Success of refuge-in-bag for *Bt*-cotton hinges on good stewardship

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The refuge deployment for *Bt*-cotton will undergo a sea change from the planting season of 2020. Stepping back, the Genetic Engineering Appraisal Committee (GEAC), Ministry of environment, Forest and Climate Change, Government of India (GoI) had approved the first transgenic single-gene *Bt*-cotton hybrids in India in 2002 and the next-generation cotton transgenics with stacked *Bt* genes (Bollgard II®) in 2006, and had recommended sowing of 20% area with non-*Bt* cotton as 'structured' refuge for both types of *Bt*-cotton. Over time, refuge planting became voluntary and cotton farmers ignored planting of refuge to maximize their returns from a field of 100% *Bt*-cotton. This turned out to be short-term gains for the farmers because, in the absence of refuge, evolutionary pressure prevailed and one of the three key bollworms, viz. the pink bollworm (PBW), gained field-resistance[1,2] to single-gene *Bt*-cotton in 2010 and Bollgard II in 2015. The stakeholders of *Bt*-cotton technology in India, following global trends, experimented with 'refuge-in-bag' (RIB)[3] in which non-*Bt* refuge seeds were blended with the main body of *Bt* seeds in a given proportion and sown. RIB, a compliance-assured new mode of refuge delivery was recommended to the regulators for approval in lieu of the 'structured' refuge for *Bt*-cotton. In 2016, the Ministry of Agriculture and Farmers Welfare, GoI[4], provided guidelines to the cotton seed industry on *Bt*-cotton seed standards for implementation of RIB from 2017, wherever ready. A complete switch-over from the structured refuge to RIB is planned by the end of 2019. Technically, 'structured' refuge is more efficient between the two refuge options, but in view of the ground reality of dismally low compliance on structured refuge planting, RIB was the next best option available and the seed industry welcomed the change. The notification specified that *Bt*-cotton with RIB (*Bt*-RIB) seed pack (475 g) shall contain a minimum of 90% and a maximum of 95% cotton seeds positive for each *Bt* transgene, implying that non-*Bt* refuge seeds shall constitute between 5% and 10% of the blend. The refuge seeds shall be of a non-*Bt* hybrid that is an isogenic version (identical in genetic composition, except for the transgene and associated genetic elements) of the corresponding *Bt* hybrid or of a non-*Bt* hybrid with similar flowering period and fibre traits as those of *Bt* hybrid. In essence, the farmer-dependent 'structured' refuge is being changed to a compliance-assured RIB mode, where the famer has no choice but to sow the refuge seeds which would be indistinguishably blended with *Bt* seeds, as against a separate packet in 'structured' refuge.

The success of sustaining the *Bt*-cotton technology, in compliance with the Government RIB directive will hinge on good seed-quality stewardship best practices of all the stakeholders. Ultimately, RIB stewardship should preserve the identity of this unique refuge delivery mechanism for *Bt*-cotton in consonance with pest behaviour and farming practices. In-built elements of integrity and quality management of *Bt* trait and seed are key to the sustenance of bollworm resistance of *Bt*-cotton and durability of technology. Specifically, high-quality standards of refuge seed production are absolutely essential to meet the key requirements of a refuge – to provide equivalence in plant phenotype, including matching bloom and boll-setting period to that of the *Bt* hybrid.

Bt-cotton seed producers also have the opportunity to rectify the past records of supply of poor-quality refuge seeds with *Bt*-cotton[5]. It is not enough that only a handful of seed producers adopt good stewardship practices; it has to be a collective effort of all those who produce *Bt*-cotton seeds, both big and small. Most importantly and as stipulated, the refuge seeds supplied should be the isogenic version of the *Bt* hybrid or at best a 'similar' hybrid with close phenological and fibre quality match. Isogenic refuge seeds would have been ideal, but the notification gave some relief in permitting 'similar' non-*Bt* hybrids in view of the challenges that some seed companies could face in retrieving non-*Bt* breeding lines, because in the past, many seed-producers had resorted to the easier-to-adopt 'forward breeding' method for *Bt* trait introgression into parental lines. The phenotype match would have to be stringently upheld because any visible mismatch would help the farmers identify and weed-out refuge plants, defeating the very purpose of RIB. Good stewardship would also ensure a match in seed size and seed density between the *Bt* and refuge seeds, which otherwise would prevent uniform distribution of refuge seeds in the packet, leading to possible clustering of refuge plants when sown. Expectedly, the production cost of quality refuge seeds would be higher because of insecticide sprays and pheromone mass-traps for bollworm management. This additional cost and apathy of the farmers to use non-*Bt* refuge were the main reasons why most seed companies were not supplying non-*Bt* refuge seeds (120 g/pack) of isogenic hybrid, when a separate packet regulation was in vogue. Seed producers at any cost should not compromise on the physical quality, germinability and phenotypic closeness, which would otherwise undermine the purpose of refuge.

The initial years of RIB implementation could see major challenges for the seed producers in identifying 'similar' non-*Bt* hybrids to the *Bt* hybrids in the market or in the approval pipeline. This gap could severely hamper the introduction of improved *Bt*-cotton hybrids for cultivation. In fact, most of the *Bt* hybrids presently popular with cotton farmers may have to be withdrawn or temporarily shelved, because of non-availability of tested non-*Bt* versions of the hybrids. One of the short-term approaches would be to carefully screen the parental (breeder/foundation) seed lots for the absence of the event/gene and bulk up the seed as a non-*Bt* parental after reconfirmation. However, this issue could considerably ease with the seed producers gradually transitioning to isogenic refuge seeds as RIB, in the coming years.

Introduction of acid/gas delinting and seed treatment/pelleting in the *Bt*-cotton era had vastly improved the germination and vigour of cotton seeds. Adopting these technologies to non-*Bt* cotton seeds, due to scale, could be a challenge to the seed companies. Any disproportionate changes in germinability and/or vigour between refuge and *Bt* seeds could entice the farmers to identify and weed out refuge plants, impacting the purpose of RIB. Further, handling of the blend from the sale of return stocks poses a challenge, as there would be no way to distinguish refuge from *Bt* seeds. Hence maintaining the germination percentage at much higher rate than the regulatory requirement is advised.

Bt seed producers should also revisit the seed quality standards in the various steps of seed production. Intended events should be clearly distinguished from the unintended constituting unapproved events (regulatory risk, if present) and approved but market-failed events (risk of gene silencing due to multiple copies of genetic elements and *Bt* cross-resistance development) in the breeding lines, breeder and foundation seed lots and *Bt* hybrid cotton seeds. Similar quality standards in seed stocks of non-*Bt* hybrids would greatly help sustain the efficacy of refuge. The breeder seed lots need to demonstrate 'high dose' expression of the *Bt* toxins and also to meet 100% gene and genetic purity, and gene equivalence at 99% confidence level. The *Bt* seeds supplied for cultivation have to meet 95% genetic purity, and 90–95% *Bt* genes purity at 95% confidence level.

Since insect resistance management (IRM) is a central piece in *Bt*-cotton stewardship, *Bt* seed producers need to reaffirm continued 'high dose' expression of *Bt* transgenes in their hybrids, especially in the years post-approval, when evaluations become internal to the seed company. Any lapse in the trait quality standards in seed production of parental lines and *Bt* hybrids would essentially jeopardize the IRM strategy of 'high dose + RIB refuge'.

On the IRM front, will RIB serve its intended purpose with the Bollgard II-resistant populations of PBW in Central and South India? This is because, in these regions, the dynamics of *Bt*-susceptible and *Bt*-resistant population levels, due to unchecked multiplication of resistant PBW on Bollgard II crop in the last few years, could be so skewed that the IRM value of the refuge could be in question. We do not have India-specific data on the resistance allele frequencies and the associated risk assessment to corroborate or deny the above. Past studies have shown impaired performance of *Bt*-resistant PBW on *Bt* cotton (Cry1Ac) plants relative to non-*Bt* plants[6] and PBW with *Bt* resistance alleles were found to be less fit than individuals without resistance alleles, implying that refuges can select against resistance even when resistance alleles are prevalent[7]. RIB would certainly work as intended in North Indian *Bt*-cotton fields (contributing to ~20% of India's cotton production) because of the relative absence of *Bt* field resistance in PBW populations.

There is a possibility that RIB in *Bt*-cotton could hasten adaptation of seed-feeding bollworms like PBW, *Helicoverpa armigera* and *Spodoptera litura* to *Bt* proteins owing to the cross-fertilization between refuge maternal × *Bt* paternal[8]. The developing F2 seeds in refuge bolls would thus be a mosaic of the *Bt* genes derived from a neighbouring *Bt*-cotton plant, resulting in the kill of susceptible larvae feeding on seeds with *Bt* trait while sparing the heterozygous *Bt*-resistant individuals. This scenario would result in the selection of heterozygous resistance and a concomitant increase in functional dominance of the *Bt* resistance alleles. However, it could be reasonable to assume that the impact of such cross-fertilization events on the IRM function of RIB would be minimal, because cotton is predominately a self-pollinating crop. Cotton pollen is heavy and sticky, and is not very amenable for wind pollination. Another perceived shortcoming of RIB is that the movement of large bollworm larvae (*H. armigera* and *S. litura*) from refuge to *Bt*-cotton plants could reduce the efficacy of the refuge[3]. In this context, if the additive 'high dose' toxicity in the tissues of the *Bt* plant can kill the migrants, then the plant would be saved from damage.

An equally important stewardship step would be to educate the ~7.5 million *Bt*-cotton farmers on the purpose and management of RIB, repeatedly and through multiple channels. In due course of RIB usage, the farmers might learn to distinguish the refuge from *Bt* plants through intrinsic phenotypic difference, or in the event of a bollworm infestation. They should be taught the value in retaining the refuge plants in spite of bollworm damage and low-yield expectation. USA and Australia have constituted Cotton Technology Stewardship Committee: a group of seed companies taking the lead to monitor resistance development and in strategizing to implement measures for the sustenance of cotton technology. At present, the responsibility of conducting *Bt* resistance monitoring studies in bollworm populations in India is with the Central Institute of Cotton Research, Nagpur in partnership with the technology providers. A consortium of *Bt* seed producers in collaboration with the governmental institutions in India could be an appropriate front to safely steward RIB-*Bt*-cotton in the years to come. Summing up, a strong collective and institutionalized RIB-*Bt*-cotton stewardship mechanism is critical at this juncture of launch for the sustained efficacy of *Bt*-cotton technology in India.

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