over the Arabian Sea in May 2001. The above threshold value of SST (26°C) does not hold good in the Arabian Sea in the hot season of May to form a tropical cyclone.

Examination of weekly SST distribution over the Arabian Sea revealed that MWP (SST ≥ 30.5°C) existed over the Arabian Sea during 2001, 2002, 2003 and 2005. The OV developed over the northern flank of LLJ[1,2,6] under the influence of MWP with the aid of a 'providing cycle' during 2001 only. Nevertheless, the LLJ must be strong enough (≥10 ms^{-1}) and lie close to the southern tip of India. Even though MWP existed in 2003 and 2005, the features relating to the LLJ at 850 hPa were absent. A strong anticyclonic circulation to the south of the LLJ axis inhibited the formation of OV. In brief, though the ocean is fully cooperative with high thermal energy in the upper part, the OVSM does not form over the MWP unless the shear line forms along the northern flank of the LLJ at 850 hPa near the west coast of India, which is a necessary condition.

1. Kershaw, R., Onset of the southwest monsoon and SST anomalies in the Arabian Sea. *Nature*, 1985, **315**, 561–563.
2. Kershaw, R., Effect of a sea surface temperature anomaly on a prediction of the onset of the southwest monsoon over India. *Q. J. R. Meteorol. Soc.*, 1988, **114**, 325–345.
3. Seetaramayya, P. and Master, A., Observed air–sea interface conditions and a monsoon depression during MONEX-79. *Arch. Meterol. Geophs. Bioklimatol.*, 1984, **A33**, 61–67.
4. Nyenzi, B. S., The influence of lower tropospheric flow on the onset of the summer monsoon over India in June 1979. *FGGE Operations Report A*, 1980, vol. 9, pp. 68–80.
5. Hui, D. Y., A case study of the formation and structure of a monsoon depression over the Arabian Sea. *FGGE Operations Report A*, 1980, vol. 9, pp. 92–113.
6. Krishnamurti, T. N., Ardanuy, P., Ramanathan, Y. and Pasch, R., On the onset vortex of the summer monsoon. *Mon. Weather Rev.*, 1981, **109**, 344–363.
7. Seetaramayya, P. and Master, A. H., Exchange of heat and momentum between ocean and atmosphere in relation to monsoon depression during MONEX-79. *Meteorol. Atmos. Phys.*, 1986, **35**, 59–63.
8. Rao, R. R. and Sivakumar, R., On the possible mechanisms of the evolution of the mini warm pool during the pre-summer monsoon season and the onset vortex in the southeastern Arabian Sea. *Q. J. R. Meteorol. Soc.*, 1999, **125**, 787–809.
9. Thadathil, P. and Gosh, K., Surface layer temperature inversion in the Arabian Sea during winter. *J. Oceanogr.*, 1992, **48**, 293–304.
10. Durand, F., Shetye, S. R., Vialard, J., Shankar, D., Shenoi, S. S. C., Ethe, C. and Cadec, G., Impact of temperature inversions on SST evolution in the south-eastern Arabian Sea during the pre-summer monsoon season. *Geophys. Res. Lett.*, 2004, **31**, L08305.
11. Shankar, D. *et al.*, Observational evidence for westward propagation of temperature inversions in the southeastern Arabian Sea. *Geophys. Res. Lett.*, 2004, **31**, L01305.
12. Shenoi, S. S. C., Shankar, D. and Shetye, S. R., On the sea surface temperature high in the Lakshadweep Sea before the onset of the southwest monsoon. *J. Geophys. Res.*, 1999, **104**, 15703–15712.
13. Reynolds, R. W. and Smith, T. M., Improved global SST analyses. *J. Climate*, 1994, **7**, 929–948.
14. Fairall, C. W., Bradley, E. F., Hare, J. E., Grachev, A. A. and Edson, J. B., Bulk parameterization on air–sea fluxes: Updates and verification for the COARE algorithm. *J. Climate*, 2003, **16**, 571–591.
15. Indian Daily Weather Reports (May 2000 and 2004), India Meteorological Department, Pune.
16. Ananthakrishnan, R., Pathan, J. M. and Aralikatti, S. S., The onset phase of southwest monsoon. *Curr. Sci.*, 1983, **52**, 155–164.
17. Laevastu, T., Rabe, M. K. and Hamilton, G. D., Mesoscale feedback from the ocean to the atmosphere in coastal and oceanic frontal regions. Environmental Prediction Research Facility (Navy) Montery, California, 1972, pp. 7–8.
18. Gray, W. M., Hurricanes: Their formation, structure and likely role in the tropical circulation. In *Meteorology over the Tropical Oceans* (ed. Shaw, D. B.), Royal Meteorological Society, 1979, pp. 155–218.

ACKNOWLEDGEMENTS. R.D. thanks CSIR, New Delhi for JRF/SRF fellowship. We thank the Director, IITM, Pune for encouragement and support. We also thank NCEP/NCAR and IMD, Pune for data and DOD, Govt of India for financial support.

Received 25 May 2006; revised accepted 30 October 2006

Genetic variability of *Helicoverpa armigera* (Hübner) attributable to cadherin gene-specific molecular markers

G. T. Gujar*, R. N. Khawale and V. Kalia

Insect Biotechnology Laboratory, Division of Entomology,
Indian Agricultural Research Institute, New Delhi 110 012, India

Cry1Ac resistance in the American bollworm, *Helicoverpa armigera* is associated with alteration in midgut cell adhesion protein, cadherin. Genetic diversity of cadherin gene was studied using molecular markers developed from cDNA sequences of *H. armigera*. Six out of eight pairs of molecular markers were able to amplify the cadherin gene in eighteen insect populations collected from different locations in India and produce a total of 218 amplicons. The maximum similarity (96%) was found for three pairs of insect populations from Rajkot and Sirsa, Akola and Bhatinda, and Faridkot and Karnal; while the minimum similarity (82%) was for the pair of insect populations from Nanded and Hyderabad. The study showed 4–18% genetic diversity in cadherin-specific gene of *H. armigera* populations which differed at least 100-fold in their susceptibility to Cry1Ac.

Keywords: American bollworm, cadherin, genetic variability, *Helicoverpa armigera*, molecular markers.

AMERICAN bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) is the most serious pest of cotton, pulses and many other agriculturally important crops in India and abroad. Indiscriminate use of pesticides for control of *H. armigera* has resulted in the development of resistance against almost all groups of conventional in-

*For correspondence. (e-mail: gtgujar@iari.res.in)

secticides, environmental pollution, loss of predators, parasites and health hazards[1]. Microbial insecticide containing δ-endotoxin (Cry proteins) from *Bacillus thuringiensis* (*Bt*) has been used as an alternate to conventional insecticides for almost 60 years[2]. Besides, genetically modified plants with *Bt* genes like *cry1Ab* and *cry1Ac* protect themselves against the lepidopterans. More than 125 million hectares of *Bt* crops, *Bt*-cotton and *Bt*-corn, aimed at controlling lepidopteran pests were grown cumulatively in the past decade and served as a great selection pressure to evolve resistance in the lepidopteran insects, especially heliothines[3]. The baseline susceptibility studies have shown higher tolerance in heliothines in recent years over the pre-*Bt* transgenic period[4,5]. And yet, target insect pests have not evolved resistance to *Bt* transgenic crops under field conditions, leading to their failure. The success of *Bt* crops is thus attributed to biological attributes of target pests, crop agronomy and *Bt* resistance-management tactics[3,6–8]. *Bt* resistance management has become mandatory in many countries, including India for the development of *Bt* crops.

Development of resistance to *Bt* toxins has been demonstrated in the laboratory for about dozen insects[9] and is also reported in *H. armigera*[10–13]. Further, resistance is primarily attributed to alteration in the target sites, viz. digestive enzyme aminopeptidase or cell adhesion proteins, cadherin-like receptors in the midgut of insect[14]. Gahan *et al.*[15] showed that resistance in the tobacco budworm, *Heliothis virescens* strain YHD2 is due to retrotransposon-mediated insertion in *HevCaLP*, a *H. virescens* homologue of the BtR175 cadherin receptor. Besides, three different alleles (*r*1, *r*2, *r*3) with deleted or truncated cadherin receptors were identified in the pink bollworm strains (AZP-R) resistant to Cry1Ac transgenic cotton[16,17]. Xu *et al.*[18] reported that disruption of cadherin gene by a premature stop codon imparted resistance to Cry1Ac in *H. armigera*. Thus, it seems that cadherin-based resistance is common and yet complicated in view of existence of several alleles which might limit the development of molecular markers for resistance monitoring. This communication reports genetic diversity of the cadherin gene in *H. armigera* collected from different locations in the country.

Larvae of *H. armigera* were collected from different places in India (Table 1). These were reared on a chickpea-based meridic diet similar to that of Nagarkatti and Prakaash[19], at 27 ± 2°C and 60–80% RH. Adults were fed with 10% honey solution and allowed to lay eggs on rough cotton cloth used for covering jars. The eggs were collected daily by changing the cloth and kept in a small container till they hatched. Third instar larvae (20–30 mg) were used for genomic DNA isolation.

About 10 to 15 larvae were randomly picked for isolation of genomic DNA separately. For isolation of DNA, individual larva was ground in liquid nitrogen using plastic homogenizer in 1.5 ml eppendorf™ tube. After grinding the larvae, 400 µl of prewarmed (60–70°C for 10 min) 2% CTAB extraction buffer [(Cetyltrimethyl ammonium bromide), 100 mM Tris-HCl (pH 8.0), 20 mM EDTA (pH 8.0), 1.4 M NaCl and 1% β-mercaptoethanol] were added and the mixture was incubated at 60–65°C in a dry bath for 1 h with gentle shaking at 10 min interval. Equal volumes of chloroform : isoamyl alcohol (24 : 1, v/v) were added and the two phases were mixed thoroughly for 30 min by gentle inversion, and centrifuged at 13,000–14,000 rpm for 15 min at room temperature. The aqueous phase was transferred to a new eppendorf tube and then 150 µl of 5 M NaCl and 0.6 volume of isopropanol were added, mixed well by gently inverting the tubes and incubated at –20°C for 1 h. The mixture was then centrifuged at 10,000 rpm for 10 min to pellet the DNA at room temperature. The pellet was washed twice with 70–80% ethanol and centrifuged at 10,000 rpm for 10 min at 4°C to pellet the DNA. The pellet was air-dried and dissolved in 50 µl of deionized sterile distilled water. The isolated DNA was treated with 25 µl of RNAase (50 µg RNAase/1000 µl TE buffer) and mixed by gentle tapping to remove RNA. The whole content was incubated at 37°C for 1 h. The isolated DNA was checked on 0.8% agarose electrophoretic gel and its purity determined by spectrophotometric analysis. All DNA samples were stored at –20°C till further use.

The primer sequences were synthesized from the cadherin cDNA sequence of *H. armigera* available in GenBank under accession number AF519180. Eight pairs of (forward and reverse) primers covering the complete sequenced region, custom synthesized by Genetix Biotech Asia Pvt Ltd, New Delhi were used to amplify the *H. armigera* cadherin (Table 2). The PCR reaction mixture contained 2.5 µl of reaction buffer with 15 mM $MgCl_2$, 200 µM dNTPs, 1 unit of *Taq* polymerase (Bangalore Genie, India), 5 pmol of each primer and 1 µl DNA (20–30 ng). Total volume of the reaction mixture was adjusted to 25 µl using sterile deionized water.

Table 1. Location of *Helicoverpa armigera* populations in India

Location	Date of collection	Host crop	Number of insect DNA for pooling
Rajkot	28/10/2005	Cotton	12
Basargaddy	02/09/2005	Cotton	10
Hisar	21/10/2005	Cotton	14
Katni	01/09/2005	Cotton	11
Sirsa	18/10/2005	Cotton	12
Khandwa	10/10/2005	Cotton	10
Karnal	09/04/2005	Chickpea	10
Hyderabad	15/10/2005	Cotton	11
Faridkot	25/04/2005	Chickpea	10
Raichur	08/10/2005	Cotton	12
New Delhi	17/03/2005	Chickpea	12
Ludhiana	25/03/2005	Chickpea	15
Panchkula	22/04/2005	Chickpea	15
Jalna	27/10/2005	Cotton	13
Hyderabad (ICRISAT)	02/09/2005	Chickpea	10
Akola	11/10/2004	Cotton	16
Bhatinda	09/09/2004	Cotton	16
Nanded	10/10/2004	Cotton	10

Table 2. Cadherin gene-specific primers used for genetic diversity of *H. armigera*

Primer code	Primer sequence	Reference
Hacad-1F	5′-ATCGGCTGGTGGATTGTTGTTC-3′	18
Hacad-1R	5′-CGCTCTGGTCTGGGTATTGCTA-3′	
Hacad-2F	5′-GTAGCAATACCCAGACCAGAGC-3′	18
Hacad-2R	5'-GCTTACTGCGTTACCCATTAGAG-3′	
Hacad-3F	5′-TAATGGGTAACGCAGT-3′	18
Hacad-3R	5′-CGACAATGCTGTAATAGGTG-3′	
Hacad-4F	5′-AAGGAGCGAGCAGTAGT-3′	18
Hacad-4R	5′-AGTGGTAACAACGCAGAGTA-3′	
Hacad-5F	5′-GTAGCAATACCCAGACCAGAGC-3′	18
Hacad-5R	5′-AGTGGTAACAACGCAGAGTA-3′	
Hacad-6F	5′-ATGAGATGGCAGTCGACGTGAGAATATTC-3′	This study
Hacad-6R	5′-GGTGTCATCGCAGTTTCCTCCATAATTTC-3′	
Hacad-7F	5′-GGTGTCATCGCAGTTTCCTCCATAATTTC-3′	This Study
Hacad-7R	5′-CGCGATGGGTGGAGATATTTGCTG-3′	
Hacad-8F	5′-CGCGATGGGTGGAGATATTTGCTG-3′	This study
Hacad-8R	5′-GCTTGCAATCGCCGGTTCAGAGTC-3′	

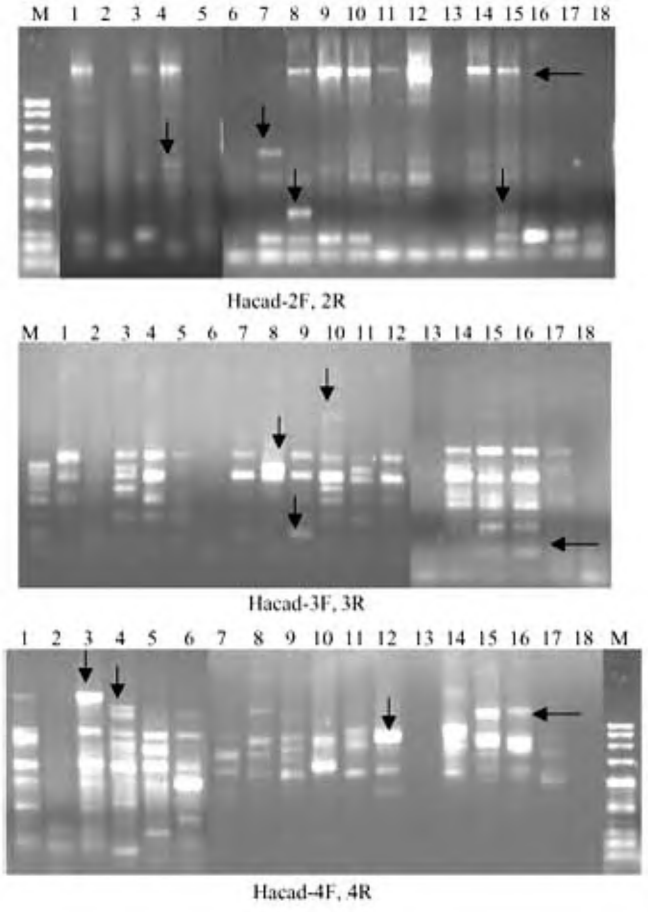

Figure 1. Agarose gel electrophoretic profile from eighteen populations of *H. Armigera*: 1, Ludhiana; 2, Punchkula; 3, New Delhi; 4, Sirsa; 5, Karnal; 6, Faridkot; 7, Katni; 8, Hyderabad; 9, Hyderabad (ICRISAT); 10, Jalna; 11, Basargaddy; 12, Rajkot; 13, Nanded; 14, Hisar; 15, Khandwa; 16, Raichur; 17, Akola; 18, Bhatinda. *Arrows indicate polymorphism.

Amplification was performed with a thermocycler (Flexigene, Techne, UK). Thermal cycles were programmed for initial denaturation at 94°C for 4 min. Each cycle consisted of denaturation for 0.30 min at 94°C, annealing for 1.00 min at 60°C and extension for 1.30 min at 72°C, with final extension after 40 cycles for 10 min. Following amplification, samples were stored at 4°C prior to electrophoresis. Amplification was repeated thrice for each primer set. PCR product was separated on 1.2% agarose gel and visualized with ethidium bromide staining. Gels were documented using Alphaimager™ documentation and analysed with AlphaEase™ software. Bands on agarose gels were scored as either present (1) or absent (0). Common band analysis was conducted using the computer program SPSS for Windows release 10, to determine the genetic distance.

All primers except the primer pairs Hacad-5F and 5R, as well as Hacad-6F and 6R, did not amplify in the DNA of any insect population. The size of the amplified product ranged from 100 to 12,000 bp. The number of bands amplified per primer ranged from 3 to 9, with a mean of 5.1 amplicons per primer. A total of 218 amplicons resulted from six pairs of primers in eighteen populations of *H. armigera* analysed. The highest number of amplicons (56) was produced by the primer pair of Hacad-3F and Hacad-3R, and 55 amplicons by the primer pair of Hacad-4F and Hacad-4R. The lowest number of 19 amplicons was produced with the primer pair Hacad-8F and Hacad-8R. Figure 1 shows the electrophoretic profile from eighteen populations of *H. armigera* with primer pairs: Hacad-2F and Hacad-2R; Hacad-3F and Hacad-3R; Hacad-4F and Hacad-4R. The electrophoretic pattern reveals polymorphism among *H. armigera* populations.

All the primers selected for the study produced unique banding patterns that could differentiate all the eighteen populations collected from different geographical cotton ecosystems. The highest similarity in cadherin gene (96%) was found for the following pairs of insect populations, viz. Rajkot and Sirsa, Akola and Bhatinda, and Faridkot and

Table 3. Genetic dis-similarity matrix among *H. armigera* populations

Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Rajkot	0																	
Basargaddy	6	0																
Hisar	6	8	0															
Katni	7	11	11	0														
Sirsa	4	10	8	11	0													
Khandwa	7	11	9	12	9	0												
Karnal	5	9	11	6	9	12	0											
Hyderabad	12	10	14	13	16	11	15	0										
Faridkot	7	9	11	8	9	14	4	15	0									
Raichur	10	12	12	7	12	7	9	12	11	0								
New Delhi	9	11	15	12	11	10	12	7	14	13	0							
Ludhiana	5	9	7	8	7	6	8	13	8	9	10	0						
Panchkula	16	14	16	11	16	15	13	12	15	10	17	17	0					
Jalna	7	9	9	12	7	8	12	13	14	11	8	8	17	0				
Hyderabad (ICRISAT)	5	9	9	8	9	6	8	11	12	7	12	8	13	8	0			
Akola	7	11	7	6	11	10	6	13	6	9	12	8	15	10	8	0		
Bhatinda	9	9	9	8	13	14	6	15	4	11	16	10	13	12	12	4	0	
Nanded	14	12	14	13	12	15	11	18	11	12	17	15	10	11	15	11	7	0

Karnal. The first two pairs of insect populations were separated by more than 1000 km, while the last one (Faridkot and Karnal) was geographically close. The lowest similarity in the cadherin gene (82%) was found for the pair of insect populations Nanded and Hyderabad, which were separated by about 400 km. Perusal of the proximity matrix data presented in the Table 3 showed 4–18% diversity among the populations due to the cadherin gene-specific markers.

Bt Cry toxin resistance in insects is being extensively studied at the molecular level with an aim to sustain the technology for crop protection[20]. Insects are able to resist Cry toxicity due to various mechanisms, viz. alteration in protoxin processing, toxin binding and repair of midgut, besides behavioural modifications. Among these, toxin binding and subsequent changes are the primary mechanisms of resistance most widely studied. Cry toxins bind with many protein receptor molecules, such as aminopeptidase-N, alkaline phosphatases, cadherins, glycolipids and glycosylated glycoprotein. Activation of immune response is also reported in *H. armigera*.

Among the Cry toxin-binding molecules, cadherin, a cell adhesion protein, was first reported as binding to Cry1A toxins in *Manduca sexta*[21]. Cadherins are high molecular weight, transmembrane proteins involved in calcium-dependent cell aggregation, sorting and cell-to-cell maintenance of contact in the midgut. The cadherin gene is linked tightly to the BtR1 locus in *M. sexta*[21], BtR175 locus in *Bombyx mori*[22] and BtR4 locus in the YHD-2 strain of *H. virescens*[15,23]. The cadherin protein at the BtR1 locus of *M. sexta*[21], equivalent of HevCaLP protein of *H. virescens*, has two toxin-binding regions, TBR1 and TBR2, at which domain II of Cry1 toxins binds[24,25]. Alterations in cadherin gene that imparts resistance to Cry toxins, have been found to be due to insertion of a retrotransposon in *H. virescens*[15], deletions of three different kinds in the AZP-R strain of *Pectinophora gossypiella*[16], and addition of stop codon in *H. armigera*[18].

Studies carried out by us using primers reported by Xu *et al.*[18] showed that some primers did not amplify the DNA. Further, using primers of Xu *et al.*[18] and others developed by us in this study, we found that *H. armigera* populations showed 4–18% dis-similarity, while these populations showed 100-fold variation in their susceptibility to Cry1Ac (unpublished data). Our studies also involved primers which are longer in their base sequences and are used in forward and reverse pairs to amplify the specific genomic region. Reproducibility of amplification profile was consistent when checked repeatedly. The cadherin cDNA sequence of *H. armigera* (GenBank accession no. AF519180) shows 95% similarity with cadherin *BtR-5* allele of *H. armigera* (AY647974), 95% similarity with midgut E-cadherin sequences of *H. armigera* (AY351904) and 98% similarity with *BtR-r1* allele of *H. armigera* (AY647975). Despite the high sequence similarities of *H. armigera* cadherin genes, significant diversity of cadherin-specific gene in *H. armigera* populations was observed in the present study.

The profile of the PCR products is quite different from the one reported by Xu *et al.*[18]. Difference in the amplification profile was due to development using the exon sequence of the DNA, i.e. cDNA from mRNA, while genomic DNA also contained intron sequences and hence expected amplicons differed from those observed.

Using RAPD primers, Zhou *et al.*[26] reported low genetic distance (0.0029 ± 0.00078 SD) and high gene flow (Nm = 16.2) resulting in high similarity among the Israeli and Turkish populations of moths of *H. armigera*. Fakrudin *et al.*[27] showed similarity coefficients ranging from 0.22 to 0.42 for the genome of *H. armigera* populations from the South Indian cotton ecosystem and found a geographic relationship. Heterozygosity for *H. armigera*

populations from twenty locations in China ranged from 0.62 to 0.91 using microsatellite markers[28]. Besides, Scott *et al.*[29] found wide variation in gene structuring of insect populations due to migration in the Darling Downs, Australia between 1999 and 2003, using microsatellite markers. It is quite natural to expect wide genetic diversity using RAPD and microsatellite markers, unlike cadherin-specific diversity. No geographic relationship could be found for the most diverse or close pairs of insect populations, possibly due to biological and ecological factors, including migration. Yet, it suggests that diversity may limit development of specific markers for monitoring homozygous-resistant alleles in the insect populations. Diversity in the cadherin gene may necessitate designing of molecular markers for *Bt* Cry toxin resistance useful to local insect populations and their utilization along with other methods of resistance monitoring.

1. McCaffery, A. R., Resistance to insecticides in Heliothine, Lepidoptera; a global view. *Philos. Trans. R. Soc. London, Ser. B*, 1998, **353**, 1735–1750.
2. Romeis, J., Meissle, M. and Bigler, F., Transgenic crops expressing *Bacillus thuringiensis* toxins and biological control. *Nature Biotechnol.*, 2006, **24**, 63–71.
3. Bates, S. L., Zhao, J.-Z., Roush, R. T. and Shelton, A. M., Insect resistance management in GM crops: Past, present and future. *Nature Biotechnol.*, 2005, **23**, 57–62.
4. Ali, M. I., Luttrell, R. G. and Young III, S. Y., Susceptibilities of *Helicoverpa zea* and *Heliothis virescens* (Lepidoptera: Noctuidae) populations to Cry1Ac insecticidal protein. *J. Econ. Entomol.*, 2006, **99**, 164–175.
5. Kalia, V., Archana Kumari, Mittal, A., Singh, B. P., Nair, R. and Gujar, G. T., Temporal variation in susceptibility of the American bollworm, *Helicoverpa armigera* to *Bacillus thuringiensis* (*Bt*) var. *kurstaki* HD-73 or its Cry1Ac toxin or *Bt* cotton. *Pestic. Res. J.*, 2006, **18**, 47–50.
6. Tabashnik, B. E., Breaking the code of resistance. *Nature Biotechnol.*, 2001, **19**, 922–924.
7. Tabashnik, B. E. *et al.*, Insect resistance to transgenic *Bt* crops: Lessons from the laboratory and field. *J. Econ. Entomol.*, 2003, **96**, 1031–1038.
8. Gujar, G. T., Will *Bt*-cotton remain effective in India? *Nature Biotechnol.*, 2005, **23**, 927–928.
9. Gujar, G. T., *Heliothis/Helicoverpa* resistance to *Bacillus thuringiensis* resistance: Management strategies. In *Heliothis/Helicoverpa Management, Emerging Trends and Strategies for Future Research* (ed. Sharma, H. C.), Oxford & IBH, New Delhi, 2005, pp. 275–288.
10. Kranthi, K. R., Kranthi, S., Ali, S. and Banerjee, S. K., Resistance to Cry1Ac-δ-endotoxin of *Bacillus thuringiensis* in a laboratory selected strains of *Helicoverpa armigera* (Hübner). *Curr. Sci.*, 2000, **78**, 1001–1004.
11. Akhurst, R. J., James, W. J., Bird, L. J. and Beard, C., Resistance to the Cry1Ac-δ-endotoxin of *Bacillus thuringiensis* in the cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae). *J. Econ. Entomol.*, 2003, **96**, 1290–1299.
12. Meng, E., Shen, J., Zhou, W. and Cen, H., Long-term selection for resistance to transgenic cotton expressing *Bacillus thuringiensis* toxin in *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). *Pest Manage. Sci.*, 2003, **60**, 167–172.
13. Chandrashekar, K. and Gujar, G. T., Development and mechanism of insect resistance to *Bacillus thuringenesis* endotoxin Cry1Ac in the American bollworm, *Helicoverpa armigera* (Hübner). *Indian J. Exp. Biol.*, 2004, **42**, 164–173.
14. Ferré, J. and Van Rie, J., Biochemistry and genetics of insect resistance to *Bacillus thuringienesis*. *Annu. Rev. Entomol.*, 2002, **47**, 501–533.
15. Gahan, L. J., Gould, F. and Heckel, G., Identification of a gene associated with *Bt* resistance in *Heliothis virescens*. *Science*, 2001, **293**, 857–860.
16. Morin, S. *et al.*, Three cadherin alleles associated with resistance to *Bacillus thuringienesis* in pink bollworm. *Proc. Natl. Acad. Sci. USA*, 2003, **100**, 5004–5009.
17. Tabashnik, B. E., Liu, Y. B., Unnithan, D. C., Carriere, Y., Dennehy, T. J. and Morin, S., Shared genetic basis of resistance to *Bt* toxin Cry1Ac in independent strains of pink bollworm. *J. Econ. Entomol.*, 2004, **97**, 721–726.
18. Xu, X., Yu, L. and Wu, Y., Disruption of cadherin gene associated with resistance to Cry1Ac δ-endotoxin of *Bacillus thuringiensis* in *Helicoverpa armigera*. *Appl. Environ. Microbiol.*, 2005, **71**, 948–954.
19. Nagarkatti, S. and Prakaash, A., Rearing of *Heliothis armigera* (Hübner) on an artificial diet. *Tech. Bull. Commonw. Inst. Biol. Control*, 1974, **17**, 169.
20. Griffitts, J. S. and Aroian, R. V., Many roads to resistance: How invertebrates adapt to *Bt* toxins. *BioEssays*, 2005, **27**, 614–624.
21. Vadlamudi, R. K., Weber, E., Ji, I. H., Ji, T. H. and Bulla, L. A., Cloning and expression of a receptor for an insecticidal toxin of *Bacillus thuringiensis*. *J. Biol. Chem.*, 1995, **270**, 5490–5494.
22. Nagamatsu, Y., Koike, T., Sasaki, K., Yoshimoto, A. and Furukawa, Y., The cadherin-like protein is essential to specificity determination and cytotoxic action of the *Bacillus thuringienesis* insecticidal Cry1Aa toxin. *FEBS Lett.*, 1999, **460**, 385–390.
23. Heckel, D. G., Gahan, L. C., Gould, F. and Anderson, A., Identification of a linkage group with a major effect on resistance to *Bacillus thuringiensis* Cry1Ac endotoxin in the tobacco budworm. *J. Econ. Entomol.*, 1997, **90**, 75–86.
24. Dorsch, J. A. *et al.*, Cry1A toxins of *Bacillus thuringiensis* bind specifically to a region adjacent to the membrane-proximal extracellular domain of BT-R(1) in *M. sexta*: Involvement of a cadherin in the entomopathogenicity of *Bacillus thuringiensis*. *Insect Biochem. Mol. Biol.*, 2002, **32**, 1025–1036.
25. Gomez, I., Dean, D. H., Bravo, A. and Soberon, M., Molecular basis for *Bacillus thuringiensis* Cry1Ab toxin specificity: Two structural determinants in the *Manduca sexta* Bt-R1 receptor interact with loops alpha-8 and 2 in domain II of Cry1Ab toxin. *Biochemistry*, 2003, **42**, 10482–10489.
26. Zhou, X., Faktor, O., Applebaum, S. W. and Coll, M., Population structure of the pestiferous moth *Helicoverpa armigera* in the Eastern Mediterranean using RAPD analysis. *Heredity*, 2000, **85**, 251–256.
27. Fakrudin, B., Prakash, S. H., Krishnareddy, K. B., Vijaykumar, Badri Prasad, P. R., Patil, B. V. and Kurruvinashetty, M. S., Genetic variation of cotton bollworm, *Helicoverpa armigera* (Hübner) of South Indian cotton ecosystem using RAPD markers. *Curr. Sci.*, 2004, **87**, 1654–1657.
28. Ji, Y.-J., Zhang, D.-X., Hewitt, G. M., Kang, L. and Li, D.-M., Polymorphic microsatellite loci for the cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae) and some remarks on their isolation. *Mol. Ecol. Notes*, 2003, **3**, 102.
29. Scott, K. D. *et al.*, Assessing moth migration and population structuring in *Helicoverpa armigera* (Lepidoptera: Noctuidae) at the regional scale: Example from the Darling Downs, Australia. *J. Econ. Entomol.*, 2005, **98**, 2210–2219.

ACKNOWLEDGEMENTS. Thanks are due to Dr P. S. Bhattacharya, J.K. AgriGenetics Pvt Ltd, Hyderabad for providing funds; Dr B. Fakrudin, University of Agricultural Sciences, Dharwad and Dr K. V. Prabhu, Division of Genetics, Indian Agricultural Research Institute (IARI), New Delhi for comments; and the Director, IARI for providing necessary infrastructure and encouragement.

Received 30 May 2006; revised accepted 28 September 2006