Identification of a gene associated with *Bt* resistance in the lepidopteran pest, *Heliothis virescens* and its implications in *Bt* transgenic-based pest control

J. Nagaraju

Agriculture feeds 6 billion humans everyday, and within 50 years will have to feed a population of approximately 9 billion (UN forecast). It is estimated that 1/3 of the world's agricultural production is lost to insect pests, pathogens and weeds. Among the insect pests, the order Lepidoptera represents a diverse and important group, since it embraces most agronomically important insects which include the world's most damaging crop pests that belong to Noctuidae. The Lepidoptera also includes economically important silk-secreting insects that belong to Bombycidae and Saturniidae. The noctuids encompass the heliothines, *Helicoverpa armigera* in Africa, Southern Europe, Asia, Australia and the Pacific, *H. zea* and *Heliothis virescens* in the Americas, which are three of the world's major crop pests. The heliothines are commonly referred to as cotton bollworm, tobacco bollworm, corn earworm, etc. depending upon their host crop. The lepidopteran pest larvae cause extensive damage to cotton, potato, tobacco, tomato, maize, sunflower, beans, citrus and other crops. The control of agriculture pest populations is achieved mainly by the application of chemical insecticides. Biological control methods (parasites, parasitoids and entomopathogens such as bacteria and viruses) are also part of integrated crop protection strategies. Genetically engineered crops producing their own built-in insecticides are emerging as an increasingly popular tool for controlling lepidopteran pests, while reducing the need for potentially dangerous chemical pesticides. The common soil bacterium, *Bacillus thuringiensis* (*Bt*) produces crystal-containing proteins that are toxic to certain insects, but are harmless to most other organisms, including beneficial insects[1]. Genes encoding *Bt* toxins have been incorporated and expressed in crop plants, thus providing environmentally benign control of insect pests[2]. The lepidopteran pest larvae are the primary targets of more than 99% of the currently deployed *Bt*-producing transgenic plants[3]. The risk of resistance development by the lepidopteran pest larvae is the most

serious threat to the continued efficacy of *Bt* toxins.

In the recent issue of *Science*, David Heckel and his colleagues[4] from the Department of Genetics, University of Melbourne, Australia, Clemson University and North Carolina University, USA, have identified a recessive gene that confers much of the resistance to *Bt* toxin in the tobacco budworm, *H. virescens*, a key pest of cotton and other crops. This is an important finding, considering the fact that this lepidopteran pest is the primary target of recently commercialized transgenic *Bt* cotton, which kills all budworm moths, except rare individuals that contain a pair of recessive genes for resistance, by providing the insecticidal Cry1Ac toxin from *Bt*. Although *Bt*-resistant populations of *H. virescens* have not yet been observed in the field, the previous studies by Gould and his colleagues[5,6] established that 1.5 of every 1000 moths carry one of the genes for resistance to the *Bt* toxin. Based on this frequency of resistance, the researchers predicted that it would be likely to take about 10 years of *Bt* resistance in budworm moths to become a problem, when *Bt* cotton is widely planted. The study reported in *Science* led to the identification of a single major gene (*BtR-4*) which is responsible for 40 to 80% of Cry1Ac resistance levels in the line YHD2, a resistant strain developed in the laboratory by selection with toxin-impregnated diet. The previous studies by Heckel and his colleagues[7] have assigned *BtR-r* to the linkage group 9 (LG 9).

In the study reported in *Science*, the authors have further localized the *BtR-4* on LG 9 by analysing 11 polymorphic markers that scanned a total genetic length of 105 centimorgans (cM) on a segregating backcross family of 48 progeny of a hybrid male and a YHD2 female. The scanning of LG 9 for resistance QTLs (quantitative trait loci) using interval mapping[8] indicated the likely location of *BtR-4* on LG 9. They have further shown that the *BtR-4* is a null allele of the cadherin superfamily gene. The functional copy of the cadherin gene is known to encode a cell adhesive protein which binds to Cry1Aa with high affinity and brings about cell lysis in *Bombyx mori* cells[9]. The authors elegantly show that the resistant allele of this gene is created by the insertional inactivation of the functional allele of the cadherin gene by a deleted copy of the retrotransposable element, *Hel-1*. The *H. virescens* larvae, which carry disrupted allele of the cadherin gene in homozygous condition develop resistance to the *Bt* toxin Cry1Ac, since *Bt* toxin in cadherin-deficient larvae is unable to bind to midgut cells of heliothine larvae to execute cell death.

The findings reported by Heckel and colleagues[4] provide an efficient tool to detect recessive resistant alleles in heterozygous condition. This finding is a major advancement since conventional bioassay-based monitoring methods, which score the number of moths resistant to *Bt* toxin, are not sensitive enough to detect resistant individuals that carry recessive alleles in homozygous condition, because of extreme rarity of resistant homozygotes in the populations. Since heterozygotes could be genotyped efficiently, the monitoring of frequency of resistant alleles in the population will indicate whether the problem is looming, well before resistant homozygotes become frequent enough to cause uncontrollable outbreaks, the study reports.

In India, where *Bt* cotton is still undergoing field trials, it is very important to ascertain the existence of resistant alleles in the heliothine pest population before and after the introduction of *Bt* cotton. Although the existence of additional genes that confer resistance cannot be ruled out, as shown by Aroian and colleagues in *Caenorhabditis elegans* in the same issue of *Science*[10], the study reported by Heckel and colleagues[7] provides what seems to be a major gene for *Bt* resistance, which could be used for monitoring *Bt* resistance in the pest populations. Preservation of DNA samples of representative pest populations prior to introduction of *Bt* cotton and the subsequent comparison of resistant allele frequencies in the populations after the introduction of *Bt* cotton, will certainly provide informed tools for the efficient use of *Bt* transgenics in pest control.

1. Schnepf, E. *et al.*, *Microbiol. Mol. Biol. Rev.*, 1998, **62**, 755–806.
2. Schuler, T. H., Poppy, G. M., Kerry, B. R. and Denholm, I., *Trends Biotechnol.*, 1998, **16**, 169–175.
3. James, C., *Int. Serv. Acquisit. Agric-Biotechnol. Appl. Briefs*, 1998, **8**, 1–43.
4. Gahan, L. J., Gould, F. and Heckel, D. G., *Science*, 2001, **293**, 857–860.
5. Gould, F., Anderson, A., Reynolds, A., Bumgarner, L. and Moar, W., *J. Econ. Entomol.*, 1995, **88**, 1545–1559.
6. Gould, F. *et al.*, *Proc. Natl. Acad. Sci. USA*, 1997, **94**, 3519–3523.
7. Heckel, D. G., Gahan, L. J., Gould, F. and Anderson, H., *J. Econ. Entomol.*, 1997, **90**, 75–86.
8. Lander, E. S. and Botstein, D., *Genetics*, 1989, **121**, 185–199.
9. Nagamatsu, Y., Koike, T., Sasaki, K., Yoshimoto, A. and Furukawa, Y., *FEBS Lett.*, 1999, **460**, 385–390.
10. Griffits, J. S., Whitacre, J. L., Stevens, D. E. and Aroian, R. V., *Science*, 2001, **293**, 860–864.

ACKNOWLEDGEMENTS. I thank Dr Seyed E. Hasnain for a critical reading of the manuscript.

J. Nagaraju is in the Laboratory of Molecular Genetics, Centre for DNA Fingerprinting and Diagnostics, ECIL Road, Nacharam, Hyderabad 500 076, India (e-mail: jnagaraju@www.cdfd.org.in).