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HETEROSIS AND CORRELATION ANALYSIS OF EARLINESS-RELATED MORPHOLOGICAL AND BIOCHEMICAL TRAITS IN UPLAND COTTON (*GOSSYPIUM HIRSUTUM* L.)

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SUMMARY

Cotton (*Gossypium hirsutum* L.) is a major global fiber crop. Early-maturing cotton offers the advantage of avoiding stresses by shortening the growth period. This study evaluated the heterotic potential and correlation of morphological and biochemical traits for earliness in upland cotton. The crossing of eight parental genotypes used a half-diallel mating design; the resultant F_1, along with parents, underwent evaluation for earliness. The cross VH-327 × CRIS-613 (-19.55% and -19.98%) displayed a maximum significant negative heterobeltiosis for days to flowering and squaring. SAS-2 × Coker Wild (53.11%) exhibited the higher heterotic response for superoxide dismutase activity, whereas Ghauri-2 × Coker Carolina Queen showed the maximum significant positive heterobeltiosis of 21.39% for peroxidase activity. For catalase, Cyto-124 × SAS-2 (6.58%), Cyto-124 × CRIS-613 (7.59%), and Ghauri-2 × Coker Wild (9.84%) demonstrated a significant positive heterobeltiosis. The correlation results revealed a significant positive association between days to squaring and flowering, while superoxide dismutase had a negative correlation with days to flowering at the phenotypic level. Overall, VH-327 × CRIS-613 and Ghauri-2 × Coker Wild had desirable results for earliness and yield-related traits and can be favorable for the breeding of early-maturing and high-yielding cotton genotypes.

Keywords: Upland cotton (*G. hirsutum* L.), heterobeltiosis, antioxidant enzymes, early maturity, diallel, correlation

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Key findings: In upland cotton (*G. hirsutum* L.), the cross combination VH-327 × CRIS-613 exhibited desirable heterosis for earliness-related traits, while Ghauri-2 × Coker Carolina Queen and Ghauri-2 × Coker Wild provided valuable resources for targeted breeding programs, particularly for antioxidant enzymes and yield-related traits.

INTRODUCTION

Cotton (*Gossypium hirsutum* L.) holds a significant position as a cash and fiber crop across the globe, providing raw material for industry (Zafar *et al.,* 2025). It grows across more than 50 countries worldwide and accounts for approximately 90% of the cotton production globally (Shah *et al.,* 2020; Attiea, 2020). However, its long growing season exposed it to various biotic and abiotic stresses, leading to a reduction in yield. Short-season is an ecotype of cotton, distinguished as a comparatively shorter growth cycle, being recognized as short-season cotton (Song *et al.,* 2012). The development of early-maturity cotton is a major breeding objective, as early-maturing cotton efficiently uses resources, i.e., light and heat. This leads to an increase in crop production under specified climatic conditions and helps lower late-season risks of insects and pests, thereby improving economic returns by reducing input costs (Song *et al.,* 2012; Qi *et al.,* 2023).

Previous researchers have used various morphological plant characteristics as markers to assess earliness in cotton, such as days to first square, vertical flowering interval, and boll maturation period (Rana *et al.,* 2021). Understanding the genetic basis of biochemical characteristics, such as antioxidant enzyme activities, i.e., catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD), is important in progressing biochemical-assisted breeding approaches and can serve as biochemical markers for earliness (Habib *et al.,* 2013). Despite limited research focused on genetic studies of biochemical traits related to earliness, Yu *et al.* (2005) studied the antioxidant enzyme genetics in short-season cotton. For short-season cotton (SSC), premature senescence is a significant limiting factor that lowers yield and fiber quality. Premature senescence has an association with the levels of chlorophyll, methane dicarboxylic aldehyde (MDA), and antioxidant enzyme activity. For this reason, it is crucial to study the activities of enzymes to develop high-quality short-season cotton (Song *et al.,* 2014). A half diallel cross is a common method for estimating GCA and SCA for earliness traits (Mahdy *et al.,* 2018). It helps to identify the best crosses for the development of early-maturing cotton varieties (Shakeel *et al.,* 2012). Utilizing a half diallel will help to study the genetics of antioxidant enzymes in cotton cultivars; hence, the selection of parents can be according to biochemical characteristics.

Heterosis is a complex quantitative phenomenon widely used for yield and hybrid improvement in several crops, including cotton. An effort has occurred to identify the superior performance of progeny as compared with their parents, which significantly increases crop yield. Remarkably, it played a key role in hybrid seed and supported the green revolution (Dirbas *et al.*, 2024). Over the past few decades, cotton breeders have shown an increased interest in heterosis. Cotton economic and fiber traits have gained improvement through heterosis breeding (Khan *et al.,* 2025). Cotton genetic diversity limitations push breeders to develop hybrids with superior heterosis. Earliness-related characteristics and their heterosis have received assessment from breeders and identified hybrids with a short maturation period (Rana *et al.,* 2021; Imtiaz *et al*., 2022). Parents, which are genetically diverse, can potentially show more hybrid vigor. Explaining heterosis can refer to additive, dominant, and epistatic gene action, as it is difficult to explain this phenomenon based only on a single factor, such as one gene, a group of genes, or a single physiological phenomenon (Labroo *et al.,* 2021).

The correlation coefficient is recognizably effective for assessing the relationship between components crucial for selection (Isong *et al.,* 2017). The objectives of

the current research were to explore the heterotic potential and correlation of morphological and biochemical traits for earliness in hybrids of upland cotton.

MATERIALS AND METHODS

Experimental design and materials

The conduct of the experiment occurred at the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad (N 31° 25' 46.8048", E 73° 4' 14.3112") during the 2022 and 2023 growing seasons. Early- and late-maturing genotypes of upland cotton (*G. hirsutum* L.) were specimens used to develop genetic material. Eight parental genotypes comprising two high-yielding (VH-327 and Cyto-124), two low-yielding (Coker Carolina Queen and Coker Wild), two early-maturing (SAS-2 and CRIS-613), and two late-maturing (Ghauri-2 and Coker-8310) were selected. Parental genotypes sowing continued in a greenhouse, with the crossing done at the flowering stage in a half-diallel mating design to develop F_1. In the next season, 28 crosses and eight parental genotypes entailed growing in a randomized complete block design with three replications. Each experimental unit consisted of a single 3.0 m row with 10 plants, covering an area of 2.25 m². Plant spacing was 0.30 m apart within rows, with a 0.75 m spacing between rows. Irrigation was applied at regular intervals (10–15 days) to maintain the crop environment, with special attention at reproductive stage to avoid water stress. All the performed standard agronomic and plant protection practices sought to maintain the crop. Data recording for various traits came from five selected plants.

Observed morphological and biochemical traits

Data recorded included earliness-related morphological, yield, fiber quality, and biochemical traits. Recording of days to squaring (DTS), days to flowering (DTF), and days to boll opening (DTBO) was the number of days from sowing to the appearance of the first square, the first flower, and the first cracked boll, respectively. The boll maturation period (BMP) calculation is as follows:

$$\text{BMP} = \text{Days to first boll opening} - \text{Days to first flowering}$$

Mean maturity date (MMD) calculation was

$$\text{MMD} = (W_1H_1 + W_2H_2 + \ldots + WnHn) / (W_1 + W_2 + \ldots + Wn)$$

Where W is the weight of seed cotton, H is the number of days from planting to harvest, and n is the consecutive periodic harvest number.

Plant height (PH) measurement was from the first cotyledonary node to the apex of the plant with the help of a meter rod and recorded in centimeters. The first fruiting node (FFN), as determined, was by counting nodes from the first node above the cotyledonary node until the first fruiting branch appeared. The number of bolls per plant (NBP) was harvested during each picking session, attained counting and documentation for each plant. Boll weight (BW), calculated in grams, was by dividing the plant's total seed cotton yield by the number of bolls collected from that plant. Seed cotton yield (SCY) per plant (grams) underwent determination by weighing and combining seed cotton collected from all pickings. Lint percentage (LP) calculation followed the formula below:

$$\text{Lint percentage} = \text{Weight of lint} / \text{Weight of seed cotton} \times 100$$

Calculating lint per boll (LPB) ensued by dividing the weight of lint obtained after ginning by the total number of bolls. Fiber parameters, including staple length (mm), fiber fineness (micronaire), fiber strength (g/tex), and uniformity (%), were assessed employing the Spin Lab HVI-900. Cotton samples, after ginning, had the lint obtained to quantify the fiber parameters.

The biochemical parameters of superoxide dismutase (Wang *et al*. 1983), peroxidase, and catalase (Liu *et al.* 2009) incurred testing in the parental genotypes and the resultant F_1 hybrids. For the enzymatic

assay, the collected top leaves from a growing plant were at the flowering stage before being stored at -80 °C.

Enzyme extract

Frozen leaves received grinding and homogenization with the help of a pestle and mortar, adding 0.05 M sodium phosphate buffer (pH 7.5). The mixture sustained centrifugation at 10,000 g for 20 min, with the supernatant utilized as the enzyme extract.

Superoxide dismutase: Preparing a reaction mixture used 150 µl of nitro blue tetrazolium (NBT), 3.9 µl of riboflavin, 780 µl of methionine, 225 µl of ethylene diamine tetra acetic acid (EDTA), 150 µl of phosphate buffer, 50 µl of the sample, and 1.64 ml of distilled water. Sample absorbance was sought to measure at 560 nm by a spectrophotometer to quantify the activity of the enzyme.

Peroxidase: A reaction mixture earlier prepared contained sodium-potassium buffer (100 mM), 1 mL of hydrogen peroxide (100 mM), 1 mL of guaiacol (0.2%), and 50 µL of enzyme extract, and then quantified the absorbance at 470 nm using a spectrophotometer.

Catalase: The reaction mixture had ingredients of 1.5 mL of 0.05 M sodium phosphate buffer, 1 mL of deionized water, 0.3 mL of 0.1 M H_2O_2, and 0.2 mL of enzyme extract. The absorbance at 240 nm sought to measure the catalase activity using a spectrophotometer.

Statistical analysis

Data analysis was through the analysis of variance to test the variability of traits among genotypes (Steel *et al.,* 1997). Griffing's half-diallel analysis (8 × 8) with fixed effects aimed at developing cross combinations (Griffing, 1956). Heterobeltiosis estimation sought to study the heterotic potential for hybrid development (Fonseca and Patterson, 1968). The formula for heterobeltiosis is expressed as:

$$\text{Heterobeltiosis (\%)} = (F_1 - BP)/BP \times 100$$

Where F1 = mean performance of the hybrid, and BP = mean performance of the better parent.

The study of genotypic and phenotypic relationships continued through the correlation analysis (Kwon and Torrie, 1964).

RESULTS

Analysis of variance

The ANOVA revealed significant differences among genotypes for all traits, except for mean maturity date (Table 1), demonstrating the presence of considerable variation within the experimental material. The mean sum of squares due to genotypes exhibited significant results for traits, including days to squaring, days to flowering, days to boll opening, and boll maturation period. The mean maturity date showed the presence of less variation among genotypes. Yield and fiber-related traits were also highly significant for genotypes. Enzymatic activities SOD, POD, and CAT also exhibited notable variation among genotypes.

Descriptive statistics

The mean value for days to squaring was slightly lower in crosses (57 days) than in parents (60 days), with grand mean of 58 days. Crosses took fewer mean days to flower (66) than parents (71), with a grand mean of 67 days. The results showed crosses reached boll opening earlier, with a mean of 105 days, than the 108 days in parents, while the overall mean was 105 days. The mean time for boll maturation appeared to be 47 days. This reduction in boll opening time and boll maturation period among crosses indicated that hybridization promoted earliness, a desirable attribute in cotton for the timely harvesting of the crop.

Mean values signified that crosses had slightly higher mean values for FFN. The mean seed cotton yield (SCY) of the crosses (96.3 g) was slightly higher than that of the parents (93.6 g), with an overall grand mean of 95.7 g.

Table 1. Analysis of variance for earliness-related, yield-related, fiber-related, and biochemical traits in *Gossypium hirsutum* L. genotypes (parents and crosses).

	DF	DTS	DTF	DTBO	BMP	MMD	PH	FFN	NOB	BW	SCY	LP	LPB	F. L	F.F	F.S	F.U	SOD	POD	CAT
REP	2	0.66^{NS}	1.39 ^{NS}	203.37**	205.79**	522.35**	13.04^{NS}	1.81^{NS}	38.28^{NS}	0.16^{NS}	39.05 ^{Ns}	241.14**	0.099**	2.02**	3.09**	3.66**	2.40**	271^{NS}	0.03^{NS}	0.12^{NS}
GENO	37	29.64**	66.31**	76.82**	73.67**	4.92^{NS}	639.21**	9.62**	37.70**	0.21**	96.91**	64.25**	0.54**	6.53**	1.22**	22.76**	12.33**	34678**	17.71**	8.77**
ERROR	70	8.64	7.98	14.96	21.59	3.7	52.05	2.31	12.33	0.089	20.72	17.06	0.014	0.15	0.08	0.29	0.12	224	0.03	0.99

Significant = * when probability is 0.05, highly significant = ** when probability is 0.01, Non-significant = ^{NS}, REP = replication, Geno = genotype, DTS = days to squaring, DTF = days to flowering, DTBO = days to boll opening, BMP = boll maturation period, MMD = mean maturity date, PH = plant height, FFN = first fruiting node, NOB = number of bolls, BW = boll weight, SCY = seed cotton yield, LP = lint percentage, LPB = lint per boll, F.L = Fiber length, F.F = fiber fineness, F.S = fiber strength, F.U = fiber uniformity, SOD = Superoxide dismutase, POD = Peroxidase, and CAT = Catalase.

Table 2. Descriptive statistics (mean, standard deviation, standard error, and coefficient of variation) for earliness-related, yield-related, fiber-related, and biochemical traits in *Gossypium hirsutum* L. genotypes (parents and crosses).

Statistical parameters	DTS	DTF	DTBO	BMP	MMD	PH	FFN	NOB	BW	SCY	LP	LPB	F. L	F.F	F.S	F.U	SOD	POD	CAT
Mean parents	60	71	108	48	121	129.7	7.4	35.2	2.4	93.6	32.9	0.8	25.6	4.1	24.7	82.3	302.6	13.0	7.3
Mean crosses	57	66	105	47	121	133.3	8.1	31.7	2.7	96.3	32.3	1.0	24.8	4.8	24.3	82.4	340.8	12.4	6.5
Grand mean	58	67	105	47	121	132.5	7.9	32.5	2.6	95.7	32.4	0.9	24.9	4.6	24.4	82.4	332.3	12.5	6.7
Standard deviation	3.26	4.73	5.18	4.94	1.33	15.07	1.84	3.60	0.27	6.21	4.62	0.42	1.48	0.66	2.83	2.06	108.39	2.44	1.74
Standard error	0.52	0.75	0.86	0.75	0.23	2.46	0.31	0.53	0.04	1.11	0.69	0.08	0.23	0.10	0.42	0.36	19.55	0.38	0.28
CV	5.60	6.98	4.89	10.31	1.10	11.37	23.20	11.08	10.25	6.48	14.24	44.92	5.96	14.27	11.59	2.50	32.61	19.46	26.02

DTS = days to squaring, DTF = days to flowering, DTBO = days to boll opening, BMP = boll maturation period, MMD = mean maturity date, PH = plant height, FFN = first fruiting node, NOB = number of bolls, BW = boll weight, SCY = seed cotton yield, LP = lint percentage, LPB = lint per boll, F.L = Fiber length, F.F = fiber fineness, F.S = fiber strength, F.U = fiber uniformity, SOD = Superoxide dismutase, POD = Peroxidase, and CAT = Catalase.

Grand mean values of lint percentage and lint per boll were 32.4% and 0.9 g. The grand mean fiber length was 24.9 mm; cross combinations exhibited relatively lower mean values than parents. The grand mean values of fiber fineness, strength, and uniformity were 4.6 micronaire, 24.4 g/tex, and 82.5%, respectively. Overall, the average activity of superoxide dismutase (SOD) was 332.3 U/g, while peroxidase (POD) and catalase (CAT) recorded mean values of 12.5 and 6.7 U/g, respectively (Table 2).

Heterosis

Parent-wise and overall average heterobeltiosis

The average heterobeltiosis of cross combinations, derived from each female parent and the overall average of crosses underwent calculations for yield, earliness, fiber, and biochemical traits in *Gossypium hirsutum* L. (Table 3). VH-327 showed consistency as a female

Table 3. Parent-wise average of cross combinations and overall average heterobeltiosis for earliness-related, yield-related, fiber-related, and biochemical traits in *Gossypium hirsutum* L.

Female parent	DTS	DTF	DTBO	BMP	MMD	PH	FFN	NOB	BW	SCY	LP	LPB	F. L	F.F	F.U	F.S	SOD	POD	CAT
VH-327	-8.76	-11.78	-0.98	5.52	-0.36	-21.18	3.38	-16.59	5.55	-2.54	-28.37	-14.41	-4.65	18.83	0.12	-11.82	23.24	-33.69	-25.83
Cyto-124	-5.75	-7.12	-11.40	-24.89	0.20	-7.86	2.07	-16.44	7.48	-2.87	-8.86	30.82	-2.14	-11.09	-0.77	0.55	-6.25	-7.72	-15.28
Ghauri-2	-6.54	-10.07	-6.21	-8.61	0.03	2.99	1.47	-10.69	6.92	7.44	7.96	63.86	-8.88	13.94	-1.76	-9.29	-11.26	-9.00	-19.98
Coker-8310	-4.93	-5.03	-4.45	-7.03	-0.22	13.88	-10.99	-12.47	-1.74	1.96	-4.78	-1.35	-16.43	5.80	-1.91	-7.16	14.49	-12.48	-13.08
SAS-2	-13.45	-16.40	-7.59	-3.31	-1.33	-1.77	-4.11	-20.12	10.68	-1.34	-10.79	2.64	0.20	13.36	-0.45	-3.64	22.26	-25.17	-8.58
CRIS-613	-3.09	-3.15	-1.17	-7.77	-0.59	21.92	-43.21	-16.78	3.10	5.83	5.97	10.89	-13.91	0.15	-5.03	-15.26	-26.63	-23.17	-43.65
Coker Carolina Queen	-10.16	-11.18	-5.29	-7.74	-1.08	3.41	19.63	6.51	-2.90	12.20	-14.10	-14.80	-6.94	18.22	-2.95	-12.17	-32.58	6.94	-41.93
Overall mean	-7.32	-9.37	-5.52	-7.68	-0.30	-2.96	-2.84	-14.48	5.24	1.07	-9.49	14.74	-6.77	7.74	-1.23	-7.44	3.85	-17.57	-20.70

DTS = days to squaring, DTF = days to flowering, DTBO = days to boll opening, BMP = boll maturation period, MMD = mean maturity date, PH = plant height, FFN = first fruiting node, NOB = number of bolls, BW = boll weight, SCY = seed cotton yield, LP = lint percentage, LPB = lint per boll, F.L = Fiber length, F.F = fiber fineness, F.S = fiber strength, F.U = fiber uniformity, SOD = Superoxide dismutase, POD = Peroxidase, and CAT = Catalase.

Table 4. Estimates of heterobeltiosis utilizing half-diallel analysis for different morphological and biochemical traits in *Gossypium hirsutum* L.

Cross combinations	DTS	DTF	DTBO	BMP	MMD	PH	FFN	NOB	BW	SCY
VH-327 × Cyto-124	-8.64*	-9.40**	-5.62*	-11.55^{NS}	-0.03^{NS}	-28.75**	-13.60^{NS}	-11.69^{NS}	-6.59^{NS}	-0.55^{NS}
VH-327 × Ghauri-2	-4.00^{NS}	-12.39**	0.66^{NS}	3.15^{NS}	0.02^{NS}	-13.65**	34.68*	-19.76**	14.13^{NS}	-0.37^{NS}
VH-327 × Coker-8310	-2.05^{NS}	-5.81^{NS}	-0.24^{NS}	-1.16^{NS}	0.78^{NS}	-28.12**	57.78**	-25.22**	9.57^{NS}	-0.04^{NS}
VH-327 × SAS-2	-1.51^{NS}	-4.07^{NS}	3.37^{NS}	8.66^{NS}	-1.32^{NS}	-23.07**	-3.47^{NS}	-4.04^{NS}	-5.33^{NS}	-10.79**
VH-327 × CRIS-613	-19.55**	-19.98**	-2.78^{NS}	10.38^{NS}	-0.64^{NS}	-13.94**	-42.70**	-16.72*	21.07^{NS}	2.65^{NS}
VH-327 × Coker Carolina Queen	-12.40**	-18.54**	-0.31^{NS}	18.78*	-1.03^{NS}	-22.88**	-9.03^{NS}	-23.35**	10.46^{NS}	-1.33^{NS}
VH-327 × Coker Wild	-13.17**	-12.30**	-1.92^{NS}	10.41^{NS}	-0.28^{NS}	-17.84**	0.001^{NS}	-15.38*	-4.44^{NS}	-7.32^{NS}
Cyto-124 × Ghauri-2	-2.55^{NS}	-4.51^{NS}	-9.13**	-22.29**	2.14^{NS}	-6.73^{NS}	38.40*	-16.91*	-5.01^{NS}	-3.30^{NS}
Cyto-124 × Coker-8310	-3.55^{NS}	-14.62**	-10.89**	-24.71**	0.72^{NS}	-6.25^{NS}	-20.00^{NS}	-27.45**	30.06**	0.85^{NS}
Cyto-124 × SAS-2	-6.44^{NS}	9.51**	-19.15**	-39.49**	-0.52^{NS}	-14.20**	4.00^{NS}	-26.23**	13.75^{NS}	0.46^{NS}
Cyto-124 × CRIS-613	-4.99^{NS}	-8.92**	-11.77**	-22.75**	0.25^{NS}	-8.48*	-11.01^{NS}	-28.29**	19.00*	-1.07^{NS}
Cyto-124 × Coker Carolina Queen	-13.92**	-14.04**	-6.56*	-13.51*	-2.18^{NS}	-4.38^{NS}	-11.00^{NS}	0.09^{NS}	-8.22^{NS}	-10.05**
Cyto-124 × Coker Wild	-3.06^{NS}	-10.14**	-10.89**	-26.56**	0.80^{NS}	-7.12^{NS}	12.00^{NS}	0.16^{NS}	-4.71^{NS}	-4.11^{NS}
Ghauri-2 × Coker-8310	-0.78^{NS}	-7.14*	-1.73^{NS}	-2.82^{NS}	0.24^{NS}	6.28^{NS}	12.10^{NS}	-10.44^{NS}	10.19^{NS}	5.18^{NS}
Ghauri-2 × SAS-2	-8.95*	-9.20**	-8.59**	-9.83^{NS}	0.41^{NS}	-10.50*	-2.96^{NS}	-16.89*	1.55^{NS}	-3.43^{NS}
Ghauri-2 × CRIS-613	-0.89^{NS}	-5.63^{NS}	-5.29^{NS}	-12.44^{NS}	-0.35^{NS}	8.44*	-11.46^{NS}	-9.92^{NS}	1.00^{NS}	5.68^{NS}
Ghauri-2 × Coker Carolina Queen	-17.17**	-15.68**	-7.93**	-5.90^{NS}	-1.68^{NS}	-1.47^{NS}	-7.26^{NS}	-5.12^{NS}	11.28^{NS}	13.21**
Ghauri-2 × Coker Wild	-4.92^{NS}	-12.68**	-7.51**	-12.06^{NS}	1.52^{NS}	12.20**	16.94^{NS}	-11.07^{NS}	10.59^{NS}	16.56**
Coker-8310 × SAS-2	-7.75^{NS}	-3.85^{NS}	-10.59**	-15.53*	-2.72*	5.82^{NS}	-10.68^{NS}	-6.42^{NS}	-14.06^{NS}	-12.24**
Coker-8310 × CRIS-613	3.44^{NS}	-1.09^{NS}	-0.77^{NS}	-5.77^{NS}	1.43^{NS}	19.87**	-37.64**	-28.18**	5.79^{NS}	1.04^{NS}
Coker-8310 × Coker Carolina Queen	-11.79**	-8.93**	-0.83^{NS}	2.57^{NS}	0.52^{NS}	22.59**	-11.66^{NS}	-22.92**	5.78^{NS}	5.37^{NS}
Coker-8310 × Coker Wild	-3.61^{NS}	-6.25*	-5.61*	-9.40^{NS}	-0.11^{NS}	7.25^{NS}	16.04^{NS}	7.63^{NS}	-4.48^{NS}	13.68**
SAS-2 × CRIS-613	-10.15*	-12.12**	-4.00^{NS}	-2.18^{NS}	0.25^{NS}	2.71^{NS}	-20.90^{NS}	-23.58**	20.29^{NS}	1.03^{NS}
SAS-2 × Coker Carolina Queen	-13.82**	-19.50**	-5.41^{NS}	2.13^{NS}	-2.11^{NS}	-2.12^{NS}	31.31^{NS}	-19.17*	7.65^{NS}	-2.03^{NS}
SAS-2 × Coker Wild	-16.38**	-17.57**	-13.35**	-9.88^{NS}	-2.12^{NS}	-5.89^{NS}	-22.74^{NS}	-17.62*	4.11^{NS}	-3.01^{NS}
CRIS-613 × Coker Carolina Queen	-6.50^{NS}	1.04 ^{NS}	3.93^{NS}	2.18^{NS}	-0.94^{NS}	37.45**	-43.71**	-16.68*	8.09^{NS}	12.53**
CRIS-613 × Coker Wild	0.33^{NS}	-7.34*	-6.27*	-17.72*	-0.23^{NS}	6.38^{NS}	-42.70**	-16.88*	-1.89^{NS}	-0.88^{NS}
Coker Carolina Queen × Coker Wild	-10.16**	-11.18**	-5.29^{NS}	-7.74^{NS}	-1.08^{NS}	3.41^{NS}	19.63^{NS}	6.51^{NS}	-2.90^{NS}	12.20**

Table 4. (Cont'd.)

Cross combinations	LP	LPB	F. L	F.F	F.U	F.S	SOD	POD	CAT
VH-327 × Cyto-124	-40.62**	-32.67**	1.84^{NS}	-6.28^{NS}	3.76**	-26.01**	18.99**	-28.17**	-48.00**
VH-327 × Ghauri-2	-28.37**	-6.48^{NS}	-4.60**	36.44**	3.34**	-2.46^{NS}	47.54**	-38.50**	-20.93**
VH-327 × Coker-8310	-22.62**	3.13 ^{NS}	-13.79**	55.48**	1.90**	-11.12**	-16.78**	-21.99**	-7.60*
VH-327 × SAS-2	-22.08**	-21.77*	-5.19**	-3.09^{NS}	1.13**	0.27^{NS}	20.16**	-32.46**	-3.80^{NS}
VH-327 × CRIS-613	-36.01**	-25.22*	-1.03NS	31.61**	-3.75**	-5.36**	48.18**	-26.91**	-7.53*
VH-327 × Coker Carolina Queen	-26.00**	-5.05^{NS}	-7.98**	6.93^{NS}	-4.42**	-25.27**	40.66**	-54.34**	-53.88**
VH-327 × Coker Wild	-22.92**	-12.81^{NS}	-1.81^{NS}	10.73*	-1.13**	-12.80**	3.95^{NS}	-33.45**	-39.06**
Cyto-124 × Ghauri-2	-23.19*	-27.10**	-4.06**	-13.76**	-0.74*	19.38**	32.54**	-22.57**	-30.96**
Cyto-124 × Coker-8310	5.14^{NS}	132.32**	-10.08**	-3.64^{NS}	3.17**	23.48**	-19.63**	-5.71**	-32.26**
Cyto-124 × SAS-2	-25.32**	-15.82^{NS}	7.81**	-18.79**	-5.29**	-0.36^{NS}	-63.77**	-23.74**	6.58*
Cyto-124 × CRIS-613	6.03^{NS}	122.60**	-12.32**	-3.72^{NS}	-1.82**	-17.42**	31.02**	-1.61^{NS}	7.59*
Cyto-124 × Coker Carolina Queen	8.00^{NS}	-1.03^{NS}	0.61^{NS}	2.46^{NS}	-1.12**	-19.61**	7.83*	4.18**	-14.47**
Cyto-124 × Coker Wild	-23.83**	-26.06**	5.21**	-29.11**	1.21**	-2.17^{NS}	-25.48**	3.14**	-28.18**
Ghauri-2 × Coker-8310	12.83^{NS}	33.06*	-6.85**	52.83**	2.46**	19.11**	-33.51**	-12.93**	-4.44^{NS}
Ghauri-2 × SAS-2	-3.67^{NS}	-4.21^{NS}	-6.84**	13.27*	-2.06**	-6.96**	-43.16**	-23.19**	-18.75**
Ghauri-2 × CRIS-613	-3.59^{NS}	-2.42^{NS}	-7.38**	-8.07^{NS}	-7.55**	-20.24**	-20.18**	-6.21**	-51.65**
Ghauri-2 × Coker Carolina Queen	32.92**	183.51**	-14.40**	17.40**	-0.26^{NS}	-34.00**	-2.86^{NS}	21.39**	-34.92**
Ghauri-2 × Coker Wild	1.30^{NS}	109.34**	-8.92**	-5.72^{NS}	-1.41**	-4.37*	43.42**	-24.08**	9.84**
Coker-8310 × SAS-2	-22.18*	-24.30^{NS}	-10.91**	21.56**	-3.05**	21.23**	38.24**	-27.49**	5.59^{NS}
Coker-8310 × CRIS-613	13.43^{NS}	20.21^{NS}	-17.35**	9.13^{NS}	-1.81**	-8.87**	26.03**	-2.81**	3.08^{NS}
Coker-8310 × Coker Carolina Queen	13.88^{NS}	24.86^{NS}	-15.86**	-9.36^{NS}	-2.88**	-34.10**	-5.30^{NS}	-10.46**	-45.08**
Coker-8310 × Coker Wild	-24.23**	-26.16**	-21.58**	1.88^{NS}	0.10^{NS}	-6.91**	-1.03^{NS}	-9.17**	-15.92**
SAS-2 × CRIS-613	1.07^{NS}	21.51^{NS}	-13.76**	2.96^{NS}	-3.26**	-11.04**	-12.08**	-24.51**	2.11^{NS}
SAS-2 × Coker Carolina Queen	-12.54^{NS}	2.66^{NS}	0.33^{NS}	23.45**	-2.38**	-16.33**	25.76**	-23.98**	-18.16**
SAS-2 × Coker Wild	-20.90*	-16.25^{NS}	14.03**	13.68**	4.30**	16.44**	53.11**	-27.01**	-9.69**
CRIS-613 × Coker Carolina Queen	33.48**	43.31**	-15.80**	9.30^{NS}	-5.19**	-26.86**	-37.71**	-7.52**	-19.01**
CRIS-613 × Coker Wild	-21.55*	-21.54*	-12.02**	-9.00^{NS}	-4.87**	-3.66*	-15.55**	-38.81**	-68.28**
Coker Carolina Queen × Coker Wild	-14.10^{NS}	-14.80^{NS}	-6.94**	18.22**	-2.95**	-12.17**	-32.58**	6.94**	-41.93**

Significant = * when probability is 0.05, highly significant = ** when probability is 0.01, Non-significant = NS, DTS = days to squaring, DTF = days to flowering, DTBO = days to boll opening, BMP = boll maturation period, MMD = mean maturity date, PH = plant height, FFN = first fruiting node, NOB = number of bolls, BW = boll weight, SCY = seed cotton yield, LP = lint percentage, LPB = lint per boll, F.L = Fiber length, F.F = fiber fineness, F.S = fiber strength, F.U = fiber uniformity, SOD = Superoxide dismutase, POD = Peroxidase, and CAT = Catalase.

parent because the average of its crosses exhibited heterobeltiosis of -8.76% for DTS and -11.78% for DTF, quite higher than the overall average of DTS and DTF. Meanwhile, Cyto-124 showed higher negative heterobeltiosis than overall heterosis for DTBO and BMP. Ghauri-2 as a female parent in cross combinations expressed higher heterobeltiosis for yield-related traits, including seed cotton yield. Cross combinations derived from SAS-2 and Coker Carolina Queen gave a desirable heterobeltiosis for earliness-related traits.

Heterobeltiosis

In assessing the genetic potential of the developed hybrids, heterobeltiosis estimation occurred for all studied traits (Table 4). VH-327 × CRIS-613 and VH-327 × Coker Carolina Queen emerged as desirable cross combinations for days to flowering and squaring, as they took fewer days to square and flower and performed better than the parents. VH-327 × CRIS-613 showed (-19.55% and -19.98%), and VH-327 × Coker Carolina Queen showed (-12.40% and -18.54%) heterobeltiosis for days to squaring and days to flowering. Cross combinations Cyto-124 × SAS-2, SAS-2 × Coker Wild, Cyto-124 × CRIS-613, Cyto-124 × Coker 8310, and Cyto-124 × Coker Wild were notably better-performing cross combinations due to higher negative significant values, indicating boll opening taking fewer days. Heterobeltiosis ranged from 1% to 30.06% in hybrids for boll weight. Higher boll weight was evident for Cyto-124 × Coker-8310 (30.06%) and Cyto-124 × CRIS-613 (19.00%), as these displayed significantly higher positive values for boll weight. Heterobeltiosis ranged from 0.04% to 16.56% for SCY. Five cross combinations were distinct as superior to better parents for seed cotton yield, which were Ghauri-2 × Coker Carolina Queen, Ghauri-2 × Coker Wild, Coker-8310 × Coker Wild, CRIS-613 × Coker Carolina Queen, and Coker Carolina Queen × Coker Wild. Positive significant results were remarkable for these crosses.

Based on the results of heterobeltiosis, three hybrids (SAS-2 × Coker Wild, Cyto-124 × SAS-2, and Cyto-124 × Coker Wild) performed higher than the better parent for fiber length. For fiber fineness, three hybrids (Cyto-124 × Ghauri-2, Cyto-124 × SAS-2, and Cyto-124 × Coker Wild) showed negative heterobeltiosis. Higher heterobeltiosis for fiber strength resulted in Cyto-124 × Coker-8310, Coker-8310 × SAS-2, Cyto-124 × Ghauri-2, and Ghauri-2 × Coker-8310. For fiber uniformity, VH-327 × Coker-8310, VH-327 × Ghauri-2, VH-327 × Coker-8310, VH-327 × SAS-2, Cyto-124 × Coker-8310, Cyto-124 × Coker Wild, Ghauri-2 × Coker-8310, and SAS-2 × Coker Wild emerged as potential hybrids, as the performance of hybrids was superior to that of better parents.

Heterotic studies for enzymes superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) also took place. For SOD, 13 cross combinations showed higher activity of the enzyme than the better parent, with maximum significant positive results observed by SAS-2 × Coker Wild (53.11%), followed by VH-327 × CRIS-613 (48.18%), VH-327 × Ghauri-2 (47.54%), and VH-327 × Coker Carolina Queen (40.66%). Meanwhile, the highest negative value was noteworthy for Cyto-124 × SAS-2 (-63.77%). For POD, heterobeltiosis ranged from 1.61% to 54.34%, and only four hybrids showed higher enzymatic activity than the superior parent. They are Ghauri-2 × Coker Carolina Queen, followed by Coker Carolina Queen × Coker Wild, Cyto-124 × Coker Carolina Queen, and Cyto-124 × Coker Wild Queen, while the highest negative value was evident for VH-327 × Coker Carolina Queen. For CAT, only three cross combinations displayed positive, significant heterobeltiosis: Cyto-124 × SAS-2, Cyto-124 × CRIS-613, and Ghauri-2 Coker Wild.

Phenotypic correlation

A positive association was evident between days to squaring and flowering. It also displayed a significantly negative correlation with the boll maturation period, mean maturity date, and fiber fineness. Days to flowering had negative associations with MMD, boll weight, lint per boll, and fiber fineness. The phenotypic correlation revealed a positive association between days to boll opening and days to squaring, days to flowering, and boll maturation period. A substantial positive phenotypic association of SCY existed with plant height, boll weight, and lint per boll. SCY showed a negative association with fiber traits.

The phenotypic correlation matrix exhibited that days to squaring, flowering, and boll opening negatively influenced the fiber length (Figure 1). Fiber length and fineness also showed a negative relationship. Fiber fineness exhibited a substantial positive association with the boll maturation period, fiber strength, and fiber uniformity. Fiber-

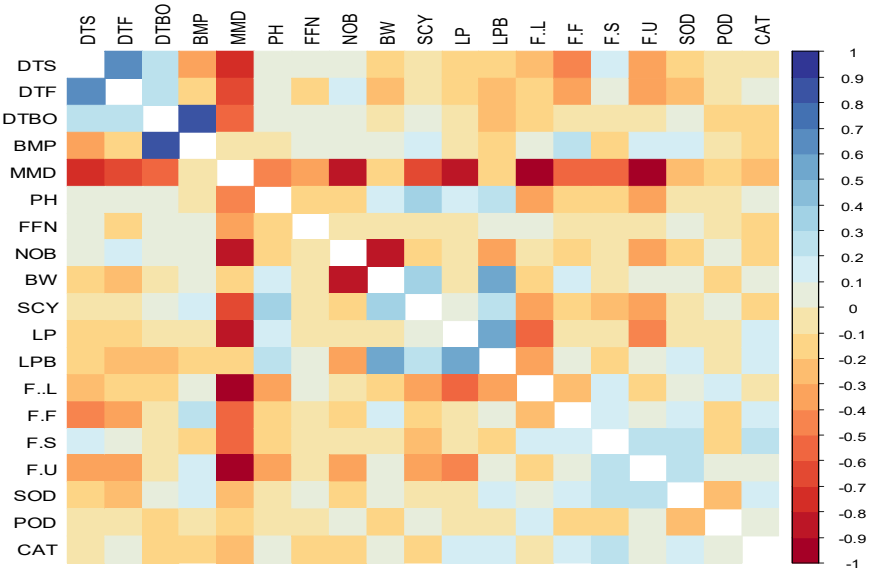

Figure 1. Phenotypic correlation matrix for different morphological and biochemical traits related to earliness in *Gossypium hirsutum* L. DTS = days to squaring, DTF = days to flowering, DTBO = days to boll opening, BMP = boll maturation period, MMD = mean maturity date, PH = plant height, FFN = first fruiting node, NOB = number of bolls, BW = boll weight, SCY = seed cotton yield, LP = lint percentage, LPB = lint per boll, F.L = Fiber length, F.F = fiber fineness, F.S = fiber strength, F.U = fiber uniformity, SOD = Superoxide dismutase, POD = Peroxidase, and CAT = Catalase.

related traits were in a negative correlation with seed cotton yield. Fiber strength revealed a significant phenotypic correlation with fiber length, fiber fineness, and fiber uniformity. Fiber strength appeared to have a positive correlation with superoxide dismutase activity.

In the case of enzymatic activities, superoxide dismutase had a positive association with fiber fineness, strength, and uniformity at the phenotypic level. Superoxide dismutase activity emerged to have a negative relationship with days to flowering and squaring. Peroxidase activity had a negative correlation with fiber strength, fineness, and superoxide dismutase activity. Catalase activity was in a positive correlation with lint percentage, fiber fineness, and fiber strength at the phenotypic level.

Genotypic correlation

Days to squaring exhibited a positive association with DTF, DTBP, and MMD. Days to boll opening exhibited a positive relationship with BMP. Seed cotton yield showed a significant positive genotypic association with the mean maturity date, plant height, boll weight, and lint per boll. It had a negative association with fiber length, fiber fineness, and fiber strength. The genotypic correlation matrix revealed that days to squaring, days to boll opening, plant height, seed cotton yield, and lint percentage significantly influenced fiber length (Figure 2).

Genotypic correlation studies revealed superoxide dismutase had a negligible association with other traits under observation. Peroxidase showed a positive association with the mean maturity date at the genotypic level. Catalase also exhibited a negative relationship with days to boll opening and boll maturation period.

DISCUSSION

Cotton production had gained improvement via hybrid development by exploiting the phenomenon of heterosis. Exploring heterosis has been a successful application in other

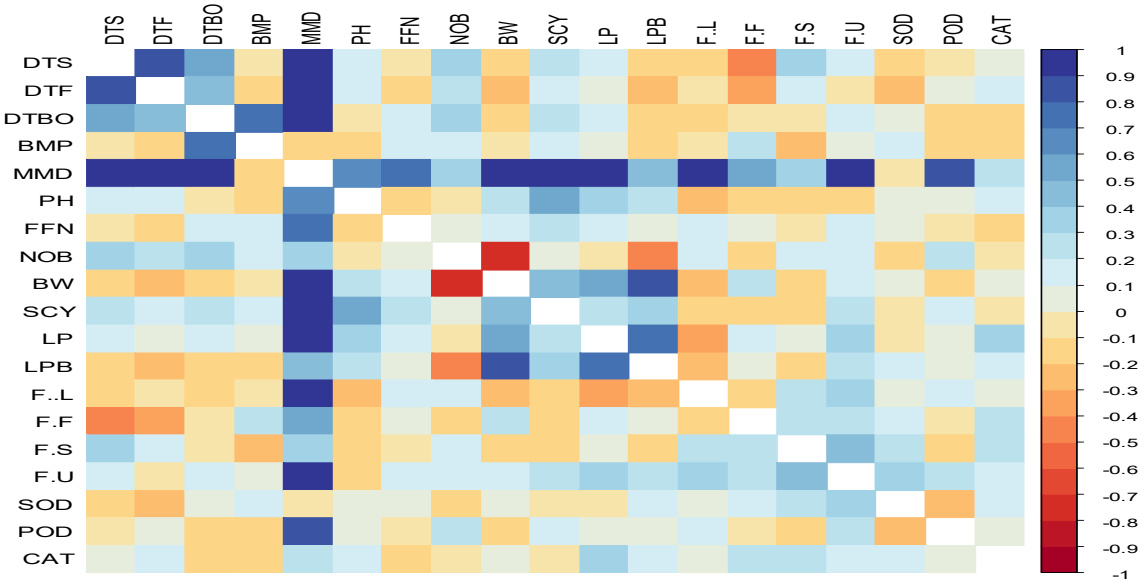

Figure 2. Genotypic correlation matrix for different morphological and biochemical traits related to earliness in *Gossypium hirsutum* L. DTS = days to squaring, DTF = days to flowering, DTBO = days to boll opening, BMP = boll maturation period, MMD = mean maturity date, PH = plant height, FFN = first fruiting node, NOB = number of bolls, BW = boll weight, SCY = seed cotton yield, LP = lint percentage, LPB = lint per boll, F.L = Fiber length, F.F = fiber fineness, F.S = fiber strength, F.U = fiber uniformity, SOD = Superoxide dismutase, POD = Peroxidase, and CAT = Catalase.

crops, i.e., rice, rape, and sorghum, which contributed to large-scale production of seeds (Zhang *et al.,* 2023; Paril *et al.,* 2024). A significant difference in the mean sum of squares showed the presence of genetic variability in the studied traits across the genotypes, suggesting scope for selection and hybrid development. Such variability is fundamental for the development of early-maturing genotypes. Dirbas *et al.* (2024) revealed significant differences among studied traits, supporting the results of the analysis of variance.

Heterosis studies helped identify cross-combinations for further utilization for crop improvement. The extent of heterosis observed in crosses indicated the potential and diversity in parents (Attiea, 2020). Cross combinations showing negative heterobeltiosis for days to squaring, days to flowering, and boll maturation period indicated early phonological progression, which is favorable for earliness. The overall trend of descriptive statistics also showed hybrids required fewer days to reach the reproductive stage, suggesting the benefits of hybrid breeding to achieve earliness in cotton. These findings align with Rana *et al.* (2021), who also observed genetics of earliness-related traits. In addition to earliness, the yield improvement is the primary objective of cotton breeding. Traits, such as the number of bolls, boll weight, and seed cotton yield, require positive heterosis. Several cross combinations yielded higher than better parents, indicating the usefulness of heterosis breeding for yield improvement (Naik *et al.,* 2020). The presence of heterosis for yield, including boll weight, was also a result reported by Reecha *et al.* (2016) and Solongi *et al.* (2019). Furthermore, fiber quality is an important feature in the hybrid development of cotton**.** SAS-2 × Coker Wild demonstrated superior fiber quality in terms of fineness and length, suggesting combining well to improve the lint. These findings were in accordance with the earlier report of Ashokkumar *et al*. (2013). Heterotic potential of identified hybrids can be useful to create variability, followed by the

selection of high-yielding genotypes. Cross combinations that showed a high degree of heterosis are desirable (Dirbas *et al.,* 2024).

Moreover, biochemical traits, such as enzymatic activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), signified associations with stresses and developmental regulation. The observed variability in enzymatic activities across hybrids suggested differential gene expression during development. Short-season cotton has an association with premature senescence. Premature senescence is in association with the levels of chlorophyll, methane dicarboxylic aldehyde (MDA), and antioxidant enzyme activity (Song *et al.*, 2014; Yu *et al.,* 2005). The cotton's maturity performance has become associated with senescence (Chen and Dong, 2016). As reported by Yu *et al.* (2005), catalase and SOD primarily contribute to early developmental stages, while POD and SOD play significant roles in later developmental stages. Previous work of Shu-xun *et al.* (2005) reported the presence of higher activities of enzymes in cotton cultivars that have short duration and premature senescence. Cross combinations, showing positive heterobeltiosis, showed the presence of higher activities of enzymes than the parents, suggesting the role of antioxidant enzymes in cellular metabolism. Some hybrids displayed more activity in SOD than parents, while for POD and CAT, they showed negative results for heterosis. Song *et al.* (2014) reported the genetics of antioxidant enzymes, reinforcing the results of this present research.

Correlation coefficients offer an understanding of the association between characters and the degree of their linear relationship (Farooq *et al.,* 2018). Positive association between days to flower and square implies that selection for one could simultaneously enhance the other to achieve earliness. A similar trend came from Farooq *et al.* (2013) and Isong *et al.* (2017). A positive significant correlation between plant height and seed cotton yield suggested that taller plants tend to yield higher; the trends agree with the findings of Baloch *et al.* (2014). Jatt *et al.'s* (2007) research results reinforced that boll weight had a positive link with SCY. Moreover, a significant correlation between earliness-related traits and enzymatic activities proposed higher enzyme activities may contribute to early development (Xia *et al.*, 2020). The presented investigation revealed considerable potential of parental lines via hybrids for earliness and associated traits, demonstrating the potential of improving genotypes for early maturity in cotton.

CONCLUSIONS

This relevant study highlighted the heterotic potential of upland cotton (*Gossypium hirsutum* L.) hybrids for morphological and biochemical traits for earliness. Among the evaluated hybrids, VH-327 × CRIS-613 and VH-327 × Coker Carolina Queen were the identified promising cross combinations for earliness by exhibiting significant negative heterobeltiosis for days to flowering and squaring. Correlation results suggested a negative association between seed cotton yield and days to squaring. These findings suggested specific cross-combinations can serve as potential breeding materials for improving both earliness and fiber quality traits.

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