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TRIPLE TEST CROSS ANALYSIS REVEALED THE ABSENCE OF EPISTASIS IN THE INHERITANCE FOR CLCuD IN COTTON

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Abstract

The modified triple test cross technique was employed to analyze the presence of dominance, additive and epistasis components contributing to the inheritance of traits and cotton leaf curl disease (CLCuD) in cotton. The study unveiled the significance of epistasis as a critical contributor in the inheritance of biochemical traits under CLCuD impact. Among epistasis the additive x additive (i) and the additive × dominance + dominance × dominance (j + l), played pivotal roles in shaping trait expression. Gene action, predominantly additive and dominance types, significantly influenced the manifestation of CLCuD, chlorophyll-b, and carotenoids. Interestingly, no indication of directional dominance was observed for these traits. Regarding chlorophyll-a, total chlorophyll, Superoxide Dismutase, Peroxidase, Catalase, and total soluble protein, a strategic approach involving recurrent selection or biparental mating is recommended, particularly starting from the F_2 generation to improve many traits. However, to harness the full potential of epistatic effects and foster the development of desired cultivars in cotton, deferring the selection of desired plants until the F_5 or F_6 generations is advisable. This deliberate delay enables the maximum fixation of epistatic effects, crucial for cultivating desired cotton cultivars resilient to CLCuD.

Key words: Cotton leaf curl disease (CLCuD), Biochemical traits, Triple test cross analysis, Epistasis, Cotton breeding.

Introduction

Cotton is one of the major cash crops of Pakistan, which is very important in the economy of the country as it is a source of foreign exchange (Afzal, 2021). It is an important component of textiles. The economic contribution of cotton crop in terms of agricultural growth and national GDP (0.6%), has demonstrated its status as a key factor in the socio-economic development of Pakistan (Maqbool *et al*., 2019). However, biotic and abiotic stress complicate its production throughout its growing season. In terms of natural stress, the most troublesome insect is whitefly which damages crops directly and indirectly as a vector of various viruses especially cotton leaf curly virus disease (CLCuD). General climatic conditions such as humidity and temperature favor the insects and diseases which cause drastically loss of yield and high cost of production (Tariq *et al*., 2020; Ali *et al*., 2022). Despite the presence of many biotic stresses, the most notorious and overall threat to cotton farming in Pakistan is a cotton leaf curl disease. Cotton leaf curl disease (CLCuD) stresses induced the reactive oxygen species (ROS), membrane toxicity and peroxidation occur. This oxidative stress can oxidize important biomolecules such as carbohydrates, proteins, lipids and DNA (Hasanuzzaman *et al*., 2013). These diseases affect chlorophyll and carotenoids and other photosynthetic pigments, ultimately affecting the integrity of the photosynthetic machinery, leaf curl, reduced chlorophyll and poor sap conductivity (Ali *et al*., 2013). The whitefly (*Bemisia tabaci*) has crucial role as vector for virus dissemination and problem got status of pandemic under the humid and high temperature condition. Many strains of virus have been identified on cotton in Pakistan; however, the most common strains are Burewala, Kokhran and Multan Virus (Khalid *et al*., 2017; Kamal *et al*., 2019). The cultivation of cotton in India and Pakistan is under economical threat due to an infestation of complex begomoviruses particularly to the Cotton Leaf Curl Multan and Kokhran strains (Haxim *et al*., 2017; Zubair *et al*., 2017).

Much research has been carried throughout the world for the development of cotton varieties that are resistant to CLCuD under wider climatic conditions to have sustainable cotton production. Indeed, the exploitation of genetics has been proven as a basic strategy against CLCuD. However, the genetics of these viruses is very complicated due to mutation. The genetic permutation of these viruses led to the CLCuD-associated viruse one of the major factors involved in determining the severity and spread of the disease. These genetic shuffling have been observed in different begomovirus strains, resulting in the emergence of new viral variants (Saleem *et al*., 2016; Farooq *et al*., 2021). Genetic engineering innovations have also been developed as preventive measures. However, in conventional breeding techniques, triple test cross analysis is a modern genetic tool that can be used to explore the genetics mechanism for CLCuD resistance in cotton. The F_1 hybrid is derived through conventional hybridization between two diverse genetic makeup lines in which one line isolated and then randomly crossed with two testers (Hassan *et al*., 2022). This technique helps to identify superior genotypes with strong combining ability, resistant traits to problematic diseases, and agronomic characteristics of an elite class nature. By performing multiple crosses between plants of various generations and studying the genetic interactions that occur in the offspring, we can explore resistant segregants from base population for the development of cotton resistant to CLCuD. Zubair *et al*., 2017, reported that epistasis is an interplay of the influence of various genes on a specific trait, contribute mostly to the inheritance pattern and phenotypic outcomes of complex traits such as CLCuD resistance. Modified triple test cross analysis helps to identify and quantify the presence and effects of epistasis and has proven important in the development of cotton varieties resistant to CLCuD (Singh & Chaudhary, 1985). Triple Test Cross analysis could also explore the contribution of additive (D) and dominant (H) genes of a given treatment, (H/D)^1/2 (the degree of dominance) and dominance direction (rs.d).

Examining the phenotypic variation between generations, TTC analysis helps to quantify additive and dominant variance components and depict the effects of epistatic effects for studied traits. Exploring the epistasis in the inheritance of CLCuD resistance gene has a great impact for breeding and possibly affecting the importance of marker assisted selection (MAS) to develop varieties of cotton which have resistance against CLCuD (Zaidi *et al*., 2019).

Materials and Method

Experimental site: Experiment was conducted in the research land allocated by Plant Breeding and Genetics department, faculty of agricultural sciences and technology, Bahauddin Zakariya University Multan, Pakistan. The experimental material was consisted of twelve genetically diverse cotton genotypes, selected on the basis of CLCuD tolerance and yield (VH – 402, FH – 490, FH – 142, BS – 2015, FH – Lalazar, BS – 20, CIM – 717, MNH – 1020, NIBGE – 12, FH – 444, NIAB -878 and Mac -07).

Experiment design: For genetic purity of genotypes, selfing was done in glasshouse and maintained growth conditions at 35/21±5°C (day/night). During normal season of cotton, FH-142 (CLuD tolerant and high yielder) and Mac-07 (CLCuD resistant) (Zaidi *et al*., 2019) were grown in the field to develop their hybrid (F_1) of (FH – 142 x Mac-07).

Table 1. Origin and category of cotton genotypes.

No.	Parent	Origin
L1	MNH – 1020	CRI, Multan
L2	FH – 444	AARI, Faisalabad
L3	BS – 2015	Bandashah Seed, Khanewal
L4	NIAB – 878	NIAB, Faisalabad
L5	FH – Lalazar	AARI, Faisalabad
L6	NIBGE – 12	NIBGE, Faisalabad
L7	BS – 20	Bandashah Seed, Khanewal
L8	FH - 490	AARI, Faisalabad
L9	CIM - 717	CCRI, Multan
L10	VH - 402	CRS, Vehari
	Tester	
No.	**Parent**	**Origin**
T1	Mac-07 (P_1)	Exotic
T2	FH-142 (P_2)	AARI, Faisalabad
T3	P1xP2 (F_1)	P1xP2 (F1)

During winter, all genotypes which were selected grown in glass house for crossing between FH - 142, Mac – 07 and (FH - 142 × Mac – 07). This parental cross (FH - 142 × Mac -07) participated as a parent (female) and all others genotypes of cotton were participated as a male donor, including MNH - 1020, BS - 2015, FH - 444, NIAB - 878, NIBGE – 12, FH - Lalazar, includes VH – 12, 402, BS - 20, CIM – 717 and FH – 490. (Table 1). The evaluation of developed breeding population was done at the experimental field of PBG department of BZU, Pakistan. Each male conducted three crosses, yielding a total of 30 crosses (10 three way and 20 individual crosses).

All 43 genotypes (30 crosses + 13 parents) were grow following randomized complete block design with three replications in the field and healthy seedlings were grown using standard cultural practices.

Leaf sample and data collection: Ninety days after emergence, fully grown Third leaf from the top of the plant with clear CLCuD symptoms (Table 2) as described by Shafiq *et al*., (2017) were used as sample for further study and data of other parameter were recorded i.e. chlorophyll-a (Chl-a), chlorophyll-b (Chl-b), carotenoids (**CARs),** total chlorophyll content (TCHL), peroxidase (POD), superoxide dismutase (SOD), catalase (CAT), and total soluble protein (TSP) were analyzed using the collected leave. Collected samples were quickly frozen in liquid nitrogen. All spectrophotometric analyzes of biochemical traits were carried out at the Genomics Laboratory, Department of Plant Breeding Genetics, Bahauddin Zakaria University, Multan, Pakistan. The assessment of these biochemical traits was calculated following the protocol mentioned by Metzner *et al*., (1965).

Chl - a: (10.30 × E663) – (0.98 × E 644)
Chl - b: (19.70 × E644) – (3.87 × E 663)
TCHL: chlorophyll a + chlorophyll b
CARs: 4.20 × E 452.50 – {(0.0264 × chlorophyll a) + (0.426 × chlorophyll b)}
E was absorbance. Lastly, leave samples were measured as mg/g.

However, the enzymatic and antioxidant activity was calculated following the methodology mentioned by Ainsworth & Gillespie, 2007, the activity of catalase and peroxidase (POD) by Chance & Maehly, 1955, superoxide dismutase (SOD) activity by Giannopolitis & Ries, 1977 and total soluble proteins activity by Bradford, 1976.

Statistical analysis

Asses the recorded data by following the triple test cross technique as mentioned by Kearsey & Jinks (1968) and extended from design III by Robinson (1948), we get a precise test for epistasis with content-wise estimates of the additive (D) and dominance (H) components. The numerous phenotypes of the TTC technique are mentioned in the model below:

$$Lijk = \mu + Gij + R\,k + Eijk$$

The model describes the value of phenotypes Lijk of a cross between line j and tester Li in kth replication. The terms μ, Gij, Rk, and Eijk represent the overall mean of a single and three way crosses, genotypic value, replication effect, and error respectively.

Detection of epistasis: The L1i+L2i–2L3i test of significance of difference (where i = number of lines) gives the information whether there is epistasis or not. Thus, L1i + L2i – 2L3i for each line and replication was first measured and then test.

Estimating the additive variance component (D): The sum of L1i and L2i for each line was measured across replications and analyzed.

Estimating the dominance variance component (H): The sum of L1i–L2i for each line was assessed separately for each replication and subjected to analysis of variance as followed.

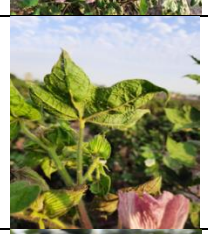
Estimation Degree of dominance: The degree of dominance was measured as (H/D)1/2, where H and D are the components of dominance and additive variance.

Coefficient of Correlation (rs,d): Correlation coefficients (rs,d) b/w sum (L1i+L2i) and genotypic variation (L1i–L2i) were measured as:

$$r_{(s.d)} = \frac{\sum XY - \sum X \sum Y/N}{\sqrt{\left(\sum X^2 - \frac{(\sum X)^2}{N}\right)} \sqrt{\left(\sum Y^2 - \frac{(\sum Y)^2}{N}\right)}}$$

Variation of sums (L1i + L2i) and differences (L1i - L2i) were used to estimate additive (D) and dominance (H) components of variation. Epistasis was estimated using variance comparison (L1i + L2i – 2L3i), where L1i, L2i and L3i denote the traits of the i^{th} offspring relative to their affected testers. Triplicate crossover analysis was performed using a modified method (Singh & Chaudhary, 1985; Khattak *et al*., 2001).

Table 2. Grading system for Cotton Leaf Curl Virus (Shafiq *et al*., (2017) 0-4 rating scale).

Severity grade	Proposed symptoms	Remarks	
0	Normal growth with no visible symptoms	Resistant	
1	The uppermost 1-4 terminal leaves exhibit curled symptoms, primarily along the veins, while the remainder of the plant shows normal growth with no signs of distress	Highly tolerant	
2	Curling symptoms, accompanied by significant vein thickening, are observed on the upper one-third of the plant, accompanied by mild stunting of the plant	Tolerant	
3	Symptoms of curling manifest on the upper half of the plant, accompanied by significant thickening of veins and moderate stunting of the plant	Susceptible	
4	Extensive vein thickening and leaf curling are observed throughout the entire plant, accompanied by severe stunting	Highly susceptible	

Results

The analysis of variance for different biochemical traits under the stress of CLCuD using Triple test cross analysis represented a significant amount of variation among studied traits expect the Chl-b, paved the way for further analysis of data. Parents, i.e. lines and testers belonging to different breeding stations and pedigree showed significant variation for all traits expect Chl-b. The presence of this variation is basic criteria for genotypes to be used in TTC assessment. Partition of the differences among genotypes into constituents presented that F_1 hybrid and both parents have observable variability for CLCuD, Chl-a, TCHL, CARs, SOD, POD, CAT and TSP and CLCuD, TCHL, SOD, POD, CAT and TSP respectively. In addition to this partitioning of parental variation into testers, lines and lines vs tester interaction also showed significant variation for CLCuD and Some off biochemical traits (Table 3). Genetic analysis of the data to detect the presence of epistasis for different biochemical traits revealed the presence of epistasis for all traits expect CLCuD. Further distribution of total epistasis into its

constituents revealed that [i]- (additive X additive) epistasis was found important for TCHL, POD and CAT. However, some were non-significant for Chl-a, CARs, SOD and TSP. Another type of epistasis [j+l] (additive x dominance) and (dominance X dominance) was also significant for TCHL, SOD and TSP (Table 4). The epistatic deviations of individual lines have been shown (Table 5) to point out the direction, comparative magnitude, and to show the lines which interacted with testers to produce significant epistatic effect.

Lines. i.e., MNH-1020 for SOD and POD; FH-444 for TCHL; BS-2015 for CARs and CAT; NIAB-878 for SOD; FH-Lalazar for Chl-a; NIBGE-12 for POD and CAT; VH-402 for SOD; BS-20 for SOD; FH-490 for Chl-a, TCHL and SOD; CIM-717 for SOD and CAT contributed a significant positive role in the total epistasis. The remaining lines were not play a significant role in the total epistasis. The value of D and H indicated the relative importance of additive and dominant genetic components. The results showed that the additive variance for D was larger in degree compared to the dominance of H for CLCuD, indicating the importance of additive gene action in the inheritance of this trait. Furthermore, the relative importance of additive and dominant gene action was definite by the dominance degree (H/D)1/2, which has value lower than 1, which confirms the participation of partial dominance in CLCuD expression. The dominance direction (rs.d) value was non-significant, indicating that the alleles were dispersed among the testers, so there was no evidence of any directional dominance for CLCuD (Table 6).

Table 3. Mean squares of different biochemical traits.

Source	d.f.	CLCuD	Chl-a	Chl-b	TCHL	CARs	SOD	POD	CAT	TSP
Replication	2	0.001	0.004	0.009	0.001	0.003	5.212	0.017	0.689	0.002
Genotypes	42	0.081**	0.015**	0	0.033**	0.007**	39.779**	1.570**	4.384**	0.018**
Hybrids	29	0.063**	0.001	0	0.027**	0.001	38.568**	0.937*	3.879**	0.016**
Parents	12	0.414**	0.046**	0	0.047**	0.016**	32.289**	2.262**	2.159**	0.012*
Lines	9	0.002**	0.037**	0	0.036**	0.009**	3.86	0.289	2.498**	0.005
Testers	2	2.003**	0.039**	0	0.039**	0.008*	46.563**	7.191**	0.356	0.050**
L1+L2_vs_F1	1	0.603**	0.004	0	0.005	0.001	67.869**	2.881*	0.039	0.030*
L1 vs L2	1	2.708**	0.074**	0	0.074**	0.015**	25.256**	11.501**	0.673	0.071**
Lines vs testers	1	1.902**	0.143**	0	0.164**	0.093**	259.606**	10.150**	2.716*	0.003
Hybrids vs parents	1	0.170**	0.053**	0	0.014	0.088**	164.778**	11.649**	45.729**	0.139**
Error	84	0.007	0.008	0	0.007	0.002	3.497	0.485	0.671	0.006

d.f Stand for Degree of Freedom, CLCuD denotes Cotton Leaf Curl Disease, Chl-a represents Leaf Chlorophyll-a, Chl-b stands for Leaf Chlorophyll-b, TCHL denotes Leaf Total Chlorophyll, CARs represents Carotenoids, SOD stands for Superoxide, POD represents Peroxidase, CAT indicates Catalase and TSP denotes Total Soluble Protein. The symbols "*", indicating $p<(0.05)$, and "**", indicating $p<(0.01)$, represent the respective significance levels respectively

Table 4. Analysis of variance of detect epistasis for different biochemical traits in cotton genotypes.

Source	Mean squares								
	d.f	CLCuD	Chl-a	TCHL	CARs	SOD	POD	CAT	TSP
Total epistasis	10	0	0.008*	0.346**	0.001	179.452**	7.807*	21.470**	0.126*
Epistasis i type	1	0	0.006	1.051**	0.004	492.383	11.055*	138.409*	0.218
Epistasis j + l type	9	0	0.009	0.268**	0.001	144.682**	7.446	8.477	0.116*
Epistasis i type × Replications	2	0	0.001	0.007	0.002	71.585	0.501	5.97	0.081
Epistasis (j+l) type × Replications	18	0	0.004	0.005	0.002	21.304	3.129	4.275	0.045
Total epistasis × Replications	20	0	0.003	0.005	0.002	26.332	2.866	4.445	0.049

d.f Stand for Degree of Freedom, CLCuD denotes Cotton Leaf Curl Disease, Chl-a represents Leaf Chlorophyll-a, Chl-b stands for Leaf Chlorophyll-b, TCHL denotes Leaf Total Chlorophyll, CARs represent Carotenoids, SOD stands for Superoxide, POD represents Peroxidase, CAT indicates Catalase and TSP denotes Total Soluble Protein. The symbols "*", indicating $p<(0.05)$, and "**", indicating $p<(0.01)$, represent the respective significance levels respectively

Table 5. Individual cotton lines showing epistasis.

Source	CLCuD	Chl-a	TCHL	CARs	SOD	POD	CAT
MNH-1020	0.002	0.001	0.003	0.002	8.394*	3.197*	2.318
FH-444	0.005	0.004	0.015*	0.003	1.823	0.871	0.691
BS-2015	0.001	0.003	0.008	0.005*	15.430*	2.147	17.842*
NIAB-878	0.002	0.004	0.009	0.001	18.577*	2.529	0.077
FH-Lalazar	0.043	0.010*	0.01	0.003	16.703*	0.053	0.312
NIBGE-12	0.005	0.003	0.002	0.001	4.212	4.057*	6.079*
VH-402	0.006	0.005	0.002	0.001	27.656*	1.317	2.666
BS-20	0.007	0.003	0.002	0.001	78.452*	4.12	1.675
FH-490	0.012	0.007*	0.007*	0.003	77.646*	2.332	4.829
CIM-717	0.009	0.006	0.006	0.003	14.428*	8.036	7.960*

CLCuD denotes Cotton Leaf Curl Disease, Chl-a represents Leaf Chlorophyll-a, Chl-b stands for Leaf Chlorophyll-b, TCHL denotes Leaf Total Chlorophyll, CARs represents Carotenoids, SOD stands for Superoxide, POD represents Peroxidase, CAT indicates Catalase and TSP denotes Total Soluble Protein. The symbols "*", indicating $p<(0.05)$, and "**", indicating $p<(0.01)$, represent the respective significance levels respectively

Table 6. Estimates of additive (D) and dominance (H) variance, degree of dominance (H/D)1/2 and direction of dominance (rs.d) for various traits.

Traits	D	H	$(H/D)^{1/2}$	rs.d
CLCuD	2.018**	0.786	0.117	-0.045

*, ** = Significant at 0.05 and 0.01 levels of probability, respectively

Discussion

It is imperative for a plant breeder to device an effective breeding strategy, yielding successful modification in genetic architecture of plants. In this scenario only exploring the additive and dominant components of variation could be biased and will not yield any encouraging results due to the involvement of epistasis in the inheritance of traits. Therefore, it is really a worth it for plant breeders to choose a biometrical technique, which is fully capable to explore additive, dominant as well as epistatic effects. Among the different biometrical techniquesTriple test cross analysis (Ketata *et al*., 1976) is a fully capable and powerful tool to explore epistasis, additive (d) and dominance (H) component of variation as well as the direction of dominance. In the present study the inheritance of different biochemical traits under the CLCuD stress was explored using Modified triple test cross analysis (Khattak *et al*., 2001).

The significant amount of difference among genotypes indicated the presence of ample genetic differences among these genotypes. This variability is essential for breeding programs as it indicates the potential for selecting superior genotypes (Gonzalez *et al*., 2019). Moreover, the high mean values of hybrids serve to indicate the TTC progenies heterogeneity, which is of great importance to the study of genetic recombination. Hybrids have a very significant contribution to the improvement of CLCuD, TCHL, SOD, CAT, and TSP traits over the parental lines (Eldin *et al*., 2018). In the same way, the presence of gene action (both additive and non-additive) in the inheritance of the study traits was confirmed by the significant mean values for lines vs testers (Hassan *et al*., 2022). This showed that the testers possessed superior alleles for these traits, which made them important for hybrid development (Murray *et al*., 2019). Besides this hybrid vs parents also reveal the significant mean values defining that there is similar substantial genetic variability among the tested groups. The divergence of genetic material can be exploited in the breeding programs that the aim would be towards crop resilience to stresses and production of better yields, that can arise from CLCuD (Bakhsh *et al*., 2016). All the results showed that we can use modified TTC for all traits except Chl-b having non-significant values.

Epistasis, meaning the interplay of genes, can be discovered genetically in different biochemical characteristics of cotton genotypes, employing a TTC, which exhibited a considerable total epistasis that affirmed the existence of non-allelic gene action. This is interpreted to mean that the traits in question are not just determined by individual genes with their own independent contribution (additive effects) but also by the interactions between different genes. Such gene action may have a positive or a negative impact on the manifestation of these specific traits (Singh *et al*., 2019). The presence of vital type epistasis denotes that traits are influenced by additive × additive gene interactions, and therefore, it can be assumed that pairs of additive genes together are responsible for a part of the genetic variance. This adds a new factor in the selection process of the breeding programs (Jiang *et al*., 2020). A recurrent breeding program, which employs a cyclic selection method consisting of repeated cycles of selection, recombination, and intercrossing among a group of individuals, is especially suitable for the improvement of traits controlled by genes with additive effects. This strategy provides the breeders with options to accumulate the favorable additive effects through successive generations, thereby, it becomes a more beneficial factor in the expression of the desirable traits which are influenced by the additive × additive interactions (Hallauer and Carena, 2019). Furthermore, the presence of significant epistasis of [j+l] type showed the additive x dominance and dominance x dominance gene interactions at play. Through the lens of epistasis, this is the key to the inheritance patterns of these traits since it is a complex and convoluted mechanism that involves the combined effects of two different kinds of genes: additive and dominant genes that are responsible for the desirable traits to be expressed (Eldin *et al*., 2018). The occurrence of these traits is an important aspect for breeders in the process of creating varieties that can sustain and perform well in different ecological zones even under stressful environments like drought or pest attack (Kumari *et al*., 2021).

Through the analysis of epistatic deviations of the different lines, important epistatic deviations were observed for (MNH-1020 for SOD and POD; FH-444 for TCHL; BS-2015 for CARs and CAT; NIAB-878 for SOD; FH-Lalazar for Chl-a; NIBGE-12 for POD and CAT; VH-402 for SOD; BS-20 for SOD; FH-490 for Chl-a, TCHL, and SOD; and CIM-717 for SOD and CAT) several lines i.e. which have been listed in Table 5. These lines were responsible for the total epistasis and thus the candidates for the studied traits are confirmed as the lines. The other lines that were involved in the equation of epistasis gave no significant result; thus they were proposed as inert elements in the mechanism of the interactions. Similar to the findings of (Ketata *et al*., 1976; Khattak *et al*., 2001), who indicated that the different lineages have contributed differently to the total epistasis, these results were also reported.

The genetic decomposition of the components of the types of additive (D) and dominance (H) has elucidated that the magnitude of additive variance (D) has the greater value than that of the dominance variance (H) in CLCuD. In this way, the additive gene action is the more important mechanism for the inheritance of the trait which is also pointed out by previous studies (Iqbal *et al*., 2012; Rehman *et al*., 2019) in support of this assumption. The dominance ratio (H/D)^(1/2) underscoring less than 1 verified the fact that CLCuD is due to incomplete dominance. This finding unveiled that the alleles responsible for resistance or susceptibility are not in complete dominance, which in turn is responsible for producing a phenotype that is a blend of the two parental traits (Saeed *et al*., 2020). Furthermore, the non-significant value of the direction of dominance (rs.d) implied that the alleles were spread out among the test group, thus, the direction of dominance for CLCuD could not be applied (Sattar *et al*., 2022).

Conclusions

The outcomes of this presented study will have a high impact on cotton breeding programs. The existence of notable epistasis for main traits implies that the interactions should be considered in breeding approaches in order to attain the maximum genetic gains. The determination of specific lines, which have a positive contribution to epistasis, can help in the selection of parental lines during hybrid development. Moreover, the additive gene effects for traits like CLCuD are emphasized, thus, the use of additive-based selection methods to improve resistance to cotton leaf curl disease is supported. Further, it is stated that this study is part Ph.D. research by 1st author.

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References

Afzal, M. 2021. Gene expression evaluation of multigenic cotton (*Gossypium hirsutum* L.) against cotton leaf curl virus. *Int. J. Agri. Biol*., 26: 169-176.

Ainsworth, E.A. and K.M. Gillespie. 2007. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. *Nature Prot*., 2: 875-877.

Ali, M.M., Z. Ali, F. Ahmad, F. Nawaz, Q. Shakil, S. Ahmad and A.A. Khan. 2022. Transcript abundance of heat shock protein genes confer heat tolerance in cotton (*Gossypium hirsutum* L.). *Pak. J. Bot*., 54(1): 65-71.

Ali, I., M.N. Tahir and S. Ahmad. 2013. Cotton leaf curl disease and its spread in Pakistan. Virol. J., 10: 1-10.

Bakhsh, A., Z. Iqbal and M. Bashir. 2016. Genetic variability and heritability in upland cotton (*Gossypium hirsutum* L.). *J. Agric. Res*., 54: 367-373.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem*., 72: 248-254.

Chance, B. and A.C. Maehly. 1955. Assay of catalase and peroxidases. In: (Eds.): Colowick, S.P. & N.O. Kaplan. *Methods in Enzymology*, Vol. 2, Academic Press, pp. 764-775.

Eldin, S.M., M.H. Mohamed and H.M. Ali. 2018. Epistasis and genetic diversity in cotton under salinity stress. *Biol. Rhythm Res*., 49: 421-433.

Farooq, T., M. Umar, X. She, Y. Tang and Z. He. 2021. Molecular phylogenetics and evolutionary analysis of a highly recombinant begomovirus, cotton leaf curl multan virus, and associated satellites. *Virus Evol*., 7(2): 1-15.

Giannopolitis, C.N. and S.K. Ries. 1977. Superoxide dismutases: II. Purification and quantitative relationship with water-soluble protein in seedlings. *Plant Physiol*., 59: 315-318.

Gonzalez, A., F. Rivas and J.A. Fernández. 2019. Biochemical traits and epistasis in cotton: Implications for breeding. *Crop Sci.*, 59: 265-278.

Hallauer, A.R. and M.J. Carena. 2019. Recurrent selection methods to improve populations and conserve genetic diversity. *Crop Sci*., 59: 2781-2795.

Hasanuzzaman, M., K. Nahar, M.M. Alam, R. Roychowdhury and M. Fujita. 2013. Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. *Int. J. Mol. Sci*., 14: 9643-9684.

Hassan, S., H. Hamed and E. Amer. 2022. Estimation of genetic variance components by using triple test cross in cotton (*Gosssypium barbadense* L.). *Egypt. J. Agron*., 44: 209-220.

Haxim, Y., A. Ismayil, Q. Jia, Y. Wang, X. Zheng, T. Chen, N. Qian, Y. Liu, S. Wang, J. Han, Y. Cheng, Y. Qiy and Y. Hong. 2017. Autophagy functions as an antiviral mechanism against geminiviruses in plants. *eLife*, 6: 23897.

Iqbal, M., M.H.U. Rahman and I. Ullah. 2012. Inheritance of fiber quality parameters in upland cotton under normal and CLCuV infected conditions. *Pak. J. Agric. Res*., 25: 153-163.

Jiang, G., J. Xu and X. Zhang. 2020. Understanding the role of epistasis in complex traits: A cotton case study. *Front. Plant Sci*., 11: 431.

Kamal, H., F. Minhas, D. Tripathi, W. Abbasi, M. Hamza, R. Mustafa and I. Amin. 2019. Bc1, pathogenicity determinant encoded by cotton leaf curl multan betasatellite, interacts with calmodulin-like protein 11 (gh-cml11) in *Gossypium hirsutum* L. *Plos One*, 14: 02258-76.

Kearsey, M.J. and J.L. Jinks. 1968. A general method of detecting additive, dominance, and epistatic variance components of variability. *Heredity.*, 23: 309-320.

Ketata, H., E.L. Smith, L.H. Edwards and R.W. McNew. 1976. Detection of epistatic, additive, and dominance variation in winter wheat (*Triticum aestivum* L. em thell.)1. *Crop Sci*., 16: 1-4.

Khalid, S., M. Ur-Rehman, U. Hameed, F. Saeed, F. Khan and M. Haider. 2017. Transmission specificity and coinfection of mastrevirus with begomovirus. *Int. J. Agri. Biol*., 19: 105-113.

Khattak, G.S., M.A. Haq, M. Ashraf and T. McNeilly. 2001. Genetic basis of variation of yield, and yield components in mungbean (*Vigna radiata* L.). *Heredity*, 134: 211-217.

Kumari, V., A. Kumar, R. Kumar and S. Sharma. 2021. Epistatic interactions in cotton under biotic stress. In: *Proc. Natl. Conf. Genet*. (Ed.): Singh, R. Indian Society of Genetics & Plant Breeding, New Delhi, India, pp. 45-50.

Maqbool, M., H. Rehman, F. Bashir and R. Ahmad. 2019. Investigating Pakistan's revealed comparative advantage and competitiveness in cotton sector. *Rev. Econ. Develop. Stud*., 5: 125-134.

Metzner, H., H. Rau and H. Senger. 1965. Untersuchungen zur synchronisierbarkeit einzelner pigmentmangel-mutanten von Chlorella. *Planta*, 65: 186-194.

Murray, T.D., P.D. Kharbanda and E. Wolff. 2019. Interactions of genetic and environmental factors on cotton biochemical traits. *J. Cotton Sci*., 23: 45-58.

Rehman, M., M. Imtiaz and W. Ahmad. 2019. Genetic analysis and inheritance of agronomic traits in cotton under drought stress. *Agronomy*, 9: 682.

Robinson, H.F. 1948. The components of genetic variance in populations of biparental progenies and their use in estimating the average degree of dominance. *Biometrics*, 4: 254.

Saeed, M., A.I. Kheiralla and M.A. Mohamed. 2020. Genetic analysis of resistance to cotton leaf curl disease in cotton (*Gossypium hirsutum* L spp.) using various epistatic models. *Euphytica*, 216: 1-13.

Saleem, H., N. Nahid, S. Shakir, S. Ijaz, G. Murtaza, A. Khan and M. Nawaz-ul-Rehman. 2016. Diversity, mutation and recombination analysis of cotton leaf curl geminiviruses. *Plos One*, 11: 01511-61.

Sattar, M., M. Javed, S. Hussain, M. Babar, P. Chee, Z. Iqbal, M. Munir and S. Al-Hashedi. 2022. Mapping of quantitative trait loci (qtls) controlling cotton leaf curl disease (clcud) resistance in upland cotton. *Euphytica,* 218: 213-217.

Shafiq, M., Z. Iqbal, I. Ali, Q. Abbas, S. Mansoor, R.W. Briddon and I. Amin. 2017. Real-time quantitative PCR assay for the quantification of virus and satellites causing leaf curl disease in cotton in Pakistan. *J. Virol. Methods*, 248: 54-60.

Singh, R.K. and B.D. Chaudhary. 1985. *Biometrical Method in Quantitative Genetics Analysis*. 2nd Edition., Kalyani Publishers. New Dehli.

Singh, A.K., S. Sood and V.K. Sood. 2019. Detection of epistasis and estimation of additive and dominance components of genetic variation for fruit yield and horticultural traits in okra [*Abelmoschus esculentus* (L.) moench]. *Int. J. Curr. Microbiol. App. Sci*., 8: 747-756.

Tariq, A., M. Rizwan, M. Rafique, S. Sajid, Z. Fatima, U. Buneen and S. Haroon. 2020. Expression of oxidative enzymes in cotton plant under biotic stress. *Int. J. Res. and Stud. Pub*., 10: 494-508.

Zaidi, S., R. Naqvi, M. Asif, S. Strickler, S. Shakir, M. Shafiq and S. Mansoor. 2019. Molecular insight into cotton leaf curl geminivirus disease resistance in cultivated cotton (*Gossypium hirsutum* L.). *Plant Biotech. J*., 18: 691-706.

Zubair, M., S. Zaidi, S. Shakir, M. Farooq, I. Amin, J. Scheffler and S. Mansoor. 2017. Multiple begomoviruses found associated with cotton leaf curl disease in Pakistan in early 1990 are back in cultivated cotton. *Sci. Rep*., 7(1): 680.

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