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In-depth analysis of Bt cotton adoption: farmers' opinions, genetic landscape, and varied perspectives—a case study from Pakistan

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Abstract

Background Bt technology has played significant role in controlling bollworms and increasing cotton yield in earlier days of its introduction, a subsequent decline in yield became apparent over time. This decline may be attributed to various environmental factors, pest dynamics, or combination of both. Therefore, the present biophysical survey and questionnaire were designed to evaluate the impact of Bt cotton on bollworms management and its effect on reducing spray costs, targeting farmers with varied landholdings and educational backgrounds. Additionally, data on farmers' cultivated varieties and the prevalence of bollworms and sucking insects in their fields were recorded. Subsequently, about eleven thousand cotton samples from farmer fields were tested for *Cry1Ac*, *Cry2Ab* and *Vip3A* genes by strip test.

Results In this analysis, 83% of the farmers planting approved varieties believe that Bt technology control bollworms, while 17% hold contradictory views. Similarly, among farmers cultivating unapproved varieties, 77% agree on effectiveness of Bt technology against bollworms, while 23% disagree. On the other hand, 67% of farmers planting approved varieties believe that Bt technology does not reduce spray costs, while 33% agree with the effectiveness. Similarly, 78% of farmers cultivating unapproved varieties express doubt regarding its role to reduce spray costs, while 22% are in favour of this notion. Differences in opinions on the effectiveness of Bt cotton in controlling bollworms and reducing spray cost between farmers planting unapproved and approved varieties may stem from several factors. One major cause is the heavy infestation of sucking insects, which is probably due to the narrow genetic variation of the cultivated varieties. Additionally, the widespread cultivation of unapproved varieties (21.67%) is also an important factor to cause different opinions on the effectiveness of Bt cotton.

Conclusion Based on our findings, we propose that the ineffective control of pests on cotton crop may be attributed to large scale cultivation of unapproved varieties and non-inclusion of double and triple transgene technologies in country's sowing plan. On the basis of our findings, we suggest cotton breeders, regulatory bodies and legislative bodies to discourage the cultivation of unapproved varieties and impure seed. Moreover, the adoption of double and triple Bt genes in cottons with a broad genetic variation could facilitate the revival of the cotton industry, and presenting a promising way forward.

Keywords Bollworms, *Cry1Ac, Cry2Ab*, Cotton, Farmer's perception, Purposive sampling, Sucking insects, Unapproved varieties, *Vip3A*

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Background

Cotton (*Gossypium hirsutum* L.) is an important fibre crop also known as 'White Gold' (Ali et al. 2020; Jarwar et al. 2019). Pakistan earns major part of foreign exchange from cotton crop which contributes significantly towards economy. Pakistan is the 5th largest cotton producer and 3rd larger cotton consuming country in the world. It is an important crop for both agriculture and textile industries, and contributes about 0.6% of GDP and 3.1% of value addition in agriculture sector (Ministry of Finance, Government of Pakistan 2023). Over the time, cotton production in Pakistan has declined, due to seed adulteration, ineffective use of fertilizers and pesticides, labour mismanagement, unfavourable weather conditions, and irregular input supplies (Ali et al. 2019).

Since the introduction of synthetic insecticides, cotton producers relied heavily on those products to control insect pests. Certain factors i.e., insect resistance, secondary pest outbreaks, and pest resurgences cause an increasing application of synthetic insecticides (Trapero et al. 2016). The bollworms (*Heliothis* and *Helicoverpa* spp.) and sucking insects (*Bemisia tabaci, Empoasca* spp.) developed resistance to traditional pesticides during the 1990's (Spielman et al. 2017). Afterwards, genetically modified (GM) cotton expressing *Bacillus thuringiensis* (Bt) toxin was introduced to control lepidopteran pests (Jamil et al., 2021a, b). Resultantly, bollworms which have developed resistance against insecticides were effectively controlled and pesticide use was significantly reduced (Ahmad et al. 2019).

First official approval for general cultivation of Bt cotton in Pakistan was granted in 2010 by National Biosafety Committee within the Pakistan Environmental Protection Agency. However, substantial evidence shows cultivation of Bt cotton at farmers field prior to its official approval (Ahmad et al. 2021; Almas et al. 2023; Razzaq et al. 2021), which are *Cry1Ac* (first-generation *cry* gene) based and are primarily resistant to lepidopteran pests. In the earlier days of its introduction, the adoption of Bt technology led to a notable surge in cotton production, increasing from 8.7 million bales in 1999 to 14.61 million bales during 2004–2005, within just five to six years period (Rehman et al. 2019). Initially, both approved Bt varieties and unapproved ones showed inconsistent and potentially ineffective transgene expression due to the ineffective regulatory system overseeing commercialization of transgenic variety, releasing of new variety and distribution of seed of approved varieties (Ahmad et al. 2019). These loopholes in the system, combined with the challenges farmers face in visually assessing varieties genuineness and seed quality during purchase have contributed to the proliferation of spurious or low-quality seeds (Ali et al. 2019; Spielman et al. 2017).

Now, the area under Bt cotton cultivation is shrinking and the yield is decreased due to increased insect pest infestations (Arshad et al. 2021) owing to field evolved resistance in insects (Jaleel et al. 2020; Lei et al. 2021). Technologically advanced countries like the USA have addressed this issue of insect resistance development by adopting non-Bt cotton refuge systems and pyramiding multiple toxin genes (*Cry1Ac*, *Cry2Ab*, and *Vip3A*). However, in developing countries like China, India, and Pakistan, similar strategies were not effectively implemented, causing the field-evolved resistance in bollworms to proliferate (Jamil et al., 2021a, b; Karthik et al. 2021). Another issue faced by farmers planted Bt cotton is increased infestation of sucking pests due to reduced use of pesticides (Ali et al. 2019; Shekhawat and Hasmi 2023). Hence, it is believed that interplay of various factors i.e. increased insect pest infestation, field evolved resistance, cultivation of unapproved and substandard seeds and adverse weather conditions result in huge loss of cotton production from 14 million bales in 2004–2005 to 4.91 million bales in 2023 (Ministry of Finance, Government of Pakistan 2023).

Keeping in view of the above mentioned facts, a survey was designed to evaluate the impact of Bt technology on cotton production across fifteen core cotton growing districts of Punjab, Pakistan and to understand the multifaceted factors affecting cotton production and to find out the root cause of declining of cotton production. In total 400 farmers possessing various landholding and educational background were surveyed to document their views on Bt. cotton's efficacy against bollworms and spray cost. Additionally, 10,986 cotton samples were tested at farmer's field through strip tests to assess the purity of cotton varieties with respect to Bt (*Cry1Ac*, *Cry2Ab* and *Vip3A)* genes.

Methods

Present study was conducted at Agricultural Biotechnology Research Institute, Ayub Agricultural Research Institute, Faisalabad 38000, Punjab, Pakistan.

Survey site

The survey was carried out in core cotton growing area of Pakistan, Punjab province. The Punjab is further divided into 36 administrative units called "districts" that vary significantly in cotton production. Out of 36 districts, fifteen were selected, i.e. Faisalabad, Toba Tek Singh, Sahiwal, Pakpattan, Multan, Lodhran, Khanewal, Vehari, Muzaffargarh, Layyah, D.G. Khan, Rajanpur, Bahawalpur, R.Y. Khan and Bahawalnagar, on the basis of acreage under cotton cultivation as outlined in AMIS.PK (http://www.amis.pk/Agristatistics/DistrictWise/DistrictWiseData.aspx). Subsequently, 400 farmer fields were selected from all "Tehsils" (sub-administrative unit) with various landholdings and diverse educational backgrounds particularly in the regions with intensive Bt cotton cultivation. The GPS coordinates of each farmer's location was recorded using Latitude-Longitude App (Financept) and listed in Table 1.

Survey questionnaire

A structured questionnaire, comprising of six questions was designed to collect data regarding farmer's demographic factors, farmers' landholdings and viewpoint about effectiveness of Bt technology in controlling cotton bollworms. The questions were: 1) farmers landholding, classified as small (0–10 acres), medium (11–50 acres) or large (above 50 acres). 2) farmers educational background, stratified into uneducated, below matric, matric, bachelor degree, and masters or above qualifications. 3) the efficacy of Bt cotton in controlling bollworms (Yes, No). 4) the role of Bt technology in reducing the frequency of pesticide sprays and respective pesticide cost to farmers (Yes, No). 5) the variety cultivated by farmers (Table S1). 6) insect infestations of i.e. jassid, whitefly, aphid, thrips, mites, American bollworm (AB) and pink bollworm (PB) (low, medium and high). Infestation levels (low, medium, or high) were based on the economic threshold level (ETL) of each insect species. Infestations below the ETL were classified as "low", those comparable to the ETL as "medium", and those exceeding the ETL as "high". The reference point ETLs for various insect species were as follows: jassid (1 nymph or adult per leaf), whitefly (5 adults per leaf), thrips (8–10 adults per leaf), mites (2 adults per leaf), aphid (20 aphids per leaf), AB (4–5 eggs and larvae per 100 plants), and PB (8% infested bolls) (Ali et al. 2019; Razaq et al. 2019; Rehman et al. 2019).

Molecular analysis of *Cry1Ac, Cry2Ab* and *Vip3A* genes

Molecular analysis was performed through strip test for detection and identification of transgenes at four hundred farmer fields, and a total of 10986 samples were tested. At each field, a minimum of 25 samples were collected and tested, at least 10 samples were tested for each variety. Consequently, depending on the number of varieties cultivated by the farmers, more than 25 samples were tested in some fields. The strip tests were performed using QuickStix combo kits (EnviroLogix), which are equipped with built-in antibody coatings for the detection of *Cry1Ac*, *Cry2Ab*, and *Vip3A* transgenes. The procedure for strip test involved pressing the cap of a disposable eppendorf tube onto two leaves to obtain double leaf disc (weighing approximately 20 mg). Subsequently, the leaf samples were finely grinded with the help of disposable pestle by rubbing against the walls of eppendorf tube after adding 0.5 mL of 1X EB2 extraction buffer. The leaf extract and extraction buffer were homogenized by thorough mixing, ensuring the components were evenly combined for accurate and

Table 1 Distribution of farmers by district, geographical location, and landholdings in the study area

District name	Total farmers	Geographical location /°		Land holdings /%		
		Latitude range	Longitude range	Small	Medium	Large
Bahawalnagar	38	29.00–30.20	72.50–73.80	21	55	24
Bahawalpur	36	28.00–29.60	71.02–72.59	17	69	14
Dera Ghazi Khan	26	28.50–31.15	69.50–70.74	15	54	31
Faisalabad	12	30.82–31.20	72.73–73.18	25	42	33
Khanewal	42	30.04–30.60	71.75–72.42	09	67	24
Layyah	20	30.70–31.36	70.90–71.64	50	35	15
Lodhran	24	29.40–29.84	71.50–72.00	12	63	25
Muzaffargarh	36	29.20–30.58	70.62–71.57	25	47	28
Multan	30	29.56–30.44	71.17–72.23	16	57	27
Pakpattan	10	30.00–30.37	72.96–73.25	20	70	10
Rajanpur	28	28.45–29.70	69.70–70.61	11	60	29
Rahim Yar Khan	46	27.80–29.00	69.85–71.00	15	74	11
Sahiwal	14	30.30–30.59	72.50–72.85	07	50	43
Toba Tek Singh	04	30.60–30.80	72.30–72.60	25	50	25
Vehari	34	29.74–30.30	71.96–72.86	16	64	18
Total	**400**	**27.80–31.36**	**69.50–73.80**	**18**	**60**	**22**

reliable downstream analysis. Following that, the QuickStix combo strips were dipped in eppendorf tube containing leaf extract with arrow pointing downward. After 10 min incubation, bands were developed on strips through antigen-antibody reaction and strips were analysed for the presence of final bands, and results were recorded (Jamil et al., 2021a, b).

Data analysis

Frequency analysis of *Cry1Ac*, *Cry2Ab*, and *Vip3A* genes was performed using the "*dplyr*" package to streamline data manipulation and summarization. District-wise opinion of farmers on bollworm management and spray cost reduction were analysed using "*tidyverse*" functions. The association between Bt. technology adoption, farmers' landholding, and education was studied through a heatmap using the "*heatmap.2*" function of "*gplots*" package in R software. The data regarding varieties cultivated by farmers in each district was analysed using stacked bar-chart illustrated by "*ggplot2*" package of R software. Lastly, insect pest infestation data was analysed using "*dplyr*" and "*ggplot2*" packages (Ross et al. 2017) and Chi-square (χ^2) test was performed to check the associations between different qualitative variables using "*chisq.test*" function in R software.

Results

Survey design and farmers demographics

The purposive sampling technique was used assessing the viewpoint of farmers having diverse landholdings and differential educational backgrounds. Landholdings varied among districts showing distinct distribution of farmers with small, medium and large landholdings (Table 1). Notably, the highest proportion of large landholders was found in Sahiwal (43%) followed by Faisalabad (33%), Dera Ghazi Khan (31%) and Rajanpur (29%) district. In terms of medium landholders, district Rahim Yar Khan had the highest (74%), while district Layyah had the lowest (35%) proportions. Among small landholders, district Layyah displayed the highest (50%) while district Sahiwal having the lowest (7%) ratio. Overall, 60% of the farmers have medium, 18% owned small and 22% possessed large landholdings (Table 1).

Similarly, variability was observed among farmers on the basis of academic background (Table 2). The majority of farmers have completed matric (53%), 22% of farmers were below matric (22%), 12% farmers had bachelor degree, 7% farmers had master degree or above qualifications, and merely 6% farmers were uneducated. The district Sahiwal has highest ratio of uneducated farmers (22%) while highest proportion of farmers with below matric qualification was observed in district Dera Ghazi Khan (39%). Besides Dera Ghazi Khan, all other analysed districts have higher proportion of farmers with matric qualification, specifically, district Toba Tek Singh exhibited highest proportion (100%) followed by Pakpattan (70%). Furthermore, district Bahawalpur and district Layyah exhibited highest proportion of farmers among bachelor degree holders (25%) and master degree or above qualification (30%), respectively (Table 2).

Table 2 Distribution of academic qualifications among four hundred farmers in fifteen cotton growing districts of Punjab

District	Uneducated /%	Below matric /%	Matric /%	Bachelor degree /%	Master degree or above /%
Bahawalnagar	8	16	63	13	0
Bahawalpur	6	19	42	25	8
Dera Ghazi Khan	15	39	27	15	4
Faisalabad	8	25	50	0	17
Khanewal	2	24	50	12	12
Layyah	0	10	50	10	30
Lodhran	4	21	46	17	12
Muzaffargarh	17	28	44	5	6
Multan	3	17	57	7	16
Pakpattan	10	20	70	0	0
Rajanpur	4	32	50	11	3
Rahim Yar Khan	2	22	61	11	4
Sahiwal	22	14	64	0	0
Toba Tek Singh	0	0	100	0	0
Vehari	8	16	63	13	0
Total	**6**	**22**	**53**	**12**	**7**

Genetic landscape and cultivation patterns of varieties

The varieties planted at farmer fields were noted and verified based on tags issues by Federal Seed Certification and Registration Department (FSC&RD). The varieties planted at farmer fields were compared with the database of approved varieties from the government to identify the approved or unapproved variety. Overall, unapproved varieties were cultivated extensively covering significant area (21.67%). Moreover among approved varieties the top cultivating were IUB-13 (15.22%), BS-15 (12.61%), FH-142 (8.26%) and FH-Lalazar (8.04%). The lowest cultivated variety was MNH-886 (3.45%). In total of 7.27% area were cultivated with other approved varieties. The top 3 area cultivated unapproved cotton varieties were Bahawalnagar (40.73%), Layyah (38.24%), and Bahawalpur (32.40%). Conversely, unapproved variety was not found in Pakpattan or Toba Tek Singh (Fig. 1).

Analysing region-specific cultivation of varieties, it was observed that IUB-13 was the most cultivated variety in Bahawalnagar (12.09%), Bahawalpur (15.10%), Khanewal (19.18%), Multan (30.41%), Muzaffargarh (21.74%), Faisalabad (24.33%), Rahim Yar Khan (21.10%), and Rajanpur (18.49%). FH-142 was the preferred variety in DG Khan (22.51%) and Layyah (10.66%). FH-Lalazar was most commonly cultivated in Lodhran district (25.64%), while BS-18 dominated in Vehari (21.59%). Additionally, BS-15 was prominently cultivated in Toba Tek Singh (51%), Sahiwal (26.00%), and Pakpattan district (25.60%). Toba Tek Singh and Pakpattan districts have least diversity of cultivated varieties (Fig. 1).

Biochemical testing of Bt cotton

To understand the genetic landscape of cultivated varieties with respect to transgenes, strip tests were performed for detection and identification of *Cry1Ac*, *Cry2Ab* and *Vip3A* genes. Across fifteen districts, a total of 10,986 cotton samples were tested. The *Cry1Ac* gene was presented in varying degrees, with highest occurrence (100%) in district Lodhran, Sahiwal, Pakpattan and Toba Tek Singh. Other districts, such as Khanewal, Bahawalpur, Bahawalnagar, Faisalabad, Layyah, Multan, Rajanpur, Rahim Yar Khan and Vehari also reported more than 80% of *Cry1Ac* gene in farmer fields. In contrast, Dera Ghazi Khan and Muzaffargarh districts displayed relatively lower percentage of *Cry1Ac* gene, 69% and 78%, respectively (Table 3).

The *Cry2Ab* gene exhibited a relatively low (9%) percentage throughout the survey area, its frequency ranged from 0% in Pakpattan to 15% in Layyah and Toba Tek Singh districts. The frequency of *Cry2Ab* gene was no more than 10% in Bahawalnagar, Bahawalpur, Faisalabad, Khanewal, Lodhran, Muzaffargarh, Multan, Pakpattan, Rajanpur, Rahim Yar Khan and Sahiwal districts. Further, the third Bt gene *Vip3A*, which has broad spectrum resistance against lepidopteron pests, was not found in a single tested sample throughout the survey area. In summary, *Cry2Ab* gene was found throughout the cotton

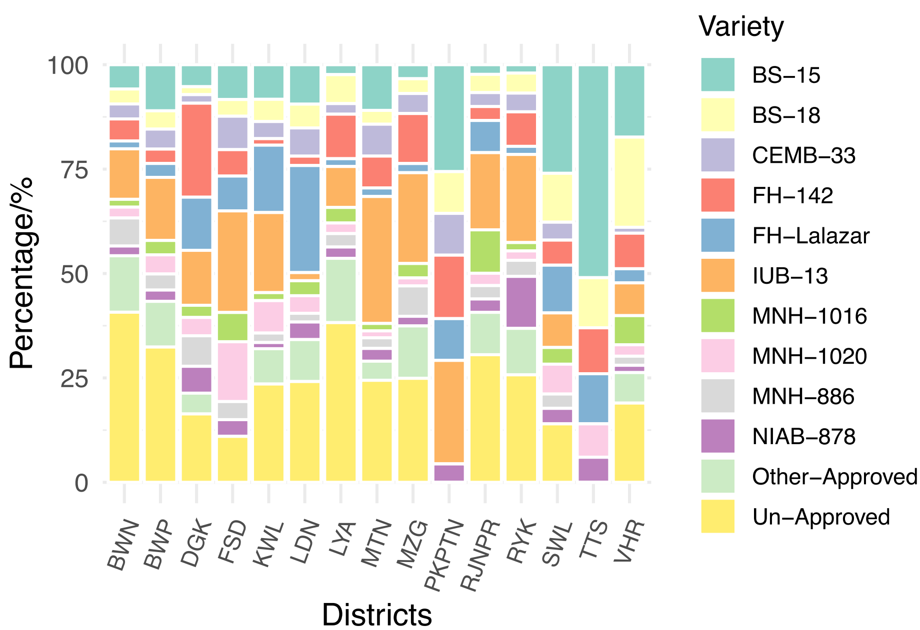

Fig. 1 Stacked bar-chart showcasing varietal diversity across fifteen cotton growing districts of cotton belt of Punjab, Pakistan; BWN; Bahawalnagar, BWP; Bahawalpur, DGK; Dera Ghazi Khan, FSD; Faisalabad, KWL; Khanewal, LDN; Lodhran, LYA; Layyah, MTN; Multan, MZG; Muzaffargarh, PKPTN; Pakpattan, RJNPR; Rajanpur, RYK; Rahim Yar Khan, SWL; Sahiwal, TTS; Toba Tek Singh, VHR; Vehari

Table 3 Genetic landscape of Bt technologies in Punjab's cotton-growing districts: frequency analysis of *Cry1Ac*, *Cry2Ab* and *Vip3A* genes

District name	Cry1Ac /%	Cry2Ab /%	Vip3A /%	Samples tested
Bahawalnagar	82	8	0	1 117
Bahawalpur	94	3	0	960
DG Khan	69	12	0	684
Faisalabad	92	8	0	300
Khanewal	95	7	0	1 147
Layyah	85	15	0	591
Lodhran	100	8	0	667
Muzaffargarh	78	10	0	1 044
Multan	93	6	0	786
Pakpattan	100	0	0	250
Rajanpur	82	4	0	779
Rahim Yar Khan	85	7	0	1 294
Sahiwal	100	10	0	350
Toba Tek Singh	100	15	0	100
Vehari	94	14	0	917
Total	**88**	**9**	**0**	**10 986**

cultivation regions, except Pakpattan, but the percentage was much lower than *Cry1Ac* gene (Table 3).

Pest dynamics at farmers' field

The pest counting was performed in the survey area for major cotton pests, i.e., AB, PB, whitefly, aphid, jassid, thrips, and mites. The PB infestation was medium level in more than 50% farmers' fields in most districts except Bahawalnagar, Der Ghazi Khan, Khanewal, Pakpattan and Vehari. Lodhran and Toba Tek Singh recorded 50% of field with low level of PB, whereas Pakpattan and Vehari recorded high level PB invasions at more than 50% fields. In case of AB, Lodhran, Muzaffargarh, Pakpattan and Toba Tek Singh exhibited low AB level at all fields. However, in Bahawalpur and Layyah, 14% and 20% fields experienced medium outbreak of AB, respectively. Notably, in Faisalabad, Layyah and Sahiwal districts, high infestation of AB was observed at 12%, 10% and 7% fields, respectively. On an average, 93% of fields from all survey regions observed low AB outbreak (Table 4).

The whitefly remains the predominant insect throughout the survey area with high outbreaks at 68% fields on the average. Five districts including Dera Ghazi Khan, Faisalabad, Muzaffargarh, Rajanpur, and Toba Tek Singh were whitefly hotspot areas, with all survey fields recorded high outbreak. Other districts like Bahawalpur, Khanewal, Multan, Layyah, Rahim Yar Khan, and Sahiwal exhibited diverse infestation patterns. Although aphid is one of the most concerned pest, 77% farmer fields reported low outbreak, particularly in Faisalabad, Sahiwal, Pakpattan, and Toba Tek Singh districts with all observed field recorded as low infestation. On the other hand, 64% fields at Bahawalpur and 44% at Muzaffargarh recorded medium level and 28% fields at district Rahim Yar Khan recorded high level outbreak (Table 4).

Apart from whitefly and aphid, jassid was another alarming threat in cotton production, showing high level of invasion at 62% farmer fields. The jassid outbreak in all fields of district Faisalabad, Muzaffargarh, Pakpattan, Rajanpur, Sahiwal, and Toba Tek Singh was at high level. The jassid infestation was also high in more than 70% fields of Bahawalnagar, Dera Ghazi Khan, and Vehari districts. The pest counting of mites revealed low infestation at 62% observed fields. Faisalabad and Pakpattan districts had low infestations in all fields, whereas Dera Ghazi Khan and Muzaffargarh districts recorded medium outbreak at 73% and 86% fields, and 50% fields of Toba Tek Singh recorded as high mites outbreak. The thrips outbreak was high at 60% farmer fields on the average, Bahawalpur, Khanewal, Lodhran, Muzaffargarh, Rajanpur, Toba Tek Singh and Vehari were recorded as high outbreak in 78%, 78%, 75%, 74%, 89%, 100% and 69% fields, respectively while all fileds in Faisalabad showed medium outbreak of thrips (Table 4).

Chi-square test on the associations between different factors

The Chi-square (χ^2) test was performed to check the association of 17 pairs of factors as detailed in Table 5. The association of transgene with varieties was non-significant, which means the type of Bt cotton either single or double transgenic cultivars is not showing distinct correlation with the approved or unapproved varieties. Moreover, farmer's education and landholding have no impact on transgene adoption. Furthermore, association of transgene was also non-significant with thrips which indicates that thrips equally affect non-Bt, single Bt gene, or double Bt gene cotton. However, association of transgene with AB, PB, whitefly, aphid, jassid and mite infestation was significant. This indicates that AB and PB attack vary with transgene and these are interlinked. Similarly, whitefly, aphid, jassid and mites infestation also vary on non-Bt., single and double gene Bt. cotton varieties (Table 5, S2).

Likewise, association of varieties with AB, aphid and mite was non-significant which reveals that there is no statistical difference of AB, aphid and mite infestation between approved and unapproved varieties. On the contrary, association of varieties with PB, whitefly, jassid and thrips was significant, indicating that infestation of PB, whitefly, jassid and thrips vary among approved and unapproved varieties (Table 5).

Table 4 Insect pest infestation at cotton farmer's field (%) in Punjab, Pakistan

District	Pink bollworm			American bollworm			Whitefly			Aphid			Jassid			Mites			Thrips		
	L/%	M/%	H/%	L/%	M/%	H/%	L/%	M/%	H/%	L/%	M/%	H/%	L/%	M/%	H/%	L/%	M/%	H/%	L/%	M/%	H/%
Bahawalnagar	16	45	39	97	3	0	0	6	94	87	13	0	3	8	89	74	26	0	24	37	39
Bahawalpur	0	61	39	86	14	0	0	100	0	25	64	11	0	100	0	75	25	0	0	22	78
Dera Ghazi Khan	23	42	35	92	4	4	0	0	100	81	19	0	0	8	92	27	73	0	12	30	58
Faisalabad	17	75	8	80	8	12	0	0	100	100	0	0	0	0	100	100	0	0	0	100	0
Khanewal	30	31	39	95	5	0	5	21	74	90	10	0	5	50	45	76	24	0	12	10	78
Layyah	35	55	10	70	20	10	15	20	65	60	40	0	30	35	35	40	50	10	15	25	60
Lodhran	50	50	0	100	0	0	12	88	0	72	28	0	12	88	0	61	25	14	0	25	75
Muzaffargarh	19	60	21	100	0	0	0	0	100	56	44	0	0	0	100	14	86	0	0	26	74
Multan	23	54	23	97	0	3	23	14	63	87	13	0	27	73	0	77	10	13	0	50	50
Pakpattan	10	10	80	100	0	0	0	20	80	100	0	0	0	0	100	100	0	0	30	20	50
Rajanpur	32	61	7	96	4	0	0	0	100	82	18	0	0	0	100	21	62	17	0	11	89
Rahim Yar Khan	7	52	41	91	9	0	33	67	0	37	35	28	41	59	0	80	20	0	24	27	49
Sahiwal	7	50	43	93	0	7	0	7	93	100	0	0	0	0	100	71	29	0	14	50	36
Toba Tek Singh	50	50	0	100	0	0	0	0	100	100	0	0	0	0	100	50	0	50	0	0	100
Vehari	15	21	64	94	3	3	3	3	94	71	16	13	0	26	74	58	29	13	16	15	69
Total	**22**	**48**	**30**	**93**	**4**	**3**	**6**	**26**	**68**	**77**	**20**	**3**	**8**	**30**	**62**	**62**	**31**	**7**	**10**	**30**	**60**

Note : L/%, M/%and H/%stands for Low, Medium and High infestation, respectively

Table 5 Chi-square test for assessing associations between various qualitative variables in Bt. cotton adoption

Factors	Chi-square statistic (χ^2)	Degrees of freedom (df)	*P*	Significant (Yes/No)
Transgene vs Varieties	2.326	2	0.312	No
Transgene vs Farmers' Education	5.229	8	0.732	No
Transgene vs Landholdings	0.725	4	0.948	No
Transgene vs American Bollworm infestation	51.632	4	1.647e-10	Yes
Transgene vs Pink Bollworm infestation	67.404	4	8.013e-14	Yes
Transgene vs Whitefly infestation	21.047	4	0.001	Yes
Transgene vs Aphid infestation	17.380	4	0.002	Yes
Transgene vs Jassid infestation	17.613	4	0.002	Yes
Transgene vs Thrips infestation	5.231	4	0.264	No
Transgene vs Mites Infestation infestation	16.123	4	0.003	Yes
Varieties vs American Bollworm infestation	0.492	2	0.782	No
Varieties vs Pink Bollworm infestation	6.430	2	0.040	Yes
Varieties vs Whitefly infestation	15.322	2	0.001	Yes
Varieties vs Aphid infestation	1.115	2	0.573	No
Varieties vs Jassid infestation	6.322	2	0.042	Yes
Varieties vs Thrips infestation	11.582	2	0.003	Yes
Varieties vs Mites Infestation	0.105	2	0.949	No

Farmer's opinion on Bt cotton

The farmer's viewpoint on efficiency of Bt technology in controlling bollworms and reducing spray cost in cotton crop was analysed and it was observed that 83% of farmers cultivating approved varieties, believed in Bt cotton's effectiveness against bollworms, while 17% hold the contrary belief. However, variation exists in different district, i.e. farmers from Bahawalpur, Faisalabad, Rahim Yar Khan, and Sahiwal unanimously agreed (100%) on Bt cotton's effectiveness against bollworms. But 50% farmers in Bahawalnagar, 33% in Toba Tek Singh and some in other districts cultivating unapproved varieties were not convinced. On the other hand, 77% of farmer cultivated unapproved varieties have faith in Bt cotton usefulness for controlling bollworms, whereas 23% expressed disbelief. All farmers cultivating unapproved varieties in Bahawalpur, Faisalabad, Rahim Yar Khan, Sahiwal and Toba Tek Singh districts unanimously believed that Bt cotton is effective against bollworms. In contrary to that, 67% farmers in Multan, 43% in Dera Ghazi Khan, 37% each in Layyah and Muzaffargarh, 33% each in Lodhran and Rajanpur, 31% in Vehari, 23% in Bahawalnagar, and 8% in Khanewal cultivating unapproved varieties are not convinced about this claim. It is evident that farmers cultivating approved varieties express higher confidence in the effectiveness of bollworm control by Bt technology as compared to those cultivating unapproved varieties (Table 6).

Similarly, examining the impact of Bt cotton on spray cost reduction revealed a complex scenario. Among farmers planting approved varieties, 33% believed that Bt technology has reduced spray costs, while majority (67%) disagree. Particularly, farmers in districts Bahawalnagar, Dera Ghazi Khan, Faisalabad, Muzaffargarh, Rajanpur, Toba Tek Singh and Vehari unanimously disagreed that Bt technology reduced the spray costs, while all farmers at Bahawalpur, Rahim Yar Khan and Sahiwal have opposite views. Likewise, among farmers cultivating unapproved varieties, 22% express confidence in reducing spray costs by introduction of Bt technology, while 78% hold opposite perspective. In Pakpattan and Bahawalpur 100% of farmers growing unapproved varieties believe in reduction of spray costs, while in Multan, Faisalabad, Khanewal, Layyah, Lodhran, Muzaffargarh, Rajanpur, Sahiwal, Toba Tek Singh and Vehari, all farmers disagreed with this notion. Overall, the analysis highlighted diverse opinions among farmers about the impact of Bt cotton on spray cost reduction (Table 6).

Discussion

In the midst of the changing agricultural technology and the persistent challenges faced by cotton farmers, our study delves into the dynamics surrounding the adoption and effectiveness of Bt cotton technology. With a focus on bollworm management and spray cost reduction, our research navigates through the perceptions and practices of farmers with diverse educational backgrounds and landholdings and revealed main factors affecting cotton farming. We unravel the complexities underlying farmer beliefs, technological advancements, and regulatory

Table 6 District-wise opinion of farmers (cultivating approved & unapproved varieties) on Bt cotton's influence on bollworm management and reduction in spray cost in Punjab, Pakistan

District	Controlled bollworms				Reduced spray cost				Total farmers
	Approved varieties		Unapproved varieties		Approved varieties		Unapproved varieties		
	Yes /%	No /%	Yes /%	No /%	Yes /%	No /%	Yes /%	No /%	
Bahawalnagar	50	50	77	23	0	100	14	86	38
Bahawalpur	100	0	100	0	100	0	100	0	36
Dera Ghazi Khan	74	26	57	43	0	100	29	71	26
Faisalabad	100	0	100	0	0	100	0	100	12
Khanewal	83	17	92	8	11	89	0	100	42
Layyah	92	8	63	37	25	75	0	100	20
Lodhran	87	13	67	33	20	80	0	100	24
Muzaffargarh	79	21	63	37	0	100	0	100	36
Multan	76	24	33	67	86	14	0	100	30
Pakpattan	83	17	75	25	50	50	100	0	10
Rajanpur	70	30	67	33	0	100	0	100	28
Rahim Yar Khan	100	0	100	0	100	0	94	6	46
Sahiwal	100	0	100	0	100	0	0	100	14
Toba Tek Singh	67	33	100	0	0	100	0	100	4
Vehari	83	17	69	31	0	100	0	100	34
Total	**83**	**17**	**77**	**23**	**33**	**67**	**22**	**78**	**400**

frameworks, aiming to chart a course towards sustainable solutions for the revitalization of the cotton crop.

We have approached farmers from all cotton growing districts of the Punjab with diverse backgrounds, i.e. possessing varying landholdings (Table 1) and different educational backgrounds (Table 2) to increase the reliability of the results (O'Connell et al. 2022). The farmers have been inquired about effectiveness of Bt technology against cotton bollworms and its impact on spray cost. Overall, 60% of the farmers have medium landholdings, 22% farmers owned large landholdings and 18% farmers possessed small landholdings (Table 1). Likewise, from education perspective, 53% farmers have matric, 22% farmers are below matric, 12% and 7% farmers have bachelor degree and master degree or above qualifications, whereas 6% farmers were uneducated (Table 2) representing a mixed population from each strata of education background and landholdings to obtain meaningful information (Swami and Parthasarathy 2020).

These farmers' opinion have been bifurcated into two categories based on cultivation of approved and unapproved varieties. The viewpoint of 83% of farmers cultivating approved varieties is that Bt cotton has controlled the bollworms effectively and 17% have opposite opinion. But among those cultivating unapproved varieties, 77% farmers think that bollworms have been controlled after introduction of Bt cotton and 23% farmers have opposite views (Table 6). These findings agrees with the study that both approved and unapproved varieties have significant Bt toxin protein level to control bollworms effectively (Spielman et al. 2017). Given that AB and PB infestation are dependent on transgenes (Table 5) and have an antagonistic relationship (Table S2), and considering that nearly all cultivated varieties (either approved or unapproved) were transgenic (Table S1), the use of these transgenic varieties is likely the primary factor in controlling bollworms (Kashif et al. 2022). Moreover, according to a previous study, unapproved varieties are as effective in controlling bollworms as approved varieties, both expressing transgenes at levels lethal to pests (Cheema et al. 2016). However, Jamil et al. (2021a, b) have contradictory viewpoint and believe that, unapproved varieties are the leading cause of resistance due to low Bt. toxin level which providing ideal environment for field evolved resistance (Ahmad et al. 2019).

In the earlier years of Bt cotton introduction, farmers were largely convinced about its efficiency to control bollworm invasions as reported in different geographies (Gore et al. 2002; Kranthi et al. 2005) and Pakistan (Arshad et al. 2009). However, with the passage of time, without adoption of some levels of refuge plants (plantation of 10% non-Bt crop as refuge) fields have evolved resistance in bollworms (Shahid et al. 2021). The situation was further aggravated due to least or no adoption of double (*Cry1Ac* and *Cry2Ab*) and triple transgene (*Cry1Ac*, *Cry2Ab* and *Vip3A*) technologies (Table 3). The double and triple transgene cotton have broad-spectrum resistance by different mode of action and corresponding receptor sites in insect gut (Chen et al. 2017; Llewellyn et al. 2007). Particularly, the *Vip3A* gene provides broad-spectrum resistance by encoding Bt toxin that disrupts the digestive system upon ingestion, ultimately leading to insect death. Unlike *Cry1Ac*, *Vip3A* gene acts through a different mode of action, making it effective against pests that may have developed resistance to *Cry1Ac*. This diversity in toxin mechanisms helps enhance the overall efficacy of Bt cotton in managing pest populations and reducing crop damage (Chen et al. 2017). Some countries swiftly adopted double and triple gene technologies in the cultivation plan, while Pakistan continues to rely solely on the initially introduced single gene (*Cry1Ac*) Bt cotton, which result in the development of resistance in the field (Tabashnik et al. 2013; Tabashnik and Carrière 2017).

Analysis of farmers' perspective about the efficacy of Bt technology in reducing spray costs has revealed that more than 50% farmers from both categories (planting approved or unapproved varieties) believe that spray cost has not been reduced upon introduction of Bt technology. Specifically, 33% of farmers cultivating approved varieties affirmed that Bt technology effectively reduces spray costs, while 67% hold a contrary viewpoint. Conversely, among farmers planting unapproved varieties, a higher percentage (78%) of farmers have expressed suspicion regarding the effectiveness of Bt cotton in reducing spray costs, with only 22% supporting this notion (Table 6). Farmers hold different views on the effectiveness of Bt cotton against bollworms and its impact on spray costs. Majority of farmers claimed that Bt cotton has successfully controlled bollworms, while they also believe that the introduction of Bt cotton has not reduced spray costs. This is attributed to the increased pressure from sucking insect pests such as whitefly, aphid, jassid, thrips, and mites (Table 4), which has led to higher spray costs instead of the anticipated reduction. The sucking pest pressure has been increased after introduction of Bt genotypes owing to the low adaptation to local agro ecological conditions (Lu et al. 2022) and narrow genetic base (Jamil et al., 2021a, b). Therefore, these varieties are more vulnerable to sucking pests compared to earlier genetically diverse varieties, thereby necessitats frequent pesticide spray and nullifys the anticipated reduction in spray costs (Arshad et al. 2009).

One significant factor influence farmers' believe on Bt technology is large scale cultivation of unapproved varieties (21.67% area). Particularly, in Bahawalnagar, Layyah and Bahawalpur districts (Fig. 1). This may be a leading cause in building farmers' perceptions about Bt. cotton's inefficiency to control bollworms and reducing spray costs, reflecting mismanagement rather than inherent flaws in the technology. Because, during the formal varietal approval process, varieties are passed through certain checks, i.e., disease & insect resistance, adaptability to different geographies, response to different climatic factors and genetic diversity from cultivated varieties (Ahmad et al. 2023a, b; Iftikhar et al. 2019). However, if a variety escape through this process and reach farmers field merely on the basis of high yield, it may be susceptible to bollworms and sucking insects (Kranthi and Stone 2020). Furthermore, approved varieties may also have mixing of non-Bt seed as reported in one of our previous study (Jamil et al., 2021a, b), supressing their genetic potential. Perhaps, all the factors explained above, underscores a deficiency on the part of cotton breeders (both public and private sectors) and regulatory bodies (such as FSC&RD), as they have not effectively regulated the supply of unapproved varieties to farmers, lacking proper check and legislative measures (Shahzad et al. 2022).

Conclusion

Different opinions among farmers on the effectiveness of Bt cotton may partly be due to cultivation of unapproved varieties. Moreover, least adoption of double and triple transgene technologies and excessive outbreaks of sucking insects particularly whitefly, jassid and thrips exacerbated the situation. To mitigate these challenges, concerted efforts from cotton breeders and regulatory bodies are imperative. Moreover, there is a need to promote and disseminate the latest Bt cotton technologies particularly *Cry2Ab* and *Vip3A* genes among farmers on large scale for dissemination of broad-spectrum resistance against bollworms.

Supplementary Information

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Supplementary Table S1. List of varieties cultivated at farmer fields along with their transgene and approval status.

Supplementary Table S2. Frequency Table showing the interaction between cotton type (BT and Non-BT) and various pest infestations, including American bollworm (AB), pink bollworm (PB), whitefly, aphid, jassid, and mite.

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Authors' contributions
Shahzad R, Jamil S, Rahman SU, and Iqbal MZ Conceived and designed the analysis; Shahzad R, Jamil S, and Chaudhry UF Collected the data; Shahzad R Chaudhry UF and Jamil S Contributed data or analysis tools; Shahzad R and Chaudhry UF Performed the analysis; Shahzad R and Chaudhry UF wrote the paper; Jamil S, Rahman SU, and Iqbal MZ proofread the manuscript. All authors read and approved the final version of the manuscript.

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Declarations

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Not applicable.

Consent for publication
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The authors declare that they have no competing interests.

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