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Influence of Transgenic (*Bt*) Cotton on the Productivity of Various Cotton-Based Cropping Systems in Pakistan

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Abstract: Cotton (*Gossypium hirsutum* L.) is an important fiber crop in Pakistan with significant economic importance. Transgenic, insect-resistant cotton (carrying a gene from *Bacillus thuringiensis* (*Bt*)) was inducted in the cotton-based cropping systems of Pakistan during 2002, and is now sown in >90% of cotton fields in the country. However, concerns are rising that *Bt* cotton would decrease the productivity of winter crops (sown after cotton), leading to decreased system productivity. This two-year field study determined the impacts of transgenic (*Bt*) and non-transgenic (non-*Bt*) cotton genotypes on the productivities of winter crops (i.e., wheat, Egyptian clover, and canola), and the overall productivities of the cropping systems including these crops. Four cotton genotypes (two *Bt* and two non-*Bt*) and three winter crops (i.e., wheat, Egyptian clover, and canola) were included in the study. Nutrient availability was assessed after the harvest of cotton and winter crops. Similarly, the yield-related traits of cotton and winter crops were recorded at their harvest. The productivities of the winter crops were converted to net economic returns, and the overall economic returns of the cropping systems with winter crops were computed. The results revealed that *Bt* and non-*Bt* cotton genotypes significantly ($p < 0.05$) altered nutrient availability (N, P, K, B, Zn, and Fe). However, the yield-related attributes of winter crops were not affected by cotton genotypes, whereas the overall profitability of the cropping systems varied among the cotton genotypes. Economic analyses indicated that the *Bt* cotton–wheat cropping system was the most profitable, with a benefit–cost ratio of 1.55 in the semi-arid region of Pakistan. It is concluded that *Bt* cotton could be successfully inducted into the existing cropping systems of Pakistan without any decrease to the overall productivity of the cropping system.

Keywords: *Gossypium hirsutum*; Egyptian clover; canola; wheat; economic analyses

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1. Introduction

Cotton (*Gossypium hirsutum* L.) is the most important fiber crop grown in Pakistan. It serves as a foundation for Pakistan's economy [1]. Pest infestations exert negative effects on cotton productivity, as >160 insect pests infest cotton at various growth stages [2,3]. Subsequently, cotton farmers incur significant amounts of money on pesticides to combat the pest infestation [2,4,5]. Farmers in Pakistan spend ~$300 million every year on pest control, and most of the pesticides (~80%) are sprayed in the cotton crop [6,7]. The extensive use of pesticides causes significant environmental and human health hazards [8,9]. The cultivation of transgenic, insect resistance crops can decrease the use of pesticides [10].

The use of genetically modified (GM) crops is drawing significant attention in Pakistan, due to their insect resistance abilities [11]. Transgenic cotton (commonly known as *Bt* cotton) is one of the greatest examples of GM crops, which are bollworms-resistant [12]. *Bt* cotton can reduce the damage caused by specific insects, and enhance crop productivity because of its resistance to bollworms [13]. The reduced use of insecticides and lower pest infestation are two major benefits of GM crops [14–16]. Numerous studies have shown that GM crops with *Bt* Cry proteins are resistant to various lepidopterans [17,18]. *Bt* cotton could help farmers in reducing the use of pesticides [2,5,19]. Therefore, *Bt* cotton is considered to be environmentally friendly [20,21].

There are increasing concerns on the potential negative consequences of GM crops on the soil health and productivity of other crops [22–24]. Numerous studies have revealed that the cultivation of GM crops increases the levels of *Bt* toxins in the soil [25–27]. *Bt* toxins are naturally present in the soil, and the recurrent planting of GM crops raises their concentration in the soil and alters the composition and behavior of soil microorganisms [28,29]. *Bt* toxins produced by GM crops enter the soil and could exert negative impacts on the productivity of other crops [30]. The cultivation of the *Bt* cotton line 'GK19' increased the accumulation of *Bt* proteins in the soil under salinity stress [31]. Increased levels of *Bt* toxins might exert negative impacts on agroecosystems [29] and alter the chemical composition of the root zone [27,32]. Similarly, toxins–microbe interactions significantly influence soil properties and nutrient availability. The changing rhizosphere conditions due to the cultivation of GM crops increased soil phosphorus (P) availability [33]. Furthermore, the cultivation of GM crops increases the available N and the oxidative metabolism in soil because of the enhanced activities of urease and dehydrogenase enzymes [34].

The induction of *Bt* cotton in the cotton-based cropping systems of Pakistan is being criticized due to the associated negative impacts. However, its introduction had favorable consequences on the environment and on farmers' health [35]. Cases of pesticide poisoning in farmers have decreased in China, India, and Pakistan after the introduction of *Bt* cotton [36,37]. However, the impacts of *Bt* cotton cultivation on the productivity of subsequent winter crops and the overall productivity of cropping systems have been less explored in Pakistan. Cotton is followed by wheat crop in the cotton-based cropping systems of Pakistan [38]. However, the recurrent cultivation of the same crops caused significant weed and insect infestation problems. It is suggested to include alternative crops (other than wheat) in the cotton-based cropping system of the country [38]. However, the impacts of *Bt* cotton on the productivity of alternative crops are still unknown.

This study assessed the impacts of *Bt* cotton cultivation on nutrient availability, the yield-related traits of different crops (wheat, Egyptian clover, and canola) sown after cotton, and the overall productivity of the cropping systems, including these crops. It was hypothesized that the cultivation of *Bt* cotton would reduce the yield of winter crops; however, the overall productivity of the cropping systems would not be affected. It was further hypothesized that cotton-based cropping system with *Bt* genotypes would have higher economic returns compared to those having non-*Bt* genotypes.

2. Materials and Methods

2.1. Experimental Site

This field study was conducted at CCRI (Central Cotton Research Institute), Multan (30.2° N, 71.43° E, and 122 meters above sea level), Pakistan, during 2016–2017 and 2017–2018. The soil of the experimental site was analyzed to assess the nutrient availability and the physico-chemical characters before and after the experiments. The soil texture was silt–clay–loam. The soil physico-chemical properties are given in Table 1.

2.2. Experimental Treatments

This experiment consisted of two factors, i.e., cotton genotypes (*Bt* and non-*Bt*) and winter crops. The *Bt* genotypes included in the study were 'CIM-616' (Bt_1) and 'GH Mubarik' (Bt_2), while the non-*Bt* genotypes were 'CIM-620' (non-Bt_1) and 'N-414' (non-Bt_2). Similarly, wheat (*Triticum aestivum* L.), Egyptian clover (*Trifolium alexandrinum* L.), and canola (*Brassica napus*) were winter crops that were sown after cotton harvest. The cultivation of winter crops resulted in three possible cropping systems, i.e., cotton–wheat, cotton–Egyptian clover, and cotton–canola. These systems may further be classified into to *Bt* and non-*Bt* cotton-based cropping systems. The *Bt* and non-*Bt* cotton genotypes were sown in May, whereas winter crops were sown in November following the harvest of the cotton crop (Table 2). The experiment was laid out according to two factorial design, where cotton genotypes were kept in main plots (6 m × 10 m), whereas winter crops were randomized in the sub-plots (2 m × 10 m). All treatments had three replications, and the experiment was repeated for two years. The sub-plots were regarded as being experimental units, and each experimental unit had three replications, as described above.

Table 1. Physicochemical characteristics of the soil in the experimental area before the initiation of the experiments.

Soil Properties	Unit	2016–2017	2017–2018
Organic matter content	%	0.59	0.56
Total nitrogen (N)	kg ha^{-1}	22.12	22.23
Available phosphorus (P)	kg ha^{-1}	18.02	18.08
Available potassium (K)	kg ha^{-1}	245.15	249.15
pH		8.17	8.19
EC	dS m^{-1}	4.96	5.00
Silt	%	54.15	54.00
Sand	%	25.75	26.10
Clay	%	20.10	19.90

Table 2. The production practices used for the cultivation of cotton and winter crops used in study.

Crops Name	Genotype Name	Planting Time *	Seed Rate (kg ha^{-1})	Fertilizer NPK (kg ha^{-1})	R × R (cm)	P × P (cm)	Harvesting Time
Cotton	GH Mubarik and CIM-616 (*Bt*) CIM-620 and CIM-554 (non-*Bt*)	08 and 10 May	25	250-175-125 (*Bt*) 200-145-100 (non-*Bt*)	75	20	Last picking in October
Wheat	Galaxy-2013	13 and 16 November	125	130-100-62	25		21 and 23 April
Canola	Hyola-420	12 and 13 November	5	90-60-50	30	4-5	6 and 10 April
Egyptian clover	Anmol berseem	9 and 11 November	25	22-115-0			Last cutting in April

Different dates in planting and harvesting time column indicate the dates in first and second years of the experiment, R × R = row to row spacing, P × P = plant to plant spacing. *, the first and second dates denote the planting time of the respective crop during 1st and 2nd year of the study, respectively.

2.3. Crop Husbandry

Cotton and winter crops were planted following the recommendations in production technology provided by the local agriculture extension department (https://www.agripunjab.gov.pk/, accessed on 12 January 2015). The recommendations followed in the study are given in Table 2. Irrigation was applied according to the moisture needs of the crops. All crop protection measures were taken to protect crops from insect and disease infestation. All crops were harvested when they reached physiological maturity.

2.4. Data Collection

2.4.1. Soil Properties

Particle size distribution was determined using the hydrometer method [39]. Soil EC was recorded using a digital EC meter following the standard procedures detailed by Dellavalle [40]. A digital pH meter was used to measure the soil pH from a saturated soil paste [40].

2.4.2. Nutrient Availability

Soil nitrogen (N) availability was measured spectrophotometrically with a segmented-flow system. The phosphorus (P) was determined using the vanadomolybdate method, potassium (K) through flame photometry, and zinc (Zn) and iron (Fe) through atomic absorption spectrophotometry [41]. Soil organic matter was measured using a loss-on-ignition protocol, as introduced by Hoogsteen et al. [42].

2.4.3. Weed Infestation

Weed infestation was evaluated 45 days after the sowing of cotton and winter crops. The weeds present in a l m^2 quadrat were counted from each experimental unit at three different places. The weeds were identified, grouped into narrow and broad-leaved, and their densities were computed. The density of each experimental unit was averaged from different locations within a replication.

2.5. Morphological and Yield-Related Traits

2.5.1. Cotton

The number of sympodial and monopodial branches were counted from 10 randomly selected plants in each experimental unit and averaged. The weights of 10 opened bolls from a single plant were measured using a sensitive balance from 10 randomly selected plants in experimental unit, and averaged. Three manual pickings were performed from each experimental unit to record the seed cotton yield. The seed cotton yields of three pickings were added and converted to seed cotton yield per hectare, using a unitary method. The cotton stalks were harvested and left in the field for two weeks. Afterwards, the stalks were weighed to record the total biomass (biological yield), and they were expressed in kg ha^{-1}. The harvest index was estimated by dividing the seed cotton yield to biological yield, and this was expressed as a percentage.

2.5.2. Wheat

The number of productive (spike-bearing) tillers present in a 1 m^2 area were counted. The lengths of 10 randomly selected spikes were recorded from four central rows in each treatment and averaged. The number of grains were counted from 10 randomly selected spikes and averaged. The weight of 1000 grains from three random samples in each treatment was measured using a sensitive balance. The grain yield per plot was measured on a sensitive balance when the seed reached the required moisture level, i.e., 12%, and converted to t ha^{-1}.

2.5.3. Canola

The number of siliques per plant were counted from three randomly selected plants in each experimental unit. Ten random siliques were opened, and the number of grains in them were counted and averaged. The weight (g) of 1000 seeds from randomly sampled seeds per plot was measured on a sensitive balance. The mature crop was harvested, sundried, and threshed manually to record the seed yield, which was converted into kg ha^{-1}. The biological yield was recorded by weighing the total above ground biomass harvested from four central rows of each experimental plot, and this was converted into kg ha^{-1}.

2.5.4. Egyptian Clover

All plants within the experimental unit were harvested during each cut, and weighed to record the fresh forage yield. A pre-weighed amount of fresh forage was oven-dried, and fresh forage yield was converted into dry forage yield using a unitary method. The fresh and dry forage yields were converted into t/ha. Crude protein was measured via a near-infrared spectroscopy system [43].

2.6. Economic Analysis

The profit abilities of different cotton genotypes via winter crops interactions were computed following CIMMYT [44]. The input costs and outputs obtained in monetary terms were calculated. The costs regarding land rent, irrigation, labor, seeds, fertilizers, pesticides, sowing, harvesting, etc., were computed. The existing market prices of the produce were used to compute the gross income. These expenses were deducted from the gross income to obtain the net income. The benefit–cost ratio (BCR) was computed by dividing the net economic returns with the expenses incurred.

2.7. Statistical Analysis

The collected data for the nutrient availability, weed density, and yield-related parameters of different crops were checked for normality using the Shapiro-Wilk normality test [45]. The parameters having non-normal distributions were normalized using the Arcsine transformation technique to meet the normality assumption of the Analysis of Variance (ANOVA). The differences among the years were tested, which were significant; therefore, the data of both years were analyzed, presented, and interpreted separately. A two-way ANOVA was used to test the significance among the treatments, and the means were compared using a least significant difference post hoc test at 95% probability, where ANOVA denoted significant differences [46]. The interactive effects of cotton genotypes and winter crops were significant for most of the studied traits. Therefore, the interactive effects were presented and interpreted. A one-way ANOVA was used to analyze the data on the yield-related traits of cotton. All statistical computations were performed on SPSS statistical software, version 21.0 [47].

3. Results

3.1. Nutrient Availability

Soil nutrient availability and organic matter content were significantly altered by cotton genotypes via winter crops interaction during both years (Table 3). Wheat sown after the *Bt* cotton genotype 'GH-Mubarik' had the highest available N during each year, while canola sown after the non-*Bt* genotypes 'N-414' and 'CIM-620' resulted in the lowest available N during both years (Table 3). Egyptian clover sown after the non-*Bt* cotton genotype 'CIM-620' during the first year, and the *Bt* genotype 'CIM-616' during the second year, recorded the highest P, whereas the lowest values were recorded for wheat sown after the non-*Bt* genotype 'N-414' during both years (Table 3). The Egyptian clover sown after the non-*Bt* genotype 'N-414' resulted in the highest available K, which was statistically similar to the wheat cultivation after the *Bt* genotype 'GH-Mubarik'. The lowest available K was noted for canola sown after the *Bt* cotton genotypes during the first year, and the non-*Bt* genotype 'N-414' during the second year (Table 3). Egyptian clover following the non-*Bt* genotypes resulted in the highest available Zn during both years, while the lowest Zn was recorded for canola sown after the *Bt* genotype 'GH-Mubarik' during both years (Table 3). The interactive effects of wheat and the non-*Bt* genotype 'CIM-620' resulted in the highest available Fe during each year, while the lowest Fe was recorded for Egyptian clover sown after the non-*Bt* genotype 'CIM-620' during first year, and canola sown after the non-*Bt* genotype 'N-414' during the second year (Table 3). The highest organic matter content was recorded for wheat sown after the *Bt* genotypes during both years, while canola sown after the non-*Bt* genotypes resulted in the lowest value of soil organic matter during both years (Table 3).

Table 3. The influences of cotton genotypes from winter crops interaction on nutrient availability and soil organic matter contents in the soil after the harvest of winter crops.

Treatments	2016–2017			2017–2018		
	Wheat	**Egyptian Clover**	**Canola**	**Wheat**	**Egyptian Clover**	**Canola**
			Available nitrogen (kg ha^{-1})			
CIM-616 (Bt_1)	0.17 ± 0.001 a–c	0.15 ± 0.003 c–e	0.14 ± 0.001 de	0.18 ± 0.003 ab	0.16 ± 0.005 b–d	0.16 ± 0.001 b–d
GH-Mubarik (Bt_2)	0.19 ± 0.003 a	0.14 ± 0.002 de	0.16 ± 0.001 b–d	0.19 ± 0.002 a	0.16 ± 0.004 b–d	0.17 ± 0.003 a–c
CIM-620 (NBt_1)	0.18 ± 0.002 ab	0.15 ± 0.001 c–e	0.14 ± 0.004 de	0.18 ± 0.001 ab	0.14 ± 0.003 cd	0.14 ± 0.002 d
N-414 (NBt_2)	0.16 ± 0.004 b–d	0.15 ± 0.002 c–e	0.13 ± 0.002 e	0.18 ± 0.002 ab	0.16 ± 0.002 b–d	0.16 ± 0.002 b–d
LSD ($p \leq 0.05$)		0.020			0.020	
			Available phosphorous (kg ha^{-1})			
CIM-616 (Bt_1)	19.36 ± 0.02 a–e	19.50 ± 0.03 a–c	19.38 ± 0.02 a–e	19.40 ± 0.01a–c	19.59 ± 0.07 a	19.28 ± 0.04 b–d
GH-Mubarik (Bt_2)	19.20 ± 0.04 de	19.52 ± 0.02 ab	19.24 ± 0.05 de	19.10 ± 0.02 de	19.42 ± 0.04 ab	19.14 ± 0.06 de
CIM-620 (NBt_1)	19.28 ± 0.06 c–e	19.58 ± 0.07 a	19.30 ± 0.04 b–e	19.18 ± 0.06 de	19.48 ± 0.02 ab	19.20 ± 0.05 c–e
N-414 (NBt_2)	19.18 ± 0.04 e	19.40 ± 0.03 a–d	19.40 ± 0.03 a–d	19.08 ± 0.05 e	19.30 ± 0.03 a–d	19.30 ± 0.04 a–d
LSD ($p \leq 0.05$)		0.10			0.12	
			Available potassium (kg ha^{-1})			
CIM-616 (Bt_1)	394 ± 6.1 b–d	400 ± 4.3 ab	394 ± 3.3 e	402 ± 2.2 a–c	404 ± 2.2 a–c	406 ± 3.2 a–c
GH-Mubarik (Bt_2)	388 ± 5.3 de	396 ± 6.1 a–c	394 ± 3.2 e	408 ± 6.1 a	402 ± 3.1 a–c	404 ± 2.6 a–c
CIM-620 (NBt_1)	390 ± 3.4 c–e	398 ± 3.3 ab	386 ± 8.3 de	400 ± 3.4 bc	402 ± 3.4 a–c	402 ± 2.7 a–c
N-414 (NBt_2)	392 ± 2.4 b–e	402 ± 1.2 a	390 ± 4.5 c–e	400 ± 3.3 bc	406 ± 4.3 ab	400 ± 2.3 c
LSD ($p \leq 0.05$)		7.58			6.30	
			Available zinc (kg ha^{-1})			
CIM-616 (Bt_1)	1.46 ± 0.01 d	1.60 ± 0.03 b	1.44 ± 0.03 de	1.56 ± 0.04 cd	1.60 ± 0.02 bc	1.48 ± 0.02 ef
GH-Mubarik (Bt_2)	1.46 ± 0.02 d	1.58 ± 0.02 bc	1.40 ± 0.02 e	1.50 ± 0.04 de	1.62 ± 0.02 b	1.42 ± 0.03 f
CIM-620 (NBt_1)	1.58 ± 0.02 bc	1.66 ± 0.01 a	1.44 ± 0.04 c	1.60 ± 0.03 bc	1.68 ± 0.04 a	1.56 ± 0.04 cd
N-414 (NBt_2)	1.58 ± 0.01 bc	1.68 ± 0.02 a	1.56 ± 0.02 bc	1.58 ± 0.02 bc	1.68 ± 0.05 a	1.58 ± 0.02 bc
LSD ($p \leq 0.05$)		0.04			0.06	
			Available iron (kg ha^{-1})			
CIM-616 (Bt_1)	7.62 ± 0.12 d–f	7.42 ± 0.10 fg	7.72 ± 0.09 c–e	7.68 ± 0.11 de	7.78 ± 0.13 c–e	7.78 ± 0.10 c–e
GH-Mubarik (Bt_2)	7.82 ± 0.14 b–e	7.68 ± 0.11 de	7.84 ± 0.11 b–d	7.90 ± 0.12 bc	8.04 ± 0.17 ab	7.80 ± 0.14 c–e
CIM-620 (NBt_1)	8.14 ± 0.19 a	7.32 ± 0.09 g	7.94 ± 0.11 a–c	8.20 ± 0.16 a	7.96 ± 0.11 bc	7.80 ± 0.13 c–e
N-414 (NBt_2)	8.04 ± 0.11 ab	7.56 ± 0.14 ef	7.60 ± 0.09 ef	7.96 ± 0.10 bc	7.84 ± 0.12 b–d	7.62 ± 0.12 e
LSD ($p \leq 0.05$)		0.24			0.20	
			Soil organic matter (%)			
CIM-616 (Bt_1)	0.59 ± 0.02 a	0.53 ± 0.04 c–e	0.51 ± 0.02 de	0.62 ± 0.01 ab	0.62 ± 0.01 ab	0.59 ± 0.01 cd
GH-Mubarik (Bt_2)	0.58 ± 0.03 ab	0.53 ± 0.04 c–e	0.51 ± 0.02 de	0.60 ± 0.02 bc	0.63 ± 0.01 a	0.60 ± 0.01 bc
CIM-620 (NBt_1)	0.57 ± 0.04 a–c	0.52 ± 0.03 de	0.49 ± 0.01 ef	0.59 ± 0.02 cd	0.59 ± 0.01 cd	0.57 ± 0.01 d
N-414 (NBt_2)	0.58 ± 0.02 ab	0.54 ± 0.04 b–d	0.45 ± 0.02 f	0.58 ± 0.01 cd	0.58 ± 0.02 cd	0.60 ± 0.01 bc
LSD ($p \leq 0.05$)		0.04			0.03	

Means followed by different letters significantly differ ($p \leq 0.05$) from each other. The values of different traits are means of three replications ± standard errors of means. NS = non-significant; *Bt* = transgenic genotypes; N*Bt* = conventional or non-transgenic genotypes.

3.2. Weed Density

The densities of broadleaved, narrow-leaved, and total weeds were significantly affected by the interactive effect of cotton genotypes and winter crops. Overall, *Bt* genotypes recorded lesser weed infestation compared with the non-*Bt* genotypes included in the current study (Figure 1).

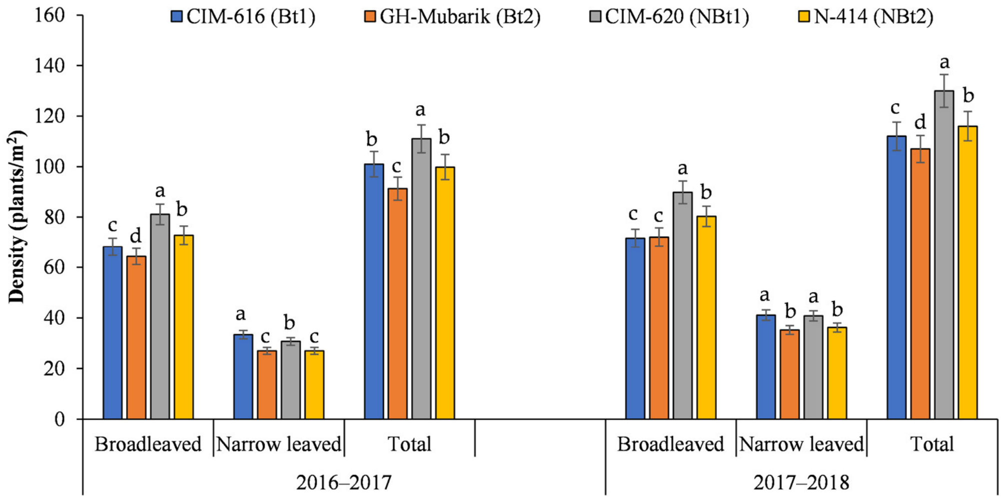

Figure 1. The densities (±standard errors of means, n = 3) of broadleaved, narrow-leaved, and total weed species recorded in different cotton genotypes. Here, Bt and NBt stand for *Bt* and non-*Bt* genotypes.

Similarly, the wheat crop had the highest density of narrow-leaved, broadleaved, and total weeds during both years (Figure 2). Wheat sown after the non-*Bt* genotype 'CIM-620' had the highest density of broad-leaved weeds, whereas the lowest density was observed in Egyptian clover and canola crops sown after both *Bt* genotypes (Table 4).

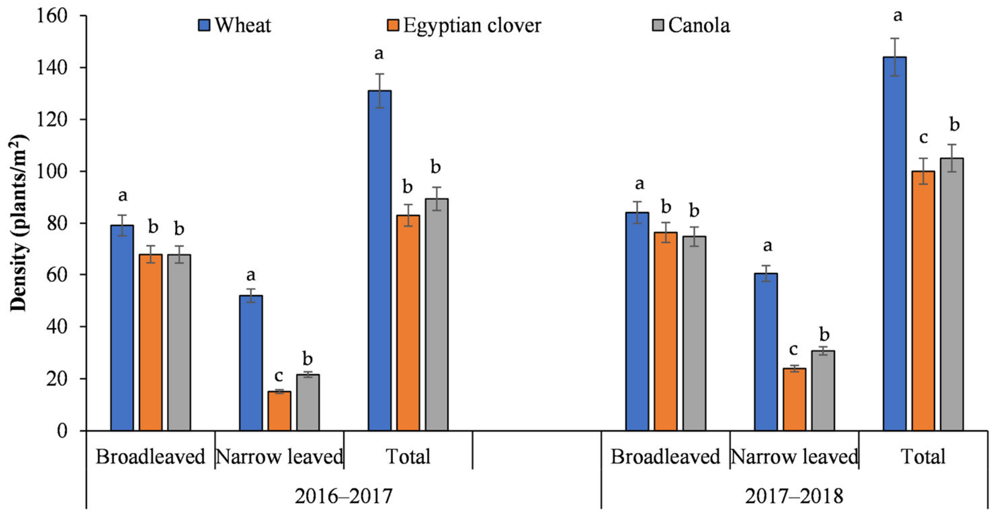

Figure 2. The impacts of different winter crops on weed density (±standard errors of means, n = 3) in different winter crops included in the study.

Similarly, the lowest density of narrow-leaved weeds was recorded for Egyptian clover sown after the *Bt* genotype 'GH-Mubarik' and non-*Bt* genotype 'CIM-620' during both years, whereas the highest density was noted for wheat sown after the *Bt* genotype 'CIM-616' and the non-*Bt* genotype 'CIM-620' during both years (Table 4). Likewise, the highest density of total weeds was recorded in wheat crop sown after the non-*Bt* genotype 'CIM-620', whereas the lowest density was recorded in Egyptian clover sown after the *Bt* genotypes during both years (Table 4).

Table 4. Interactive effects of different cotton genotypes and winter crops on the densities of broadleaved, narrow-leaved, and total weeds recorded in winter crops.

Treatments	2016–2017			2017–2018		
	Wheat	Egyptian Clover	Canola	Wheat	Egyptian Clover	Canola
			Broadleaved weeds density (m^{-2})			
CIM-616 (Bt_1)	81.3 ± 3.1 b	61.0 ± 3.1 f	62.3 ± 2.8 f	83.0 ± 3.4 cd	66.7 ± 3.9 g	65.0 ± 3.1 g
GH-Mubarik (Bt_2)	68.7 ± 4.3 e	60.3 ± 2.4 f	64.0 ± 2.9 f	74.3 ± 2.6 ef	69.3 ± 5.2 fg	72.7 ± 3.4 ef
CIM-620 (NBt_1)	90.3 ± 3.4 a	77.7 ± 2.8 bc	75.3 ± 2.4 cd	97.0 ± 6.3 a	88.7 ± 2.8 b	83.7 ± 5.1 bc
N-414 (NBt_2)	76.0 ± 2.7 cd	72.7 ± 3.3 de	69.7 ± 1.4 e	82.0 ± 4.7 cd	81.0 ± 3.0 cd	78.0 ± 3.1 de
LSD ($p \leq 0.05$)		4.42			5.57	
			Narrow-leaved weeds density (m^{-2})			
CIM-616 (Bt_1)	57.0 ± 2.2 a	16.0 ± 3.6 e	27.3 ± 2.1 c	64.0 ± 4.6 a	24.0 ± 3.4 e	35.3 ± 4.4 c
GH-Mubarik (Bt_2)	48.0 ± 3.3 b	12.7 ± 3.4 ef	20.3 ± 3.4d	55.7 ± 3.3 b	22.0 ±3.3 e	28.0 ± 3.2 d
CIM-620 (NBt_1)	56.0 ± 4.5 a	11.7 ± 2.6 f	24.3 ± 2.9 c	66.3 ± 4.1 a	21.7 ± 4.5 e	34.3 ± 4.0 c
N-414 (NBt_2)	46.7 ± 2.6 b	20.0 ± 2.9 d	14.3 ± 1.7 ef	55.7 ± 4.8 b	28.0 ± 3.7 d	25.0 ± 4.2 de
LSD ($p \leq 0.05$)		3.47			3.53	
			Total weeds density (m^{-2})			
CIM-616 (Bt_1)	138 ± 6 b	77.0 ± 8 h	89.7 ± 3 f	147 ± 10 b	90.7 ± 5 h	100 ± 5 g
GH-Mubarik (Bt_2)	116 ± 5 d	73.0 ± 11 i	84.3 ± 2 g	130 ± 11 d	91.3 ± 6 h	100 ± 6 g
CIM-620 (NBt_1)	146 ± 10 a	89.3 ± 4 f	99.7 ± 8 e	163 ± 19 a	110 ± 8 f	118 ± 11 e
N-414 (NBt_2)	122 ± 4 c	92.7 ± 5 f	84.0 ± 4 g	137 ± 6 c	109 ± 7 f	103 ± 5 g
LSD ($p \leq 0.05$)		3.84			5.43	

Means followed by different letters significantly differ ($p \leq 0.05$) from each other. The values of different traits are means of three replications ± standard errors of means. NS = non-significant; *Bt* = transgenic genotypes; N*Bt* = conventional or non-transgenic genotypes.

3.3. Yield-Related Attributes of Cotton

Various yield-related traits of cotton were significantly affected by genotypes, except for the number of monopodial branches and the harvest index (Table 5).

The *Bt* genotype 'CIM-616' and non-*Bt* genotype 'CIM-620' recorded the highest number of sympodial branches during the first year, whereas both *Bt* genotypes recorded the highest number of sympodial branches during the second year. The non-*Bt* genotypes had the lowest number of sympodial branches during the second year (Table 5). The *Bt* genotype 'CIM-616' recorded the highest seed cotton yield during the first year, while it was not affected by genotypes during the second year (Table 5).

3.4. Yield-Related Attributes of Wheat

Yield-related traits of wheat crop were significantly impacted by different cotton genotypes with some exceptions, i.e., grain yield during the first year, and harvest index during both years. Overall, the wheat crop planted after the non-*Bt* genotypes recorded higher values for the number of productive tillers, number of grains per spike, 1000-grain weight, and grain and biological yields (Table 6). Wheat sown after the *Bt* genotypes recorded lower values for the number of productive tillers, number of grains per spike, 1000-grain weight, and grain, and biological yields, compared to non-*Bt* genotypes during both years (Table 6).

Table 5. The influences of different *Bt* and non-*Bt* genotypes on yield-related attributes of cotton crop.

Treatments	2016	2017	2016	2017	2016	2017
	Monopodial Branches (Plant^{-1})		**Sympodial Branches (Plant^{-1})**		**Boll Weight (g)**	
CIM-616 (Bt_1)	1.78 ± 0.2	1.78 ± 0.3	23.6 ± 0.9 b	26.0 ± 1.1 a	3.2 ± 0.1 a	3.2 ± 0.04 a
GH-Mubarik (Bt_2)	1.67 ± 0.3	1.67 ± 0.4	22.3 ± 0.8 b	25.6 ± 1.2 a	3.1 ± 0.1 ab	3.1 ± 0.02 b
CIM-620 (NBt_1)	1.89 ± 0.3	1.89 ± 0.2	25.0 ± 1.4 a	23.0 ± 0.8 b	3.0 ± 0.05 bc	3.0 ± 0.08 b
N-414 (NBt_2)	1.67 ± 0.4	1.67 ± 0.3	22.0 ± 1.1 bc	24.0 ± 1.2 b	2.9 ± 0.04 c	2.9 ± 005 c
LSD ($p \leq 0.05$)	NS	NS	1.49	1.29	0.15	0.07
	Seed cotton yield (kg ha^{-1})		Harvest index (%)			
CIM-616 (Bt_1)	2892 ± 141 a	2832 ± 221	32.7 ± 2.12	32.4 ± 1.9		
GH-Mubarik (Bt_2)	2685 ± 123 b	2635 ± 213	29.5 ± 2.21	29.4 ± 3.1		
CIM-620 (NBt_1)	2645 ± 129 b	2563 ± 303	33.9 ± 2.39	31.2 ± 2.2		
N-414 (NBt_2)	2613 ± 147 b	2570 ± 309	32.3 ± 2.53	31.1 ± 2.6		
LSD ($p \leq 0.05$)	150.14	NS	NS	NS		

Means followed by different letters significantly differ ($p \leq 0.05$) from each other. The values of different traits are means of three replications ± standard errors of means. NS = non-significant; *Bt* = transgenic genotypes; N*Bt* = conventional or non-transgenic genotypes.

Table 6. Yield-related parameters of wheat crop sown after the harvest of transgenic and non-transgenic cotton genotypes.

Treatments	2016–2017	2017–2018	2016–2017	2017–2018	2016–2017	2017–2018
	Productive Tillers (m^{-2})		**Grains (Spike^{-1})**		**1000-Grain Weight (g)**	
CIM-616 (Bt_1)	189 ± 15 ab	191 ± 17 ab	55.7 ± 1.9 b	56.0 ± 2.0 b	36.2 ± 1.8 c	36.8 ± 1.4 c
GH-Mubarik (Bt_2)	181 ± 11 b	176 ± 14 b	53.3 ± 1.7 b	53.9 ± 1.8 c	37.9 ± 1.7 bc	37.9 ± 1.6 bc
CIM-620 (NBt_1)	197 ± 10 a	201 ± 14 a	59.5 ± 1.2 a	58.8 ± 1.4 a	40.2 ± 1.5 a	40.7 ± 1.3 a
N-414 (NBt_2)	202 ± 12 a	202 ± 16 a	59.1 ± 1.4 a	58.1 ± 1.6 a	39.8 ± 1.6 ab	39.6 ± 2.3 ab
LSD ($p \leq 0.05$)	14.4	14.4	2.4	1.7	2.0	1.8
	Grain yield (t ha^{-1})		Biological yield (t ha^{-1})		Harvest index (%)	
CIM-616 (Bt_1)	5.82 ± 0.8	5.95 ± 0.2 b	17.6 ± 0.5 bc	15.7 ± 0.4 bc	33.1 ± 1.2	37.8 ± 2.1
GH-Mubarik (Bt_2)	5.98 ± 0.7	5.92 ± 0.1 b	17.1 ± 0.6 c	15.3 ± 0.5 c	34.9 ± 1.6	38.8 ± 2.2
CIM-620 (NBt_1)	6.30 ± 0.7	6.26 ± 0.2 a	18.2 ± 0.6 ab	16.4 ± 0.6 ab	34.6 ± 1.8	38.2 ± 2.1
N-414 (NBt_2)	6.21 ± 0.8	6.31 ± 0.2 a	18.7 ± 0.5 a	16.9 ± 0.5 a	33.2 ± 2.0	37.4 ± 2.4
LSD ($p \leq 0.05$)	NS	0.25	0.86	0.86	NS	NS

Means followed by different letters significantly differ ($p \leq 0.05$) from each other. The values of different traits are means of three replications ± standard errors of means. NS = non-significant; *Bt* = transgenic genotypes; N*Bt* = conventional or non-transgenic genotypes.

3.5. Yield-Related Attributes of Canola

Yield-related traits of canola were not affected by different cotton genotypes except for seed yield during the first year, where the crop sown after non-*Bt* genotypes recorded higher values compared to the *Bt* genotypes (Table 7).

Table 7. Yield-related traits of canola sown after different transgenic and non-transgenic cotton genotypes.

Treatments	**2016–2017**	**2017–2018**	**2016–2017**	**2017–2018**	**2016–2017**	**2017–2018**
	Siliques (Plant^{-1})		**Seeds (Silique^{-1})**		**1000-Seed Weight (g)**	
CIM-616 (Bt_1)	106 ± 22	105 ± 7 ab	26.7 ± 2.3	26.9 ± 3.7	2.77 ± 0.3	2.73 ± 0.4
GH-Mubarik (Bt_2)	103 ± 12	102 ± 9 b	24.0 ± 3.4	25.0 ± 4.0	2.90 ± 0.4	2.85 ± 0.3
CIM-620 (NBt_1)	109 ± 6	109 ± 7 ab	25.2 ± 3.6	25.8 ± 3.1	2.87 ± 0.6	2.90 ± 0.2
N-414 (NBt_2)	112 ± 16	113 ± 8 a	26.3 ± 4.1	27.0 ± 3.3	2.83 ± 0.5	2.93 ± 0.5
LSD ($p \leq 0.05$)	NS	8.4	NS	NS	NS	NS
	Biological yield (kg ha^{-1})		Seed yield (kg ha^{-1})		Harvest index (%)	
CIM-616 (Bt_1)	4800 ± 343	5271 ± 234	1650 ± 212 b	1797 ± 158	34.4 ± 2.2	34.1 ± 3.4
GH-Mubarik (Bt_2)	5132 ± 412	5070 ± 267	1700 ± 223 b	1833 ± 123	33.2 ± 3.1	36.2 ± 2.1
CIM-620 (NBt_1)	5233 ± 345	5345 ± 312	1950 ± 201 a	1850 ± 112	37.4 ± 2.6	34.7 ± 3.3
N-414 (NBt_2)	4876 ± 321	5478 ± 434	1900 ± 198 a	1900 ± 121	39.1 ± 2.8	34.7 ± 3.1
LSD ($p \leq 0.05$)	NS	NS	197.7	NS	NS	NS

Means followed by different letters significantly differ ($p \leq 0.05$) from each other. The values of different traits are means of three replications ± standard errors of means. NS = non-significant; *Bt* = transgenic genotypes; N*Bt* = conventional or non-transgenic genotypes.

3.6. Yield-Related Attributes of Egyptian Clover

The yield-related traits of Egyptian clover were significantly affected by different cotton genotypes during both years (Table 8). The crop sown after non-*Bt* genotypes recorded higher values for the total forage yield, dry matter content, and crude protein during both years of the study, compared to the *Bt* genotypes (Table 8).

Table 8. The influence of various cotton varieties on yield-related traits of Egyptian clover.

Treatments	**Fresh Forage Yield (t ha^{-1})**		**Dry forage Yield (t ha^{-1})**		**Crude Protein (%)**	
	2016–2017	**2017–2018**	**2016–2017**	**2017–2018**	**2016–2017**	**2017–2018**
CIM-616 (Bt_1)	28.3 ± 1.21 b	30.7 ± 1.98 b	2.91±0.11 b	3.62 ± 0.09 b	21.0 ± 1.2 b	20.6 ± 1.7 b
GH-Mubarik (Bt_2)	28.2 ± 1.26 b	32.0 ± 2.02 b	2.97 ± 0.17 b	3.72 ± 0.16 ab	20.2 ± 1.6 b	20.3 ± 2.4 b
CIM-620 (NBt_1)	34.1 ± 2.34 a	34.8 ± 1.12 a	3.50 ± 0.21 a	3.87 ± 0.11 a	24.0 ± 2.1 a	22.3 ± 1.6 ab
N-414 (NBt_2)	32.3 ± 2.31 a	33.2 ± 1.63 ab	3.35 ± 0.18 a	3.76 ± 0.12 ab	23.7 ± 1.9 a	23.6 ± 1.2 a
LSD ($p \leq 0.05$)	1.96	2.63	0.18	0.17	2.44	2.52

Means followed by different letters significantly differ ($p \leq 0.05$) from each other. The values of different traits are means of three replications ± standard errors of means. NS = non-significant; *Bt* = transgenic genotypes; N*Bt* = conventional or non-transgenic genotypes.

3.7. Economic Returns/System Productivity

Economic analysis showed that the *Bt* genotypes–wheat cropping system resulted in the highest net benefits, whereas the non-*Bt* genotypes–canola cropping system recorded the lowest net benefits (Table 9). Similarly, the *Bt* genotypes–wheat cropping system resulted in the highest benefit–cost ratio (BCR), while the non-*Bt* genotypes–canola cropping system resulted in the lowest BCR (Table 9).

Table 9. The impacts of different transgenic and non-transgenic cotton genotypes on system productivity of various cotton-based cropping systems.

Treatments	2016–2017				2017–2018			
	TE	GI	NI	BCR	TE	GI	NI	BCR
Bt_1 × Wheat	1563.59	2607.75	1044.16	1.67	1563.59	2531.58	967.99	1.62
Bt_2 × Wheat	1563.59	2516.55	952.96	1.61	1563.59	2423.59	860.00	1.55
NBt_1 × Wheat	1629.85	2572.23	942.38	1.58	1629.85	2466.93	837.09	1.51
NBt_2 × Wheat	1629.85	2564.07	934.22	1.57	1629.85	2491.97	862.12	1.53
Bt_1 × Canola	1500.76	1979.11	478.35	1.32	1500.76	2009.13	508.37	1.34
Bt_2 × Canola	1500.76	1910.59	409.83	1.27	1500.76	1923.63	422.87	1.28
NBt_1 × Canola	1567.02	1966.87	399.85	1.26	1567.02	1904.60	337.59	1.22
NBt_2 × Canola	1567.02	1925.86	358.85	1.23	1567.02	1926.66	359.65	1.23
Bt_1 × Egyptian clover	1621.02	1989.23	368.21	1.23	1621.02	2016.24	395.22	1.24
Bt_2 × Egyptian clover	1621.02	1893.59	272.57	1.17	1621.02	1957.23	336.21	1.21
NBt_1 × Egyptian clover	1687.28	2010.18	322.90	1.19	1687.28	1987.73	300.45	1.18
NBt_2 × Egyptian clover	1687.28	1953.95	266.68	1.16	1687.28	1954.70	267.42	1.16

BCR = benefit–cost ratio; Bt_1 = CIM-616; Bt_2 = GH-Mubarik; NBt_1 = CIM-620; NBt_2 = N-414; *Bt* = transgenic cotton; N*Bt* = non-transgenic cotton; TE= total expenditure; GI= gross income; NI= net income, the values of TE, GI, and NI are in US$.

4. Discussion

The results of the current study indicated that nutrient availability, yield-related attributes of winter crops, and overall system productivity were significantly affected by the cotton genotypes. *Bt* cotton genotypes improved the nutrient availability and system productivity, which are directly linked to the higher fertilizer input and the better yield of the *Bt* genotypes. Increased fertilizer use after the introduction of *Bt* genotypes have increased crop yields because of better pest control [48]. Furthermore, farmers started applying more fertilizers after the induction of *Bt* genotypes in the existing cropping systems of Pakistan [49]. Cotton yields and related benefits may also be affected by numerous factors, such as changes to irrigation systems, crop production technologies, agronomic practices, farmer training, or weather fluctuations, etc. [48]. Earlier studies have reported that nutrient availability may vary across *Bt* and non-*Bt* genotypes because of the differences in their nutrient requirements and absorption [33]. Therefore, the results of the current study regarding nutrient availability are in agreement with the earlier studies.

The highest P, N, Zn, Fe, and organic matter contents were recorded from the soil cultivated with winter crops after the *Bt* genotypes. Wheat crop sown after *Bt* genotypes resulted in higher values of available N, Fe, and organic matter contents, whereas Egyptian clover following *Bt* genotypes resulted in higher P, K, and Zn contents. Nutrient uptake is dependent on plants and their genetic makeup. Several factors affect the nutrient uptake capacity of plants [50]. These factors include root surface area, and the type and quantity of root exudates released in the rhizosphere and microbial communities [50]. Moreover, the plant characteristics and interactive effect between roots and soil microorganisms also play significant roles in nutrient uptake [51]. The quantity of nutrients available to plants depends mainly upon their availability in the root zone [52]. Genetically modified (GM) crops can disrupt soil nutrient cycles due to changes in the root zone [53]. The quantity and quality of root exudates affects microbial activity, which alters the solubility of mineral or fixed P, and P availability [2,21,27]. The availability of soil nutrients is significantly affected by the cultivation of *Bt* cotton. Growing *Bt* cotton also decreased the available N and K, while increasing Zn and P [29,54]. However, our study indicated that the available N was increased in the soil cultivated with *Bt* genotypes. The *Bt* genotypes received higher amounts of nutrients, which can be linked to the increased nutrient availability. Similarly, the availabilities of K, Zn, and P also increased in the treatments with *Bt* genotypes. Higher root biomass-mediated exudation is responsible for the enhanced availability of Zn and Fe in *Bt* cotton cultivated soil, compared to non-*Bt* cotton [55,56].

Weed infestation exerts significant negative impacts on crop yields [57,58]. Weed infestation decreases cotton yield by 0.26 to 66%, depending on the weed species and their densities [59]. The cultural practices used in the existing cropping systems of Pakistan encourage the growth of several weed species [60]. The recurrent cultivation of *Bt* genotypes may result in the proliferation of specific weed species. Different genotypes significantly vary in their weed competitive ability [61]. Several earlier studies have reported that cotton genotypes significantly differ in their competitive ability with weeds [62,63]. The weed competitive abilities of these cultivars were linked with their potential to establish a crop canopy. The cultivars which developed a dense canopy in a shorter period were more competitive. However, these studies did not include any *Bt* genotypes. Low weed density was recorded in the soil cultivated with *Bt* genotypes in the current study. Weed competition of *Bt* genotypes in the current study could be linked with its quicker canopy development, compared to non-*Bt* cotton genotypes.

Wheat and Egyptian clover sown after non-*Bt* genotypes had better yield-related traits. However, the yield-related traits of canola were not affected by cotton genotypes. The lower yields of winter crops in the fields cultivated with *Bt* genotypes can be linked with the increased levels of *Bt* toxins in the soil and the higher nutrient consumption by cotton plants. Moreover, the improvement in the yield-related traits of winter crops in the fields cultivated with non-*Bt* genotypes can be linked to the low *Bt* toxin levels in these soils. It has been observed that toxins produced in the aerial parts and roots of *Bt* cotton may cause soil pollution upon their release [34,64,65]. *Bt* toxins released from the plants become absorbed or bound to the soil particles, and then they become safe from degradation from other microorganisms that are present in the soil [66]. The recurrent cultivation of *Bt* cotton in the same field increases the level of *Bt* toxins in the soil, which can change the activity and composition of the soil microbes and the soil biochemical nature [29,67–69].

The *Bt* cotton–wheat cropping system revealed the highest net income and benefit–cost ratio (BCR). The highest productivity of this system was due to a higher production of *Bt* cotton and wheat. The *Bt* genotypes produced the highest yield due to a lower rate of insect infestation, compared with non-*Bt* genotypes. However, wheat yield was higher in the fields cultivated with non-*Bt* genotypes, and a lesser number of sprays in *Bt* cotton for pest management decreased the input costs. This eventually reduced expenses, which resulted in a higher net income and BCR than with non-*Bt* cotton.

The results confirmed the hypothesis of the study, where *Bt* genotypes exerted negative impacts on the yield-related traits of winter crops (with some exceptions) and improved the overall system productivity. Therefore, *Bt* genotypes can be included in the cotton-based cropping systems without any decrease in the productivity and economic returns.

5. Conclusions

The results of the current study indicated that nutrient availability, weed infestation, the yield-related traits of winter crops, and the system productivity of various cotton-based cropping systems were significantly affected by *Bt* and non-*Bt* cotton genotypes. Overall, *Bt* genotypes had higher yields than non-*Bt* genotypes. The soil cultivated with *Bt* genotypes resulted in higher N, P, and Zn availabilities. The yield-related traits of winter crops were negatively affected by the *Bt* genotypes. Economic analysis indicated that *Bt* cotton, followed by wheat, resulted in the highest economic returns and benefit–cost ratios. Therefore, *Bt* cotton can be successfully inducted in the cotton–wheat cropping systems of semi-arid regions in Pakistan in order to obtain higher economic benefits.

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