab proteins. The GM crops are regulated by GEAC. Different companies are released different *Bt* hybrids. Till now, the Genetic Engineering Approval Committee approved more than 111*Bt* cotton hybrids. *Bt* cotton cultivation is increased year by year. The *Bt* events such as MON 531, CryX and MON15985. The cultivation of *Bt* in north and south part increased up to 96 percent till end of 2014 (Jayalalitha *et al*., 2015) [14].

The pyramid is a strategy contacting two toxin proteins which interaction between two proteins such as Cry1Ac and Cry2Ab and there is no effect between of Cry2Ab on Cry1Ac while working on same pest. The proteins are work independently on insect pests. The Cry2Ab counter the resistance insect (Akhtar *et al*., 2018) [15].

Problems in Bt cotton

Globally, insect pests such as *Helicoverpa zea*, *Helicoverpa virescens* and *Pectinophora gossypiella* are major pests in cotton growing regions. These lepidopteron pest feeds initially on leaves, flowers and bolls and cause a large economic loss to farmers. *Helicoverpa zea* and *Pectinophora gossypiella* can survive and increase their generations four times in a single standing crop time period (Fleming *et al.*, 2018) [16].

Resistance in insects to transgenic cotton is a major problem in transgenic cotton regions in all over the world. Resistance is a sudden heritable change in the insect guts which cannot bind with cry toxins and these leads to control of pests in transgenic cotton growing regions is a major problem (Tabashnik *et al.* 2013) [4].

Globally, all most 17 insects was developed resistance at in-vitro condition and few insects developed resistance at field level such as *Helicoverpa zea*, *Pectinophora gossypiella*, and *Helicoverpa armigera* (Sheikh *et al.* 2017) [17]. Controlling of insect resistant by pyramid strategy and refuge strategy was widely adopting all over the world.

Resistance management

Refuge strategy

Refuge strategy is one method that delays the insect resistance and increased the cry1Ac expression levels in transgenic cotton. The combination of refuges in different percentages such as 10%, 25% 50% and 75% with *Bt* cotton were observed in recent studies (Perumalla *et al*., 2018) [18].

The studies find that the increasing expression levels of Cry1AC with increasing level of refuge cotton percentage. The expression levels of Cry1Ac in different parts such as upper leaves, lower leaves, sepals and boll bracts was increased while increasing refuge percentage. Planting refuge with alternatively with *Bt* cotton also increase the Cry1Ac expression levels compare with border refuge. The combination of 75% *Bt* with 25% showed higher expression level of Cry1Ac and gave highest yield. This combination is useful to counter insect resistance (Perumalla *et al*., 2018) [18].

Mixed refuge strategy is one the refuge strategy to control insect resistance. The present results were find that insect larvae population in 20% refuge was higher than the 20% mixed refuge strategy. Larvae population depends up on the size of refuge and refuge placement at field level (Sheikh *et al*., 2017) [17].

Pyramid strategy

This strategy emphasize on staking of two toxin Cry proteins in same transgenic cotton. Globally, the Bollgard II of *Bt* cotton contains Cry2Ab and Cry1Ac that makes a "pyramid strategy" under which plant uses two or more toxins to kill same pest. The Crystalline proteins work individually in all plant parts effectively. The effectively control of Lepidoptera pests, one protein kills the larvae and another protein counter resistance against insect pests (Arshad *et al*., 2011) [19].

The pyramid interaction between Cry1Ac and Vip3AcAa is found as there is no effect between of Vip3AcAa on Cry1Ac while controlling insect pests. The proteins are work independently on insect pests. The Vip3AcAa is a very good pyramid strategy to counter the insect resistant (Chen *et al*., 2017) [20].

The susceptible inset pest survived and exposed to the two toxins (pyramid strategy) reductant killing is reduced in insects. During, growing season of transgenic cotton *Helicoverpa zea* population was reaches up to 5% by pyramid strategy (Greenburg *et al*., 2010) [21].

Table 1: *Bt* toxin pyramids used proactively and separately from one-toxin plants or remedially and concurrent with one-toxin plants (Sheikh *et al*., 2017) [17]

Pest	Crop	Country	Toxins in pyramid	Resistance detected
Protective and separate from one toxin plants				
H. armigera	Cotton	Australia	Cry1Ac, Cry2Ab	None
H. punctigera	Cotton	Australia	Cry1Ac, Cry2Ab	None
Remedial and concurrent with one-toxin plants				
D. virgifera	Corn	USA	Cry3Bb, Cry34/35Ab	Cry3Bb
H. zea	Cotton	USA	Cry1Ac, Cry2Ab	Cry1Ac
H. zea	Cotton	USA	Cry1Ac, Cry1F	Cry1Ac
P. gossypiella	Cotton	India	Cry1Ac, Cry2Ab	Cry1Ac
S. frugiperda	Cotton	USA	Cry1F, Cry1A.105b, Cry2Ab	Cry1F

Future aspects

In transgenic cotton the future aspects would be, avoiding of insect resistance development to Cry toxins in transgenic cotton and new resistant management strategies would required to control insect resistance. New genes would identify to alternate to *Bt* genes in order to control insect pest. In cotton, lot of abiotic stress factors such as drought and nutrient availability to plants there is a need to develop abiotic stress transgenic cotton to survive in adverse conditions. Cotton is rich in fiber and oil, the transgenic cotton would develop to increase the fiber quality and improve the oil content in cotton crop. The transgenic cotton should be high and mostly in India farmers are poor, cost effective transgenic cotton would develop in India to purchase these transgenic cotton seeds in low cost.

Conclusion

After introducing of transgenic cotton, the resistance is a major problem in transgenic cotton growing regions in all over world. For controlling of insect resistance so many strategies are there, among the strategies refuge and pyramid strategies are widely used management methods. Studies observed that the combination of refuge cotton along with transgenic cotton with different percentages of refuge such as 10%. 25%, 50% and 75% along with transgenic cotton find that Cry1Ac expression was increased and there is a symbiotic effect between transgenic cotton refuge cotton. But, the yield was decreased by increasing of refuge cotton. Among different refuge percentages, the 25% refuge with 75% transgenic cotton showed second and third highest in field trails and highest yield found in both the field trails. These, combination would helpful to farmer to counter the insect resistance and get more income. In pyramid strategy also insects was developed resistance to two gene transgenic cotton (Bollgard II) and more genes then two genes would helpful to counter the insect resistance in transgenic cotton and it is difficult to insects to develop resistance to multi genes at a time.

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