0–2 longitudinal septa (Figure 2 *d*). These morphological characteristics and measurements were similar to those of *A. tenuissima*. This was further confirmed by Indian Type Culture Collection (ITCC), Indian Agricultural Research Institute, New Delhi (ID 10.826.18). The molecular identification was done by sequencing the internal transcribed spacer (ITS) region of rDNA using primers ITS1 and ITS4 (ref. 3). The generated sequences were submitted to GenBank (accession no. MH938072). Mega-BLAST analysis of sequence showed more than 99% homology with *A. tenuissima* strains MH032750 and MH864067. To fulfil Koch's postulates, conidial suspensions (5×10^5 conidia ml^{-1}) from cultures on PDA were inoculated on ten leaves of 35-day-old *Euryale ferox* plants by pin-prick method[4]. Conidial suspension was injected using modified hypodermic needle at the prick point and the leaves were covered by moist sterilized cotton swab along with sterilized aluminium foil. After five days of inoculation, the aluminium foil was removed and test leaves were exposed to natural humidity regime. Ten leaves of *E. ferox* plants were inoculated similarly with sterilized distilled water. The tested leaves were continuously monitored for the appearance of symptoms. After two weeks, typical symptoms of brown spots with concentric ring were observed on all the inoculated leaves (Figure 2 *a*), whereas the control leaves remained asymptomatic. *A. tenuissima* was specifically reisolated and identified from the inoculated symptomatic leaves, thus confirming Koch's postulates. Thus the occurrence of leaf spot on *E. ferox* may pose a serious threat to the cultivation of makhana and result in higher yield reduction.

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Bt-cotton hybrid seeds and 'refuge-in-bag' strategy

This is in response to the correspondence by Muralimohan and Mahesh[1], wherein the authors have conducted the much-needed *Bt*-trait quality analysis of dual *Bt*-gene Bollgard II™ (BG II) cotton seeds sampled from the markets of central and southern India. Their contention that the *Bt* cotton seed producers, now, need not 'purposefully mix non-*Bt* seeds' to implement 'refuge-in-bag' (RIB) because by default the proportion of non-*Bt* refuge seeds was ~5% in 93% of the BG II seeds sampled, is not correct, for the following reasons.

The Ministry of Agriculture and Farmers Welfare, Government of India, has directed that all BG II cotton seed producers should completely switch over to RIB mode of refuge delivery from the *kharif* of 2020. Accordingly, the seed producers have suitably geared-up their seed production and packaging processes to achieve this objective. Simultaneous implementation of the new seed packaging process, across all *Bt*-seed producers, would henceforth ensure correct blend of non-*Bt* refuge seeds (5–10%) with trait-validated BG II seeds (90–95%). It is hoped that the in-built quality assurance in the new packaging system would bring uniformity across all brands of BG II in terms of refuge quality and proportion in the blend, which appears to be lacking now. The current situation is reflected in the authors'[1] analyses which reveals a wide variation in *Bt*-trait levels in the sampled bags, ranging from 61% to >95%. In addition, the constituent *Bt* traits (Cry1Ac, Cry2Ab2) were found singly in about 1% of the seed samples. Hence, the BG II seed producers should implement RIB for BG II which would entail stewardship and quality management of the highest order. Here is an opportunity for the *Bt* cotton seed producers to correct the existing inadequacies in refuge proportion and quality[2].

The quality of non-*Bt* refuge seeds (supplied in a separate packet and which these authors[1] have not analysed) is a big concern. Kranthi *et al.*[3] have shown that refuge seeds often contained *Bt* traits and the refuge plants varied widely in phenotype, viz. bloom initiation, bloom period, fibre quality and yield from that of the BG II hybrid. The refuge plants should be isogenic (same parentage but lack *Bt* trait) or close hybrids with matching phenotype, enabling the farmer to get a good yield in the absence of pest incidence. In short, all the above anomalies can be set right with the new RIB system.

The authors[1] also conclude that the RIB option may not be effective in delaying evolution of *Bt* resistance in bollworms, because in their view the default presence of ~5% non-*Bt* seeds along with *Bt* seeds from the time of commercialization of BG II in 2006 did not delay the evolution of *Bt* resistance in pink bollworm (PBW).

We need to get to the basics to understand this issue. PBW is currently resistant to both toxins expressed by BG II. PBW can multiply only on conventional cotton and no other host, implying that the selection pressure to evolve *Bt* resistance would have been the greatest in this pest relative to another major bollworm like *Helicoverpa armigera*, which can multiple on more than a dozen host crops (natural refuge). Thus, it is critical to supplement refuge (structured/RIB) with integrated pest management (IPM) measures for the management of PBW. Structured refuge and IPM for *Bt*-cotton were neglected by the farmers and consequently resistance evolved. Right from the beginning, the *Bt*-technology

stakeholders and regulators had prescribed cultivation of *Bt*-cotton in the realm of IPM practices.

The evolution of resistance, first to Cry1Ac and then to Cry2Ab2 was sequential. BG II cotton formed ~27% of total *Bt*-cotton acreage in 2008, 57% in 2009 and increased to 90% and above from 2012 onwards. PBW had already evolved resistance to Cry1Ac by the time BG II occupied a fair share of cotton acreage from 2008 and beyond. It was relatively quicker to evolve resistance to the second toxin, Cry2Ab2. Thus, it would not be right to use evolution of *Bt* resistance in PBW as a simple dipstick evaluation for the efficacy of RIB. It would serve other bollworms as well.

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Response

Data have revealed that *Bt* seed packets planted by the farmers contained non-*Bt* seeds to the extent that the Government of India has recommended for deliberate inclusion[1]. Mohan seems to have wrongly assumed that we have suggested not to mix 5% non-*Bt* seeds in the bag. In contrast, we have mentioned that there was no need for a deliberate mix of 5% non-*Bt* seeds under the existing circumstances, and, further, that companies had to ensure that the extent of trait purity was greater than 95%, for justifying the addition of 5% non-*Bt* seeds.

The objective of our study was to evaluate the extent of non-*Bt* seeds in the *Bt* seed packets that are planted by the farmers. While digressing from the objective, Mohan's comments pertain to *Bt* seeds present in the non-*Bt* seed packets that are not planted by majority of Indian farmers. His comment in this regard is irrelevant.

It is a fact that the field populations of PBW have already developed resistance to both *Cry* genes. It is either failure in the implementation of resistance management strategies or an issue related to the technology that might have led to the recorded resistance. Any corrective action that is made now (like introducing RIB) holds little water.

Mohan makes an unsupported claim that evolution of resistance in PBW was first to Cry1Ac, followed by Cry2Ab2 in a sequential manner. Such presumptions hold no relevance. PBW is a target bollworm, and it has developed resistance against both Cry toxins. We have shown that the requisite quantity of non-*Bt* seeds was already present in the seed packets planted by the farmers, which raises concerns about the recommendations made in the RIB strategy for deliberate inclusion of non-*Bt* seeds for resistance management. It appears that the development of resistance might not be delayed by such deliberate inclusion. Moreover, rigorously conducted studies have shown that the RIB strategy could accelerate the rate of resistance development in target insects[2].

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