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Resistance Development in Pink Bollworm *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) to Bt Cotton and Resistance Management Strategies

Sree Latha Edpuganti*

National Institute of Plant Health Management, Rajendra Nagar, Hyderabad – 500 030

*Corresponding Author E-mail: sreelatha437@gmail.com

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ABSTRACT

Transgenic cotton producing Bacillus thuringiensis (Bt) toxins kill Lepidopteran pests and thus; can reduce reliance on insecticides to control boll worms. Through, biotechnology the naturally occurring Bt proteins are used to develop genetically modified (GM) insect-protected crops that help farmers to protect against insect damage and destruction. GM crops were first commercialized in the US, Canada, Mexico, Argentina, China, and Australia in 1996. In India Bollgard I released in 2002 and Bollgard II in 2006 in cotton crop. India, the world's biggest cotton producer, has the fifth largest area under GM crop cultivation; cotton had been sown on 111.55 lakh hectares during 2017-18. Huge area under Bt crop increased the chances of rapid evolution of resistance to Bt toxins by boll worms. When Bt cotton was released, initially it was extremely effective at killing lepidopteran pests, but inherently these pests have potential to develop resistance under intense selection pressure.

Key words: *Pink bollworm, Resistance, refuge, Bt cotton, Gene pyramiding*

INTRODUCTION

The pink bollworm PBW, a major pest in many countries including the world's three leading cotton producers - China, India, and the United States[9,10] . PBW has experienced intense selection for resistance to Bt cotton in India.

RESISTANCE - NATURAL AND EXPECTED

Resistance development in insects is a natural phenomenon. Insects develop resistance through natural selection. Pest species had been exposed to plant antibiotic components long before agriculture began. Many plants produce phyto-toxins, antibiosis as one of the mechanisms of resistance to protect them from herbivores. As a result, co-evolution of herbivores and their host plants required development of the physiological capability to detoxify or tolerate these antibiotic components produced by plants[21]. Pest populations can develop resistance to host plant resistance, chemical pesticides and even can adapt to non-chemical methods of control also. Pests with limited diets i.e., mono-phagous insects develop resistance faster, because they get exposed to higher pesticide concentrations and have fewer chances to mate with unexposed susceptible mates.

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Pests with shorter lifecycle and having more generations per year develop resistance more quickly than others. Bt is naturally occurring protein, so a small number of insects may have natural resistance to some Bt proteins. If extensive area is cultivated with crops containing Bt proteins over time, it is possible that some insects in a field could develop resistance to a Bt protein and cause significant damage or destruction. Without adequate countermeasures like refuge of Non Bt crop which produce susceptible insects, these pests can quickly evolve resistance. It is well known fact that Insect resistance to Bt proteins is natural and expected. Resistance development in bollworms of cotton is determined by many factors like the frequency with which Cry-resistance alleles are expressed, dominant or recessive nature of the resistance alleles, migration patterns of moths, the survival advantage or disadvantage that resistance allele(s) offer and finally the number of susceptible moths available for mating with moths carrying resistance gene (s)[23,24,28]. Over the years of study recessive mutations that confer resistance to Bt toxin Cry1Ac have been reported in three major lepidopteran pests, including the cotton bollworm, *Helicoverpa armigera*. Zhang *et al.*[35] reported non-recessive Bt toxin resistance conferred by an intracellular cadherin mutation in field-selected populations of cotton bollworm and identified a novel allele from cotton bollworm that is genetically linked with non-recessive resistance to Cry1Ac. Tabashnik *et al.*[23] monitored pink bollworm resistance to Bt toxin in Arizona, USA for 8 years with laboratory bioassays and reported "delayed resistance to transgenic cotton in pink bollworm" and explained that the delay in resistance is due to refuge of cotton without Bt toxin, recessive inheritance of resistance, incomplete resistance, and fitness costs associated with resistance. Heuberger *et al.*[9] reported contamination of refuge by Bt transgenes could reduce the efficacy of refuge strategy in USA. Bt contamination in refuge produce survival of pink bollworm that were resistant (rr), susceptible (ss), or heterozygous for resistance (rs) to Cry1Ac. Due to this contamination resistant population (rr) develops and contaminated refuge also confers selective advantage to rs over ss individuals and accelerates resistance development. In India, because farmers have not planted adequate refuge, field-evolved practical resistance has occurred to PBW[5,19,20,33]. Jeffrey *et al.* reported multi-toxin resistance enables PBW survival on pyramid Bt cotton containing Cry 1Ac and Cry 2 Ab

RESISTANCE – COUNTER STRATEGY

There are many strategies to delay resistance development in insects with major objective to reduce selection pressure and supply susceptible population.

Role of farmers: Growers of bt crops has to play crucial role in Insect Resistance Management (IRM) practices. Farmers who choose to grow a Bt crop must plant a "refuge" crop that does not contain a Bt trait. The primary strategy for delaying resistance is providing refuge of the host plants to pests that do not make Bt proteins i.e., non Bt crop. Refuge allows survival of insects that are susceptible to Bt proteins and reduces the chances that two resistant insects will mate and produce resistant offspring. This strategy is particularly effective for delaying resistance, that is inherited as a functionally recessive trait, because the heterozygous progeny produced by mating between resistant and susceptible adults do not survive on the Bt crops. Conversely, if inheritance of resistance is dominant, the progeny from mating between resistant and susceptible adults survive on Bt crops, and refuge is less effective for delaying resistance. Refuge provides a source of homozygous susceptible (ss) insects that can mate with rarely occurring homozygous resistant (rr) individuals. This concept was widely accepted by eminent scientist working on IRM strategies[4,13,15,22,23]. In PBW If resistance is inherited recessively, as in the laboratory-selected strains[1,14,15,22,23,24] the mating between resistant and susceptible insects produce heterozygous (rs) offspring, that are killed by Bt cotton, thus reducing the frequency of resistance alleles in a pest population. This is possible only if refuge of non Bt cotton is planted to produce susceptible PBW moths. The other strategy of IRM to delay or counter resistance, transgenic plant "pyramids" producing two or more Bt proteins that kill the same pest have been adopted extensively, because insects can develop resistance to one gene faster than to, two or more genes working in the same genotype[35]. Bollgard I cotton contains only one gene, the Cry 1Ac, from *Bacillus thuringiensis*. Bollgard II contains the Cry 2 Ab gene, in addition to Cry 1 Ac. Bollgard I offer protection against American bollworm (*Helicoverpa armigera*), Bollgard II provides season long control of key pests of cotton including *Spodoptera litura* and *Helicoverpa armigera* in India. The synergistic influence of two genes in Bollgard II would further delay the development of resistance by the pests to the two insecticidal proteins.

CHINA - IRM STRATEGIES

China, where farmers are still growing BG I and not switched to BG II cotton, small but significant increases in PBW resistance to Cry1Ac have occurred in northern China, where close to 100% of the three million ha of cotton planted yearly produces Cry1Ac as the only Bt toxin[12,31].Details of the 11-year study that tested more than 66,000 PBW caterpillars from China's Yangtze River Valley are published in the Proceedings of the National Academy of Sciences. Scientists from the U.S. and China discovered that the ingenious strategy used in China entails interbreeding Bt cotton with non-Bt cotton, then crossing the resulting first-generation hybrid offspring and planting the second-generation hybrid seeds. This generates a random mixture within fields of 75 percent Bt cotton plants out of which 25% are homozygous and 50% are hemizygous side-by-side with 25 percent non-Bt cotton plants, according to the University of Arizona, the 25 % plants produce susceptible population of PBW and reduced the development of resistance.[31].

USA- IRM STRATEGIES

Choice of genes and Gene Pyramiding: There are many choices to plant; to US farmers whenever it seems like some resistance to available traits in the market may be building up. In USA Bollgard®I carrying the Cry 1Ac gene was commercialized in 1996/97, Bollgard® II, with the Cry 1Ac and Cry 2Ab genes, in 2003/04 and WideStrike™, with Cry 1Ac+Cry 1F, in 2005/06. The purposes of adding a second gene to Bollgard® II and WideStrike™ were to broaden the spectrum of pests controlled and to delay the development of resistance to single type of toxin. Bollgard®3 cotton released by MONSANTO adds another protein — Vip3A — to the Cry1AC and Cry2AB Bt proteins which are found in Bollgard II® cotton varieties, to create a triple-mode-of-action. TwinLink® cotton technology of Bayer crop sciences offers two Bayer proprietary genes for the *Bt* proteins Cry1Ab and Cry2Ae. TwinLink® Plus offers three *Bt* proteins (Cry1Ab, Cry2Ae and Vip3Aa19) for technology durability and improved insect resistance management. WideStrike®3 of Dow Agro Sciences has WideStrike® Insect Protection features the Cry1Ac and Cry1F proteins and a vegetative insecticidal protein (Vip3A) from *Bacillus thuringiensis* (*Bt*). This type of pyramiding of Bt cotton genes, delays resistance development.

Refuge: The United States Environmental Protection Agency (EPA) mandates that growers in some regions plant refuge of non-Bt cotton in or near Bt cotton fields to delay pest resistance[30]. Refuges range from single rows of non-Bt cotton interspersed throughout Bt fields to large blocks of non-Bt cotton in or near Bt fields[1]. In 2010, the regulations were modified to include refuges planted with mixtures of Bt and non-Bt seeds that yield a random array of Bt and non-Bt plants side-by-side within fields. Seed mixtures have several advantages relative to block refuges, including elimination of the problem of farmers who do not comply with block refuge requirements. Mallet and Porter[16] proposed that when individual larvae feed on both Bt and non-Bt plants, seed mixtures can increase the dominance of resistance by increasing survival of heterozygous larvae relative to homozygous susceptible larvae.

Sterile moth technology: Tabashnik *et al.*[29,26] reported that in the United States, refuges of non-Bt cotton and mass releases of sterile moths suppressing resistance to Bt cotton have sustained PBW susceptibility to Bt toxins for two decades, helping to bring this invasive insect close to eradication in Arizona and other southwestern states. In 2005, the PBW Rearing Facility in Phoenix began cranking out pinkies for the Arizona experiment. The factory treated the moths with just enough radiation to damage the chromosomes in their reproductive cells without causing injuries that would prevent their survival in the wild. Over the course of each growing season during 2006-2010, about 2 billion PBW moths were released into Arizona's cotton fields[8].

IRM STRATEGY IN INDIA PAST, PRESENT AND FUTURE

In India the refuge supply as a non-Bt counterpart, as 120g packet separately in the Bt cotton hybrid seed bag of 450g. Mayee and Bhagirath 2013 reported that more than 90% farmers did not use non-Bt cotton packet for refuge plantings across three states of Maharashtra, Andhra Pradesh and Punjab in India. The unwillingness of farmers to plant non-Bt cotton refuge is a violation of the regulatory requirements of Bt cotton cultivation. It was observed that farmers who received pigeon pea as refuge bag planted it along with Bt cotton particularly in Maharashtra state. Many famers due to low quality of non-Bt cotton refuge seed didn't use it fearing it would attract insect-pests and would not produce desirable cotton yield. Apart from this there are many reasons for neglecting non Bt cotton seed sowing, main being that they cannot see the immediate effect of resistance development by boll worms. Many farmers are having very small land holdings of less than 5 acers and they were not ready to plant some portion with non-Bt seed.

PBW developed resistance to Bt Cotton in India - Why not *Helicoverpa* and *Spodoptera*?

Most scientists agree that the tobacco budworm and the American bollworm will eventually become resistant to the Cry1Ac protein used in current Bt cotton varieties. The tobacco budworm has a well-known reputation for developing resistance to chemical insecticides. Currently it is resistant to most conventional insecticides used on cotton. However, for the time being, it is extremely susceptible to the Cry1Ac protein in Bt cotton. The American bollworm is inherently more tolerant to this toxin, and it is likely to develop resistance faster than the tobacco budworm. Field and laboratory studies document the developed resistance of several insects to spray formulations of Bt toxins. The best-known example is the diamondback moth, a caterpillar pest that attacks cabbage and related plants. It has shown high levels of resistance to Bt sprays in Florida, Hawaii, North Carolina, Asia, and other locations[27]. It has also shown resistance to Bt transgenic plants[13]. Researchers have already developed laboratory colonies of Colorado potato beetles, European corn borers, tobacco budworms, and American bollworm that are resistant to Cry-protein. The resistant laboratory colonies of tobacco budworms and American bollworm demonstrate, these insects have the genetic potential to become resistant. Crop protection with Bt cotton is a form of host plant resistance, it is common that the resistant crops losing their protection from pests - the same fate is predicted for Bt cotton, but it has not happened in India. *H. armigera* and *S. litura* are highly polyphagous species having more than 120 host plant species like cotton, tomato, pigeon pea, chickpea, rice, sorghum, cowpea, groundnut, okra, peas, field beans, soybeans, lucerne, Phaseolus, tobacco, potatoes, maize, flax, ornamentals, wild plants, weeds and shade trees etc.,. In India other than Bt cotton remaining all conventional host plants produce susceptible populations. Even though any resistant population emerges from Bt cotton it will have adequate chances to mate with susceptible populations emerged from conventional hosts and reduces the chances that two resistant insects will mate and produce resistant offspring, so delayed the development of resistance. In contrast PBW feeds only on a few crops such as cotton, bhendi, Hibiscus, and jute which belong to *Malvaceae* family. In the absence of refuge the resistant population emerging from the huge area of Bt cotton will inter mate and produce resistant offspring. The strategy used in China of using 2nd generation hybrid seed may not be suitable to India. Indian hybrids are made with cross pollination of female and male parents based on heterotic value. In the next generation the plants segregate with respect to all characters. The plant population will become heterogeneous with respect to all physical characters including fibre and GM traits. In BG II cotton Cry 1 Ac and Cry 2Ab behaves as dominant genes under hemizygous condition in hybrids and each gene segregates independently to 3:1 ratio of 75% positives and 25 % negatives in next generation after selfing of hybrid, genotypically it is 1:2:1 of Homo positive: Hemi positive: negative for each trait, respectively. Cry 1Ac and Cry 2Ab are on different chromosomes and follow the law of Independent assortment[18]. The segregation of alleles for one gene occurs independently to that of the other gene, expresses 9:3:3:1 phenotypic ratio but genetically it is 1:2:1:2:4:2:1:2:1 which gives 9 different types of segregants. In 2nd generation after selfing of hybrid 3 out of 16 plants express Cry 2b alone without Cry 1Ac gene which is not permitted in India. The fibre also segregates and loses the uniformity which is main quality feature of Indian hybrid cotton. Among the strategies followed in USA the gene pyramiding strategy followed to certain extent in India: Bollgard I cotton contains only one gene, the Cry 1Ac, Bollgard II contains the Cry 2 Ab gene, in addition to Cry 1 Ac. As development of cross resistance is rare phenomenon, the remaining options available for future are Bollgard III, TwinLink® TwinLink® Plus, WideStrike®, WideStrike®3. Fabrick *et al*.[6] reported that PBW resistance to Cry1Ac does not confer strong cross-resistance to Cry2Ab. Mating disruption and sterile moth technologies are other options to control PBW immediately. Presently the focus and attention is needed to bring down current problem of PBW, to control and in future strict refuge strategy is to be designed and followed.

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