Bt resistance in *Helicoverpa* species: Indian policy needs urgent revision

GM crops are being cultivated for the last 15 years in many countries and culminating in the last year, GM crops were planted in more than 1 billion hectares (b ha) by nearly 15 million farmers. In India, about 6.3 million farmers cultivated *Bt* cotton last year (http://www.isaaa.org) in more than 9 million hectares (m ha) for protection against the ravages of the most devastating cotton pest, the American bollworm, *Helicoverpa armigera*. While this stupendous growth is amazing, there is a point of concern in the large-scale commercial cultivation of *Bt* cotton and other *Bt* crops. That is, the insect pests may develop genetic resistance to *Bt* toxin, due to the widespread cultivation of *Bt* crops. Evolution of insect resistance to *Bt* toxins is a worldwide phenomenon with potential for great economic burden, and can reduce the long-term effectiveness of *Bt* crops, thus making *Bt* technology less effective[1,2]. Strains of many pests have been selected for resistance to *Bt* toxins in the laboratory, whereas insects have evolved field resistance to *Bt* sprays and *Bt* transgenics in many parts of the world. A number of strategies are available to effectively address the problem of development of resistance in insect pests to *Bt* toxin[3]. The primary strategy in the field for delaying insect resistance to *Bt* crops is planting refuges of non-*Bt* crops near *Bt* crops, referred to as the high dose/refugia[1,2,4]. Two other strategies include seed mixtures of *Bt* and non-*Bt* plants[5,6], and tissue-specific expression of insect resistance[5,7]. Under ideal conditions, insect resistance to *Bt* toxins is recessive. Thus, heterozygous offspring, produced when homozygous resistant insects mate with susceptible insects, are killed by the *Bt* crops that produce high toxin doses. Models predict that resistance can be postponed substantially if the rare homozygous resistant insects surviving on a *Bt* crop mate with the more abundant susceptible insects from refuges. The strategy is called the high-dose/refuge strategy because the created plants produce *Bt* toxin concentrations high enough to kill heterozygous insects, thereby making resistance functionally recessive[2]. Stringent compliance of refugia can address the problem of *Bt* resistance development. However, in India, the larger concern is the poor compliance of the mandatory refugia in *Bt* cotton cultivation.

Real-time and practical experiences in India and worldwide suggest that the success of refugia is largely possible due to stringent farmer compliance. In the United States and many other countries, the level of compliance of 20% refuge is high among the farmers. For example, 91% farmers were found to meet the regulatory requirements for refuges associated with *Bt* corn[8]. Another study[9] of *Bt* cotton revealed that compliance with the refuge strategy was higher than 88% in five of six years from 1998 to 2003. In addition to mandating non-*Bt* crop refuges, the US Environmental Protection Agency requires monitoring for field resistance to provide early warning of resistance development[4]. In Arizona, where *Bt* cotton producing Cry1Ac has been used widely since 1997 and pink bollworm has been under intense selection for resistance, a state-wide surveillance system for resistance exists. Although the strategies implemented to delay resistance have helped sustain efficacy of *Bt* crops longer than that expected by many scientists, field-evolved resistance to *Bt* crops was reported[2,10].

However, it is hard to imagine that such a stringent compliance is practically and religiously followed in India. Voluntary planting of non-transgenic plants by the farmers is not assured, since farmers may deem it as a reduction in their profit to do so. Besides, the beneficial application of minimal insecticides during *Bt* transgenic crop (cotton) cultivation is also largely ignored. This severely compromises the premises on which *Bt* resistance management has been developed. Besides, absence of field data on mechanisms of resistant alleles, insect movements between plants, resistance against reduced doses and quantitative and population genetic structures of insect populations make the models more complex[11]. The refugia strategy becomes risky when there are more chances of the insect pests developing resistance to *Bt*. If so, what can be the next best alternative to refugia? Instead of structured refuge, seed mixtures containing seeds of both *Bt* transgenic and non-transgenic plants are the need of the hour (Figure 1). This is in response to poor compliance of the Indian farmers to grow the mandatory 20% refuge crop in *Bt* cotton fields, who do not plant the refugia at all. A major advantage of using seed mixtures is that there is no apprehension of farmers' non-compliance since the commercial packets of *Bt* seeds will also contain non-transgenic seeds premixed. In contrast to the structured refugia planting wherein, for example, for every eight rows of *Bt* cotton, two rows of non-*Bt* cotton are planted, in seed mixtures, the non-*Bt* cotton is randomly grown. By far, this is the cheapest and simplest solution, effectively achieving nearly similar result as using a structured refuge. This will force the farmers to use the non-transgenic seeds along with *Bt* seeds with 100% compliance. A caveat, however, is that the strategy of seed mixtures can become ineffective and unproductive if the frequency of resistant insect pests has already become unmanageable. But, in India, development of *Bt* resistance is not full-fledged but is on the possible horizon, although less worrying at present[12,13]. Besides, compliance of refugia is poor. Keeping these two major concerns in mind, time is now ripe to prudently opt for seed mixture strategy.

This strategy is easily enforceable by the regulatory agencies, easily implementable by all companies and organizations involved in commercializing the *Bt* transgenic technologies, and will finally enable the farmers with 100% compliance. *Helicoverpa* has an impressive track record at evolving resistance to a range of chemical groups and *Bt* across continents, including the Indian subcontinent[14]. Besides, *Helicoverpa* species are innately tolerant to Cry toxins[15]. Therefore, *Helicoverpa* cannot be taken for granted when it comes to large-scale cultivation of *Bt* cotton. Bollgard II *Bt* cotton expresses two *Bt* proteins, Cry1Ac and Cry2Ab, and is the predominant *Bt* cotton GM crop worldwide. Screening for *Helicoverpa* resistant to *Bt* should also involve resistance of *Helicoverpa* against both *Bt* proteins. There are many reports of *Helicoverpa* resistant to Cry1Ac protein as expressed by the earlier version

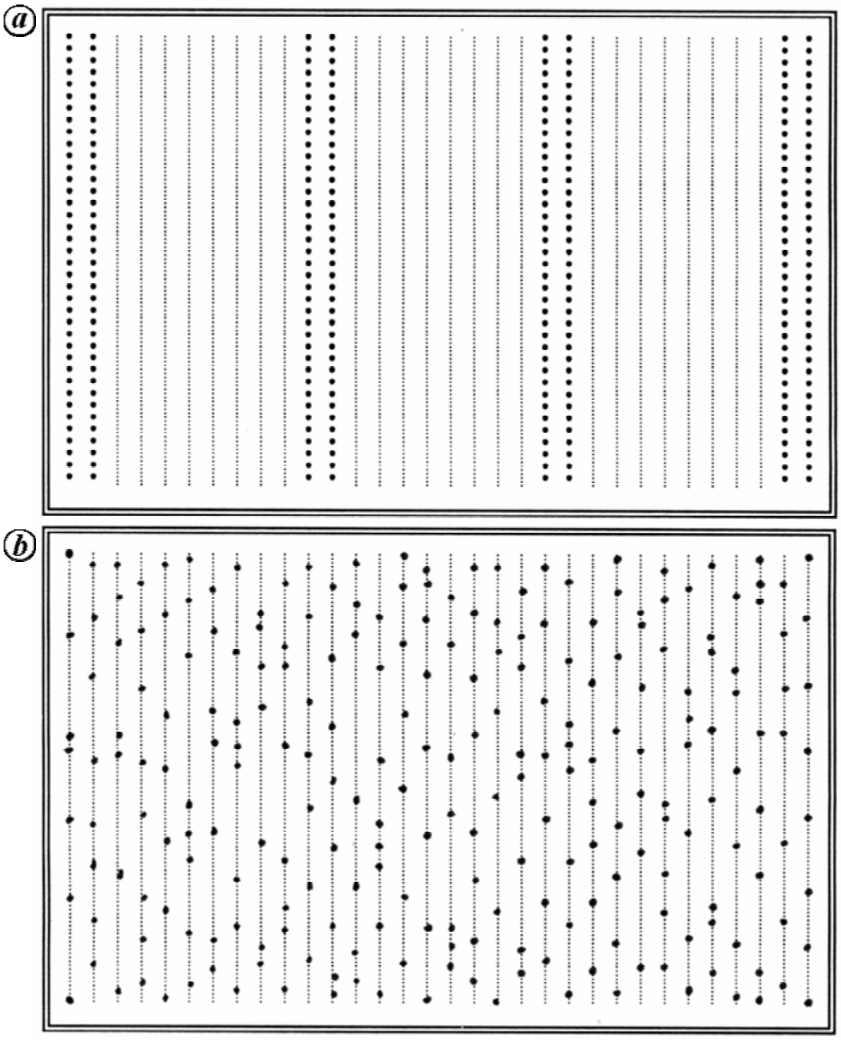

Figure 1. Methods of growing *Bt* and non-*Bt* plants for *Bt* resistance management. ***a***, Structured refugia contain two rows of non-*Bt* plants (bold dotted lines) for every eight rows of *Bt* plants; ***b***, Seed mixture strategy involves random mixing of 20% non-*Bt* plants (bold dots) among *Bt* plants.

of the *Bt* cotton, Bollgard. Now it is also understood that the frequency of Cry2Ab resistance in *Helicoverpa* is uncomfortably high[16] and increasing[17,18]. Apart from the need for holistic research on *Helicoverpa* genomics and *Bt* resistance development[19], imperative political legislation in this regard, in the best national interest, will go a long way in the management of *Bt* resistance in insect pests, including *Helicoverpa* species vis-à-vis *Bt* cotton cultivation[20,21] in India, lest the vicious cycle of *Bt* resistance ensue.

1. Gould, F., *Annu. Rev. Entomol*., 1998, **43**, 701–726.
2. Tabashnik, B. E., Gassmann, A. J., Crowder, D. W. and Carriere, Y., *Nature Biotechnol*., 2008, **26**, 199–206.
3. Hanur, V. S., *Curr. Sci*., 2008, **95**, 449–451.
4. US Environmental Protection Agency, 2007; http://www.epa.gov/oppbppd1/biopesticides/pips/Bt_corn_refuge_2006.htm
5. Gould, F., *Bioscience*, 1988, **38**, 26–33.
6. Altman, D., Wilson, F. D., Benedict, J. H. and Gould, F., *Science*, 1992, **255**, 903–904.
7. Gould, F., *Am. Sci*., 1991, **79**, 496–507.
8. National Corn Growers Association; http://www.ncga.com/news/releases/2005/January/news010605.htm
9. Carriere, Y. *et al*., *Pest Manage. Sci*., 2005, **61**, 327–330.
10. van Rensburg, J. B. J., *S. Afr. J. Plant Soil*, 2007, **24**, 147–151.
11. Mallet, J. and Porter, P., *Proc. R. Soc. London, Ser. B*, 1992, **250**, 165–169.
12. Ranjith, M. T., Prabhuraj, A. and Srinivasa, Y. B., *Curr. Sci*., 2010, **99**, 1602–1606.
13. Manjunath, T. M., *Curr. Sci*., 2011, **100**, 604–605.
14. Kranthi, K. R., Jadhav, D. R., Kranthi, S., Wanjari, R. R., Ali, S. and Russell, D. A., *Crop Prot*., 2002, **21**, 449–460.
15. Liao, C., Heckel, D. G. and Akhurst, R., *J. Invertebr. Pathol*., 2002, **80**, 50–63.
16. Mahon, R. J., Olsen, K., Downes, S. J. and Addison, S., *J. Econ. Entomol*., 2007, **100**, 1844–1853.
17. Knight, K., *Aust. Cottongrower*, 2009, **29**, 33–35.
18. Mahon, R. J. and Young, S., *J. Econ. Entomol*., 2010, **103**, 835–842.
19. Heckel, D. G., Gahan, L. J., Daly, J. C. and Trowell, S., *Philos. Trans. R. Soc. London, Ser. B*, 1998, **353**, 1713–1722.
20. Ali, M. I., Luttrell, R. G. and Young, S. Y., *J. Econ. Entomol*., 2006, **99**, 164–175.
21. Luttrell, R. G., Wan, L. and Knighten, K., *J. Econ. Entomol*., 1999, **92**, 21–32.

VAGEESHBABU S. HANUR

Division of Biotechnology,
Indian Institute of Horticultural Research,
Hesaraghatta Lake Post,
Bangalore 560 089, India
e-mail: vageesh@iihr.ernet.in