Bt-cotton: refuge in seed-mix – some clarifications on its need and viability

This note has reference to the correspondence: '*Bt* cotton: refuge in mixed bag' by Muralimohan and Srinivasa[1]. In fact, their article itself was in response to an earlier correspondence entitled '*Bt* resistance in *Helicoverpa* species: Indian policy needs urgent revision' by Hanur[2], in which the author has advocated 'refuge in mixed bag'. Since Muralimohan and Srinivasa[1] have raised certain concerns and opined against the use of 'mixed bag', I have made an attempt to address their concerns, clarify certain doubts and also explain the need for this approach.

To ensure refuge compliance as a proactive measure towards insect resistance management (IRM), a new approach has been developed wherein *Bt* and non-*Bt* seeds are pre-mixed in a recommended proportion and made available to farmers in the same packet or bag. This technique is referred to as 'refuge-in-a-bag' (RIB) in USA, where it is deployed for the *Bt*-corn products and in India as 'refuge in mixed bag'. For the sake of uniformity and better clarity, the name 'refuge in seed-mix' (RISM) appears to be more appropriate and is used in this note.

The objective of RISM in *Bt*-cotton is to ensure that non-*Bt* plants are randomly distributed among *Bt*-cotton plants in a field in a pre-decided proportion. The presence of such randomly distributed non-*Bt* plants may raise certain concerns such as: (i) potential movement of the later instar larvae of *Helicoverpa armigera* from non-*Bt* to *Bt* plants, thus causing crop damage[1,3,4], and (ii) exposure of such migrant larvae to sub-lethal dose of *Bt* protein, thereby increasing the chances of resistance development[1]. These concerns appear valid, but their actual impact needs to be examined in the Indian context from a practical perspective, especially in view of certain data available elsewhere on the adverse fate of the *Bt*-fed later instar larvae[5] and also that RISM is considered as a practical strategy towards IRM in pursuit of preserving a remarkable technology like *Bt*-cotton.

It is a well-known fact that in India, the original regulatory recommendation of planting a structured refuge, comprising 20% non-*Bt* with need-based chemical control measures against bollworms (in other countries, the option of planting 5% non-*Bt* with no control measure is also available), along the border of *Bt*-cotton field, has not been adopted by most farmers. Although the farmers are provided with the recommended quantity of non-*Bt* seeds in a separate packet along with each packet of *Bt*-cotton seeds, the non-*Bt* seeds are not planted as refuge. Instead, the farmers sow only the *Bt* seeds in the entire field as their contention is that there would be yield loss from the 'refuge' crop. With the *Bt*-cotton area significantly increasing in India from year to year since its introduction in March 2002 and in 2010 exceeded 10 million hectares (i.e. 25 million acres) constituting 92% of the total cotton acreage with little or no refuge, resistance development is a serious concern[4].

Providing a refuge of non-*Bt* cotton as a source of susceptible moths to mate with resistant insects, if any, has been the primary approach for preventing resistance to *Bt*-cotton and its mechanism and benefits are well documented[3,4,6–8]. In countries like USA and Australia, refuge compliance is mandated through an agreement with the farmers. In India, for reasons explained above, we have to find some practicable alternative solution so that farmers do not neglect refuge. It is in this context that RISM is considered a viable option[2]. In RISM, the recommended quantity of non-*Bt* cotton seeds will be premixed with the bulk of *Bt* seeds in the same packet and sold to farmers. Since the two seeds are indistinguishable, farmers will plant the 'refuge', thereby adopting resistance management strategy. It is not that RISM (i.e. 'refuge in mixed bag' or RIB) is altogether a new concept. It was discussed much earlier in USA as one of the possible strategies for IRM[9,10], as cited by Hanur[2]. *Bt*-corn products with refuge within the bag (i.e. RIB) have been registered in USA[11] and Canada[12]. However, RISM for *Bt*-cotton is being considered as a new approach in India.

Having discussed the need for RISM, let us try to examine the apprehensions expressed by Muralimohan and Srinivasa[1]. These authors as well as Hanur[2] have focused their attention more on *H. armigera* for resistance management. On a closer examination, we realize that resistance management in other bollworms such as Pink bollworm (*Pectinophora gossypiella*), Spotted-bollworm (*Earias vittella*) and Spiny bollworm (*Earias insulana*) is equally, if not more, challenging, if we consider their host range and feeding behaviour. Pink bollworm is functionally a monophagous pest of cotton, whereas both the species of *Earias* have only a limited number of alternative hosts. Therefore, these pests almost entirely depend on cotton crop for their feeding and breeding. On the other hand, *H. armigera*, although the most destructive among cotton bollworms, has more than 180 plant species as alternative hosts with at least a dozen of them being highly preferred[13]. Studies have shown that some of these alternative crops are cultivated around the same time as cotton (*Gossypium hirsutum*) in the same area in several parts of India, and that a few of these like chickpea (*Cicer arietinum*) and pigeon pea (*Cajanus cajan*) are highly preferred over cotton, thus serving as natural refuge for *H. armigera*[14]. This also applies to a polyphagous pest like the Tobacco caterpillar, *Spodoptera litura*, which is presently a sporadic pest of cotton. But the same is not true with Pink bollworm, Spotted bollworm and Spiny bollworm and, therefore, it becomes necessary to provide them with adequate cotton (non-*Bt*) crop itself as refuge to support the required susceptible populations.

The later-instar larvae of *Pectinophora* and *Earias* hardly move between plants. So, the question of their moving from non-*Bt* to *Bt* plants is hardly a concern. However, in the case of *H. armigera*, some (not all) later-instar larvae are likely to disperse from non-*Bt* to adjacent *Bt*-cotton plants, especially when there is 'crowding' on a plant. Understandably, we need to assess the consequences of such dispersal. In this context, It is worthwhile noting that a series of feeding studies conducted in USA has shown that later-instar larvae of *Helicoverpa zea*, a close relative of *H. armigera*, and also other Lepidoptera (*Heliothis virescens*, *Spodoptera frugiperda*, *Spodoptera exigua*) that were fed on dual *Bt* proteins (cry1Ac and cry2Ab as expressed in Bollgard II) showed low survivorship and plant damage, whereas the result was intermediary with those larvae fed with a single *Bt* protein (cry1Ac as in Bollgard)[5]. In India, observations on *H. armigera* that fed on *Bt*-proteins also revealed 'the older larvae may not die, but they suffer a set back in their overall growth and development. Such sick larvae feed much less'[3,4]. Considering these, the concerns expressed by Muralimohan and Srinivasa[1] that RISM may result in increased plant damage due to migrant larvae and also that it may lead to development of resistance, though valid, do not appear to be serious from a practical perspective. Nevertheless, it would be worthwhile conducting systematic studies to address all perceived implications before coming to any conclusions. It is true that such mortality, however small, would result in some reduced number of susceptible moths from the intended refuge[1] (the same is also true if general insecticides are sprayed for some reason), but it is also true that all larvae will not be affected and, therefore, some would develop into susceptible moths on the non-*Bt* plants.

Throughout their article, Muralimohan and Srinivasa[1] have assumed that the proportion of non-*Bt* seeds in RISM or 'mixed bag' will be 20%. Earlier studies elsewhere have shown that stacking of *Bt* genes, with different binding sites in the insect midgut, imparts greater insect control efficacy and also that it minimizes the probability of development of cross-resistance. Therefore, in such cases, the size of refuge can be considerably smaller[15–17]. In fact, the size of the refuge in RIB for stacked *Bt* genes in corn has been reduced to 5% (non-*Bt*) in the United States Environment Protection Agency (USEPA) registered *Bt*-corn products in USA[11], and the same recommendation has been more recently adopted in Canada[12]. Bollgard II, the two-gene *Bt*-cotton which was approved in India in 2006 and is currently predominantly cultivated (> 75%), also provides two *Bt* proteins with independent sites of action, and hence the effective refuge size would certainly be far less than 20%, perhaps around 5%.

Muralimohan and Srinivasa[1] have also expressed that 'mixed bag' (or RISM) could go against the India Seed Act, because this Act does not allow mixing of two genotypes. In reality, the India Seed Act stipulates that in hybrid cotton seed crop, 90% of plant population should be genetically homogenous and in the remaining 10% heterogeneity, out-crossed plants can constitute a maximum 1.5% and the plant population due to selfing of female lines can constitute the balance (subject of total genetic impurity of 10%)[18].

Insect resistance development is a natural phenomenon and is a genuine concern. Since implementation of structured refuge has not been satisfactory in India, RISM appears to be a practicable and viable option to ensure IRM. Considering the benefits delivered by *Bt*-cotton to over 6 million farmers and to the Indian textile economy which generates employment for several million people and large revenue, it might be prudent to adopt RISM so that this valuable technology is preserved as long as possible. It does not prevent anyone from evolving more suitable refuge management strategies or developing new products for bollworm management.

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Funds promote scientific output

Research funds are important resources for basic research, which affect scientific and social development[1] as well as the career of scientists[2]. While scientific output of basic research is measured by scientific articles, the case of scientific articles supported by research funds has also been studied[3–6]. Since August 2008, the *Web of Science* (*WoS*) has started recording funding information of publications, which provides reference data for funding analysis.

Using the *Science Citation Index* database in *WoS*, we counted 2,060,838 scientific articles and 1,165,276 funded articles in 2009–2010. Total articles and funded articles of the top 20 countries/territories (according to published articles) in the world are shown in Figure 1.

For total articles, the top 20 countries/territories accounted for 86.2% of those