

CORRESPONDENCE

the identified plants will be retested. It is expected that the mutants so identified will have compounded reproduction comprising apomictic and normal sexual mechanisms. The apomictic mutants will be easier to detect when the starting genotype is characterized by high or complete sexual sterility. The auto- and allo-triploids that are known to be nearly sterile may prove highly useful starting genotypes. To begin with, triploid genotypes of species belonging to Solanaceae, Cucurbitaceae and Brassicaceae appear to offer requisite advantages. In this regard, *Arabidopsis* (or *Brassica*), *Lycopersicon esculentum* (or *Solanum melongena*), papaya and melons appear promising herbaceous and tree species for experimentation.

1. Kaushal, P., Zadoo, S. N., Malaviya, D. R. and Roy, A. K., *Curr. Sci.*, 2005, **89**, 1092–1096.
2. Bhat, V., Dwivedi, K. K., Khurana, J. P. and Sopory, S. K., *Curr. Sci.*, 2005, **89**, 1879–1893.
3. Carman, J. G., *Biol. J. Linn. Soc.*, 1997, **61**, 51–94.

4. Crane, C. F., *The Flowering of Apomixis: From Mechanisms to Genetic Engineering*, CIMMYT and IRD, Mexico, 2001, pp. 24–34.
5. Grimanelli, D., Leblanc, O., Perotti, E. and Grossniklaus, U., *Trends Genet.*, 2001, **17**, 597–604.
6. Grossniklaus, U., *The Flowering of Apomixis: From Mechanisms to Genetic Engineering*, CIMMYT and IRD, Mexico, 2001, pp. 168–211.
7. Nogler, G. A., *Embryology of Angiosperms*, Springer-Verlag, Berlin, 1984, pp. 475–518.
8. Bharathan, G., *Am. J. Bot.*, 1996, **83**, 440–451.
9. Matzk, F., Hammer, K. and Schubert, I., *Sex. Plant Reprod.*, 2003, **16**, 51–58.
10. Quarin, C. L., Espinoza, F., Martinez, E. J., Pessino, S. C. and Bovo, O. A., *Sex. Plant Reprod.*, 2001, **13**, 243–249.
11. Bicknell, R. A., Borst, N. K. and Koltunow, A. M., *Heredity*, 2000, **84**, 228–237.
12. Leblanc, O., Grimanelli, D., Gonzalez de Leon, D. and Savidan, Y., *Theor. Appl. Genet.*, 1995, **90**, 1198–1203.
13. Nogler, G. A., *Bot. Helv.*, 1984, **94**, 411–422.
14. Sherwood, R. T., Berg, C. C. and Young, B. A., *Crop Sci.*, 1994, **34**, 1490–1494.
15. Grossniklaus, U., Spillane, C., Page, D. R. and Koehler, C., *Curr. Opin. Plant Biol.*, 2001, **4**, 21–27.
16. Vinkenoog, R. and Scott, R. J., *Sex. Plant Reprod.*, 2001, **14**, 189–194.
17. Ohad, N. *et al.*, *Plant Cell*, 1999, **11**, 407–416.
18. Koehler, C., Hennig, L., Spillane, C., Pien, S., Gruissem, W. and Grossniklaus, U., *Genes Dev.*, 2003, **17**, 1540–1553.
19. Guitton, A. E., Page, D. R., Chambrier, P., Lionnet, C., Faure, J. E., Grossniklaus, U. and Berger, F., *Development*, 2004, **131**, 2971–2981.
20. Schubert, D., Clarenz, O. and Goodrich, J., *Curr. Opin. Plant Biol.*, 2005, **8**, 553–561.

SUSHIL KUMAR

National Centre for Plant Genome
Research,
Post Box 10531,
Aruna Asaf Ali Marg,
New Delhi 110 067, India
e-mail: sushil2000-01@yahoo.co.in

***Bt*-cotton: Protein expression in leaves is most critical**

This has reference to the article by Kranthi *et al.*¹. Their conclusions ... 'Cry 1Ac expression levels were the lowest in the ovary of flowers and boll rind of green bolls, which constitute the most favoured sites of bollworm attack ... The toxin expression was clearly inadequate to confer full protection to the fruiting parts', have been widely quoted and exploited by certain NGOs to condemn this technology².

No doubt, the fruiting parts, especially squares and bolls, are the most preferred for feeding by *Helicoverpa armigera*, causing direct damage and heavy yield loss. However, what appears to have been less realized is a significant fact that larvae move to these parts after completing their initial feeding on leaves.

H. armigera starts its activity when the crop is young and still in its vegetative phase. Its feeding and reproductive activities intensify as the crop enters the reproductive phase and plenty of squares and green bolls, the most cherished food, become available. Its most preferred site for oviposition is the leaf, especially tender leaves at the upper half of the plant³, although a small number

is laid on other parts as well. A great majority of the newly hatched larvae initially feed by scraping chlorophyll in the tender leaves and, as they grow, move over to the squares and bolls for further feeding and development^{4,5}. The neonates that directly feed on squares and bolls are relatively few. Furthermore, *Bt* protein is most effective only against the early instar larvae, while the grown-up larvae (3rd instar and beyond), even if they feed on *Bt* plants, no matter whether it is flowers, squares or bolls, do not die, although they suffer a setback in their overall health. Therefore, it is most critical that in a *Bt*-cotton plant the expression of *Bt* protein should be adequate and sustained in the leaves throughout or for most part of the plant life. As a result, a large number of larvae that feed on such leaves, where the protein expression is highest, perish with little chance to advance to the next instar. Of course, if protein expression is adequate in the fruiting parts also, it will add to further mortality of the larvae hatching on them, however small their population may be.

The studies carried out to date⁶, including the recent one by by Kranthi *et al.*¹, have revealed that *Bt* protein expression is highest in leaves. This matters the most. Hence *Bt*-cotton is able to provide satisfactory control of *H. armigera* in India as in China, Australia and other old world countries where the same species occurs, and also related species in USA and other countries⁷.

If the authors had used neonates^{8,9} instead of the one-day-old larvae for bioassays, it would have been closer to reality and they would have recorded higher percentage mortality in the tissues of all parts. However, they have brought out certain issues like the importance of parental background for improvement of *Bt*-cotton which should be considered wherever possible. I believe this was their intention.

1. Kranthi, K. R. *et al.*, *Curr. Sci.*, 2005, **89**, 291–298.
2. Sahai, S., *The Hindu*, 29 August 2005, (www.genecampaign.org, 29 July 2005).
3. Patel, R. C., Patel, R. M., Madhukar, B. V. and Patel, R. B., *Curr. Sci.*, 1974, **18**, 588–589.

4. Jayaraj, S., Proc. Workshop on *Heliothis* Management, 15–20 November 1981, 1982.
5. Gore, J., Leonard, B. R., Church, C. E. and Cook, D. R., *J. Econ. Entomol.*, 2002, **95**, 763–769.
6. Greenplate, J. T., *J. Econ. Entomol.*, 1999, **92**, 1377–1383.
7. James, C., Report, ISAAA Briefs No. 32, ISAAA, Ithaca, 2004.
8. Deeba, F., Nandi, J. N., Anuradha, K., Ravi, K. C., Mohan, K. S. and Manjunath, T. M., *Entomon*, 2003, **28**, 27–31.
9. Jalali, S. K., Mohan, K. S., Singh, S. P., Manjunath, T. M. and Lalitha, Y., *Crop Prot.*, 2004, **23**, 53–59.

T. M. MANJUNATH

'SUMA', 174 G-Block,
Sahakaranagar,
Bangalore 560 092, India
e-mail: tmanjunath1939@yahoo.com

Response:

Bt-cotton: High toxin level in fruiting parts is most critical for bollworm control

Protein expression in leaves is certainly important. I agree. But to say that it is the most critical is incorrect. Fruiting parts are the most favoured feeding sites of the four bollworm species, viz. the cotton bollworm, *Helicoverpa armigera*; the pink bollworm *Pectinophora gossypiella*; the spotted bollworm, *Earias vittella* and the spiny bollworm, *Earias insulana*. High toxin expression in fruiting parts, and not leaves, is certainly most critical for effective bollworm control.

It is true that the *H. armigera* lays majority (70–80%) of its eggs on leaves of the upper canopy and neonate larvae scrape and feed on the surface of the leaf soon after hatching and get killed. However, rest of the eggs (20–30%) laid directly on squares, flowers and bolls can survive, depending on the levels of toxin expression in these parts. The issue also relates to the efficacy of Bt-cotton on the other three major pests of cotton, the pink bollworm, spiny bollworm and spotted boll-

worm. These insects lay majority of their eggs directly on fruiting parts or in their close vicinity. Neonates that hatch from eggs laid elsewhere go straight to the fruiting parts within a few hours after hatching. Therefore, toxin expression at adequate levels in fruiting parts becomes important. It must be mentioned here that Cry1Ac is at least 3–6 times more toxic to the other three bollworms compared to its toxicity on *H. armigera*. Hence, the current levels of Cry1Ac in fruiting parts are reasonably effective against the pink bollworm, spotted bollworm and spiny bollworm. But higher levels would be more preferable for effective and sustainable control of all the bollworms. More importantly, higher toxin expression in fruiting parts, especially in the boll rind can prevent bollworm larvae from reaching the developing non-Bt seeds that constitute about 25% of the total seeds present in each of the bolls on the Bt-cotton F-1 hybrid plants.

Manjunath suggests that using neonates in the bioassays would have been closer to reality. In our experience, we did not find any significant difference in Cry1Ac bioassay results of corrected mortality using neonates and one-day-old larvae. However, the major disadvantage with neonates compared to one-day-old larvae is that the former are fragile and show >10% mortality in controls, which is unacceptable; whereas, mortality of one-day-old larvae rarely exceeds 5% in controls.

As Manjunath pointed out, some NGOs¹ were trying to use our results to show that Bt-cotton is ineffective in India. Our data² show that Bt-cotton can be less effective than is commonly expected, but certainly not ineffective. The NGOs also tried to relate our data to the cases of Bt-cotton crop failures in some parts of the country. Clearly, there appears to have been some confusion between failure of the technology itself and failure of the product that was developed incorporating the technology. Failure of a particular Bt-cotton hybrid to give higher yields in a particular location can be due to innumerable factors, including genetic, environmental, biotic and abiotic stresses and not necessarily because the Bt-technology

failed to protect the crop from bollworm damage. There has been hardly any scientific proof anywhere in India thus far to show that low yields in Bt-cotton in the locations reported were due to bollworm damage. Bt-cotton was introduced in India three years ago, in March 2002. In fact, bollworm infestation over the past three years did not cause cotton crop failures either in Bt-cotton or even in non-Bt-cotton in any part of the country. Therefore, it would be incorrect to relate poor yields of Bt-cotton to the varying levels of Cry1Ac expression in the plant and erroneously conclude that the technology is ineffective.

Our effort has been to present a realistic picture of the true potential of the currently available Bt-cotton hybrids with reference to India². We believe that there is room for improvement and much can be done even with the currently available material. Under field conditions, even MECH-162, which is not really the best (in terms of Cry1Ac expression) of available Bt-cotton hybrids, has been successful in reducing bollworm populations by at least 70–80%. This is significant in terms of control efficacy and economic returns. However, our data clearly show that Cry1Ac expression could be enhanced in fruiting parts depending on the parents used in hybrid development. We suggested that seed companies should enhance their efforts to develop hybrids that have high expression in fruiting parts and high expression in leaves for the longest possible time. Most importantly, Indian researchers must intensify efforts to develop straight Bt varieties at the earliest, incorporating the best possible toxin expression in all parts of the plant.

1. Sahai, S., *The Hindu*, 29 August 2005. (www.genecampaign.org, 29 July 2005).

2. Kranthi, K. R. *et al.*, *Curr. Sci.*, 2005, **89**, 291–298.

K. R. KRANTHI

Central Institute for Cotton Research,
P.B. No. 2, Shankarnagar PO,
Nagpur 440 010, India
e-mail: krkranthi@satyam.net.in