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Research Article

Adaptability of Genetically Engineered *Bt* Cotton Varieties in Different Growing Regions of Ethiopia

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Cotton varieties that are high yielding and resistant to pests are required to improve production and productivity and to capitalize on the crop's enormous potential and its critical role in Ethiopia's expanding textile industry. Lack of improved cotton technology has forced farmers to recycle local varieties for ages which have become very susceptible to pests which are the major causes of very low productivity and quality of cotton in the country. Among major pests, bollworms (*Helicoverpa armigera* and *Pectinophora gossypiella*) account for 36–60% of yield losses. In the absence of genetically resistant or tolerant varieties, genetically engineered bollworm-resistant *Bacillus thuringiensis* (*Bt*) cotton has offered a great opportunity to reduce crop losses from bollworms. The objective of the study was to evaluate the efficacy of bollworm resistance and adaptability of *Bt* cotton varieties across cotton growing environments in Ethiopia and provide recommendations. Two *Bt* cotton hybrids (JKCH 1947 and JKCH 1050), one *Bt* OPV (Sudan), and three OPV conventional varieties (Weyito 07, Stam-59A, and Deltapine-90) were evaluated at seven different agro-ecologies using a randomized complete block design (RCBD) with three replications. Results showed significant differences among genotypes for yield and other traits. Hybrids JKCH 1947 and JKCH 1050 were the top high yielders under high and mild bollworm infestations, with mean seed cotton yield of 3.10 $t{\cdot}ha^{-1}$ each and lint yield of 1.20 and 1.19 $t{\cdot}ha^{-1}$, respectively, whereas the standard check Deltapine-90 (popular variety) recorded a mean seed cotton and lint yield of 2.3 $t{\cdot}ha^{-1}$ and 0.8 $t{\cdot}ha^{-1}$, respectively. Combined analysis showed that genotypes, environment, and the genotypes × environment interactions had a highly significant effect ($P < 0.05$) on fiber quality. Weyito 07 and the two hybrids (JKCH 1947 and JKCH 1050) had upper half mean fiber lengths in the range of 27.78 to 32.11 mm. For fiber strength, genotypes Weyito 07, JKCH 1050, Stam-59A, and JKCH 1947 had 33.50 g/tex, 28.59 g/tex, 28.00 g/tex, and 27.75 g/tex, respectively. The fiber quality values of the hybrids were within acceptable limits, with staple lengths ranging from 27.78 to 28.44 mm and fiber strengths ranging from 27.75 to 28.59 g/tex. Results show potential adaptation of the hybrids under different cotton growing environments and their superior yield performance due also to added protection of yield losses from damage by bollworms. The contrast is bigger under high insect pressure conditions due to the genetically engineered *Bt* trait compared to the conventional varieties. The effective field resistance against bollworms in most locations shows that wider use of these hybrids can enhance cotton productivity and quality in Ethiopia.

1. Introduction

Cotton (*Gossypium hirsutum* L.) is the most important industrial fiber crop in Ethiopia. The culture of cotton production and use in Ethiopia has been deeply rooted since ancient times [1]. Cotton is a major cash and industrial crop extensively grown in the lowlands under large-scale irrigation schemes as well as under rain-fed conditions on small-scale farms [2]. Medium-staple cotton (*G. hirsutum* L.) accounts for more than 95% of the total production in the country [3]. Being one of the major cash crops, cotton offers considerable employment opportunity on farms, in textile factories, and in the ginneries [4]. Ethiopia has estimated 3.0 million hectares of potentially suitable cotton growing environments for cotton production [5]. Of this, less than 3% is currently cultivated with cotton [6].

The industrial development policy of the Ethiopian government has given priority to the development of the textile and garment sector, which demands a well-functioning and competitive cotton sector [7]. In Ethiopia, the total cotton consumption of the factories is showing an increasing trend every year [8]. However, the availability and quality of raw material are not satisfying the growing demand of rapidly expanding cotton-based textile industries in the country. Despite increasing demand for raw cotton, production has been declining from time to time, particularly since 2010/11 (Figure 1) due to various problems affecting cotton production and productivity in the country.

Even though cotton is the main fiber crop that plays a key role in the economic development of many countries in the world [10], the growth, yield, and fiber quality of cotton are constrained by several abiotic and biotic factors [11–14].

Among the biotic factors are insect pests which are the primary causes of yield and quality losses. Based on a field survey conducted from 1986/87 to 1995/96, more than 60 insect and 2 mite species were reported from cotton fields in Ethiopia [15, 16]. Among these insect pests, African bollworm (ABW) (*Helicoverpa armigera*) and pink bollworm (*Pectinophora gossypiella*) were shown to be key pests of cotton, severely limiting production [15, 16].

Bollworms cause about 36–60% yield loss [17–19]. Significant efforts have been made to control the key pests through the use of chemical pesticide applications, which have proven ineffective. Producers spray a minimum of five to nine insecticidal applications without promising results, and no germplasm has proven resistant against the major pests, which are bollworms. In large farms, pest control costs amount to 30–40% of the total production costs. The repeated chemical spray not only triggers resistance development in the target pest, but it has also been noted that cotton growers usually use the most hazardous (toxic) pesticides without applying appropriate personal protection measures that have significant health and environmental effects.

Global adoption of bollworm resistance *Bt* trait-containing cotton varieties began in 1996, when the first cotton transgenic with the *Cry1Ac* transgene conferring bollworm resistance was approved for cultivation in the United States after several years of research [20, 21]. Other *Cry* genes identified in the subsequent years such as *Cry1*, *Cry2*, *Cry9*, and *Cry13* were found effective against lepidopterous pests. Whereas, *Cry3*, *Cry7*, and *Cry8* proteins were effective against coleopterans, and *Cry4*, *Cry10*, and *Cry11* were effective against dipterous pests (Pathania et al., 2019). Rapid global adoption occurred to this and other Cry gene containing varieties developed periodically including those containing double Cry genes due to the effective protection provided to the plant from pest attack and the proven safety and benefit brought to farmers and the environment. The benefits to farmers are reflected in a higher yield and lower cost without the need for the expensive and hazardous chemicals used to control bollworms. [23]. Ethiopia's Biosafety Bill was passed in 2015, and global progress has been one of the driving factors for the Ethiopian Government to acquire *Bt* cotton varieties that have been approved for cultivation by national authorities in India and Sudan and have proven safety and efficacy in insect pest control for more than ten years. The *Bt* cotton hybrids obtained from India and Sudan contain truncated *Cry1Ac* gene which has registered very high mortality of bollworms. India ranks first in cotton acreage (12.4 m·ha), occupying about 38% of the global area (32.6 m·ha) [9, 24]. *Bt* cotton is planted by 7.7 million smallholder farmers with an adoption rate of more than 96% [25]. Sudan ranks first in the sub-Saharan Africa in *Bt* cotton production, covering about 0.24 million ha [26]. This paper is the first report on the performance of two commercial genetically engineered *Bt* hybrids, JKCH 1947 and JKCH 1050, developed by JK Agri Genetics Ltd. (JKAL) in India, as well as one open pollinated *Bt* cotton variety, Sudan, tested and evaluated across Ethiopia's cotton growing regions. The two *Bt* cotton hybrids and one open pollinated *Bt* variety were selected by the Ethiopian Institute of Agricultural Research (EIAR) because of familiarity with the cultivars' performance, efficacy of the trait, and relative similarity of growing conditions where the cultivars were developed and grown. Thus, decision was made to access seed from India and Sudan for a confined field testing in Ethiopia.

2. Materials and Methods

2.1. Genotypes and Locations. Two *Bt* cotton hybrids: JKCH 1947 and JKCH 1050 engineered with a truncated *Cry1Ac* gene developed by JK Agri Genetics Ltd. (JKAL), India, and one open pollinated variety (Sudan) containing *Cry1A* gene obtained from Sudan were introduced by Ethiopian Institute of Agricultural Research (EIAR) for evaluation and release for commercial production in Ethiopia. The three *Bt* varieties and three OPV conventional cotton varieties (Weyito 07, Stam-59A, and Deltapine-90 or DP-90) that were released earlier from the National Cotton Research Program of EIAR in Ethiopia and are under current production were used in the experiment. The conventionally improved varieties were thus used as standard checks in the evaluation of the two *Bt* hybrids and an OPV.

This study was conducted during the 2017 cropping season at seven different cotton growing agro-climatic locations (Figure 2), with altitude (m.a.s.l.) and rainfall (mm

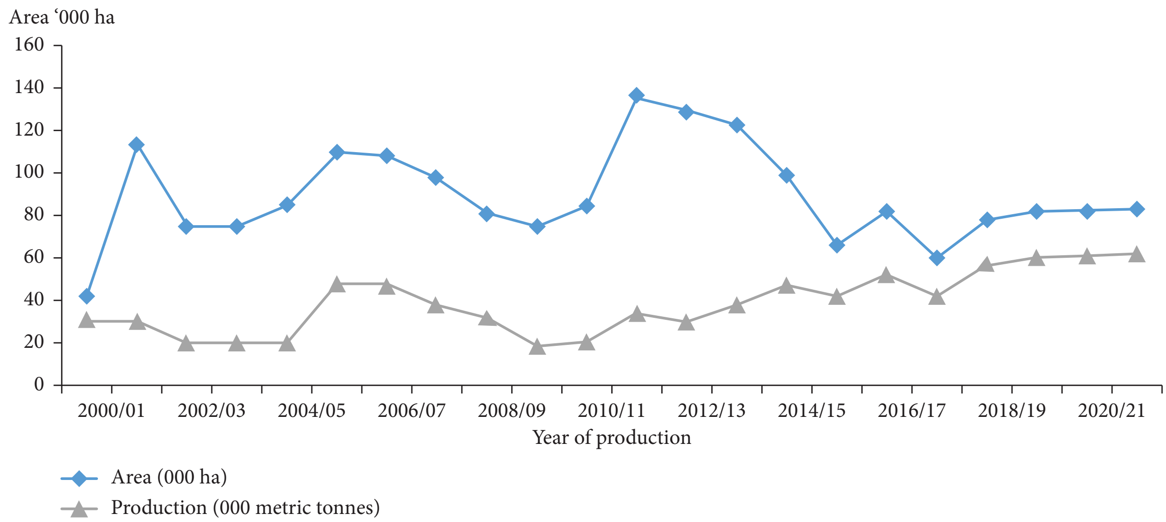

Figure 1: Cotton acreage (‘000 ha) and production (‘000 MT) in Ethiopia since 2000/01 (source: [9]).

per annum) shown in parenthesis: Werer Agricultural Research center (WARC) station (740; 500–1000); Blen (722; 500–1000); Gewane (587; 300–500); and Asayita (351; 0–300) in the Afar Region; Omorate (372; 500–1000) at Southern Nations, Nationalities, and People’s Region (SNNPR); Kamashi (1216; 1600) at Benishangul–Gumuz Region; and Humera (699; 500–1000) in Tigray Region. The respective maximum and minimum average annual temperatures (°C) of Werer, Blen, Gewane, Asayita, Omorate, Kamashi, and Humera were (35.2, 16.8), (34.9, 16.6), (31.5, 14.7), (39.6, 20.0), (37.9, 21.9), (28.2, 13.1), and (34.9, 20.6), respectively. The trials were conducted under rain-fed conditions at Kamashi and Humera and under irrigation system in all the remaining locations.

2.2. Experimental Design and Management. The trial with the six genotypes was arranged in a randomized complete block design with three replications at all seven locations. The spacing was 90 cm × 60 cm between rows and plants for the two *Bt* cotton hybrid varieties (JKCH 1050 and JKCH 1947) and 90 cm × 20 cm between rows and plants for the remaining four OPV cotton genotypes, with a plot size of 5 rows × 5 meters × 0.9 meters = 22.5 m^2. A total of 175 kg of nitrogen (N) and 80 kg of phosphorus (P) per hectare of fertilizer rate were applied to the plots. Nitrogen was applied in three splits at the sowing, squaring, and peak flowering stages. Phosphorus was applied as a basal dose at planting. No chemical pesticides were applied for bollworm control. Special measures similar to a confined field trial were taken for the field trials to meet any additional requirements of safety procedures.

2.3. Variables and Data Recorded. All important agronomic and insect pest data were collected at field level from each plot of all the different genotypes, including standard checks and locations. Data collected include African bollworm (ABW) (*Helicoverpa armigera*) and pink bollworm (*Pectinophora gossypiella*) infestation larvae count, number, and damage to fruiting bodies (squares, flowers, and bolls) at intervals, number of damaged bolls per plant, and number of larvae per plant in 10 randomly selected plants per plot. Yield and quality data included seed cotton and lint yield, ginning percentage, yield components (boll numbers and boll weight), and fiber characteristics such as fiber length, strength, and fineness, and other agronomic and morphological characteristics such as flowing date, boll setting and boll opening date, and plant height were collected. Ginning percentage was calculated as weight of lint/weight of seed cotton × 100.

2.4. Statistical Analysis. Data were analyzed using SAS software to evaluate the adaptability, yield, and quality response of cotton cultivars carrying the *Cry1Ac* traits versus the conventional varieties in different locations. For statistical analysis, the locations were treated as random and the genotypes were treated as fixed effects, and a mixed-effects model ANOVA was used. The following ANOVA model has been used to test the performance of genotype (G) at each location or environment (E): $Yij = \mu + Gi + Bj + eij$, where Yij = observed value of genotype *i* in block *j*; μ = grand mean of the experiment; Gi = the effect of genotype *i*; Bj = the effect of block *j*; and eij = error effect of genotype *i* in block *j*. After testing the homogeneity of error variance for each location, a combined analysis of variance was performed over seven locations. The variance for variety was broken down into three components: G, E, and GXE interaction (GEI) effects using the equation: $Yijk = \mu + Gi + Ej + GEij + Bk\ (j) + eijk$, where Yijk = observed value of genotype *i* in block *k* of environment *j*, μ = grand mean of the experiment, Gi = the effect of genotype *i*, Ej = environment or location effect, GEij = the interaction effect of genotype *i* with environment *j*, Bk (*j*) = the effect of block *k* in location *j*, and eijk = error (residual) effect of genotype *i* in block *k* of environment *j*.

3. Results

3.1. Yield-Related Parameters (Plant Height, Boll Number per Plant, and Boll Weight). The results of the analysis of variances showed that boll number per plant (BOLPP) varied

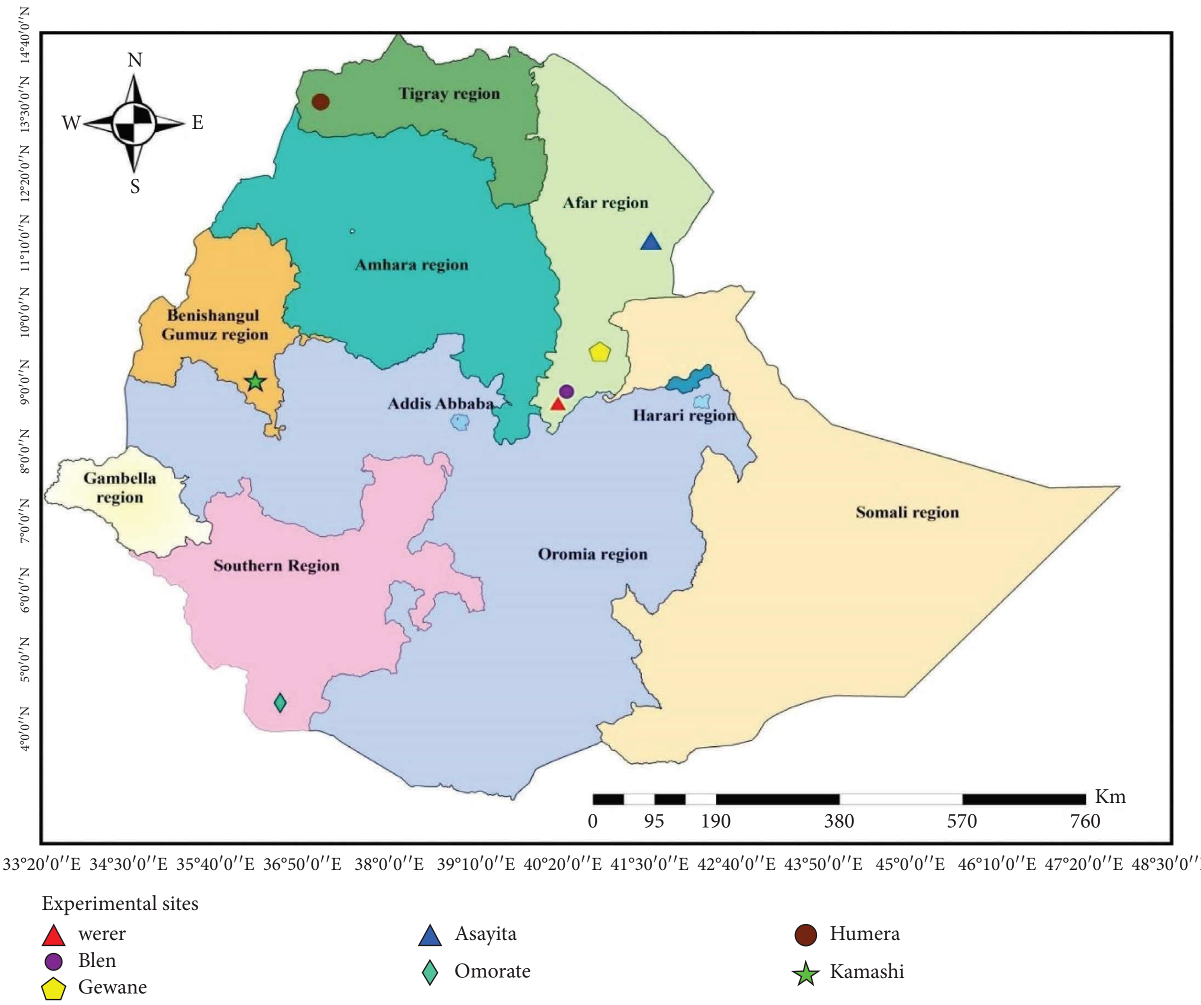

Figure 2: Experimental sites for the *Bt* cotton and non-*Bt* conventional variety evaluation in the cotton growing regions of Ethiopia.

highly significantly ($P < 0.05$) among tested cotton genotypes in all the locations except Kamashi. The mean performance of BOLPP of tested cotton genotypes across the environment ranged from 6.5 to 57.8. The highest mean BOLPP (57.8) was recorded at Gewane, while the lowest mean BOLPP (6.5) was recorded at Kamashi. The *Bt* cotton hybrids JKCH 1050 and JKCH 1947 scored the highest BOLPP throughout all the locations (Table 1).

The analysis of variance for boll weight (BOLWT) indicated significant differences ($P < 0.05$) among genotypes at all locations except at Humera, where no statistical difference was observed among genotypes (Table 1). The results ranged from 4.02 to 6.03 grams. The two GM hybrid *Bt* cotton varieties had higher mean performance for BOLWT than the standard checks in all locations except Humera.

3.2. Seed Cotton Yield and Ginning Percentage. Results show that seed cotton yield (SCY) was significantly different ($P < 0.05$) among genotypes in four out of seven locations (Blen, Gewane, Kamashi, and Werer) (Table 2). The mean SCY among genotypes ranged from 0.30 $t{\cdot}ha^{-1}$ for Weyito 07 (locally released variety) to 4.8 $t{\cdot}ha^{-1}$ in JKCH 1947, and both yields were recorded at the same location, Gewane. Such contrasting records occurred at Gewane because of a severe pink bollworm infestation at this site during the trial season, which affected the performance of all the conventional varieties (Figures 3(a)–3(c) and 4(a)–4(c)). There was a change in performance rank of genotypes from one location to the other suggesting the presence of crossover interaction (Table 2). For example, genotype JKCH 1947 had high SCY at Gewane (4.77 $t{\cdot}ha^{-1}$) followed by Blen (3.54 $t{\cdot}ha^{-1}$), Omorate (3.3 $t{\cdot}ha^{-1}$), and Kamashi (1.81 $t{\cdot}ha^{-1}$), while genotype JKCH 1050 had high SCY at Humera (1.17 $t{\cdot}ha^{-1}$). The genotype from Sudan was superior at Asayita (4.45 $t{\cdot}ha^{-1}$) and genotype Weyito 07 was superior at Werer (3.8 $t{\cdot}ha^{-1}$) (Table 2). SCY was also very low under rain-fed conditions for all the varieties, but the two *Bt* hybrid varieties performed better under rain-fed conditions.

Genotype variation for ginning percentage (GINPCT) has shown significant variation among genotypes (Table 2). JKCH 1947 had the highest GINPCT (42.8%) at Omorate, and 36.0% at Asayita, whereas JKCH 1050 was superior at Werer (39.86%). Both *Bt* cotton hybrid varieties had the highest GINPCT consistently across the test environments.

Table 1: Performance of *Bt* cotton and local improved genotypes for boll number per plant (BOLPP) and boll weight (BOLWT) at seven irrigated and rain-fed locations in Ethiopia.

Variety	Irrigated						Rain-fed				Irrigated			
	Asayita		Blen		Gewane		Humera		Kamashi		Omorate		Werer	
	BOLPP	BOLWT (gm)	BOLPP	BOLWT (gm)	BOLPP	BOLWT (gm)	BOLPP	BOLWT (gm)	BOLPP	BOLWT (gm)	BOLPP	BOLWT (gm)	BOLPP	BOLWT (gm)
Sudan	22.5b	4.5ba	15.1bc	4.7bac	50.8a	4.6b	6.8c	4.1a	12.2ba	5.2b	14.3b	3.6ba	17.1b	4.3bc
JKCH 1947	48.4a	5.0a	27.8a	5.0a	56.2a	5.5a	17.7b	3.7a	15.2a	5.7ba	24.5a	4.0a	32.6a	5.0a
JKCH 1050	46.0a	4.9a	25.4a	5.2a	57.8a	5.0ba	21.1a	4.1a	15.0a	5.5ba	26.1a	3.9a	36.1a	5.2a
Weyito 07	27.2b	3.9b	21.0ba	3.6c	22.0b	3.2c	9.0c	3.9a	13.2ba	4.7c	13.4b	3.1b	20.7b	4.0c
Stam-59A	22.8b	4.7a	11.9c	3.8bc	22.3b	3.2c	6.5c	3.6a	11.4ba	5.2b	13.1b	3.7ba	19.7b	4.7ba
DP-90	22.8b	4.5ba	9.3c	4.9ba	31.3b	3.1c	7.1c	4.2a	10.5b	6.0a	17.6ba	3.4b	16.6b	4.8ba
Mean	31.6	4.6	18.4	4.5	40.0	4.1	11.3	3.9	12.9	5.4	18.2	3.6	23.8	4.7
CV	13.5	7.7	26.7	14.0	23.5	8.4	13.3	9.4	17.1	5.6	27.1	8.8	10.1	8.4
Significance	***	*	**	*	**	***	***	NS	NS	**	*	*	***	*
LSD	7.62	0.65	8.94	1.16	17.11	0.63	2.75			0.55	8.97	0.58	4.37	0.71

*Significant at $P < 0.05$; **highly significant at $P < 0.01$; ***very highly significant at $P < 0.001$. NS = non-significant. Means that do not share the same letter are significantly different at 5% level.

Table 2: Mean seed cotton yield ($t \cdot ha^{-1}$) and ginning percentage of *Bt* cotton and local improved genotypes at different cotton growing areas in Ethiopia.

Variety	Locations													
	Irrigated						Rain-fed				Irrigated			
	Asayita		Blen		Gewane		Humera		Kamashi		Omorate		Werer	
	A ($t \cdot ha^{-1}$)	B (%)	A ($t \cdot ha^{-1}$)	B (%)	A ($t \cdot ha^{-1}$)	B (%)	A ($t \cdot ha^{-1}$)	B (%)	A ($t \cdot ha^{-1}$)	B (%)	A ($t \cdot ha^{-1}$)	B (%)	A ($t \cdot ha^{-1}$)	B (%)
Sudan	4.5a	35.4ba	2.1b	41.8a	3.4b	40.1a	0.8b	36.7a	1.2c	40.4ba	2.7ba	40.2b	2.5c	37.9ba
JKCH 1947	4.2a	36.0a	3.5a	42.4a	4.8a	38.0ba	0.9b	36.9a	1.8a	41.4ba	3.3a	42.8a	2.9bc	38.1ba
JKCH 1050	4.0a	35.2ba	3.4a	41.1ba	4.7a	38.6ba	1.2a	36.0a	1.8a	41.6a	3.0ba	42.3a	3.3ba	39.9a
Weyito 07	4.4a	31.1d	2.1b	35.6d	0.3c	36.7ba	1.0ba	33.5b	1.7ba	37.6c	3.0ba	35.2d	3.8a	33.8d
Stam-59A	4.4a	33.2c	1.8b	38.9bc	0.4c	36.2b	0.9ba	33.8b	1.4bc	40.8ba	2.5ba	36.7c	3.5ba	36.0bc
DP-90	4.7a	34.2bc	2.5b	38.4c	1.0c	35.2b	1.1ba	36.7a	1.4bc	39.6b	2.3b	37.9c	3.2bac	35.5dc
Mean	4.4	34.2	2.6	39.7	2.4	37.5	1.0	35.6	1.5	40.2	2.8	39.2	3.2	36.9
CV	17	2	17.5	3.26	22.3	5.2	15.9	3.11	12.5	2.6	17.3	1.9	13.4	3.3
Significance	NS	***	**	***	***	NS	NS	*	*	**	NS	***	*	**
LSD		1.24	0.82	2.35	0.98			2.02	0.35	1.88		1.4	0.78	2.18

*Significant at $P < 0.05$; **highly significant at $P < 0.01$; ***very highly significant at $P < 0.001$. NS = non-significant; A = seed cotton yield; B = ginning percentage (%). Means that do not share the same letter are significantly different at 5% level.

(a) (b) (c)

Figure 3: Cotton showing full bloom and contrasting performance between *Bt* cotton hybrids ((a) JKCH 1050 and (b) JKCH 1947) and a widely grown cultivar DP-90 (c) at experimental plots at Gewane, Ethiopia.

(a) (b) (c)

Figure 4: Pink bollworm infestations on standard checks: (a, b) before boll opening and (c) at boll opening stage at Gewane in rift valley in Ethiopia.

The *Bt* cotton OPV from Sudan had comparable results with the hybrid varieties across test environments. The conventional varieties recorded low GINPCT almost consistently across the test environments, except at Gewane, Humera, and Kamashi (Table 2).

3.3. Infestation of Bollworms. Natural infestation records showed that damage due to bollworms was very high at Gewane, followed by Blen, where infestations occurred lately in the growing season. A high number of damaged bolls per plant occurred in all the conventional varieties at the two locations, but more so at Gewane, which is also a high potential area for cotton production. Damaged bolls per plant were low on the *Bt* cotton varieties due to an effective field resistance level against the bollworm (Figures 5(a) and 5(b)). Although records showed some infestation on the *Bt* varieties, this did not result in damaged bolls or yield loss. At both locations, the number of bolls per plant was higher for all varieties due to favorable growing conditions, but most of the matured bolls in the conventional varieties were affected by bollworms and were not open or only partially open. Natural bollworm infestations were very low in the remaining five locations. Overall, the damage due to pink bollworm larvae on conventional varieties at Gewane and Blen was high and led to very low yields and poor fiber qualities (Tables 2 and 3; Figures 5(a) and 5(b)).

3.4. Lint Yield. In terms of lint yield (LNTY), there were significant differences ($P < 0.05$) among the mean performances of the tested genotypes at Blen, Gewane, Kamashi, and Omorate but non-significant at Asayita, Humera, and Werer (Table 3). Nevertheless, the highest mean lint yield was recorded at Asayita followed by Werer, Omorate, and Blen. The mean LNTY across environments ranged from 0.3 $t{\cdot}ha^{-1}$ at Humera to 1.8 $t{\cdot}ha^{-1}$ at Gewane. The minimum LNTY was recorded at Humera because of shortage of rainfall during the season, and the maximum LNTY was recorded at Gewane for favorable environment during the cropping season. JKCH 1947 had high mean performance of 1.8 $t{\cdot}ha^{-1}$ at Gewane, 1.5 $t{\cdot}ha^{-1}$ at Blen, 1.4 $t{\cdot}ha^{-1}$ at Omorate, and 0.8 $t{\cdot}ha^{-1}$ at Kamashi. JKCH 1050 had high mean performances of 1.3 $t{\cdot}ha^{-1}$ and 0.4 $t{\cdot}ha^{-1}$ at Werer and Humera, respectively.

Genotype DP-90, a widely grown variety used as a standard check, recorded a numerically high lint yield (1.6 $t{\cdot}ha^{-1}$) at Asayita, but this was not statistically different from the other tested genotypes. The mean lint yield across locations showed both JKCH 1947 and JKCH 1050 to be

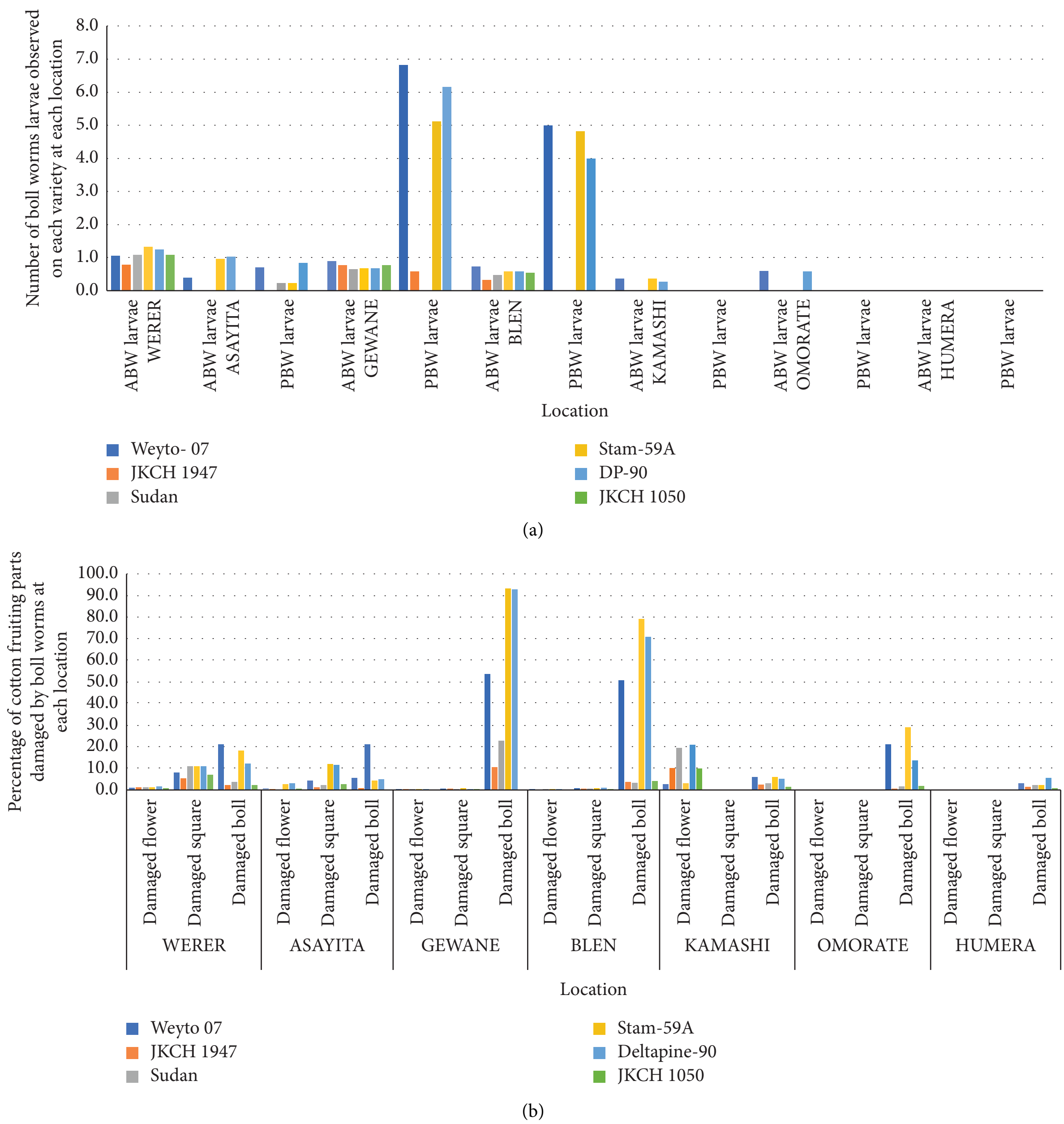

FIGURE 5: Bollworm infestation level on each variety across all testing locations in Ethiopia: (a) number of bollworm larvae infesting cotton fruiting parts; (b) percentage of cotton fruiting parts damaged by bollworms.

superior (1.2 and 1.9 t·ha^{-1}, respectively), followed by *Bt* OPV from Sudan (0.94 t·ha^{-1}).

3.5. Combined ANOVA. Results of the combined ANOVA indicated highly significant variation among genotypes, environments, and GEI for BOLPP, BOLWT, SCYLD, GINPCT, and LNTY. Genotypes JKCH 1050 and JKCH 1847 recorded higher BOLPP (32.5 and 31.7, respectively) as well as higher BOLWT (4.84 and 4.83 g, respectively) than conventional standard checks.

A combined ANOVA indicated highly significant variation among genotypes, environments, and GEI, such that JKCH 1947 (3.06 t·ha^{-1}) and JKCH (3.05 t·ha^{-1}) expressed higher SCYLD than the standard checks Weyito 07 (2.33 t·ha^{-1}), DP-90 (2.30 t·ha^{-1}), and Stam-59A (2.12 t·ha^{-1}), while the *Bt* OPV from Sudan (2.44 t·ha^{-1}) gave comparable seed cotton yield with the standard checks across locations.

Overall ANOVA showed significant differences among genotypes, environments, and GEI for GINPCT and LNTY, such that JKCH 1947 and JKCH 1050 had higher mean GINPCT of 39.37 and 39.23, respectively, than the standard checks (Table 4). JKCH 1947 and JKCH 1050 also had the highest LNTY, 1.20 t·ha^{-1} and 1.19 t·ha^{-1}, respectively. The non-*Bt* local OPVs had LNTY of 0.84 t·ha^{-1} (DP-90), 0.79 (Weyito 07), and 0.76 t·ha^{-1} (Stam-59A) (Table 4).

Table 3: Mean performance of lint yield of tested *Bt* cotton and local improved genotypes at different cotton growing areas in Ethiopia.

Variety	Lint yield (t ha^{-1}) Locations							
	Asayita	Blen	Gewane	Humera	Kamashi	Omorate	Werer	Mean
Sudan	1.6a	0.9b	1.4b	0.3c	0.5b	1.1bc	0.9b	0.9
JKCH 1947	1.5a	1.5a	1.8a	0.3bc	0.8a	1.4a	1.1ba	1.2
JKCH 1050	1.4a	1.4a	1.8a	0.4a	0.7a	1.3ba	1.3a	1.2
Weyito 07	1.4a	0.8b	0.1c	0.4bac	0.6ba	1.0bc	1.3a	0.8
Stam-59A	1.5a	0.7b	0.1c	0.3bc	0.6b	0.9c	1.3a	0.8
DP-90	1.6a	1.0b	0.4c	0.4ba	0.6b	0.9c	1.1ba	0.8
Mean	1.5	1.0	0.9	0.3	0.6	1.1	1.2	1.0
CV	17.8	19.6	23.6	14.4	14.3	16.1	13.4	
Significance	NS	**	***	NS	*	*	NS	
LSD		0.367	0.399		0.160	0.321		

*Significant at $P < 0.05$; **highly significant at $P < 0.01$; ***very highly significant at $P < 0.001$. NS = non-significant. Means that do not share the same letter are significantly different at 5% level.

3.6. Quality Performance at Individual Location. Length, strength, and micronaire are the three most important properties of fiber qualities. For the fiber properties analyzed, that is, fiber micronaire, strength, and length (upper half mean length (UHML)), the acceptable ranges are shown in Table 5. Accepted values for length >27.4 mm, strength >28 g/tex, and micronaire 3.5–4.9 are described by Pretorius et al. [28] (see also Table 5). Cotton lint with a micronaire below 3.5 is usually considered immature and weak [29]. Micronaire values greater than 4.9 are less desirable as the fiber becomes too coarse for spinning. The various fiber quality results are discussed below.

3.7. Micronaire. Genotypes significantly varied ($P < 0.05$) for micronaire values at all locations except Humera and Omorate (Table 6). All micronaire values were in the acceptable range according to Chaudhry and Guitchounts [29] and the Ethiopian cotton quality specification (Table 5), except for *Bt* OPV from Sudan (5.36) and DP-90 (5.01) at Asayita, which are in the discount range as the fiber became too coarse for spinning. At Gewane, the standard checks Weyito 07 (2.79), Stam-59A (2.73), and DP-90 (2.76) had micronaire values below the acceptable range, which are considered as immature and weak fibers [29] and less desirable for spinning. In this study, micronaire values were significantly affected by the environment, which is in agreement with previous studies by Pretorius et al. [28].

3.8. Length Uniformity. Length uniformity is the ratio between the mean length of fiber and the upper half mean length expressed in percentage. Low uniformity values are a function of fibers that are more easily broken. Significant differences ($P < 0.05$) were found among genotypes for the uniformity index only at locations Blen and Gewane, where low percentages were recorded for standard checks DP-90, Stam-59A, and the *Bt* OPV from Sudan at Blen (Table 7).

3.9. Fiber Length and Strength. In terms of cotton fiber length and strength, genotypes showed significant differences ($P < 0.05$) at all locations except Omorate (Table 8). Variety Weyito 07 recorded long fiber length and very strong fiber strength that exceeded all the other varieties, including the two *Bt* cotton hybrid varieties, at all locations except Omorate. However, the fiber length of JKCH 1947 was in the acceptable range at all locations except Blen, Humera, and Omorate. The fiber length of JKCH 1050 was in the acceptable range at all locations except Kamashi. The fiber strength of JKCH 1947 was in the acceptable range at Asayita, Blen, Gewane, Kamashi, and Werer but below the acceptable range at Humera and was very marginal at Omorate. Fiber strength of genotype JKCH 1050 was within the acceptable range in all locations except at Humera.

3.10. Combined Analysis of Fiber Quality Traits. Results showed highly significant differences ($P < 0.05$) among genotypes, environment, and GEI for fiber quality parameters. According to Pretorius et al. [28], environments affected micronaire more significantly ($P < 0.05$). However, all micronaire values of genotypes in this study were in the acceptable range according to the Ethiopian cotton quality grading system specification (Table 5) and standards referred to by Chaudhry and Guitchounts [29]. Combined analysis indicated that genotypes, environment, and GEI had a very highly significant effect ($P < 0.05$) on UHML (Table 9). The fiber lengths of genotypes Weyito 07 (32.11 mm), JKCH 1050 (28.44 mm), and JKCH 1947 (27.78 mm) were in the acceptable range, while the fiber lengths of the other remaining genotypes, including the widely cultivated variety DP-90, were in unacceptable range or marginally acceptable (Table 5). In terms of fiber strength, only the genotypes Weyito 07 (33.50 g/tex), JKCH 1050 (28.59 g/tex), and Stam-59A (28.00 g/tex) were in the acceptable range according to Pretorius et al. [28], but they were considered marginally optimum by the Ethiopian cotton quality specification. Generally, most fiber quality values of the *Bt* cotton hybrids in this study were within acceptable ranges. Since they are competitive with or better in fiber quality than the standard checks, their added merits in yield and quality, in addition to their effective protection against bollworms, make them the best candidates for further expansion by farmers. The GEI,

TABLE 4: Overall mean of cotton genotypes for yield and yield-related traits tested across seven locations in Ethiopia.

Variety	Plant height (cm)	Boll no./plant	Boll weight (gm)	Seed cotton yield (t·ha^{-1})	Ginning percent	Lint yield (t·ha^{-1})	Stand count
Sudan	93.8cb	19.8b	4.4ba	2.4ba	38.9a	0.9ba	43.2b
JKCH 1947	111.7a	31.7a	4.8a	3.1a	39.4a	1.2a	29.8c
JKCH 1050	114.7a	32.5a	4.8a	3.1a	39.2a	1.2a	28.7c
Weyito 07	110.0a	18.1b	3.8c	2.3ba	34.8c	0.8b	56.5a
Stam-59A	98.9b	15.4b	4.1bc	2.1b	36.5b	0.8b	59.9a
DP-90	86.6c	16.5b	4.4ba	2.3ba	36.8b	0.8b	56.1a
Mean	102.6	22.3	4.4	2.6	37.6	1.0	45.7
CV	10.2	21.9	9.1	18.3	3.2	18.8	23.6
R^2	0.89	0.928	0.86	0.93	0.91	0.93	0.79
Genotype	***	***	***	***	***	***	***
Environment	***	***	***	***	***	***	***
Geno * ENV	***	***	***	***	***	***	Ns
LSD	10.549	6.5524	0.4761	0.923	1.23	0.343	8.3326

*Significant at $P < 0.05$; **highly significant at $P < 0.01$; ***very highly significant at $P < 0.001$. NS = non-significant. Means that do not share the same letter are significantly different at 5% level.

Table 5: Ethiopian cotton quality grading system specifications.

Specification	Grade		
	A	B	C
Staple length (mm)	≥28.5	27 – 28.5	25
Micronaire/fineness	3.5–4.2	4.3–4.9	3.2–3.4 and >5
Strength (g/tex)	≥29	26–28.9	25–25.9
Average sticky points	0–10	11–20	21–32
Short fiber content (%)	≤10	11-12	13-14
Trash content (%)	<3.5	3.5–4.5	4.6–5
Moisture content (%)	<8	<8	≤8
Maturity ratio (%)	≥85%	81–84	75–80
Length uniformity ratio (%)	≥83	81-82	76–80
Color grade	11 – 1 up to 21 – 1	21 – 1 up to 31	41 – 1 up to 51 – 4
Contamination (g/bale)	≤5	11	10–15

Source: adapted from Tiliksew et al. [27].

Table 6: Mean performance of cotton genotypes for micronaire at different cotton growing locations in Ethiopia.

Variety	Asayita	Blen	Gewane	Humera	Kamashi	Omorate	Werer
Sudan	5.36a	4.33a	3.78a	3.30bc	4.51a	4.15a	4.66ba
JKCH 1947	4.23dc	4.18a	3.99a	3.74ba	3.83b	4.20a	4.25bc
JKCH 1050	4.47dc	4.02a	3.77a	3.45bac	4.04b	3.79a	4.11c
Weyito 07	4.08d	3.13b	2.79b	3.05c	3.04c	3.81a	3.56d
Stam-59A	4.59bc	4.19a	2.73b	3.42bac	4.50a	3.91a	4.25c
DP-90	5.01ba	4.13a	2.76b	3.92a	4.65a	3.77a	4.78a
Mean	4.62	4.00	3.30	3.48	4.09	3.94	4.27
CV	5.98	5.10	4.29	9.21	5.12	6.86	5.28
Significance	**	***	***	NS	***	NS	**
LSD	0.50	0.37	0.26		0.38		0.41

*Significant at $P < 0.05$; **highly significant at $P < 0.01$; ***very highly significant at $P < 0.001$. NS = non-significant. Means that do not share the same letter are significantly different at 5% level.

Table 7: Mean performance of cotton genotypes for uniformity index at seven cotton growing locations in Ethiopia.

Variety	Locations and uniformity index values expressed as a percentage						
	Asayita	Blen	Gewane	Humera	Kamashi	Omorate	Werer
Sudan	83.4a	79.5b	81.9ba	79.6c	81.8ba	80.6b	81.7ba
JKCH 1947	83.6a	81.0b	83.0a	80.3bac	83.8a	82.1ba	81.6b
JKCH 1050	83.7a	81.8ba	82.4ba	81.5ba	79.5b	82.2a	82.9ba
Weyito 07	84.6a	83.8a	81.0bc	81.9a	84.1a	81.7ba	83.3a
Stam-59A	83.0a	80.2b	79.2dc	79.8bc	80.8ba	81.9ba	82.6ba
DP-90	83.6a	81.6ba	77.7d	79.5c	82.6ba	81.6ba	81.4b
Mean	83.7	81.3	80.9	80.4	82.1	81.7	82.3
CV	1.33	1.63	1.32	1.16	2.50	1.04	1.12
Significance	NS	*	**	NS	NS	NS	NS
LSD		2.41	1.94				

*Significant at $P < 0.05$; **highly significant at $P < 0.01$; ***very highly significant at $P < 0.001$. NS = non-significant. Means that do not share the same letter are significantly different at 5% level.

on the other hand, suggests that genotypes, including the *Bt* hybrids, responded differently to environment. However, across most locations, JKCH 1947 and JKCH 1050 had shown good adaptation and statistically similar performance for most parameters.

3.11. AMMI Biplot Analysis for Lint Yield. The results of AMMI analysis for mean LNTY are presented as genotype by environment interaction biplot in Figure 6. The plot captures GEI effects and distributes all the seven environments into four different sectors. The small angle between

Table 8: Mean performance of cotton genotypes for upper half mean length and strength at seven locations in Ethiopia.

Variety	Asayita		Blen		Gewane		Humera		Kamashi		Omorate		Werer	
	UHML	Str	UHML	Str	UHML	Str	UHML	Str	UHML	Str	UHML	Str	UHML	Str
Sudan	28.0cb	29.7b	26.3c	26.8b	28.5cb	28.4a	26.0c	22.5b	27.2cb	28.9cb	26.9a	25.6a	26.6d	26.4c
JKCH 1947	28.2cb	31.1b	26.7c	26.6b	28.4cb	27.8ba	27.3cb	24.5b	28.5b	30.2b	27.3a	25.2a	28.0cb	28.9b
JKCH 1050	28.3cb	31.5b	28.2b	27.2b	29.3b	28.8a	28.7b	25.8b	27.1c	29.3cb	29.1a	29.2a	28.3b	28.2cb
Weyito 07	34.0a	40.3a	33.8a	35.1a	31.6a	30.7a	31.5a	31.9a	32.4a	34.5a	29.0a	27.4a	32.6a	34.6a
Stam-59A	27.2c	32.3b	26.4c	26.2b	27.1c	25.2bc	26.1c	25.4b	27.4cb	29.3cb	26.8a	28.3a	28.4b	29.4b
DP-90	28.9b	29.6b	26.5c	26.1b	27.2c	22.6c	27.0cb	23.0b	27.5cb	27.5c	26.5a	25.0a	27.5c	27.6cb
Mean	29.1	32.4	28.0	28.0	28.7	27.2	27.8	25.5	28.4	30.0	27.6	26.8	28.6	29.2
CV	2.4	6.4	2.7	2.5	3.4	6.0	3.6	9.1	2.7	4.3	5.8	9.5	1.5	4.4
Significance	***	**	***	***	**	**	**	*	***	**	NS	NS	***	***
LSD	1.3	3.8	1.4	1.3	1.8	3.0	1.8	4.2	1.4	2.3	2.9	4.6	0.8	2.4

*Significant at $P < 0.05$; **highly significant at $P < 0.01$; ***very highly significant at $P < 0.001$. NS = non-significant; UHML = upper half mean length (mm); Str = strength. Means that do not share the same letter are significantly different at 5% level.

Table 9: Overall mean performance of cotton genotypes on quality parameters.

Variety	Mic	UHML	UI	Str
Sudan	4.30a	27.06c	81.21b	26.90cb
JKCH 1947	4.06a	27.78cb	82.20ba	27.75b
JKCH 1050	3.95a	28.44b	82.00ba	28.59b
Weyito 07	3.35b	32.11a	82.92a	33.50a
Stam-59A	3.94a	27.05c	81.09b	28.00b
DP-90	4.15a	27.30c	81.13b	25.92c
Mean	3.96	28.29	81.76	28.44
CV	6.10	3.36	1.52	6.32
Genotype	***	***	***	***
ENV	***	***	***	***
ENV × genotype	***	***	***	***
LSD	0.38	0.98	1.26	1.78

*Significant at $P < 0.05$; **highly significant at $P < 0.01$; ***very highly significant at $P < 0.001$. NS = non-significant; Mic = micronaire; UHML = upper half mean length; UI = uniformity index; Str = strength. Means that do not share the same letter are significantly different at 5% level.

environments Kamashi (K) and Werer (W) indicated the similarity of the two environments for lint yield. The large angle between environments Humera (H) and Gewane (G) indicated the two environments' dissimilarity. Among the locations, Gewane (G) provided the most favorable season, and JKCH 1947 and JKCH 1050 interacted with Gewane, but JKCH 1050 somehow favored at Blen. Weyito 07 (d) and Stam-59A (e) were favored at Werer and Kamashi, whereas Deltapine-90 (f) performed its best at Humera and Asayita. Among the seven environments, Omorate and Kamashi were the least favorable for all varieties.

3.12. GGE Biplot for Cotton Lint Yield. To know which genotype performed well where, the GGE biplot was generated based on the cotton LNTY, with the seven environments falling into four sectors with different cultivars, which indicated the presence of crossover GEI. Blen, Omorate, Kamashi, and Humera were grouped under one mega-environment while Gewane, Werer, and Asayita were each under a separate mega-environment.

Generally, JKCH 1050 and JKCH 1947 performed well at all environments except at Asayita and Werer (Figure 7). Genotypes e, d, and f were the low yielding at all environments as they are far from all environments.

4. Discussion

Adaptability of cotton varieties carrying the *Bt* trait, that is, their fitness to the different growing locations, is a prerequisite for its effectiveness in protecting the plant from damage by the bollworms [30]. Therefore, after the efficacy tests for the bollworm-resistant*Bt* trait, the national adaptability trial for the *Bt* cotton hybrids (JKCH 1947 and JKCH 1050) and *Bt* containing open pollinated variety was compared with improved and widely grown conventional local varieties across different cotton growing agro-ecologies in Ethiopia. The study across different agro-ecologies also allowed for the confirmation of previous efficacy findings that the *Bt* gene *Cry1Ac* protein in the two Indian hybrids and the *Cry1A Bt* trait in the Sudanese OPV variety are adequately expressed in Ethiopia's various cotton growing environments.

For bollworm infestations, at Gewane and Blen, which are among the most favorable for cotton production as well as for pest growth, infestation of both African bollworm (ABW) and pink bollworm (PBW) during the study period was higher than any of the other locations (Figures 4(a)–4(c), 5(a), and 5(b)). Nevertheless, *Bt* hybrids, JKCH 1947 and JKCH 1050, including the open pollinated *Bt* variety, have shown 4–10 folds increase having seed cotton yield range of 3.4–4.8 t ha-1 which is more than the conventional varieties, which had seed cotton yield range between 0.3–1.0 t ha-1 at Gewane. The performance at Gewane was a genuine reflection of the whole evaluation, which showed a phenomenally superior performance of the *Bt* varieties. The *Bt* gene was adequately expressed in the two hybrids and the OPV in the cotton growing environments and had sufficiently protected against boll damage by both the African and pink bollworms. The level of protection in the two hybrids was better than that in the OPV from Sudan. Cotton yield was higher in *Bt* cotton hybrids due to the high number

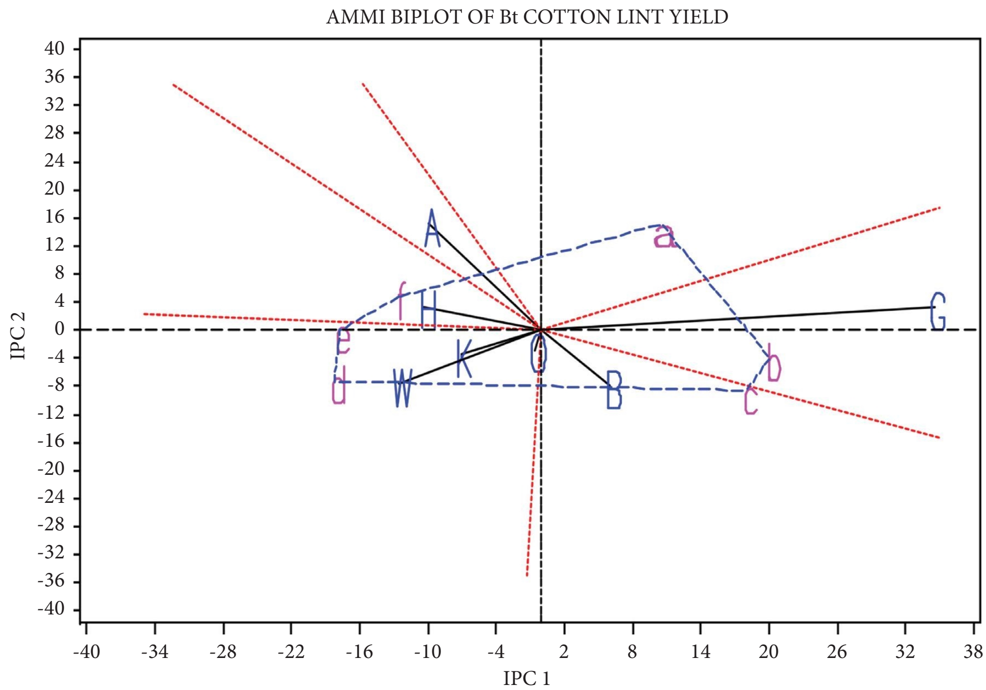

Figure 6: AMMI biplot for mean lint yield of cotton genotypes (lower case) and environments (upper case).

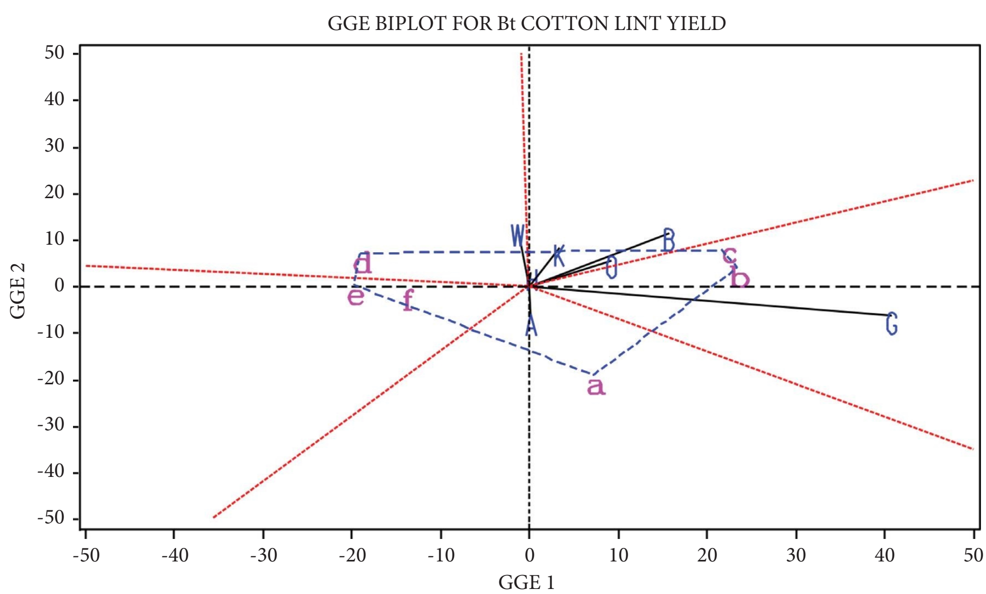

Figure 7: GGE biplot of lint yield of cotton genotypes based on the G × E analysis (lower and upper case letters showing means for genotypes and environments, respectively).

of bolls per plant and significantly less damaged bolls where infestation occurred. At Gewane and Blen, where infestation was high, the larvae population for both ABW and PBW in the *Bt* containing varieties was checked at a minimum, which effectively reduced damage due to the pests. As a result, without the use of insecticides, the *Bt* cotton hybrids enabled higher boll retention through effective pest control and protected potential yield losses due to bollworm damage. Several previous works have also shown that *Bt* cotton hybrids not only give significantly higher yields but also realize a significant reduction in chemical insecticide spray over their non-*Bt* cotton varieties [31, 32]. The efficacy performance was similar both under rain-fed and irrigated conditions, giving sufficient protection against bollworms.

The results of the mean comparison tests for the agronomic performance of *Bt* cotton hybrids, *Bt* OPV, and non-*Bt* open pollinated varieties showed that hybrids had better plant height, number of bolls, boll weight, seed cotton yield, ginning percentage, and lint yield almost in all locations when compared to the non-*Bt* conventional varieties. The results are in agreement with the findings of other researchers who concluded that F1 hybrids performed better for the majority of the traits [33, 34]. In all the seven experimental sites and under both irrigated and rain-fed conditions, JKCH 1947 and JKCH 1050 had up to 33% yield advantage over the most widely cultivated variety, Deltapine-90. However, comparison in the absence of pest incidence as it happened in most locations is bound to underestimate the potential advantage of the *Bt* hybrid cotton varieties against bollworms. The ginning percentage of the two *Bt* hybrids (39.2–39.4%) was very comparable among each other but higher than the local conventional varieties (34.8–37.6%). Similar superior results have been reported for hybrid derived *Bt* cotton varieties in earlier studies [35, 36]. Seed cotton yield was heavily affected by the type of production, i.e., rain-fed or irrigated. All varieties significantly underperformed in rain-fed conditions, while the hybrids had relatively higher seed cotton yields.

The analysis of variance for most variables showed that boll number per plant, boll weight, seed cotton yields, ginning percentage, and lint yield were significantly affected by genotype, environment, and genotype by environment interaction (GEI). The *Bt* cotton hybrids JKCH 1050 and JKCH 1947 had a higher average boll number per plant, boll weight, seed cotton yield, and ginning percentage than the conventional controls. Results further demonstrated that 80.30%, 56.80%, and 27.10% of the variation in cotton seed yield were mainly due to the variation in bolls per plant, plant height, and boll weight, respectively [37]. The final yield of a cotton cultivar is determined by the number of bolls per plant, plant height, and the morphological framework of the plant, which is influenced directly or indirectly by the growing conditions and its genetic ability to perform in the given environmental conditions [38, 39]. The hybrids outperformed the different non-hybrid cotton genotypes in yields and other physiological traits under normal and heat stress, supporting the concept of hybrid adaptability across stress environments [40, 41]. Other findings reported that hybrids were found to be more stable and performed better than conventional genotypes under optimal and deficit water (irrigation) conditions and concluded that the genotypic and phenotypic variances for various traits were greater under water deficit conditions than under the optimal irrigation regime [42]. This is true because the two *Bt* cotton hybrids have performed better at all locations and have also expressed their higher yield potential even under water stressed, rain-fed, and other stress conditions. This has proven the competitive and promising nature of the hybrids containing *Bt* trait for high yield and strong adaptability to stressed growing conditions. On the other hand, the significant influence of rain-fed growing condition on the yield of all genotypes shows the importance of drought tolerant varieties for such agro-ecologies and farming conditions.

Fiber characteristic values for fineness (micronaire) showed that genotypes, environment, and the genotypes × environment interaction had a very highly significant effect. This result is in agreement with previous works which reported that micronaire was affected more by environments [28]. But, all micronaire values of the tested cotton varieties in this study were in the acceptable range according to Chaudhry and Guitchounts [29]. For fiber length, uniformity, and strength, genotypes, environment, and the genotypes × environment interaction had a very highly significant effect. The two hybrids and the standard checks under all environments show that the *Bt* hybrids had closely similar fiber quality with UHML and fiber strength values being exceeded only by Weyito 07, which had 32.11 mm and 33.5 g/tex, respectively, compared to 27.78 mm and 28.44 mm of fiber length and 27.75 g/tex and 28.59 g/tex of fiber strength, respectively, for JKCH 1947 and JKCH 1050. But all fiber characteristic values of the two *Bt* cotton hybrid varieties were not within the acceptable range of the quality standards issued by the Ethiopian Textile Industry Development Institute (ETIDI) in Ethiopia (Table 5).

The AMMI and GGE models were used to analyze lint yield data for the tested cotton varieties in seven environments. Environment as a main effect contributed to most of the variability for this trait. Both JKCH 1947 and JKCH 1050 recorded the highest mean lint yield and interacted and performed best at most environments. Another study of lint yield trait in cotton by Shahzad et al. [43] also reported that hybrids outperformed in a wide range of environments than inbred lines. Among the seven environments, Gewane was the most favorable. The two *Bt* hybrids, JKCH 1947 and JKCH 1050, performed well at all environments except at Asayita and Werer, and other non-*Bt* cotton varieties were low yielding at all environments as they are far from all environments on GGE biplot.

These results showed that the *Bt* containing hybrid varieties are well adapted to the cotton growing agro-ecologies in Ethiopia. This could be due to the broad adaptability of the two *Bt* cotton hybrid varieties. Studies with the same *Bt* cotton hybrids from JK Agri Genetics Ltd., India, have shown good adaptability of both *Bt* hybrids (JKCH 1050 and JKCH 1947) and successful commercialization in the cotton growing environments in Sudan [44] and Eswatini [31, 32]. The superior performance of the *Bt* cotton varieties in terms of number of bolls per plant and seed cotton yield across locations compared to the standard local varieties in Ethiopia is attributable to the successful control of bollworms without any chemical pesticide spray and the adaptability of the varieties to the different cotton agro-ecologies in the country. The adapted hybrids and similar *Bt* products appear to be a great promise for the potential opportunity of cotton improvement in Ethiopia. There is a need however to strategize the seed access issue by smallholder farmers through forming strong public private partnerships to strengthen the current poor cotton seed system [45, 46]. This is very critical in view of the growing role of the textile industry in the country's economy. More work is needed to test more varieties, including second-generation*Bt* cotton products, as well as the introgression of such traits into the most adapted local varieties, for better results. Moreover, the possibility of resistance development of targeted species and potential emergence of challenges from currently less important pests need to be seriously considered through integrating appropriate resistance management strategies [47, 48]. The health benefits of *Bt* cotton due to significantly reduced chemical insecticide use are an added benefit to the growers over the non-*Bt* cotton varieties that require extensive chemical insecticide application [31, 32, 49].

5. Conclusion

The transgenic *Bt* cotton hybrids provided superior protection against bollworm infestation, resulting in a higher number of bolls per plant, which helped to increase cotton yield across all tested environments. The use of the hybrid *Bt* cotton varieties not only resulted in significantly higher yields but also showed a reduced insecticidal usage which has huge implication for large acreage cotton production. As a result, the two *Bt* cotton hybrids (JKCH 1947 and JKCH 1050) were recommended for wider commercial use in Ethiopia due to their safety, superior performance in controlling bollworms, broader adaptability, and higher yields. Maximizing benefits from a sustained use of the resistant hybrids can be achieved through integrating appropriate resistance management strategies along with a wider adoption of the technologies.

Data Availability

The data that support the findings of this study are available from the corresponding author upon request

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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