Modelling adaptability of cotton bollworm, *Helicoverpa armigera* (Hübner) to *Bt*-cotton in India

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A stochastic model '*Bt*-Adapt' was developed to simulate the rate of resistance development of *Helicoverpa armigera* to Cry1Ac under Indian farming conditions. The model integrates genetic and ecological parameters of *H. armigera* in relation to its response to the Cry1Ac expressing *Bacillus thuringiensis* (*Bt*)-cotton. Simulation analysis showed that relative survival rate of the Cry1Ac-resistant homozygous (RR), heterozygous (RS) and homozygous susceptible (SS) *H. armigera* genotypes on *Bt*-cotton, was the most important factor influencing resistance development. In the order of significance, the other factors that had the greatest impact on resistance development were the relative proportion of area under *Bt*-cotton, dominance of the resistant allele and initial frequency of resistant alleles in field populations. The extent of population reduction in *Bt*-cotton and non-*Bt* crops due to pest control, was found to have a significant impact on the rate of resistance development. Simulation studies showed that cultivation of *Bt*-cotton in 10, 20, 30 and 40% of the total area under cotton, is likely to result in resistant allele frequency reaching 0.5, which would be adequate to cause crop failure, after 54, 25, 16 and 11 years respectively, if no pest control measures were adopted in both *Bt*-cotton and non-*Bt* crops. With a pest control efficacy of 0.9 in *Bt*-cotton and 0.5 in non-*Bt* crops, it would take 70 and 45 years for resistant allele frequency to reach 0.5 with the *Bt*-cotton area at 30 and 40% respectively. Based on the simulation analysis, resistance management strategies are proposed with emphasis on reducing populations of *H. armigera* that survive *Bt*-cotton and enhancement of area of alternate host crops that are as attractive as cotton to *H. armigera*, to be used as trap crop or intercrop refuges.

GENETICALLY modified cotton genotypes incorporating a crystal (Cry) toxin producing *cry1Ac* gene derived from *Bacillus thuringiensis* (*Bt*), were introduced in India for commercial cultivation in the year 2002. The transgenic crop, now popularly called *Bt*-cotton, represents the state-of-the-art in pest management and holds great promise in controlling the cotton bollworm, *Helicoverpa armigera* (Hübner), which has developed resistance to all the commonly used insecticides in the country[1]. The technology is expected to provide cotton growers with significant ecological and economic advantages. It is now widely acknowledged the world over that the benefits accrued from *Bt*-cotton outweigh risks substantially. However, one of the primary concerns of deployment of genetically engineered insect-resistant crops in a developing country like India, is the durability of resistance[2]. Studies[3,4] showed that *H. armigera* has a high capacity to develop resistance to Cry1Ac under laboratory selection. Development of bollworm resistance to *Bt*-toxins is considered to be an inevitable evolutionary eventuality, considering the intense selection pressure that *Bt*-cotton is likely to impose on the insects due to constitutive expression of toxins throughout the plant for the entire growing season. A progressive increase in the concentration of resistance-conferring alleles in pest populations due to sustained selection pressure, results in a concomitant decrease in the pest control efficacy of the transgenic crop. Ultimately, a complete control failure is expected when the frequency of resistant alleles in the pest population reaches 0.5. Resistance management strategies aim to conserve susceptible populations through a spatial or temporal restriction of toxin deployment. Preservation of susceptible alleles indefinitely would mean enforcing severe restrictions on the use of the technology that may curtail the benefits significantly. Hence a rational deployment of *Bt*-cotton to ensure at least 10–15 years of bollworm control efficacy, would be in the best interests of the technology and the farming community[5].

The development of insect resistance to toxins expressed in transgenic crop plants is affected by a number of interacting influences. Significant amongst these are genetic factors such as initial resistant allele frequencies, additive genetic variance, dominance, mode of inheritance, relative survival rates of the RR, RS and SS genotypes on the toxic and non-toxic plants, and all factors influencing Hardy–Weinberg equilibrium. Other factors such as relative host preference, natural survival, insecticide survival,

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random mating, mating synchrony between resistant and susceptible genotypes, relative fitness of the genotypes and accessible abundance of non-toxic hosts, will have a significant impact on the dynamics of resistant allele changes. Resistance development is thus a complex phenomenon, which is governed by several variables. Modelling remains one of the few alternatives for exploring region-wide resistance to transgenic crops[6]. Simulation models can integrate population genetics and population dynamics so as to assess the rate of development of resistance in field populations under any defined conditions.

Many input parameters used in models are either inherently variable or are unmeasured in the real world[5]. Hence model outputs cannot be treated as predictive. They assist in the identification of parameters that have the largest effects on resistance development. Once the critical factors and conditions responsible for rapid development of resistance are properly identified through simulation, it would then enable the development of proactive resistance management strategies. Implementation of such well-defined strategies can ensure a rational spatial exposure of pests to the toxin, so that a steady source of sufficient susceptible alleles is made available to dilute the frequency of resistant alleles. The stochastic model '*Bt*-Adapt' being proposed herein, takes all relevant genetic and ecological variables into cognizance to simulate rate of resistance development of *H. armigera* to Cry1Ac under Indian farming conditions.

Materials and methods

The initial average density of moths emerging from each of the host crops was designated as X_i ($i = 1 \sim n$), with i denoting the ith type of crop, i.e. *Bt*-cotton, non-*Bt*-cotton, pigeonpea, chickpea, sunflower and other host crop farms in the cotton ecosystem. $X_1' \ldots X_n'$ are the redistributed initial densities of moths in each of the respective crops based on ovipositional preference and local movement. The proportion of population that emigrates from the site of emergence was represented by r. The oviposition preference indices on each of the crops, denoted as S_i ($i = 1 \sim n$) was derived from relative population densities occurring on the ith type of crop. The frequency of resistant allele (p) and the susceptible allele ($q = 1-p$) was designated as p_i ($i = 1 \sim n$) and q_i ($i = 1 \sim n$) respectively, for insect populations in the ith type of crop. The frequencies of resistant and susceptible alleles after redistribution were denoted as p_i' and q_i' in the ith type of crop. The area under each of the crops in hectares was designated as A_i ($i = 1 \sim n$) for the ith type of crop. It is assumed that insects would have attained equilibrial density prior to being exposed to *Bt*-cotton. The recursion equations for the redistributed initial densities and initial frequencies of resistant allele of the insect in any of the ith type of crops would be

$$X_i' = (1-r)A_iX_i + rS_i\sum_{i=1}^{n} A_iX_i, \tag{1}$$

$$p_i' = \frac{(1-r)A_iX_ip_i + rS_i\sum_{i=1}^{n} A_iX_ip_i}{X_i'}. \tag{2}$$

The net increase in population density is a function of fecundity (F), natural survival rate of eggs (α_i), natural survival rate of larvae (β_i), survival rate after insecticide exposure (λ_i) and survival rate after exposure to *Bt*-cotton. If R and S are alleles for resistance and susceptibility respectively, the survival rates of the three genotypes on *Bt*-cotton are defined[7] as L for RR (resistant homozygotes), $Lh + (1-h)K$ for RS (heterozygotes) and K for SS (susceptible homozygotes). Dominance of the resistant allele was represented by h. Values of h range between 0 for fully recessive and 1 for dominant. The recursion equations for surviving insect density and resistant allele frequency after exposure to *Bt*-cotton would be:

$$X_i'' = \{Lp_i'^2 + [Lh + K(1-h)]2p_i'q_i' + Kq_i'^2\}X_i'F\alpha_i\beta_i\lambda_i, \tag{3}$$

$$p_i'' = \frac{\{Lp_i'^2 + [Lh + K(1-h)]p_i'q_i'\}X_i'F\alpha_i\beta_i\lambda_i}{X_i''}. \tag{4}$$

Each year, three generations of the insect would be exposed to *Bt*-cotton and the subsequent populations survive on other non-*Bt* alternate host crops or enter into diapause in some regions such as north India. Based on published reports[8–12], we considered random mating to occur freely throughout the cropping season within an area with a radius of 40 km (generally representative of a district) and migration to occur once a year to an extent to allow intermating of moths between districts clustered within an area with a radius of 250 km. The initial frequency of the resistant allele in each of the districts prior to being exposed to *Bt*-cotton would be:

$$p_i''' = \frac{(1-r)A_i'Y_i'p_i^j + r/n\left(\sum_{i=1}^{n} A_i'Y_i'p_i^j\right)}{(1-r)A_i'Y_i' + r/n\left(\sum_{i=1}^{n} A_i'Y_i'\right)}, \tag{5}$$

where Y_i', A_i' and p_i^j ($i = 1 \sim n$) are defined as the average density of moths, total area of the host crops harbouring the emigrating population, and frequency of resistant allele (p) respectively, in the ith district out of the n districts clustered within an area with a radius of 250 km, just preceding the emigration.

We used published data on life tables[13–30], emigration rates[9], relative abundance of *H. armigera* on alternate host crops[19,25,27,30] and district-wise cropping systems and

area under various crops[31,32], to model population dynamics of *H. armigera*. Biological parameters of 42 days mean generation time, 1000 eggs per pair of moths and 0.5 as emigration rate from the site of natality, were used as default values.

The software for simulation was written in Visual Basic 6 and operated in Windows 2000. The model integrates all input parameters and simulates the changes that would occur in resistant allele frequency in field populations of each district in any of the three north, central and south zones of India. We tested a range of input values for factors that are likely to have the greatest impact on the development of resistance. The range chosen was within the likelihood of normal occurrence. Significant amongst these are the percentage area under *Bt*-cotton, initial frequency and dominance of the resistant allele, and survival of the susceptible and resistant genotypes on *Bt*-cotton. Default parameters and the range used for testing are presented in Table 1. The time taken for resistant allele frequency to reach 0.5 in the field populations was assessed with variable parameter combinations simulated for the existing cropping systems of North, Central and South India, which were considered as three replicate situations within the country. The following combinations of parameters were tested for varying extent of area of *Bt*-cotton (relative to the total area under cotton cultivation in India) within a range of 10–90% for the entire period of simulation, keeping all other parameters at default for the simulation.

(i) Variable dominance levels (h) of resistant allele at 0.2, 0.3. 0.4, 0.5, 0.6, 0.7 and 0.8.
(ii) Variable initial frequency of resistant allele (p_i) at 0.0005, 0.001, 0.002 and 0.003.

Table 1. Genetic parameter default values and range tested

Description	Parameter	Default	Range tested
Initial frequency of R allele	P		
North India		0.00075	0.0005–0.003
Central India		0.0015	0.0005–0.003
South India		0.0013	0.0005–0.003
Dominance of R allele	h	0.42	0.2–0.8
Survival of SS genotype on *Bt*-cotton at three phases of the crop	Ki (i = 1~3)		
Early squaring phase		0.005	0–0.01
Peak flowering phase		0.01	0.005–0.02
Boll formation phase		0.05	0.01–0.09
Survival of RR genotype on *Bt*-cotton at three phases	Li (i = 1~3)		
Early squaring phase		1.0	0.85–1.0
Peak flowering phase		1.0	0.85–1.0
Boll formation phase		1.0	0.85–1.0

(iii) Three factorial analysis with varying survival levels of 0.85, 0.9, 0.95 and 1.0 for RR genotypes, varying survival rates of 0.001 to 0.5 for SS genotypes and variable extent of area under *Bt*-cotton.
(iv) Variable pest control efficacy on non-*Bt*-cotton at five levels of efficacy at 0.2, 0.4, 0.5, 0.6 and 0.8, with no pest control activity in *Bt*-cotton. Variable pest control efficacy on *Bt*-cotton at 0, 0.2, 0.4, 0.5, 0.6, 0.8 and 0.9, with a presumed constant efficacy of 0.5 in non-*Bt*-cotton.

Analysis of variance was carried out using methods described by Gomez and Gomez[33].

Results

The simulated dynamics of resistant allele frequency in field populations of the north, central and south zones of India is presented in Figure 1. We used probabilistic parameters of 30% area under *Bt*-cotton, presuming pest control efficacy of 0.5 in non-*Bt*-cotton and 0.2 in *Bt*-cotton, with other parameters at default for the simulation. At the conditions described, the frequency of the R allele would reach 0.5 in 36, 31 and 33 generations in north, central and south India respectively. Because three generations of *H. armigera* are exposed to *Bt*-cotton each year, it is expected that a control failure would occur after 12, 10 and 11 years in the north, central and south zones respectively.

Dominance levels of the R allele

Dominance levels of the resistant allele are critical for expression of resistance in the heterozygous genotypes. Inheritance of resistance was determined through Cry1Ac bioassays on Cry1Ac resistant and susceptible strains, and their F-1 and backcross progeny. Resistance was found to be inherited as incompletely dominant (0.42) monogenic trait[34]. The impact of dominance levels of the resistant R allele on the rate of resistance development as influenced by the selection pressure imposed by the extent of area under *Bt*-cotton was examined.

The results (Figure 2) clearly indicate that both factors 'increase in area' and 'dominance levels of resistant allele' had a significant (F = 109.2; df = 6, 12; P = <0.0001) impact on resistance. At the default parameters, with a value of dominance at 0.4, a constant area of 10% *Bt*-cotton would result in resistant allele frequencies reaching 0.5 after 54 years. Constant areas of 20, 30, 40, 50, 60, 70, 80 and 90% would cause a similar effect after 25, 16, 11, 8, 6, 4, 3 and 2 years respectively. With a second probable scenario in which the area under *Bt*-cotton would increase consistently from 10 to 80% at increments of 10% every year, with dominance value at 0.4, the resistant allele would reach a frequency of 0.5 after 8 years. The prob-

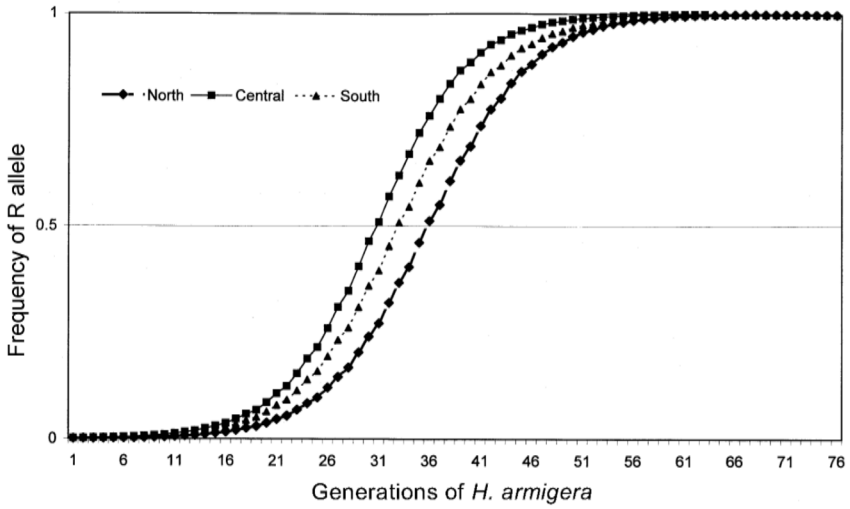

Figure 1. Simulated dynamics of R allele frequency in field populations of *Helicoverpa armigera* as influenced by 30% area under *Bt*-cotton, with pest control efficacy of 0.5 and 0.2 in *Bt*-cotton and non-*Bt* crops respectively.

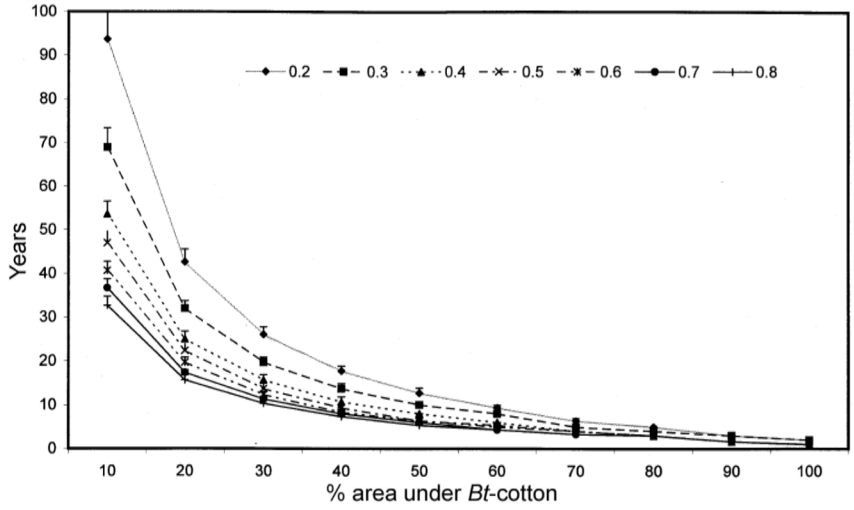

Figure 2. Simulated time for R allele frequency to reach 0.5 at variable dominance levels.

able rate of increase in the frequency of the resistant allele (data not being presented) was likely to be the highest in districts (Mysore, Gadag, Dharwad, Shimoga, Krishna, Guntur, Nalgonda, Karimnagar, Warangal, Tirunelveli, Jalgaon, Khargone, Khandwa, Mehsana, Surat, Bhatinda, Faridkot and Sriganganagar), which had relatively lesser acreage of alternate host crops for *H. armigera*. However, it is important to note that the impact of the acreage of alternate crop becomes more significant only if it is as attractive as cotton to *H. armigera* simultaneously during the cotton season. Generally, pigeonpea, sorghum, pearl millet and other crops serve as alternate host crops to *H. armigera* only at a time when cotton crop starts becoming less attractive.

Initial frequency of resistant alleles

The resistant allele frequency was estimated (Kranthi *et al.*, unpublished) using 278 isofemale lines through the F-2 screening method described by Andow and Alstad[35]. The initial frequencies of Cry1Ac resistance-conferring alleles were found to be 0.75×10^{-3}, 1.15×10^{-3} and 1.3×10^{-3} in North, Central and South India respectively. The rate of resistance would accelerate at higher initial frequencies of the resistant allele (Figure 3). We simulated the temporal increase in frequency as a function of the initial frequency of resistant allele at 0.0005, 0.001, 0.002 and 0.003, with all other parameters at default. There was a significant ($F = 485.1$; df = 3, 6; $P = < 0.0001$) decrease

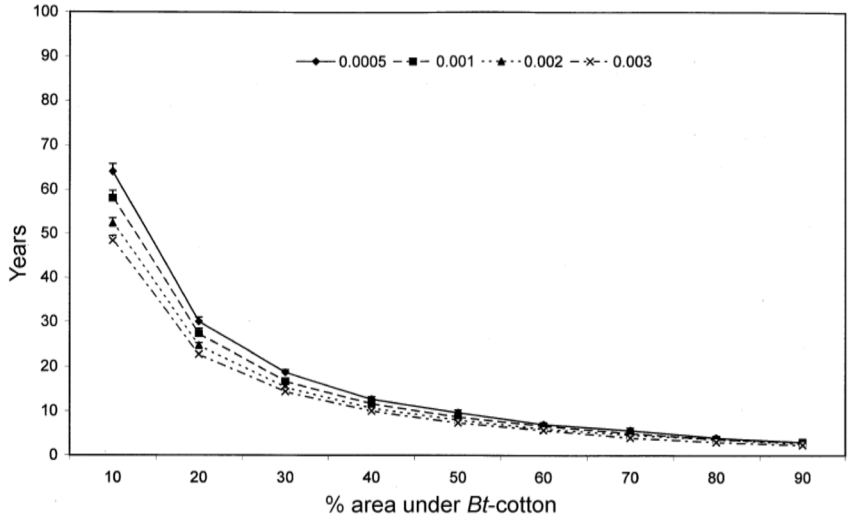

Figure 3. Influence of initial frequency of R allele on time required for the frequency to reach 0.5.

in the time required for the resistant allele to reach 0.5 at each incremental increased frequency tested. For example, at initial frequencies of 0.0005, 0.001, 0.002 and 0.003 with 10% *Bt*-cotton area, it would take 63, 57, 52 and 48 years for the resistant allele frequency to reach 0.5. However, at higher proportions of *Bt*-cotton area at more than 40%, the rate of resistance development was not significantly different at the variable frequencies tested.

Larval survival on Bt-cotton as influenced by gene expression

The expression of Cry1Ac in *Bt*-cotton is variable and declines progressively over crop growth[34]. Larval survival on *Bt*-cotton depends on insect genotype and crop phenology. We did three factorial analysis with varying survival levels of 0.85, 0.9, 0.95 and 1.0 for RR genotypes, varying survival rates of 0.001 to 0.5 for SS genotypes and varying extent of area under *Bt*-cotton from 10 to 90%, with other parameters at default, to assess the relative importance of incremental changes in each of the parameters in contribution to resistance (Figure 4 *a–d*). The extent of survival of the RR and SS genotypes on *Bt*-cotton had a significant effect (for RR survival, $F = 37.8$; df = 3, 6; $P = 0.0003$); (for SS survival, $F = 32.8$; df = 3,24; $P = < 0.0001$) on the rate of resistance development. The extent of area under *Bt*-cotton, relative to non-*Bt*-cotton, had a significant ($F = 148.5$; df = 8, 240; $P = <0.0001$) influence on the rate of resistance development. Resistance development was slower if: (i) the survival of SS genotypes was higher, (ii) survival of RR genotypes was lesser on *Bt*-cotton and (iii) proportion of *Bt*-cotton area was less. For example, with survival rates of 1.0 for RR genotypes, and variable survival rates of SS on *Bt*-cotton, the resistance development time ranged from 2 to 15 and 15 to 68 years at 90–40 and 30–10% *Bt*-cotton area respectively. Whereas with survival rates of 0.85 for RR genotypes, the resistance development time ranged from 3 to 200 and > 200 years at 90–40 and < 30% *Bt*-cotton area respectively. It was clear that with any of the three parameters being varied within the testing range, it would require at least more than 10 years for resistant allele frequency to reach 0.5 at < 40% *Bt*-cotton area.

Pest control efficacy

If the levels of pest control were none or equal, simultaneously, in both *Bt* and non-*Bt*-cotton, with area under *Bt*-cotton at 10, 20, 30 and 40% it would take 54, 25, 16 and 11 years respectively, for the frequency of resistant allele to reach 0.5. We varied the pest control efficacy on non-*Bt*-cotton at five levels of efficacy at 0.2, 0.4, 0.5, 0.6 and 0.8, with area under *Bt*-cotton varying from 10 to 90% and other parameters at default levels (Figure 5 *a*). It was found that resistance development would accelerate significantly ($F = 574.1$; df = 4, 8; $P = <0.0001$) with increasing levels of pest control in non-*Bt*-cotton. For example, with area under *Bt*-cotton at 10, 20, 30 and 40%, the time required for resistant allele frequency to reach 0.5 would decrease to 31, 15, 10 and 7 years respectively, if pest control efficacy in non-*Bt*-cotton would be at 0.5 and 13, 7, 5 and 4 years respectively, at an efficacy of 0.8. However, the simulation would hold good only if pest management measures are not taken up to reduce populations of *H. armigera* in *Bt*-cotton fields. Concomitantly, the extent of pest control measures in *Bt*-cotton fields to reduce the *Bt*-cotton surviving populations of *H.*

armigera, in comparison to the control efficacy in non-*Bt* fields ($F = 1573.9$; df = 6, 12; $P = < 0.0001$), would determine the rate of resistance development (Figure 5 *b*). At a pest control efficacy presumed to be at 0.5 in non-*Bt*-cotton fields and 0.2 in *Bt*-cotton, the resistant allele frequency would reach 0.5 after 35, 17, 12 and 8 years in *Bt*-cotton in areas with 10, 20, 30 and 40% *Bt*-cotton respectively. At a higher efficacy level of 0.9 in *Bt*-cotton and 0.5 in non-*Bt*-cotton, it would take >100 years for areas with less than 20% *Bt*-cotton and 70, 45, 32 and 23 years in areas with 30, 40, 50 and 60% *Bt*-cotton respectively. Hence, it is important to consider the initiation and improvement of pest management strategies to minimize bollworm populations surviving on *Bt*-cotton, so as to ensure a delay in the development of resistance.

Discussion

The model enables the integration of simulated ecological and genetic factors to assess the rate of resistance development. It also helps in identifying the key factors that contribute significantly to resistance development. In the order of significance, the most important factors that appeared to have a significant impact on the rate of resistance development were: (i) survival rates of RR, RS and SS genotypes on *Bt*-cotton, (ii) relative reduction of *H. armigera* populations in *Bt* and non-*Bt* fields through pest management options, (iii) area under *Bt*-cotton relative to non-*Bt*-cotton and other alternate host crops of *H. armigera*, (iv) dominance of the resistant allele and (v) initial frequency of the resistant allele.

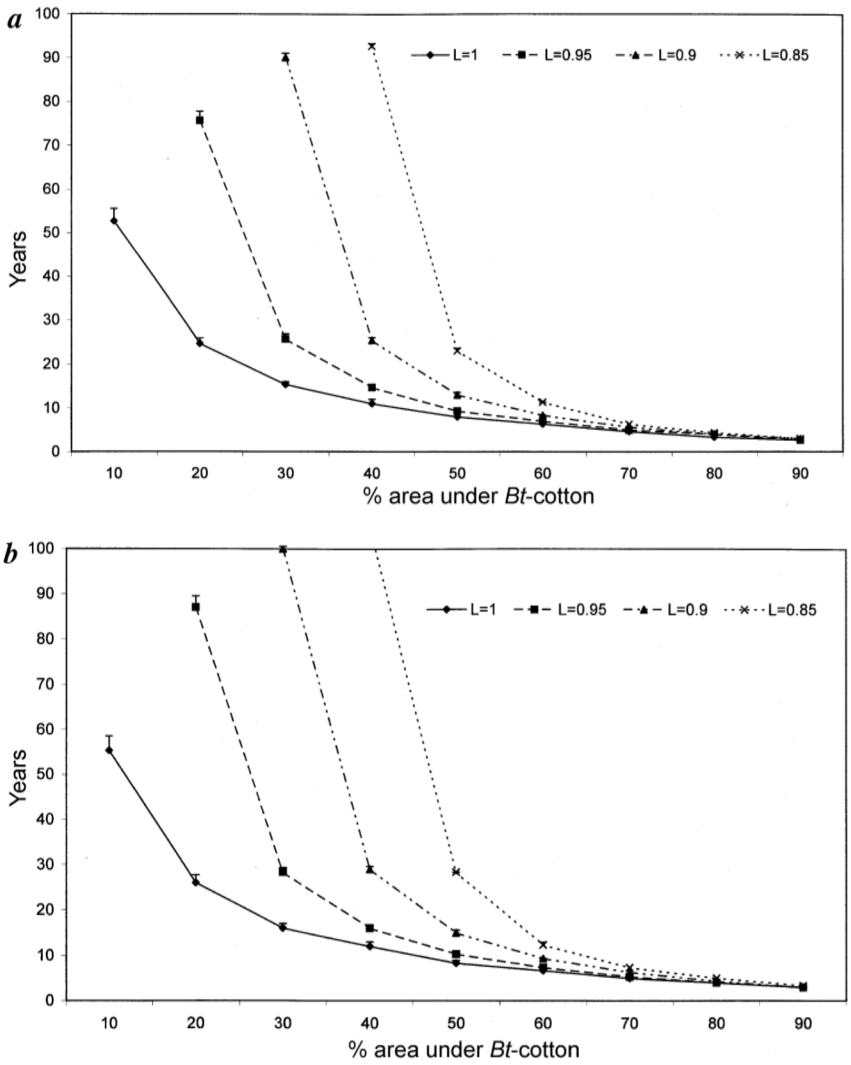

Figure 4. Time required for R allele frequency to reach 0.5 in field populations at variable survival levels of SS genotype on *Bt*-cotton. Survival of SS at 0.001, 0.005 and 0.01 (***a***), 0.01, 0.05 and 0.1 (***b***) of 1st, 2nd and 3rd generations of *H. armigera* respectively, on *Bt*-cotton.

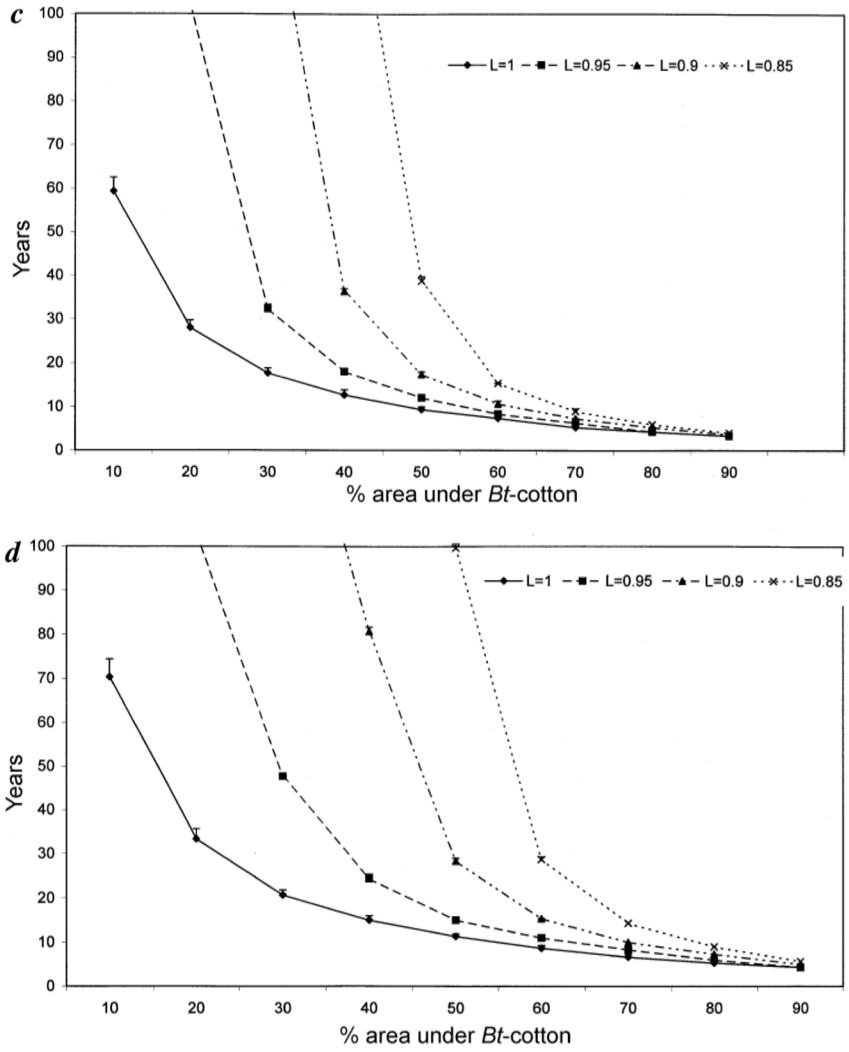

Figure 4. Time required for R allele frequency to reach 0.5 in field populations at variable survival levels of SS genotype on *Bt*-cotton. Survival of SS at 0.05, 0.1 and 0.2 (***c***) and 0.1, 0.2 and 0.5 (***d***) of 1st, 2nd and 3rd generations of *H. armigera* respectively, on *Bt*-cotton.

The intensity of selection pressure is a direct function of the area covered by *Bt*-cotton. The cropping systems of each of the cotton-growing districts, primarily the acreage of alternate host crops of *H. armigera*, the proximity of these to the cotton fields and the relative oviposition preference of *H. armigera* on these crops, would determine the extent of conservation of susceptible alleles and the subsequent dilution of resistant allele frequency. In the cotton-growing zones of India, *H. armigera* completes 7–8 generations per year with three generations on cotton[15,21,27]. In the three North Indian states of Haryana, Rajasthan and Punjab, *H. armigera* occurs initially on chickpea, sunflower and vegetable crops from February to July and starts infesting cotton in August. Peak populations are noticed during September–November. During this period there are few alternate hosts except small farms of bhendi, beans and redgram, mostly attracting the residual generation from cotton. Facultative pupal diapause was reported in winter months[26,28,29]. In the central cotton belt of India comprising Maharashtra, Madhya Pradesh and Gujarat, *H. armigera* populations start appearing on cotton in August, with two successive peaks during mid-September and late October. Though pigeonpea and jowar are cultivated either as solo or intercrop with cotton, they are preferred by *H. armigera* only after cotton becomes unsuitable as a host crop by mid-November. In South India, the pest has a different seasonal biology in the three different cotton-growing states of Karnataka, Tamil Nadu and Andhra Pradesh. In Karnataka and Andhra Pradesh, *H. armigera* appears first on cotton during early September and continues to thrive until early December. Pigeonpea is the main alternate host crop,

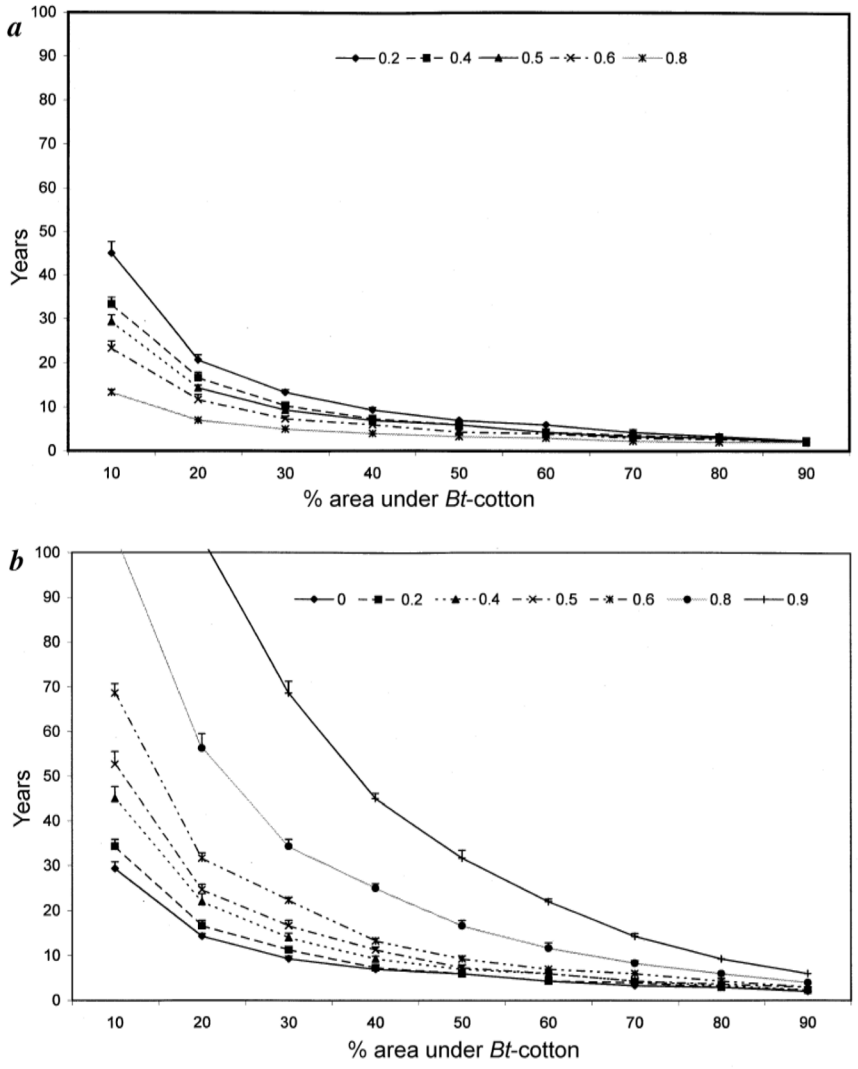

Figure 5. Time required for R allele frequency to reach 0.5 with varying pest control efficacies in non-*Bt* fields (***a***) and *Bt*-cotton fields (***b***).

which attracts populations simultaneously only after November. The pest survives on pigeonpea, sunflower and chillies in December. In Tamil Nadu, peak infestation of *H. armigera* occurs on cotton and legumes in December. Chickpea and pigeonpea are the main alternate host crops available simultaneously alongside cotton for major part of the season. The cropping systems in North and South India, appear to play a major role in delaying resistance development compared to the conditions in Central India. In the context of resistance management, it is important to have a cropping system that would ensure the presence of alternate host crops of *H. armigera* at least during the peak infestation period. It would be useful, therefore, to explore the options of cultivating other non-*Bt* crop plants as intercrops, if their flowering synchronizes with the peak infestation period of *H. armigera* on cotton and if these were as attractive as cotton, and ideally more attractive than cotton. Also, patches of trap crops such as African marigold cultivated in the vicinity of *Bt*-cotton crop, may be helpful in conserving the susceptible alleles. The strategies of simultaneous alternate host crops assume more significance in light of the fact that the extent of area under these would determine the extent of conservation of susceptible alleles, and susceptible population is a resource that can be depleted if proper care is not exercised. It is also important to consider that the refuge method can be more useful when used in combination with other IPM (Integrated Pest Management) strategies to enhance the effectiveness of both. After completing the initial three generations on cotton, *H. armigera* completes 4–5 generations on other host crops. The resistant allele frequency attains Hardy–Weinberg equilibrium in the

field populations and remains unchanged in the absence of any fitness cost that may be associated with the resistant allele. However, if any of these alternate host crops are converted to *Bt*-transgenics, the insect populations may be subjected to additional selection pressure, which would then cause changes in the resistant allele frequency.

Simulation models can have important implications for resistant management strategies. The only resistance management strategy that has thus far been recommended for *Bt*-cotton in India is cultivation of 20% non-*Bt*-cotton as a border row crop that is expected to serve as refuge for susceptible genotypes. However, use of conventional insecticides is permissible on the 20% non-*Bt* refuge crop, which may then reduce the proportion of susceptible *H. armigera* genotypes, the extent of which would depend on the pest control efficacy. If the refuge strategy was not followed with an area of 40% under *Bt*-cotton, it is expected that the frequency of resistance allele would reach 0.5 in 11 years, with no pest control measures being taken up in either *Bt* or non-*Bt*-cotton areas. Generally, non-*Bt* crops are subjected to pest control operations. With an estimated pest control efficacy of 0.5 in non-*Bt* crops, if the area under *Bt*-cotton was 40%, it would take only 7 years for resistance to develop. With a 20% unsprayed refuge, resistance development can be delayed by at least 2–3 years in either of the cases, i.e. with or without pest control action in non-*Bt* crops.

'Refuge' is one of the most favoured resistance management option preferred all over the world. The strategy ensures that an appropriate area of non-*Bt* crop is cultivated in the vicinity of the *Bt*-transgenic crop so as to ensure the survival of susceptible insect genotypes. The presence of these would then ensure the dilution of resistance alleles through gene flow from the refuge into the *Bt*-surviving insects. The susceptible genotypes when mated with the survivors from transgenic plants would result in heterozygous progeny which would express susceptibility, especially if the resistant alleles are recessive in nature. The strategy relies on several conditions. Mainly, the alleles should be recessive or the expression levels of the toxin in transgenic plants should be adequate to kill heterozygous larvae. Other conditions include that mating is random, frequency of resistance alleles is rare and that there is no fitness cost associated with the resistance allele. However, for the Indian situation, it may be required to redefine the parameters to strengthen the concept of refuge. This is necessary because, neither is the resistance allele recessive, nor is the *Bt*-expression adequate to kill all heterozygous larvae (Kranthi *et al*., unpublished data). Moreover, the frequency of resistant alleles is not rare. Mating asynchrony due to different development times of larvae on *Bt* and non-*Bt*-cottons may be yet another matter of concern. The crop maturation period is also different for some of the *Bt* and non-*Bt*-cottons. Thus, the ecological genetics of *H. armigera* resistance to Cry1Ac in India may be different from that occurring elsewhere in the world. Importantly, the cropping systems in India are unique with several crops occurring in a limited region, thereby forming a spatial mosaic for resistance management. This is advantageous for *Bt*-cotton, especially since alternate hosts of *H. armigera* are also available simultaneously with cotton in some parts of the country. One complicating factor particularly relevant to the *Bt*-cotton in India, wherein the technology is available only in the form of hybrids, is that the bolls of *Bt*-cotton hybrids have F-2 seeds, thus having 25% non-*Bt* seeds. This is an important food source that could serve as inbuilt refuge for susceptible genotypes to survive if they manage to penetrate the boll rind, which in some cases expresses low concentrations of Cry1Ac that may not be adequately toxic to susceptible genotypes. But, it is possible that these non-*Bt* seeds may also facilitate enhanced survival of the RS heterozygous genotypes, thus contributing to enhanced rate of resistance development. For random mating to occur, between resistant and susceptible genotypes, it is important that the *Bt* and non-*Bt* crops are situated in close proximity within the dispersal range of *H. armigera*.

Mark-recapture experiments[9] conducted in India showed indications of an obligatory mass exodus of almost the entire (93%) cohort of moths from a 1.7 ha natality site examined. The study also showed that a proportion of moths travelled at least 3.5 km during a single night from the site of origin, with majority distribution within a 900 m range. The high rate of localized migration/dispersal favours intermixing of resistant and susceptible genotypes and helps in rapid dilution of resistant allele frequency in the field populations. It also results in a spatial spread of resistant alleles. Hence the relevance of a border row for resistance management is not well understood. The 20% refuge could serve the purpose of conserving susceptible alleles, even if grown as a patch at a site with proximity of 900 m or for practical purposes, approximately 1 km near *Bt*-cotton fields. Dispersal[9,11] and flight potential studies[10] showed that *H. armigera* commonly dispersed to 3–6 km per night, with a capability of flying distances of over 20 km, without wind assistance, on the first night of eclosion. Patterns of insecticide resistance[8,36–39] in *H. armigera* were not found to vary significantly within an area with radius of about 40–50 km, generally designated as districts. This indicates that a large-scale gene flow keeps occurring at least within the cropping season, which may be confined to a reasonably large acreage. Based on these published reports, we considered random mating to occur freely throughout the cropping season within an area with a radius of 40 km, which would be representative of an average area of a district. Evidence indicates that *H. armigera* is a facultative migrant[11,12] with demonstrated capabilities of migrating 250 km, generally in search of food after the cropping season[8,10,12]. We considered random mating between moths emerging from districts clustered within an area with a radius of 250 km once a year pre-

ceding their exposure to cotton. Hence resistance management programmes need to be designed as area-wide strategies to influence panmictic populations within a relatively larger area.

In USA, refuge in *Bt*-cotton was defined as 20% sprayed or 4% unsprayed non-*Bt* crop within 1.6 km of the *Bt* crop to allow survival of at least 500 susceptible moths per each of the resistant surviving insect from *Bt*-cotton fields. The strategy was an outcome of simulation models developed specifically for *H. virescens* in USA[40]. The strategies are most appropriate for *H. virescens* in USA, wherein the resistant allele is recessive and the *Bt*-cotton confirms to a high dose definition, which are two of the most important prerequisites for a refuge to be effective. The regulatory authorities in Australia have defined a restriction on the overall area of *Bt*-cotton that is not supposed to increase beyond 30% of the total area under cotton[41]. In India, an area of sprayed 20% refuge of non-*Bt* with *Bt*-cotton (based on the US strategy) can be seen as a strategy that has been arbitrarily derived in the absence of well-defined resistance management options that could be drawn from scientific data from Indian work. For logistic reasons, the recommended strategy of five border rows of non-*Bt* around a one acre *Bt*-field is yet to be readily accepted by the Indian farmer. Based on the current study, it can be surmised that the most useful and practical options for resistance management under the Indian conditions would involve strategies that can enforce a significant negative impact on the *H. armigera* populations surviving on *Bt*-cotton. Apparently, any fitness deficit associated with resistance would have the greatest impact on resistance development. Alternatively, if manual picking or the use of insecticides in *Bt*-cotton would result in the reduction of RR and RS genotype numbers to levels similar to those which would be due to even a minimum fitness deficit of 0.1, it would be possible to retard the rate of resistance development significantly. Larval growth on *Bt*-cotton is slower and thus majority of larvae are generally less than the third instar stage. Biopesticides such as HaNPV (*H. armigera* nuclear polyhedrosis virus), neem seed kernel extracts or any other effective insect growth-regulating chemicals may be more useful to manage the resistant genotypes surviving on *Bt*-cotton, as these would be effective on younger larvae apart from being eco-friendly.

Currently, *Bt*-cotton is available in India only in the form of *Gossypium hirsutum* hybrid varieties. Hybrid cotton occupies 40% area under cotton in the country, with rest of the 30, 18 and 12% area under varieties of *G. hirsutum*, *G. arboreum* and *G. herbaceum* respectively. Presuming that the entire hybrid cotton is converted to *Bt*-cotton, it would still occupy only 40% of the total area under cotton in India. But, it is pertinent to note that the development of resistance would be largely influenced by the relative proportion of area under *Bt*-cotton in localized regions such as districts or clusters of districts. It is likely that some regions may develop into 'hot-spots' of resistance within 3–4 years of introduction of the technology, if the area under *Bt*-cotton hybrids increases beyond 70–80%. However, it is also likely that the resource-poor majority farmers, mainly of the rainfed regions of the country, may not be able to afford the high-priced *Bt*-cotton seeds, which is currently four times the cost of the corresponding non-*Bt* hybrid seeds, thus contributing to the maintenance of a sizeable non- *Bt*-cotton crop. But, if the economic benefits of *Bt*-cotton hybrids are proven to be sustainable and consistently higher enough to outweigh the technology costs, it is possible that the dryland farmer may not hesitate in adopting *Bt*-cotton as an economically feasible option.

In general, the area under *Bt*-cotton did not increase beyond 40% in any of the major cotton growing countries such as USA, China or Australia, even 6–7 years after its first commercial release[41] in 1996. With 40% *Bt*-cotton area in India, it would take at least 11 years for *H. armigera* Cry1Ac resistant allele frequency to reach 0.5, which would cause difficulties in pest control with *Bt*-cotton. But generally regular pest control operations are taken up in non-*Bt* crops, with occasional sprays on *Bt*-cotton. Use of effective insecticides in *Bt*-cotton fields will reduce insect populations that survive Cry1Ac toxin and thus represent resistant genotypes. The potential linkage of alleles conferring resistance to Cry1Ac and other conventional insecticides used for bollworm management, is yet to be examined. Any such association could confer cross-resistance and can be detrimental for the long-term sustainability of the Cry1Ac-based *Bt*-cotton technology as well as for the insecticide. More importantly, the utility of the insecticide in controlling bollworms in *Bt*-cotton fields would be severely hampered, as the frequency of resistant alleles to either of the toxins increases. Thus far, there have been no indications to suggest that alleles conferring resistance to conventional insecticides and Cry toxins share the same linkage group in any of the lepidopteran species tested. Hence, it may be possible to use various pest management strategies, including a few selected insecticides to ensure reduction in bollworm populations surviving on *Bt*-cotton. If control efficacy with insecticides was presumed to be 0.5 in non-*Bt* crops and 0.2 in *Bt*-cotton, it would then take 8 years for resistant allele frequency to reach 0.5 with 40% *Bt*-cotton area. If it were presumed that only 75% of the area under hybrid varieties would be *Bt*-cotton, then, with a pest control efficacy of 0.5 in non-*Bt*-cotton and 0.2 in *Bt*-cotton, it would take 11 years for the resistance allele frequency to reach 0.5. Hence, even with the entire hybrid cotton area being converted to *Bt*-cotton, the technology would still be effective for at least 8 years. However, if it is ensured that 50% of the *Bt*-cotton surviving population of *H. armigera* is controlled either through insecticide use or by manual picking (pest control efficacy at 0.5), it would be possible to extend the efficacy of the technology by a fur-

ther 3 years at 40% area under *Bt*-cotton. With a pest control efficacy of 0.9 in *Bt*-cotton with 0.5 in non-*Bt* crops, it would take 70 and 45 years for resistant allele frequency to reach 0.5 with the *Bt*-cotton area at 30 and 40% respectively. It would be an understatement to suggest that one of the most important strategies in *Bt* resistance management would be to reduce the *Bt*-cotton-surviving population of *H. armigera* through any pest management practices. The extent of reduction in the surviving population, which represents resistant genotypes, would determine the longevity of the technology utilization. Therefore, strategies that would enable extending the usefulness of *Bt* technology would be: (i) Use eco-friendly methods such as cultural control or hand-picking of surviving bollworms in *Bt*-cotton fields. Biopesticides that are neem-based or HaNPV would be useful to manage younger larvae on 60–90-days-old crop. Alternatively, conventional insecticides such as endosulfan, thiodicarb, quinalphos and chlorpyriphos, or new molecules such as spinosad, emamectin benzoate, novaluron or Indoxacarb can be used on 90 and 120-day-old crop to reduce populations of resistant genotypes. (ii) Identify and use attractive synchronous alternate host crops for *H. armigera*, which could be used as intercrop or trap crop refuges. (iii) Avoid use of *Bt*-based biopesticides that may contribute to selection of a broad-spectrum resistance to several useful *Bt* genes of interest. (iv) Use alternate genes that do not share common resistance mechanisms as those of Cry1Ac, in transgenic plants either in rotation or alternation or mixtures. Finally, resistance development would be slower if the *Bt*-cotton technology is targetted more for areas which are identified as hot-spot problematic zones for *H. armigera* management. Though this is difficult to implement without a stringent policy restriction, it makes meaningful sense to focus efforts properly in addressing an intractable problem in problematic zones using a powerful, eco-friendly technology such as *Bt*-cotton. Pest management in the rest of the regions can still rely on IRM (Insecticide Resistance Management)-based IPM systems that have been proven to be economically viable, effective and useful for the small-scale farming systems in the country.

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