

Whether one is getting good students, as is the case with the engineering and medical colleges, or whether one is not getting even average students, as is the case with a lot of UG science colleges in different parts of the country, the teachers do not have any say in the matter. On the other hand, teachers have certain academic needs that can only be fulfilled with enrichment programmes. Since a good number of teachers are more than fifty years old and took to teaching as the sole vocation, they are in a way becoming less productive and a sense of frustration has crept in. Many of these teachers are PhDs and have done some research work in their twenties or thirties; they have drifted away from research activities since then. It is indeed a matter of regret

that most of the members in the UG teacher community feel that the UGC Refresher Courses have failed to serve their purpose. So the enrichment of teachers and the process of motivating them is lacking. It is as if teachers are being stamped as poor just because they do not receive good students. The UG physics teacher community all over India has a rich and high quality of human resources. The subject may not be drawing the brightest of the students for the time being, but the intellectual capability and the need for its enrichment among teachers do remain.

If different research institutes in the country could take up an initiative and fund such programmes, the organizational aspects may be taken care of by the colleges. The content of this sort of pro-

grammes needs to be chalked out with suggestions from different quarters and keeping in mind the necessity of the teachers of physics, where the knowledge horizon is fast-expanding.

If, say, after five years owing to some reason good students start pouring into the UG physics, chemistry or mathematics classes, they should get an inspired bunch of teachers who have kept the fire burning all along. The enrichment programme is about all that.

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On the variability of Cry1Ac expression in commercialized *Bt* cotton varieties in India

This is with reference to the article by Kranthi *et al.*¹. Though the authors observed that 'the *Bt* transgenic technology has thus far proven itself to be one of the most environment-friendly methods of bollworm management', their major conclusion was that the quantitative levels of Cry1Ac *Bt* toxic protein in the floral and fruiting parts of eight varieties of *Bt* cotton that they tested during the 2003 season, 'are clearly inadequate to confer full protection to the fruiting parts'. This unwarranted conclusion, based on their experiments and results, is being used as a lightening rod to denigrate the performance of *Bt* cotton in India²⁻⁶. Kranthi and coworkers rebutted implications made out by the critics of *Bt* technology^{7,8}, and adduced that they are reading the article out of context and are completely misinterpreting it.

This article would not affect the scientific community's perception of the potential of *Bt* technology. *Bt* technology is being unnecessarily maligned, since the Central Institute for Cotton Research (CICR), Nagpur is a public institution under the ICAR regime, and the authors are termed 'Government Scientists' as Gospel truth, and conclusions in this paper are being projected as those of the Government of India. Several Non-Governmental Organizations (NGO) have demanded withdrawal of all *Bt* cotton varieties and take action against the purvey-

ors of the *Bt* cotton seeds based on the article by Kranthi *et al.*

The objective of our note is to point out certain basic flaws in the paper by Kranthi *et al.*

1. There is not a single crop variety anywhere in the world that has performed uniformly, throughout the range of its cultivation and season after season. Temporal, spatial and geographical variations in gene expression are universal phenomena. Quantitative variation is natural not only for Cry1Ac protein, but for all other compounds, in not just plants, but in all organisms. Inter-varietal, inter-plant and intra-plant quantitative variability of Cry1Ac protein has been studied for a long time before the first *Bt* cotton variety was approved in the US and other countries and commercial permits were granted. The authors have just once again confirmed what is already well known in gene expression and regulation literature.

2. Kranthi *et al.* have grown plants in an area of 150 m² that does not represent the cotton field situation anywhere (not even that of a subsistence farmer) and the size of a crop field has an influence on the density of pest populations. Experiments were conducted in plastic cups in an insectary that also does not simulate field conditions. The worms had no choice

but to eat plant parts offered, while under field conditions there are alternative hosts.

3. The authors have used excised parts of plants, which makes a significant deviation from the field situation. The saliva of the insects acts as a chemical trigger for the mobilization of defence chemicals or stimulators such as jasmonates, and may be even proteins (protein hormones move within plant systems). Of course, no one has tested if Cry1Ac protein also is thus mobilized. Isolated plant parts are denied the natural systemic defence triggers and responses.

4. Extraction of Cry1Ac protein from tissues for estimation purpose is a challenging exercise and this is not adequately described. As the plant forms mature leaf and boll rind, tissues become fibrous and some resinous and/or triterpenoidal compounds and tannins do accumulate, making the extraction of proteins from such tissues difficult and incomplete.

5. The bioassay with detached *Bt* cotton plant parts at best serves a qualitative purpose, but is not a robust and reliable means to gather data for determining the 'critical expression level in the plant'. The main shortcoming in this method is the lack of knowledge on the rate of degradation of Cry1Ac in plant parts after they were detached from the plant.

6. The detached plant parts were kept at room temperature for 24 h, for the in-

sects to feed on. If there were considerable *in situ* degradation of Cry1Ac during the assay period of 24 h, larvae would be able to survive on the tissues even if the plant parts were from 'high Cry1Ac expressing' plants. A whole plant assay, involving artificial infestation of plants with one-day-old larvae, could have yielded more reliable data.

7. The authors have noted that 'despite variability in toxin expression, the pest control properties are unlikely to be affected significantly at least until the crop becomes 100–115 days old'. Most of the eggs are laid on the leaves. After flowering, only a small proportion of egg-laying occurs on the floral parts. Thus predominant pest pressure comes from the worms that hatch from the eggs laid on the leaves and worms mostly feed on the middle canopy. Larvae start feeding the moment they hatch and their early feeding is on the leaves, which the authors accept have the highest and more than adequate quantities of Cry1Ac toxin. Consequently, most of the pre-flowering crop of worms is killed by the time of flowering. Only the flowering time hatchings matter and these are rarely devastatingly high.

8. The American bollworm moth (ABW, *Helicoverpa armigera*) lays eggs predominantly on the shoot regions in the upper half of the *Bt* cotton plant. Neonates feed on the shoot and other tender parts, mature into II/III instar and then migrate to lower parts of the plant to attack the reproductive structures.

9. The spotted bollworm moth (SBW, *Earias vittella*) also lays eggs predominantly in the apical terminal shoot and is often referred to as 'shoot-borer'. Similar to the ABW, only the II/III instar larvae reach the green bolls to bore and feed on the internal tissue.

10. Only the pink bollworm moth (PBW, *Pectinophora gossypiella*) tends to lay eggs on the flowers and green bolls. Neonates bore through the boll rind and the main source of food for the growing PBW larvae is the developing cotton seed.

11. The developing cotton seed may form the food for the grown-up ABW or SBW, but not for the neonate stages of these insects. Neonate stages feed on shoot regions and mature parts. In rare instances, neonates also feed on pollen if the eggs happen to be laid on the square or flower.

12. Even the authors' results show that though some worms survived, the larvae

'on all parts were stunted with a weight reduction of 48.8–98%', compared to the larvae on non-*Bt* cotton plants. This is significant and cannot give the larvae on *Bt* plants full score of damage potential.

13. The quantity of the Cry1Ac protein in the ovary and fruiting parts is not a significant issue. It is important to have high expression levels in the shoot/young leaves, because neonates of both ABW and SBW feed on these before migrating to the reproductive structures.

14. What is important is that there are only few worms on the post-pollination flowers, due to high levels of toxin in the leaves. The residual pest pressure has to be controlled by pesticide application at this phase of the crop.

15. The authors are concerned about the hemizygous condition of the *Bt* event used in India, while it is homozygous elsewhere. Whether a transgenic event is hemizygous or homozygous would make some quantitative difference, but there are no indications in the literature that this is statistically significant, which was the basis for using hemizygous events.

16. Studies of evaluation of Bollgard varieties that came up for commercial approval were conducted by the ICAR, also involving CICR. The results of these trials were taken into account by the GEAC for the approval of 20 varieties of Bollgard till now. None of these studies appear to have indicated that any of the approved Bollgard varieties were inadequate in the task of bollworm control.

17. It is the problem of management, if farmers are not properly advised on the number and timing of pesticide applications to support the defence offered by Cry1Ac protein. Choice of the parental material is certainly an important issue, but inter-varietal differences are not alarmingly high.

18. The authors have noted that 'Since the *Bt* transgenic technology has thus far proven itself to be one of the most environment-friendly methods of bollworm management, it is in the interests of the technology itself that researchers, technology providers and administrators ensure that it must be provided to farmers in a form which gives the best possible returns for the investment'. The ICAR and CICR should take serious note of this advice.

Conclusions of the authors, based on this limited one-season (2003) study, conducted using controlled methodology,

cannot be extrapolated to situations under farmers' field conditions. It is unwise on the part of NGOs to use the flawed findings of Kranthi *et al.*¹ to demand a blanket ban on *Bt* cotton throughout the country just because it supports their ideological convictions.

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Response:

Shantharam and Rao raise interesting points and make a number of criticisms on our article¹. We can deal quickly with their negative points. To begin with, I would like to reiterate that our methodology is robust and results are reliable, and stand vindicated by two recent publications by independent peer research groups from China² and Australia³, wherein the data mirror our findings on the temporal decline of Cry1Ac and the low levels of expression in fruiting parts of *Bt* cotton.

The authors are critical of our plot size of 150 m², which is large or larger than areas used for similar studies^{2–5} carried out in USA, Australia and China, wherein either ELISA was carried out on plants grown in plots far less than 150 m² in

area and/or 'no-choice' bioassays were conducted with isolated plant parts such as leaves, squares, flowers and bolls, almost exactly as described in our article. This was not a field trial to evaluate pest densities as Shantharam and Rao point out, and the Cry1Ac content in the plants parts would not have changed if the plot size had been 150 ha! The protocols followed are standard. A total of 900 plants (300 per replicate) is more than adequate for a study of this kind. Insect bioassays conducted in plastic cups as a 'no-choice' test on isolated plant parts are standard 'textbook' methods that have been most commonly used all over the world for experiments of this nature. The 'no-choice' tests are conducted to ensure that the insects feed on the food provided, so that toxicity can be assessed. Cry1Ac toxin degradation in excised plant parts could have been an issue and therefore before conducting the bioassays, we examined the Cry1Ac toxin degradation over 36 h in isolated leaves and bolls. There was no significant degradation of Cry1Ac in detached plant parts at least over the 24 h period at room temperature. There are several publications by reputed groups²⁻⁵, including those from Monsanto, wherein bioassays were carried out using isolated plant parts that were changed not every 24 h as we did, but once every 48 h in some cases, and in others with no change at all even for 7d. There have never been any issues on these publications or the methods reported.

It is well known that matrix effects may play a minor role in sample extraction for ELISA. Like our peer groups elsewhere in the world, we have standardized precise extraction buffers and protocols for cotton tissue matrix and used ELISA to quantify the toxins. Our methods have been repeatedly validated with plant samples internally spiked with known quantities of Cry1Ac and we are confident of the accuracy of the values presented in the article.

The data therefore are good and the methods used entirely acceptable internationally. Ever since the publication of our article, I have received letters of appreciation from several peer groups spread across the globe.

The conclusions made in our article were actually based on a three-year study. Our original manuscript of over 32 pages had nine tables and five graphs, containing the complete data. At the journal editor's request and the advice of four referees

(including one sub-editor) we condensed the article, presenting only one-year data, which was representative of the results of the full study. The additional material can be made available if required, but the overall conclusions will not be affected.

Of more concern is the suggestion that our article aimed to reflect negatively on *Bt* technology. We concluded that 'the toxin expression in the boll-rind, square bud and ovary of flowers was clearly inadequate to confer full protection to the fruiting parts'. This does not indicate that the current *Bt* plants are not making important contribution to bollworm control and we did not suggest this. We had stated previously that *Bt* technology had the capability of reducing bollworm infestation by 60–90% under field conditions⁶. On these issues, I have already responded in detail to Suman Sahai⁷ and Manjunath⁸ on the implications of variable Cry1Ac expression in *Bt* cotton plants for bollworm control.

We are well aware of the distribution of egg-laying and feeding sites for cotton bollworms and that certain key tissues have inadequate Cry1Ac to control some stages of *Helicoverpa armigera*, especially during certain growth stages of the plants. Indeed the material would hardly have passed through the registration process without these issues being discussed. It is also common knowledge now that expression in different tissues and at different growth stages will vary. What we set out to do was to quantify this for the Indian *Bt* hybrids. All stages of the bollworm can be found on various parts of *Bt* cotton plants⁹. It is important to know, for resistance management and effective control purposes, which insect stages can survive, where and when, and whether this differs with the particular hybrid. In India, as elsewhere, there is significant survival of *H. armigera* larvae on *Bt* cotton in the field, thereby warranting supplemental insecticide sprays. Our studies have shown that, so far, this is mainly due to inadequate Cry1Ac expression in particular tissues and times of the year and not because of the evolution of significant resistance, though this may change.

Bt cotton receives insecticide sprays against bollworms wherever it has been deployed. To state a few examples, the average number of insecticide sprays against *H. armigera* in Australia on Bollgard (Ingard) during 1998–99 was 7.4 as against 13 on non-*Bt* cotton¹⁰. In the previous and succeeding years the average

was 5.4 and 5.1, compared to 9.7 on non-*Bt* cotton. It is important to note that decisions to spray insecticide in Australia are made based on economic threshold levels. Following the introduction of *Bt* cotton in 1996, a total average of at least 2.4 insecticide sprays were made to control bollworms on *Bt* cotton across all cotton-producing states in USA, thus reducing the overall insecticide applications by 50–60%. The situation has not been different in any other part of the world, including India.

The current Cry1Ac cotton provides a valuable benefit in bollworm control but it is not perfect, and these imperfections have implications. The Model T-Ford was a technical and economic breakthrough conferring considerable benefits, but let us not pretend it was a Rolls Royce!

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