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GENETIC ANALYSIS OF *BT* AND NON-*BT* COTTON FOR VARIOUS QUANTITATIVE TRAITS

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SUMMARY

This study sought to identify the optimal general and specific combiners and the nature of gene action concerning morphological, yield, and fiber traits under the line-by-tester mating design. It utilized six *Bt*-gene-containing parental lines and three non-*Bt* testers, along with their 18 F_1 hybrids in cotton (*Gossypium hirsutum* L.), with experiments held at the Sindh Agriculture University, Tandojam, Pakistan. Eight characteristics, such as days to first flowering, plant height, sympodial branches per plant, bolls per plant, seed cotton yield per plant, fiber strength, and insect mortality (%), sustained scrutiny. The notable disparities in line-by-tester interactions revealed the significance of specific combining ability in hybrids, indicating the role of dominant genes in the manifestation of traits. The substantial mean squares attributed to lines and tester parents confirmed that the general combining ability (GCA) reflects the dominant influence of additive genes on the traits. The GCA estimation indicated the *Bt* lines IUB-13, FH-901, and Koonj, along with the non-*Bt* testers Chandi-95 and CRIS-585, emerged as the most effective general combiners for the studied traits. The nine hybrids comprised parental genotypes with high × high, high × low, and low × low GCA effects for earliness, yield, and fiber quality traits.

Keywords: Cotton (*G. hirsutum* L.), *Bt*-cotton, line × tester, GCA, SCA, insect mortality, earliness, seed cotton yield and fiber traits

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Key findings: Among inbred lines, such as IUB-13, FH-901, and Koonj, and testers Chandi-95 and CRIS-585, become most recognized effective general combiners in cotton (*G. hirsutum* L.). They should be favorable for use in hybridization programs to develop superior cotton hybrids.

INTRODUCTION

A crop of great importance, growing cotton (*Gossypium hirsutum* L.) is widespread in over 80 nations because of its socioeconomic value and products. According to Statista 2024, the top seven nations—China, India, the United States, Brazil, Australia, Turkey, and Pakistan—account for 80% of global cotton production. The cotton crop provides 0.70% of Pakistan's GDP annually in foreign exchange profits (GOP, 2023). A primary goal of cotton breeding is to create high-yielding cultivars by hybridizing genotypes with favorable general combining ability for seed cotton yield and fiber quality, followed by the selection of promising combinations from segregating populations (Balci *et al*., 2023). Despite its economic worth, cotton cultivation faces challenges like drought, heat, and salinity stresses; weed competition; diseases; and pest infestation during its life cycle (Verma *et al.,* 2024). Transgenic cotton, often known as *Bt* cotton, serves as a notable example of genetically modified crops exhibiting resistance to bollworms (Cheema *et al*., 2021). The *Bt* cotton reduces damage from specific insects and enhances crop output owing to its resistance to bollworms (Lv *et al*., 2022).

Assessing genetic variation and combining ability serves as a valuable breeding tool for evaluating the breeding potential of specific populations or parents, thereby aiding cotton breeders in the application of appropriate breeding techniques (Abdel-Aty et al., 2023; Han *et al*., 2020). The concept of combining ability acts as a valuable standard in breeding processes for assessing the performance of lines in hybrid combinations. The findings indicated that the variance in specific combining ability (SCA) was greater than the general combining ability (GCA) concerning the number of days to first flowering (Imran *et al*., 2015). The investigation into gene action related to earliness traits signified that, except for the first fruiting node, the additive gene action demonstrated a significantly greater influence than the non-additive gene action (Mohammed *et al*., 2015).

Patel (2020) also documented the impact of meteorological factors on the pink bollworm population, finding that the pink bollworm larvae first emerged in the fifth week of September and remained active until the third week of January. The highest population's recording surfaced in the third week of December. The insecticidal effectiveness of *Bt* cotton in Pakistan's South Punjab region was successful in its examination by analyzing the *Cry1Ac* gene and *Bt* protein concentration of five *Bt* cultivars. It was evident that the insecticidal efficiency of *Bt* cotton was considerably diminished as a result of the combined abiotic stressors of high temperature (HT) and waterlogging. The *Bt*-protein content's quantitative analysis and the probe of the performance of the *Cry1Ac* gene under HT stress used ELISA (Enzyme-Linked Immuno-Sorbent Assay). The stress conditions led to a pest (whitefly) attack on the plant, which subsequently resulted in a decrease in yield (Ahmad *et al*., 2021). Thus, the presented study sought to determine the combining ability of parents and hybrids for early-maturing and high-yielding cotton genotypes.

MATERIALS AND METHODS

This investigation aimed to assess the combining ability of six locally developed and approved transgenic varieties alongside three non-transgenic high-yielding upland cotton varieties concerning various quantitative traits. Experiments commenced at the Seed Production and Development Center, Sindh Agriculture University, Tandojam.

The sown seeds of 18 F_1 hybrids, originating from nine parent varieties, used a randomized complete block design with four repetitions at the Seed Production and

Development Center, Sindh Agriculture University, Tandojam, Pakistan (25°26′10.6″N 68°32′32.7″E). The collected data underwent analysis to evaluate the combining ability in F_1 hybrids for earliness, yield, and fiber characteristics, as estimated by Kempthorne (1957) and later utilized by Singh and Choudhry (1985).

Estimation of GCA effects

The estimates of the general combining ability (GCA) used the following formula:

$$(a)\ \boldsymbol{Females}: gi = \frac{xi..}{mr} - \frac{x..}{fmr}$$

$$(b)\ \boldsymbol{Males}: gj = \frac{xj..}{fr} - \frac{x..}{fmr}$$

Where:
f = Number of female parents,
m = Number of male parents,
r = number of replications,
$x_i..$ = sum of F_1s ensuing from crossing ith female with all the males,
$x_j..$ = sum of total crosses of jth male with all the females, and
x.. = sum of total crosses

Estimation of SCA effects

The specific combining ability (SCA) effects' estimation has the following formula:

$$\text{Sij} = \frac{xij}{r} - \frac{xi..}{mr} - \frac{xj..}{fr} + \frac{x..}{fmr}$$

Where:
xij = Total of F_1 ensuing from crossing ith female with jth male.

Meteorological and soil conditions

According to natural and climatic conditions, the research area belongs to the sub-tropical zone, characterized by normal atmospheric precipitation and high summer and normal winter air temperatures. Soil physico-chemical characteristics of the experimental soil are available in Table 1a.

RESULTS AND DISCUSSION

General and specific combining ability effects

The general (GCA) and specific combining ability (SCA) effects for all qualities appear in Tables 1b to 4. The results and discussions pertaining to each trait had further elucidations below.

Days to first flowering

Parents demonstrating increased negative GCA effects for days to first flowering are advantageous for developing early-maturing hybrids. Four out of the six transgenic insect-

Table 1a. Soil physico-chemical properties of experimental area.

Soil properties	Depths (cm)		
	0-15	15-30	30-45
Textural class	Silty clay (Sand 9%, Silt 35%, Clay 56%)	Silty clay (Sand 8%, Silt 40%, Clay 52%)	Silty clay (Sand 10%, Silt 33%, Clay 57%)
pH (1:2)	8.0	8.0	8.1
EC (1:2.5) (dS m^{-1})	0.45	0.50	0.40
Organic Matter %	0.80	0.50	0.45
Total N%	0.040	0.030	0.030
Available P (mg kg^{-1})	2.7	3.8	2.9
Extractable K (mg kg^{-1})	220	210	190

resistant lines (IUB-13, FH-142, Koonj, and FH-901) exhibited negative GCA effects, while the other two lines (MNH-886 and NIAB-878) displayed unacceptable positive GCA effects regarding the days to the opening of the first flower (Table 1). Among the non-*Bt* male parents, CRIS-585 exhibited markedly acceptable GCA effects, followed by Shahbaz, respectively (Table 2). These results suggested the above parents and hybrids should be beneficial in the breeding program for developing varieties and hybrids with early maturity.

Among the 18 F_1 hybrids examined, eight exhibited favorable negative SCA effects regarding the days to the first flower's opening, but 10 showed unfavorable positive SCA effects (Table 3). The most pronounced adverse SCA effects were evident in the F_1 hybrids IUB-13 × CRIS-585 and Koonj × Shahbaz, followed by FH-142 × CRIS-585 and FH-901 × CRIS-585. Our results were in agreement with Kumar *et al*. (2013) and Sivia *et al*. (2017), who identified substantial general combining ability impacts for days to first flowering, seed index, and lint weight, paralleling outcomes of this study. Similarly, the study findings were consistent with those of Chakholoma *et al*. (2022) and Sivia *et al*. (2017). They observed that non-additive gene action contributed to controlling the character when there was a greater magnitude of SCA variance than GCA in days to first flowering.

Plant height

The GCA and SCA variations for plant height were considerable, indicating the involvement of both additive and non-additive genes in its inheritance, which indicates that improving plant height could be through simple selection in lateral generations. Four *Bt* lines (IUB-13, FH-901, Koonj, and NIAB-878) exhibited positive GCA effects, which were undesirable for the development of dwarf varieties (Table 1). Meanwhile, two lines (FH-142 and MNH-886) showed the desired negative GCA effects, which were more desirable for developing medium-tall cotton genotypes (Table 1b). Among the three non-transgenic insect-resistant testers, Shahbaz had markedly unfavorable positive GCA impacts, whereas the male Chandi-95 displayed a considerable negative desirable GCA value for plant height (Table 2).

From the 18 F_1 hybrids examined, 16 exhibited significant positive SCA effects on plant height, ranging from 4.76 to 18.76, while two crosses had negative SCA effects, between -0.24 and -5.24 (Table 3). In contrast to our findings, Chakholoma *et al*. (2022) documented substantial positive GCA and SCA effects for plant height. Moreover, Chapara *et al*. (2020) discovered the hybrid GSHV 179 × L 765 with a notable positive SCA, and Arain *et al*. (2015) noted hybrids exhibiting favorable negative SCA estimations for this trait. In light of the above results and findings of another researcher, negative GCA and SCA effects should be choices for the development of medium-tall cotton genotypes.

Sympodial branches per plant

Sympodial branches have a unique position in the cotton plant, other than monopodial branches, because these branches are favorable as fruiting branches. The GCA estimations for the number of sympodia plant^{-1} presented in Table 1b indicated that two lines (MNH-886 and FH-142) demonstrated negative GCA effects, whereas four lines showed significant positive GCA effects. Among the three testers, CRIS-585 and Shahbaz expressed favorable GCA effects (Table 2). The results implied that lines IUB-13 and NIAB-878, together with testers MNH-886 and FH-142, were effective general combiners due to their elevated GCA estimations. It makes them valuable for hybridization and selection programs aimed at developing new breeding materials with enhanced sympodial branches.

Table 1b. General combining ability (GCA) effects for earliness, seed cotton yield, and its components in six lines of cotton.

Lines	Days to 1st flowering	Plant height	Sympodial branches plant^{-1}	Bolls plant^{-1}	Seed cotton yield plant^{-1}	Fiber strength
FH-142	-0.76**	-7.62**	-1.64**	-1.60**	-5.23**	-1.01**
MNH-886	2.31**	-3.70**	-2.69**	-2.60**	-11.09**	-0.87**
IUB-13	-2.02**	5.30**	1.31**	1.40**	15.27**	0.90**
FH-901	-0.32**	3.30**	0.79**	1.20**	4.05**	0.63**
NIAB-878	1.31**	1.13*	0.93**	0.27**	-3.03**	0.74**
Koonj	-0.52**	1.60**	1.31**	1.33**	0.04	-0.40**
S.E. (gi)	0.04	0.46	0.02	0.08	0.87	0.02
S.E. (gi - gj)	0.08	0.92	0.03	0.04	1.74	0.04

*, ** Declared significant when GCA effects were twice and thrice equal or greater than S.E. (gi.), respectively.

Table 2. General combining ability (GCA) effects for earliness, seed cotton yield, and its components in three testers of cotton.

Testers	Days to 1st flowering	Plant height	Sympodial branches plant^{-1}	Bolls plant^{-1}	Seed cotton yield plant^{-1}	Fiber strength
Chandi-95	0.79**	-0.84**	-0.68**	-0.77**	-9.05**	-0.52**
Shahbaz	-0.35**	0.63*	0.04**	0.33*	0.38	0.29**
CRIS-585	-0.44**	0.21	0.64**	0.43*	8.67**	0.23**
S.E. (gi)	0.02	0.23	0.01	0.16	0.43	0.01
S.E. (gi - gj)	0.04	0.46	0.02	0.08	0.87	0.02

*, ** Declared significant when GCA effects were twice and thrice equal or greater than S.E. (gi.), respectively.

Table 3. Specific combining ability (SCA) effects for days to first flowering, plant height, and sympodial branches plant^{-1} in 18 crosses of cotton.

F$_1$ hybrids	Days to 1st Flowering	Plant height	Sympodial branches plant^{-1}
FH-142 × Chandi-95	0.95**	-5.24**	-1.70**
FH-142 × Shahbaz	-0.25*	-0.24	1.30**
FH-142 × CRIS-585	-3.05**	8.01**	2.45**
MNH-886 × Chandi-95	1.95**	4.76**	0.30**
MNH-886 × Shahbaz	0.95**	2.76*	-1.70**
MNH-886 × CRIS-585	3.95**	6.76**	0.30**
IUB-13 × Chandi-95	-0.05	9.76**	1.30**
IUB-13 × Shahbaz	-2.05**	18.76**	5.30**
IUB-13 × CRIS-585	-4.05**	12.76***	4.30**
FH-901 × Chandi-95	0.80**	14.01**	3.20**
FH-901 × Shahbaz	1.20**	10.76**	3.53**
FH-901 × CRIS-585	-3.05**	10.51**	2.63**
NIAB-878 × Chandi-95	1.45**	6.76**	3.45**
NIAB-878 × Shahbaz	1.95**	12.76**	2.48**
NIAB-878 × CRIS-585	0.45**	9.26**	3.83**
Koonj × Chandi-95	-0.55**	15.66**	3.30**
Koonj × Shahbaz	-4.05**	9.76**	3.30**
Koonj × CRIS-585	2.95**	4.76**	4.30**
S.E. (gi)	0.12	1.37	0.05
S.E. (gi - gj)	0.24	2.75	0.09
Range of SCA effects	-0.05 to -4.05	-0.24 to -5.24	0.30 to 5.30

*, ** Declared significant when SCA effects were twice and thrice equal or greater than S.E. (gi.), respectively.

The SCA effects for sympodia plant^{-1} indicated the 16 crosses exhibited positive values between 0.30 and 5.30, whereas only two hybrids showed negative values of -1.70 (Table 3). The current study revealed significant mean squares for GCA and SCA regarding sympodia plant^{-1}, indicating both additive and non-additive genetic contributions to the inheritance of this characteristic. Further researchers like Kumar *et al.* (2013) observed certain inbreds demonstrated superior general combining ability estimates for sympodia. Chapara *et al.* (2020) and Kumar *et al.* (2013) also reported a substantial positive SCA for the number of sympodia, highlighting the role of both additive and dominant genes in the inheritance of sympodia plant^{-1}. This is contingent upon the GCA and SCA of the parental lines in the cross combinations.

Bolls per plant

The total number of bolls plant^{-1} is a crucial yield-contributing trait in cotton. The mean squares for GCA and SCA were significant, indicating the involvement of both additive and dominant genes in the inheritance of bolls plant^{-1}. For six *Bt* lines, four lines (IUB-13, Koonj, FH-901, and NIAB-878) exhibited positive GCA effects, while the remaining two displayed negative GCA effects for bolls plant^{-1} (Table 1b). Among the three non-*Bt* testers, two (CRIS-585 and Shahbaz) demonstrated positive GCA effects and produced the highest number of bolls plant^{-1}, while one exhibited negative GCA effects for bolls plant^{-1} (Table 2).

In 14 out of 18 F_1 hybrids, the range of positive and desirable SCA effects for bolls plant^{-1} varied from 0.10 to 8.10, while four crosses exhibited negative SCA effects, ranging from -1.50 to -5.90 (Table 4). The hybrids with the most dominant genes and that were, therefore, favorable for hybrid development were FH-142 × CRIS-585, FH-901 × CRIS-585, Koonj × Shahbaz, and IUB-13 × Shahbaz. These hybrids also had the utmost values of positive SCA effects. These results were comparable to those of Khamdullaev *et al*. (2021), who observed non-additive types of gene action for controlling the character. Such findings proposed improving the bolls plant^{-1} is necessary with simple selection in further segregating generations. Similarly, Ullah *et al.* (2022) confirmed the substantial influence of additive and non-additive genes on bolls plant^{-1} in cotton.

Table 4. Specific combining ability (SCA) effects for bolls per plant, seed cotton yield per plant, and fiber strength in 18 crosses of cotton.

F_1 hybrids	Bolls plant^{-1}	Seed cotton yield plant^{-1}	Fiber strength
FH-142 × Chandi-95	-5.90**	-18.75**	-1.88**
FH-142 × Shahbaz	0.10	-5.35*	1.9.2**
FH-142 × CRIS-585	8.10**	51.23**	1.50**
MNH-886 × Chandi-95	1.10**	9.55**	-0.88**
MNH-886 × Shahbaz	1.10**	3.65	1.22**
MNH-886 × CRIS-585	-2.90**	-3.65	1.62**
IUB-13 × Chandi-95	2.10**	-5.15	3.12**
IUB-13 × Shahbaz	6.10**	48.45**	1.82**
IUB-13 × CRIS-585	3.10**	45.35**	2.32**
FH-901 × Chandi-95	5.20**	10.46**	2.22**
FH-901 × Shahbaz	-2.60**	-2.66	2.22**
FH-901 × CRIS-585	8.10**	47.17**	2.02**
NIAB-878 × Chandi-95	5.00**	22.42**	2.32**
NIAB-878 × Shahbaz	4.40**	7.98**	2.05**
NIAB-878 × CRIS-585	-1.50**	3.33*	2.40**
Koonj × Chandi-95	2.10**	12.85**	1.12**
Koonj × Shahbaz	7.10**	35.85**	1.62**
Koonj × CRIS-585	1.90**	-5.75*	0.62**
S.E. (gi)	0.23	2.61	0.06
S.E. (gi - gj)	0.47	5.21	0.12
Range of SCA effects	0.10 to 8.10	3.33 to 51.23	0.06 to 3.12

*, ** Declared significant when SCA effects were twice and thrice equal or greater than S.E. (gi.) respectively.

Seed cotton yield per plant

Among six *Bt* lines, IUB-13 exhibited highly significant positive GCA effects, while FH-901 and Koonj had moderate positive benefits. Conversely, MNH-886 displayed extremely significant negative GCA effects, with FH-142 following for seed cotton yield plant^{-1} (Table 1b). For three testers, only one (CRIS-585) gave promising GCA estimates for seed cotton yield plant^{-1}, and Shahbaz exhibited a positive but non-significant result. Conversely, Chandi-95 showed considerably negative GCA impacts for seed cotton yield plant^{-1} (Table 2). The participation of a parent with an elevated GCA is likely to enhance the prevalence of advantageous alleles.

Six F_1 hybrids showed negative SCA effects, while 12 of the 18 F_1 hybrids under study provided favorable SCA effects (Table 4). FH-142 × CRIS-585, IUB-13 × Shahbaz, FH-901 × CRIS-585, and IUB-13 × CRIS-585 were the hybrids with the ultimate significant positive SCA estimations for yield plant^{-1}, demonstrating their superiority for seed cotton yield plant^{-1} and pointing to improved performance of these cross-combinations. Most hybrids exhibiting favorable SCA effects may result from the strong GCA of the parents. It suggests the dominance of additive genetic effects, as noted by researchers like Khamdullaev *et al*. (2021) and Ullah *et al*. (2022), who reported positive GCA and SCA effects for seed cotton yield plant^{-1}.

Fiber strength

Among the lines, IUB-13, NIAB-878, and FH-901 had considerably positive GCA effects, but the other three lines (FH-142, MNH-886, and Koonj) displayed significant negative GCA effects (Table 1). Among the testers, Shahbaz and CRIS-585 had remarkable positive GCA effects (Table 2), while Chandi-95 demonstrated the most pronounced negative GCA effect for fiber strength.

From the 18 F_1 hybrids, 16 had positive SCA effects for fiber strength, ranging from 0.62 to 3.12, whereas only two hybrids showed negative SCA effects. The top-three scoring hybrids were NIAB-878 × CRIS-585, IUB-13 × CRIS-585, and NIAB-878 × Chandi-95. These hybrids should be applicable in hybrid crop development. Results suggested such lines and testers giving positive GCA effects should be considerations for further hybridization programs, while the hybrids should be effective in hybrid crop development with improved fiber strength (Table 4). Elfeki *et al*. (2024) further reported the positive GCA and SCA influences on fiber strength, while the non-additive gene effects governed fiber fineness, fiber length, and fiber strength.

Insect mortality

The enhanced expression of *Cry1Ac* in *Bt* transgenic cotton is essential for effectively managing lepidopteran pests, particularly bollworms. Notwithstanding a uniform gene inclusion event, the presented investigation confirmed distinct variation in the *Cry1Ac* quantitative levels and a seasonal decline in expression across the examined hybrids. At 30 days after sowing (DAS), the *Cry1Ac* content was high. Subsequently, the level of expressiveness decreased over the course of the season, reaching its lowest point at 120 DAS.

The Cry1Ac protein, however, did not become undetectable. At 30 DAS, the dangerous concentration of Cry1Ac in the leaves of 18 F_1 hybrids in the investigation varied from 1.020 to 2.555 µg/g dry weight. The hybrids NIAB-878 × Chandi-95, Koonj × CRIS-585, MNH-886 × Shahbaz, and NIAB-878 × CRIS-585 exhibited the highest Cry1Ac hazardous levels, measuring 2.555, 2.393, 2.031, and 1.386 µg/g, respectively (Table 5). The top 10 hybrids were choices for additional bioassay studies and insect mortality percentages at 60, 90, and 120 DAS. A significant disparity in the potentially deleterious concentrations of Cry1Ac in the upper canopy leaves was prominent at 60 DAS across 10 hybrids with varying parentage combinations (Figure 1). The hybrid NIAB-878 × Chandi-95 demonstrated the premier gene expression at 1.408 µg/g dry weight in the upper canopy leaves, followed by 1.365 µg/g in Koonj × CRIS-585, 1.361 µg/g in MNH-886 × Chandi-95, and 1.338 µg/g in NIAB-878 ×

Table 5. Immunostrip test and quantification of *Cry1Ac* toxin level in 18 F_1 hybrids of cotton at 30 days after sowing.

F_1 hybrids	Quantitative Immunostrip (MON 531)	ELISA *Cry1Ac* (µg/g) 30 days after sowing
FH-142 × Chandi-95	+ve	1.126
FH-142 × Shahbaz	+ve	1.278
FH-142 × CRIS-585	+ve	1.025
MNH-886 × Chandi-95	+ve	1.334
MNH-886 × Shahbaz	+ve	2.031
MNH-886 × CRIS-585	+ve	1.140
IUB-13 × Chandi-95	+ve	1.154
IUB-13 × Shahbaz	+ve	1.047
IUB-13 × CRIS-585	+ve	1.064
FH-901 × Chandi-95	+ve	1.039
FH-901 × Shahbaz	+ve	1.050
FH-901 × CRIS-585	+ve	1.049
NIAB-878 × Chandi-95	+ve	2.555
NIAB-878 × Shahbaz	+ve	1.020
NIAB-878 × CRIS-585	+ve	1.386
Koonj × Chandi-95	+ve	1.027
Koonj × Shahbaz	+ve	1.042
Koonj × CRIS-585	+ve	2.393

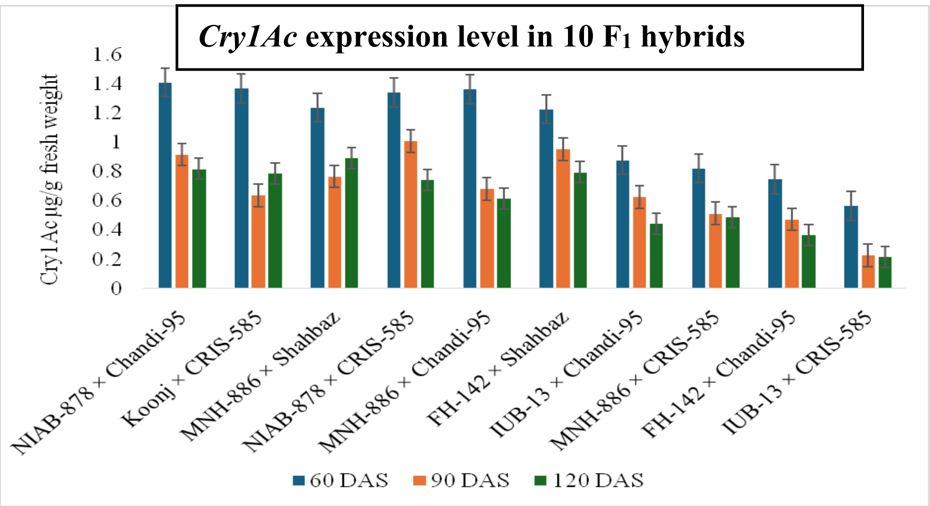

Figure 1. *Cry1Ac* expression level in 10 F_1 hybrids.

CRIS-585. The results further demonstrate that the other combinations displayed gene expressions in upper canopy leaves of less than 1.235 µg/g. At 90 days, only one hybrid, NIAB-878 × CRIS-585, revealed a gene expression level of 1.005 µg/g, which was markedly superior to other hybrid combinations, followed by FH-142 × Shahbaz at 0.951 µg/g and NIAB-878 × Chandi-95 at 0.914 µg/g (Figure 1). At 120 DAS, all hybrids exhibited gene expression levels below 1.0 µg/g, with a peak of 0.891 µg/g in hybrid MNH-886 × Shahbaz, followed by 0.816 µg/g in NIAB-878 × Chandi-95 and 0.794 µg/g in FH-142 × Shahbaz, respectively. Gene expression levels were significantly different among the hybrids (Figure 1). The interaction effect of age intervals and hybrids was statistically significant. The hybrids NIAB-878 × Chandi-95 and NIAB-878 × CRIS-585

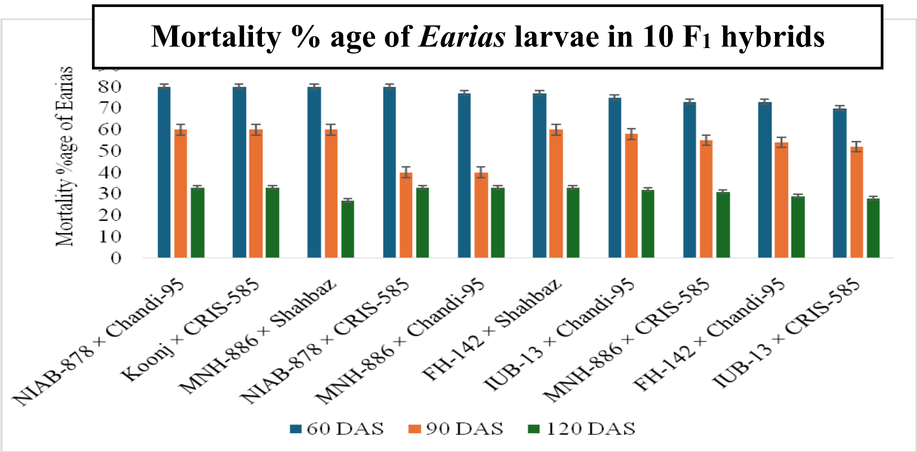

Figure 2. Mortality % age of *Earias* larvae in 10 F_1 hybrids.

unveiled higher gene expression levels of 1.05 µg/g and 1.03 µg/g, respectively. The subsequent entries were FH-142 × Shahbaz, MNH-886 × Shahbaz, and Koonj × CRIS-585, exhibiting gene expression levels of 0.99, 0.96, and 0.93 µg/g, respectively (Figure 1). The decline in expression across the season has been well documented, and the new findings corroborate previous studies. Numerous scientific experiments reported that the expression of plant cry toxins varied widely.

Comparing terminal canopy leaves to middle and lower canopy leaves, Jamil *et al*. (2021) found the former expressed an extreme level of intensity. According to Soberon *et al*. (2018) and Jamil *et al*. (2021), they observed that the leaves, squares, flowers, and bolls have the highest levels of Cry protein expression. This explains why *H. armigera* larvae prefer the upper canopy leaves because they are succulent and also contain a high amount of Cry endotoxin. Furthermore, it was proven that the expression pattern changes with the season, peaking at the start and falling off at the end. The species *Earias* spp. was a choice for this study due to its perceived high level of harm and resilience, enabling it to endure a broader range of environmental conditions, including optimal humidity and temperature levels seen in Pakistan. Figure 2 illustrates the mortality percentage of *Earias* spp. in bioassays, utilizing young bolls at 60, 90, and 120 DAS. In comparison to other hybrids, the hybrids NIAB-878 × Chandi-95, Koonj × CRIS-585, MNH-886 × Shahbaz, and NIAB-878 × CRIS-585 exhibited a statistically significant mortality rate of 80% at 60 DAS (Figure 2). At 90 DAS, the mortality rate declined to 60% in NIAB-878 × Chandi-95, Koonj × CRIS-585, MNH-886 × Shahbaz, and FH-142 × Shahbaz; still, it remained relevantly elevated in these hybrids compared with others. At 120 DAS, the mortality rate declined to a concerning range of 27% to 33%. The hybrids NIAB-878 × Chandi-95, Koonj × CRIS-585, NIAB-878 × CRIS-585, MNH-886 × Chandi-95, and FH-142 × Shahbaz displayed considerably higher death rates than the other hybrids (Figure 2). The overall seasonal mean mortality percentage appeared to be statistically higher for the hybrids NIAB-878 × Chandi-95, Koonj × CRIS-585, FH-142 × Shahbaz, and MNH-886 × Shahbaz. It was evident that the mortality percentage of *Earias* spp. larvae decreases with time as the amount of *Bt* toxin in the fruiting bodies gradually decreases (Figure 2).

Our results were equivalent to Riaz *et al*. (2021), who reported that the endotoxin that the *Cry* genes make also affects the degree of insect control; it was at its maximum early in the season and gradually declined as time passed. A reduction in the amount of toxin may not only result in inadequate control over the target insects but also exacerbate resistance to the Cry protein.

CONCLUSIONS

Among *Bt* lines, IUB-13, FH-901, and Koonj, along with the non-*Bt* testers Chandi-95, CRIS-585, and F_1 hybrids FH-142 × CRIS-585, MNH-886 × Chandi-95, and IUB-13 × Chandi-95, emerged superior. Therefore, these parental lines may be chosen for hybridization programs to create advantageous hybrids. Specific combining ability and heterotic effects were essential metrics for assessing the suitability of F_1 hybrid development.

REFERENCES

Abdel-Aty MS, Sorour FA, Yehia WM, Kotb HM, Abdelghany AM, Lamlom SF, Shah AN, Abdelsalam NR (2023). Estimating the combining ability and genetic parameters for growth habit, yield, and fiber quality traits in some Egyptian cotton crosses. *BMC Plant Biol.* 23(1): 121.

Ahmad SJN, Majeed D, Ali A, Sufian M, Aslam Z, Manzoor M, Ahmad JN (2021). Effect of natural high temperature and flooding conditions on Cry1Ac gene expression in different transgenic Bt cotton (*Gossypium hirsutum* L.) cultivars. *Pak. J. Bot.* 53(1): 127-134.

Arain BT, Baloch MJ, Sial P, Arain MA, Baloch A (2015). Estimation of heterosis and combining ability in F_1 hybrids of upland cotton for yield and fiber traits: Upland cotton yield and fiber traits. *Biol. Sci.* 58(3): 132-139.

Balci S, Cinar VM, Unay A (2023). Estimating gene action and combining ability for yield and fiber quality in cotton (*Gossypium hirsutum* L.). *Genetika.* 55(1): 61-69.

Chakholoma M, Nimbal S, Jain A, Kumar M (2022). Combining ability analysis for yield and fiber quality traits in American cotton (*Gossypium hirsutum* L.) genotypes. *Ekin J. Crop Breed. Genet.* 8(1): 61-69.

Chapara R, Rani SM, Satish Y (2020). Combining ability studies in cotton (*Gossypium hirsutum* L.) for yield and fiber quality parameters. *IJCS.* 8(2): 523-527.

Cheema HMN, Khan AA, Noor K (2021). *Bt* Cotton in Pakistan. Genetically Modified Crops in Asia Pacific*;* CSIRO Publishing: Clayton, Australia, 91.

Elfeki FE, Hamed MF, El-Twab RM (2024). Estimation of genetic parameters for some agronomic and technological quantitative traits in cotton using line x tester analysis. *Menoufia J. Plant Prod.* 9(4): 229-240.

Government of Pakistan-GOP (2023). Economic Survey of Pakistan. Economic Advisory Wing: Islamabad, Pakistan.

Han YY, Wang KY, Liu ZQ, Pan SH, Zhao XY, Zhang Q, Wang SF (2020). Research on hybrid crop breeding information management system based on combining ability analysis. *Sustainability,* 12(12): 4938.

Imran M, Saif-ul-Malook SA, Nawaz MA, Ahabaz M, Asif M, Ali Q (2015). Combining ability analysis for yield related traits in sunflower (*Helianthus annuus* L.). *American-Eurasian J. Agri. Environ. Sci.* 15(3): 424-436.

Jamil S, Shahzad R, Rahman SU, Iqbal MZ, Yaseen M, Ahmad S, Fatima R (2021). The level of Cry1Ac endotoxin and its efficacy against *H. armigera* in *Bt* cotton at large scale in Pakistan. *GM Crops & Food*. 12(1): 1-17.

Kempthorne O (1957). An Introduction to Genetic Statistics. XVII, 545, Oxford: Wiley. 1957.

Khamdullaev S, Nabiev S, Azimov A, Shavkiev J, Yuldashov U (2021). Combining ability of yield and yield components in upland cotton (*G. hirsutum* L.) genotypes under normal and water-deficit conditions. *Plant Cell Biotechnol. Mol. Biol.* 22(35&36): 176-186.

Kumar M, Nirania KS, Sangwan RS, Yadav NK (2013). Combining ability studies for yield and quality traits in upland cotton (*Gossypium hirsutum* L.). *J. Cotton Res.* 27(2): 171-174.

Lv N, Liu Y, Guo T, Liang P, Li R, Liang P, Gao X (2022). The influence of *Bt* cotton cultivation on the structure and functions of the soil bacterial community by soil metagenomics. *Ecotoxicol. Environ. Saf.* 236: 113452.

Mohammed R, Are AK, Bhavanasi R, Munghate RS, KaviKishor PB, Sharma HC (2015). Quantitative genetic analysis of agronomic and morphological traits in sorghum (*Sorghum bicolor* L*.). Front. Plant Sci.*6: 945.

Patel Y (2020). Effect of weather parameter on the population of pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera-Gelechiidae) in Cotton*.J. Curr. Microbiol. Appl. Sci*. 9: 1476-1481.

Riaz S, Johnson JB, Ahmad M, Fitt GP, Naiker M (2021). A review on biological interactions and management of the cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae). *J. Appl. Entomol*. 145(6):467-498.

Singh RK, Chaudhary BD (1985). Biometrical Methods in Quantitative Genetic Analysis. Kalyani Publishers. New Delhi, pp. 57.

Sivia SS, Siwach SS, Sangwan O, Lingaraja L, Vekariya RD (2017). Combining ability estimates for yield traits in line x tester crosses of upland cotton (*Gossypium hirsutum*). *Int. J. Pure Appl. Biosci*. 5(1): 464-474.

Soberon M, Monnerat R, Bravo A (2018). Mode of action of cry toxins from *Bacillus thuringiensis* and resistance mechanisms. *Microbial Toxins*, 1.

Statista (2024). Distribution of global cotton production by country. 2024. https://www.statista.com/statistics/263055/cotton-production-worldwide-by-top-countries/. Accessed March 14, 2024.

Ullah A, Shakeel A, Ahmed HG, Naeem M, Ali M, Shah AN, Wang L, Jaremko M, Abdelsalam NR, Ghareeb RY, Hasan ME (2022). Genetic basis and principal component analysis in cotton (*Gossypium hirsutum* L.) grown under water deficit condition. *Front. Plant Sci.* 13: 981369.

Verma SK, Kimar R, Sain SK, Singh A, Bagri S, Paul D (2024). Emerging challenges in cotton cultivation and strategies for improving productivity in the north zone of India. *J. Cotton Res. Develop.* 38(1): 7-18.