Release of insecticidal transgenic crops and gap areas in developing approaches for more durable resistance

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Transgenic cultivars expressing d-endotoxin coding genes of Bacillus thuringiensis are being grown globally on about 12 million hectares this year. Agriculture in India can benefit substantially by adopting transgenic insecticidal cultivars since, in contrast to the world average of 30%, of the total chemical pesticides used in India 75% are employed against insects. No other biological approach, as safe as and yet as effective as the Bt technology is presently known to control agricultural pests. The question at the center stage is to expedite the commercial release of Bt transgenics and also make a parallel effort to devise knowledge-based strategies aimed at achieving longer durability of crop resistance to insect pests. Plant breeders have encountered similar situations in the past for improving crops against insects and other diseases. This article identifies the gap areas where research efforts are needed to develop strategies for enhancing the durability of crop resistance.

GLOBALLY, 39.9 m hectares was under cultivation of transgenic crops in 1999 – nearly 72% of it being in USA. In terms of distribution by traits, 71% of this area was under genetically engineered crops expressing alien genes for herbicide tolerance[1]. The second position at 22% was taken by insect-resistant transgenic crops – primarily corn and cotton that expressed the δ-endotoxin coding genes (*cry*) of the soil bacterium *Bacillus thuringiensis* (*Bt*). Transgenics for insect resistance have been drawing special attention in India also since 20–30% of the crop productivity is lost due to damage by insect pests. The Department of Biotechnology and the Indian Council of Agricultural Research have been sponsoring researches aimed at the development of insecticidal transgenic cultivars. Some of the multinational agribiotech companies have conducted limited field trials in India during the last two years, aimed at collecting data required to facilitate the release of transgenic crops expressing *cry* genes.

Though several proteins that interfere in insect development have been reported, δ-endotoxin-based transgenic crops (*Bt* transgenics) provide the best choice presently. Unlike metabolic inhibitors (proteinases and amylase inhibitors, lectins, cholesterol oxidase, etc.), δ-endotoxins cause mortality to target insects to the extent of 100% at ppm level. These are not toxic to vertebrates and are highly specific to target hosts. Though the first *Bt* transgenic plants were reported in 1987 by three groups working independently, it took nearly ten years to take one of the *cry* genes in an agronomically acceptable genetic background and develop the technology to a stage that it could be released for commercial cultivation. Since the first release in 1996, the increase in area under transgenic *Bt* crops from about 1 m hectare to 11.8 m hectares in 1999 testifies the economic advantage (besides a substantially lower application of synthetic pesticides) that farmers have experienced. Nevertheless, commercial cultivation of *Bt* crops has been limited to the mainland USA (excluding Hawaii) and southern parts of Australia, pending further data on ecological safety, and China. Having got this outstanding opportunity of developing transgenic *Bt* crops for resistance to insects pests, after a substantial effort and investment in R&D through the last 10–15 years, it is important to learn how to manage widespread adoption of *Bt* crops without leading to rapid emergence of resistance in target insects. The question at the center stage is to expedite the commercial release of *Bt* transgenics and devise knowledge-based strategies to ensure long durability of the *Bt* technology. This article aims at summarizing broad issues related to the emergence of resistance to analyse how the opportunity offered by *Bt* technology can be made more lasting. It also pins down major gap areas in which scientifically proven data are required to resolve some of the diametrically opposite views that often draw the *Bt* technology into controversy.

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Genetic basis of insect resistance to δ-endotoxins

Several mutants of a variety of insects have been isolated for resistance to different δ-endotoxins. An extensive review on this aspect was published by Tabashnik[2]. Most of the reported mutants were obtained in the laboratory after several generations of feeding on δ-endotoxin-supplemented diet. In some cases, resistant isolates were obtained from fields with a history of application of *Bt*-based microbial formulations. As of now, no resistant mutant has been reported from fields cultivated with transgenic *Bt* cultivars. It is probably too early to expect the emergence of resistance at this stage, since area under transgenic *Bt* crops has been substantial only during the last three years. Globally, in 1999, it was only 9.6 and 2.1 m hectares under *Bt* corn and cotton respectively. Assuming an average of 5 generations of target pest per season in one year, an exposure for 15 generations may be insufficient to make the resistance visible, specially if it is genetically recessive. Growers and academics in the US and Australia are understood to be working with seed companies to monitor the possible emergence of resistance. Insect populations collected voluntarily throughout the cotton belt are sent for susceptibility analysis. If *Bt* transgenics are to be released with an inbuilt durability plan, it is desirable to lay pheromone traps in different agroclimatic zones in India within migratory and mating distance of target insects and determine their baseline susceptibility. This needs to be done during different cropping seasons before and after the release of transgenic cultivars. As discussed later, suitable strategies need to be evolved to ensure a check on any increase in the frequency of alleles that may enhance the tolerance of insect pests to δ-endotoxins.

Questions related to the number of genes, allelic dominance, cross resistance and gene frequency need to be addressed for individual target pests to design strategies for durability of *Bt* crops. For instance, *Bt*-resistant strains of *Plutella* isolated from four geographical locations – Hawaii, Pennsylvania, Philippines and Malaysia, have been compared[3]. Biochemical analysis suggested that the first three may be similar but not identical. Competition binding experiments concluded that *Plutella* receptor(s) may have two binding sites for the δ-endotoxin CryIAa. Alteration in a common site in the Hawaii (NQ-QA) and the Pennsylvania (PEN) isolates gave cross resistance to CryIAa, IAb, IAc and IF. A different mutation gave resistance to CryIA group of δ-endotoxins but not to IF in the Philippines (PHI) strain. Information on host range of different δ-endotoxins and cross resistance in target insects is invaluable in deciding the *cry* genes that should be expressed together in a transgenic cultivar to achieve durable resistance.

In most of the cases, resistance against *Bt* has been reported as recessive. The receptor mutations are presumably recessive because the same receptors may be required for a yet unknown but indispensable physiological function. Dominant alleles for resistance may nevertheless be found, once a larger spectrum of mutants becomes available. For instance, a mutation leading to the formation of a new gut protease that could degrade δ-endotoxins would presumably be dominant. Incompletely dominant mutations have been reported for *Ostrinia*[4], *Plutella*[3], *Heliothis*[5] and *Leptinotarsa* (cf. Tabashnik[2]). Such events may be rare but warrant attention for designing strategies for the management of resistance to *Bt*. The presently recommended strategy of refugia (discussed later) can fail altogether if emergence of resistance through dominant allele is of common occurrence in a target insect or against a target toxin. Thus, genetic basis and mechanism of resistance need to be understood so that strategies that will succeed against the problem of dominance can be adopted. In the event of dominant resistance, an alternative toxin (or even chemical pesticide) can be employed in parallel or sequentially.

Frequency of resistance alleles in natural population

Frequency of occurrence of resistance allele(s) in natural populations of target insects is an important aspect that has not been examined sufficiently. The published literature gives the frequency of resistance alleles, ranging from 0.1 in *Plutella*[6] to 0.001 in *Heliothis*[7]. Such values for other insects are not available and may vary depending upon insect species, target toxin and past exposure to *Bt*. Though resistance may predominantly be recessive, isolation of homozygous recessive lines (rr) in the laboratory can facilitate the estimation of frequency of r in natural population by paired matings. For instance, it will take only 1000 paired matings with rr to detect resistance allele even if it occurs at the frequency of 0.001. Such standard homozygous-resistant lines need to be developed for major Indian pests and can be valuable in monitoring any increase in frequency of resistance allele, as the cultivation of *Bt* transgenics is promoted. Regular monitoring for any shift in baseline susceptibility and possible emergence of resistance is desirable if large-scale commercial release of *Bt* crops has to last 10–15 years, and hopefully beyond, through timely synergism with other insect management practices.

Baseline data on population susceptibility

The principles of population genetics can predict the number of generations it would take for homozygous recessive (rr) resistant individuals to become visible, given the frequency of such an allele in population. Tabashnik[2] collated a large amount of published data

showing up to 700-fold differences in sensitivity (LC_{50}) of geographically different populations of *Plutella*, 2 to 7-fold variation in *Heliothis virescens*, 13 to 16-fold variation in *Helicoverpa zea*, 2 to 42-fold variation in *Plodia interpunctella*, etc. Such variations in basal level of susceptibility to *Bt* have been reported, primarily for the populations in the US where microbial formulations of *Bt* were employed on horticultural and forest crops through the last several years. In Chinese populations of *H. armigera*, up to 100-fold differences in mortality against CryIAc have been recorded[8]. After this article was accepted for publication, we noticed very useful data published by Gujar *et al.*[9] reporting 2 to 5-fold variation in the *Bt* susceptibility of *H. armigera* collected from a dozen different locations from Punjab to Tamil Nadu. Population sensitivity was not very high (LC_{90} being 545 ppm for HD73), considering that mortality to the extent of 98% is desirable to ensure durable release of transgenic cultivars without necessitating a high level of refugia (discussed later). Several hundred-fold differences in the mortality dose of local *H. armigera* isolates determined in different Indian studies[10,11] may be due to differences in methodological details. However, comparative toxicity data on various target pests, generated through harmonized laboratory bioassays and also, as far as possible, actual data using transgenic *Bt* plants are desirable to develop sound strategies for durable field stability of *Bt*-cultivars.

At a biochemical level, population differences in sensitivity could occur because of differences in affinity of the receptors for toxins[6,12–15], lower density of receptors or binding sites[13,16], decreased solubilization or activation of toxins in the insect gut[17], reduced insertion of toxins in epithelial membrane, reduced pore formation, etc. Varying levels of resistance can also develop due to additive effect of independent recessive genes, as reported for *Heliothis virescens*[5,16] and *Plutella xylostella*[2]. Additive multiple loci and incompletely recessive or co-dominant alleles can influence susceptibility of insects to endotoxins. Development of baseline data on the susceptibility of insect species and populations within migratory and mating distance and mortality curves for heterozygotes are desirable for predicting the rate at which homozygous recessives can appear in population. Assuming that the allele for resistance to endotoxins is recessive (r), it occurs in natural population at the frequency of 0.001 and that a transgenic *Bt* cultivar causes mortality of heterozygotes (Rr) to the extent of 90%, the principles of population genetics can be employed to predict the number of generations it would take for the frequency of r to increase from 0.001 to 0.5. The rr types will become visible in fewer generations if Rr are killed to the extent of 90% instead of 98%. A 10% lower killing of *Helicoverpa* heterozygotes can lead to the appearance of resistance several fold faster. This over-simplified example illustrates the value of developing experimental data on baseline susceptibility, number of alleles, allele frequency and genetic basis of resistance to ensure durable large-scale release of transgenic cultivars.

The refugia concept

It is easy to visualize that the development of homozygous recessives can be delayed as long as 100% of the heterozygotes (Rr), being susceptible, are killed by feeding on the *Bt* crop. In practice, some heterozygous susceptible individuals can escape killing and mate with another Rr, leading to the emergence of homozygous resistant (rr) individuals. Rapid emergence of rr can be delayed if these can be prevented from coming across rr and Rr types. This is possible if a sufficiently large Hardy Weinberg population (RR, Rr, rr at a frequency of p^2, $2pq$ and q^2, respectively, with p being $1 - q$, i.e. 0.999 and q being 0.001, viz. the frequency of the recessive resistance allele in the population) can be maintained and mates randomly with the rare rr in field. This can be achieved by growing a non-transgenic cultivar in close vicinity of the transgenic cultivar. The rare rr that may emerge at a probability of 0.000001 ($q^2 = 0.001 \times 0.001$) in transgenic field would then end up mating with the normal population growing on non-transgenic crop. This strategy of growing a parallel non-transgenic (refugia) crop as an assurance to delay the emergence of homozygous recessive resistant phenotypes is a powerful design. However, it has several untested assumptions. The unknowns lead to the uncertainty of deciding the extent to which refugia should be included. The suggestions vary[18] from 4% to 50% – the former being a possible underestimate and the latter being an over assuring but economically lean proposition. This section would analyse the gap areas in knowledge that need to be addressed to give a sound logic to the issue of refugia, the complications in implementing the refugia strategy in the poorly managed Indian agriculture and the possible alternatives.

The assurance of refugia rests on the following assumptions:

1. The transgenic cultivars kill susceptible heterozygotes nearly completely.
2. The resistance is recessive and frequency of the resistance allele is rare.
3. A sizable susceptible population is available within effective mating distance.
4. The resistant insects that emerge on *Bt* crop mate randomly with the susceptible population.

Each of the above assumptions has untested components. Transgenic cultivars that express the *Bt* genes constitutively at a whopping 0.1 to 0.2 per cent of the

total soluble protein have been developed in several cases. It is desirable to develop baseline susceptibility data for locally prevalent agricultural pests within mating and migratory distances in zones where target crops are grown or alternative insect hosts are prevalent. Cotton belts in India are affected by *H. armigera* rather than *Heliothis virescens*. The latter is killed 95 to 100% by Bollgard (CryIAc) cotton[19] developed by Monsanto, St. Louis. However, mortality data on field efficacy of transgenic cultivars against *H. armigera* and the evolution of resistance are not available. Such data are required to confirm the validity of results generated by laboratory bioassays. The problem that emerged in the Texas belt following the first release of transgenic cotton in 1996 was related to the escape of *H. zea* which is known to be less susceptible to CryIAc and is more comparable to *H. armigera*. It was (an often misunderstood) a case of escape (due to lower susceptibility and build up of population on the earlier corn crop) rather than the emergence of genetic resistance. Another species that infests cotton crop as a major alternative pest especially in southern India is *Spodoptera litura* (tobacco caterpillar). Transgenic cotton to be released in India needs to cause nearly complete killing of *S. litura* as well as *H. armigera* heterozygotes at the resistance locus, if the refugia has to be effective. The CryIAc, in spite of being highly toxic to *Heliothis* sp., causes some morbidity but no mortality of *Spodoptera* sp. (our unpublished data; Hofte and Whiteley[20]). Other cotton bollworms like *Pectinophora gossypiella* and *Earias vittella* (spotted bollworm) are known to be highly sensitive to CryIAc[21]. Susceptibility data for insects collected from cotton-growing geographical regions of the country need to be developed. In any case, with *Spodoptera* waiting to take over and *H. armigera* expected to show less than 95% killing, the life of CryIAc Bollgard cotton may be uncertain in the absence of relevant data. In such a situation, it is not possible to rationalize the extent of refugia required to manage possible emergence of resistance. Roush[22] predicted by computerized simulation modelling, the effect of the extent of refugia on the number of generations required for the frequency of the recessive resistance allele r to reach or exceed 50%. Assuming the initial frequency of r at 0.001, employment of 10 per cent refugia with transgenic crop that killed 98% of heterozygotes, r was predicted to exceed the frequency of 0.5, after 40 generations that may take 6 to 8 years. Another 2 to 5 years may additionally be allowed for resistance to become visible at regional and national levels. Other assumptions being the same, if the target insect is less susceptible, resistance development can become a threat much faster. At a heterozygote mortality of 90%, resistance can be delayed for not more than 10 generations, i.e. 2 years with 10% refugia. At 90% mortality, in order to delay resistance by 40 generations, the refugia required would be at the high level of 50%. The modelling suggests that the initial frequency of resistance allele in insect population is less important to the durability of a transgenic crop than the mortality of heterozygotes.

The predictions suggest that transgenic crops to be released should cause a heterozygote mortality of 95 to 100% to ensure that the problem of resistance does not emerge before 8 to 10 years with a refugia of not more than 20%. In practice, this level of mortality may not be achievable – less so when a variety of target insects infest a given crop, as in the case of cotton. Under such a situation, pyramiding of two or more genes is the alternative.

Pyramiding of genes in transgenic crops

Achieving 95 to 100% mortality of both *Helicoverpa* and *Spodoptera* in cotton may require the deployment of two different genes. A novel chimeric *cry* gene highly effective against local isolates of *S. litura* has been designed and synthesized (Tuli *et al.*, unpublished) in our laboratory. Transgenic tobacco plants expressing this gene give 100 per cent mortality of 1st to 5th instar larvae of *Spodoptera* after 4 h of feeding on transgenic leaves (Table 1). While the already commercialized CryIAc is expected to kill *Helicoverpa* to the extent of 90% or more, the endotoxin coded by the chimeric *cry* gene developed in our laboratory, retards its growth. It is desirable to determine the synergistic effect of the two proteins on geographically distant populations of the two target pests, and to develop a commercializable cultivar of cotton expressing both the genes. Though these two toxins are expected to operate through independent receptors, it is essential to exclude their cross

Table 1. Insecticidal effect of feeding the larvae of *Spodoptera litura* on *Bt* transgenic tobacco leaf for different time periods

Hours of feeding on leaf of *Bt* transgenic plant NB 516-8	Larval mortality (%) after indicated duration of shifting from NB 516-8 to non *Bt* tobacco leaf			
Four-day-old larvae	48 h	96 h	120 h	144 h
1 h	25	75	80	85
2 h	35	80	90	90
4 h	60	95	100	100
8 h	75	100	100	100
Eight-day-old larvae				
1 h	10	20	20	20
2 h	20	20	30	40
4 h	60	60	70	100
8 h	70	70	90	100
16 h	90	90	100	100

Four- or eight-day-old larvae were fed on the leaves of a transgenic plant for the indicated period of time. At each time point, ten larvae were shifted to leaves of a non *Bt* plant to follow mortality.

resistance experimentally. Once such data become available, transgenic crops with two killer genes can give a lasting resistance without a heavy demand of refugia. For example, the model by Roush[22] predicts that even at the low 50% mortality of the heterozygotes for each toxin-related locus, a transgenic cultivar with two genes pyramided and 15% refugia can delay resistance by 40 generations. At 70% mortality, even 7% refugia is predicted to delay the frequency of r from exceeding 0.5 by more than 80 generations. However, presently not many candidate genes that meet such criteria are known. Non-*Bt* genes (proteinase inhibitors, lectins, cholesterol oxidase etc.) delay the development of insects rather than kill them. Any new candidates may take another ten years to reach a commercializable stage, as it happened for *Bt*. Thus, it is important to benefit as much as possible, from the known *Bt* genes by intensifying studies on aspects related to resistance development following their use, singly, or in combinations and with and without other inhibitors and toxins.

Refugia requirements being less stringent, it may be prudent to eventually recommend pyramided transgenics for commercialization in India. It may virtually be impossible to expect Indian farmers with small holdings to risk themselves with a single crop and grow refugia around the *Bt* crop. If the refugia is to be sprayed with synthetic pesticides (as is done in 20% refugia-single gene strategy in the US), the whole management practice may perhaps become too complicated to implement. Unless all the farmers grow transgenic *Bt* cultivar within mating distance of a target insect, the non-*Bt* crops in neighbourhood fields can provide an unintended refugia. Thus, refugia requirement can perhaps be relaxed, provided the pattern of cultivation of *Bt* lines can be regulated to ensure that neighbouring fields within mating distance of target insects act as refugia.

Complexities associated with the application of refugia

As mentioned in the foregoing discussion, in the absence of experimental data, recommendations on the extent of refugia required are based on predictions and computer simulation models. To illustrate the variations in recommendations made by different expert groups, some details related to *Bt* cotton can be examined. The Union of Concerned Scientists, USA recommended 50% of total (*Bt* + non-*Bt*) acerage as refugia if synthetic pesticides were to be sprayed and 16% if pesticides were not to be sprayed on refugia. The corresponding figures recommended by the US Environmental Protection Agency (EPA) were 20% and 4%, respectively. The wide discrepancy between the figures for *Bt*-cotton and corn prompted the environmental activists in February 1999 to file a lawsuit against EPA having permitted the transgenics with low refugia. Recently, the EPA revised its resistance management plan[18]. The new plan, irrespective of the pesticide sprays, directs 20% refugia in regions where a single *Bt* crop – cotton or corn, is grown and 50% in areas where agroclimatic conditions permit farmers to take both the crops (as would be the situation in India). A higher refugia in dual crop areas is recommended because insects like *Helicoverpa* can thrive on both and therefore, resistant individuals that emerge on corn can then multiply on cotton, in the following season. A flat 20% refugia, now recommended by the EPA and 30% in Southern Australia needs to be substantiated with experimental data. In the US, during the earlier years, about half the growers are understood to have used the 80/20 (with pesticide) option and the other half, the 96/4 (without pesticide) option with *Bt*-Bollgard cotton as per the earlier refugia plan. Though detailed calculations on economic returns from *Bt* cotton and the refugia component are not available, the yield loss in the unsprayed refuge could easily be 50%. Despite the refugia and crop loss, average advantage for Bollgard is claimed at US$ 100 to 400 per hectare by Monsanto. It is desirable to conduct multilocational field trials in India to develop data on yield advantage and economic gain so that *Bt* crops can be recommended to farmers.

A knowledge-based approach, substantiated with field data may point to additional variables that can influence the extent and placement of refugia. Migratory and mating habits of pests can determine if the refugia can be provided to farmers in the form of mixed seeds or if it needs to be structured in separate plots. Since the corn pest, i.e. *H. zea* is more migratory than the cotton pests, i.e. *Spodoptera*, *Heliothis* and other bollworms, it may be good to have refuge immediately adjacent in case of *Bt* cotton, while it should be located a few hundred metres away in case of *Bt* corn plots. In poorly migratory insects like potato tuber moth, mixed refugia may be acceptable. In the case of a mixed refugia, the migration of larvae from transgenic to non-transgenic plants within a field may make refugia ineffective if a brief period of feeding on *Bt* plants is enough to cause mortality or break in the life cycle. On the transgenic plants developed in our laboratory, 4 h of continuous feeding is enough to cause 100 per cent mortality of the larvae of *Spodoptera* in all instar stages (Table 1). However, about 50% of the *Spodoptera* larvae fed on mixed *Bt* and non-*Bt* leaves of tobacco escape mortality (Table 2) due to migration. However, in spite of escape from mortality, they are retarded in growth and fail to complete metamorphosis. A few adults that emerge, fail to lay eggs. The few eggs that are laid, fail to hatch or may be infertile. The results suggest that simplification of refugia in the form of seed mixtures may not be feasible, in the case of highly migratory pests since susceptible insects may not reach the next generation in spite of

Table 2. Effect on life cycle of *Spodoptera litura* larvae fed on *Bt* transgenic leaf mixed with control leaf (refugia)

Type of leaf fed	% larval survival on 4th day	% pupation of surviving larvae	% adult emergence of surviving pupae	% egg laying pairs	Egg hatching
Non-*Bt* leaf	100	100	100	100	100
Bt transgenic	0	–	–	–	–
Non-*Bt* + transgenic	47	57	75	50	0

Each treatment comprised three replications with ten larvae each. Eight-day-old larvae were marked with colour and released on tobacco leaves of control or *Bt* transgenic plants, given with or without mixing, as indicated. The larvae migrated among *Bt* and non-*Bt* leaves within a given replicate. Life cycle was followed till egg hatching, as indicated.

refugia being available. Moreover, in the case of insects that migrate even once in their life cycle, 10% mixed refugia would actually mean that only 1% of the insect population would have spent its complete life cycle on non-*Bt* plants. If 50% of the larvae move once, 10% seed mixture will effectively be equivalent to 5.5% of separated–structured refugia. This needs to be considered while designing strategies based on mixed refugia.

Further, greenhouse experiments reported by Liu *et al.*[23] demonstrated that *Bt*-resistant larvae of *Pectinophora gossypiella* take 5 to 6 days longer to develop on *Bt* cotton than the wild type or the resistant larvae take on non-*Bt* cotton. This developmental asynchrony can favour non-random matings that could reduce the expected benefits of the refuge strategy. However, field level experiments are required to explore, if in a case like this, mixed refugia may actually be preferable over structured refugia since the former may improve overlap between generations and therefore facilitate random mating. On the other hand, it can be argued that despite preferential mating among resistant strains, it is uncertain if fitness of the slower developing adults and their progeny will be sufficiently good to survive overwintering and thus actually enhance the evolution of resistance. As discussed earlier, our results (Table 2) suggest that developmentally lean larvae may not be sufficiently fit to push themselves through the exigencies of survival and therefore, may not make it to the next generation. Aspects like these suggest that the real risk of emergence of resistance may be substantially lower than what has been commonly predicted. There is a need to take these into consideration and correct the earlier predictions by doing multi-locational field experiments involving artificial infestation of transgenic cultivars.

Conclusion

Widespread acceptance and unprecedented increase in land area under *Bt* crops suggest endorsement of the technology by the farmers in USA. This is presumably indicative of the economic advantage that *Bt* cultivars bring to the cultivators, besides environmental and health benefits due to non use of chemical pesticides. However, it is desirable to address the above issues related to the emergence of resistance, to ensure that the success of *Bt* technology will be long-lived. The genetics of development of resistance in target species of insects needs to be critically understood. Strategies for delaying the emergence of resistance need to be substantiated with actual field experiments. The implementation of refugia may get simplified after actual data become available from field experiments and in view of other regional, social and managerial factors. The fitness cost of resistance, variations in mortality of different species of target pests, larval migration and adult mating behaviour in field need to be considered in designing the extent and the type of refugia and other resistance management strategies.

For a country like India, pyramiding of genes along with refugia provided as seed mixture is certainly a more easily implementable strategy. Till cultivars with pyramided genes become available, reaping the benefits of *Bt* transgenics may be employed along with other pest management strategies, including insect viruses (NPV) and chemical pesticides. Single gene transgenics that cause more than 95% mortality of target pests may be permitted with 10% mixed refugia for poorly migratory pests. For migratory pests, crops in the fields of a large majority of farmers who may not grow *Bt* crop can provide refugia, if the pattern of cultivation can be monitored and regulated. Such a release may be permitted for a limited period of three years with mandatory monitoring of possible emergence of resistance. Monitoring may be entrusted to entomological laboratories supported with corpus fund to be provided by transgenic seed companies. Hence, the first priority should be to harness economic benefits of insect control through *Bt* transgenics by permitting time-limited release and generating data during this period by monitoring the fields where transgenics are grown. After acceptance of this manuscript, we noticed the study by Kranti *et al.*[11] who reported the emergence of 76-fold resistance in Indian isolates of *H. armigera* after challenge with CryIAc for 10 generations in the laboratory. As discussed in earlier sections, determination of the frequency of such new mutations and the nature of their inheritance are extremely important in devising the strategies for pest

management. However, the survival of already existing resistant insect population[9] is a greater threat to the breakdown of *Bt* transgenic cultivars. The strategy therefore calls for regular monitoring of pest population in fields with transgenic crops, and the reduction of already existing recalcitrant population through the application of carefully selected pesticides and alternative approaches, like NPV. These reports re-emphasize the need for a parallel work on employing the two-toxin strategy. It is reasonable to predict that if *Bt*-resistant insects are identified sufficiently early by alert monitoring, it should be possible to develop new transgenics well in time to cope with the situation. Parallel examples of first working for resistance, followed by work on durable resistance can be found in breeding for rust resistance in the Indian wheat breeding efforts.

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