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Line × tester analysis for yield and its attributed traits in upland cotton (*Gossypium hirsutum* L.)

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Abstract

Cotton production in our country is declining due to produced cultivars that are not well-adapted to changing environmental conditions. New high-yielding, climate-resilient cotton genotypes are needed to enhance our economy. The objective of the study was to assess the effects of gene action, combining ability and heterosis on seed cotton yield and its associated traits. Five lines and three testers of upland cotton (*Gossypium hirsutum* L.) were used to produce fifteen F_1 hybrids using a line × tester mating design. The research was conducted in a randomized complete block design with three replications at Cotton Research Station, Ayub Agricultural Research Institute (AARI), Faisalabad in the Kharif season of 2020-21. The mean squares of all the traits were found significant. The results showed that among the female lines; FH-414 performed best for plant height, days to 50% flowering, boll weight, fiber strength, and fiber length while FH-490 was found best for seed cotton yield and GOT%. Testers concluded that EYE-111 was an excellent general combiner in terms of plant height, monopodial branches, days to maturity, bolls per plant, days to 50% flowering, seed cotton yield, and fiber quality. The cross combinations i.e., FH-492 × CIM-602 for plant height, FH-414 × CIM-602 for fiber fineness and fiber length, and FH-492 × NIAB-SANAB-M for seed cotton yield showed good SCA effects. FH-414 × CIM-602 and FH-415 × EYE-111 depicted maximum heterosis for fiber traits and seed cotton yield, respectively. FH-ANMOL × EYE-111 had shown maximum heterosis for sympodial branches and days to 50% flowering, FH-414 × CIM-602 for GOT% and fiber fineness. all the characters were regulated by non-additive kind of gene action. The above-mentioned genotypes having GCA and SCA effects could be further exploited for hybrid/variety development programs to cope with unexpected climatic conditions.

Keywords: Combining ability, Cotton revival, GCA, Heterosis, Heterobeltoisis, SCA

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Introduction

Cotton is the world’s most important fiber crop and is commonly known as silver fiber (Ali et al., 2012; Zia et al., 2015; Munir et al., 2018). The upland cotton (*G. hirsutum* L.) is generally known as “American cotton” and is cultivated in about 90% of the area throughout the globe (Wang et al., 2020). Cotton is widely grown in China, India, the United States, the Middle East and Australia (Tantuway & Patil, 2020). Cotton is said to be the backbone of Pakistan’s economy (Zia et al., 2018a; Zia et al., 2022; Abbas et al., 2022; Khan et al., 2023). It contributed 0.8% to the GDP of the country and 4.1% of the total value added to agriculture (Government of Pakistan [GOP], 2021-22). Since Pakistan's independence, scientists have studied and developed new varieties with the best traits, yet per-unit seed cotton yield is still poor (Zia et al., 2018b; Makhdoom et al., 2019). The overall area of cotton has increased, but the production remained below as compared to last year. The decrease in production was due to the use of poor-quality seeds, inadequate water availability, fluctuation in weather during flowering (Singh et al., 2022) and less water available during the crucial plant development stages (Government of Pakistan [GOP], 2021-22). It is very important to develop high-yielding and well-adapted cultivars under climate change scenarios to fulfill future requirements (Shuli et al., 2018; Shoukat et al., 2020; Shaukat et al., 2021; Arif et al., 2022).

Cotton breeders are constantly using different breeding methods to develop high fiber-yielding varieties by utilizing diversified genetic resources of cotton (Khokhar et al., 2018). However, it is important to understand the various cotton components that increase yield before beginning any breeding effort to boost seed cotton yield (Nizamani et al., 2017, Soomro, 2020). A thorough understanding of yield components like bolls per plant, boll weight, lint percentage etc. and how they are inherited is critical for making informed breeding decisions. The knowledge of combining ability, obtained through biometrical tools like line x tester analysis, provides key insights into the gene action governing yield and component traits. Information on general combining ability (GCA) helps identify parents with superior breeding value, while specific combining ability (SCA) indicates hybrid combinations with optimum trait expression. Such knowledge empowers breeders to choose ideal parents and crosses for harnessing both additive and non-additive genetic variances. This enables more rapid genetic gains from selection and hybridization. Therefore, a good grasp of combining abilities and inheritance patterns is pivotal before initiating any breeding endeavor to improve seed cotton yield (Khokhar et al., 2018).

A biometrical tool called line × tester analysis provides details on the variances in combining ability and the effects of the genotypes (Sajjad et al., 2016) regarding parent genotypes and their crosses which could be useful for a future breeding program (Abdel-Aty et al., 2022). Plant researchers widely used line × tester for early generation selection (Usharani et al., 2016; Sivia et al., 2017). GCA aids in the identification of potential parental genotypes for hybridization and subsequent selection in a population that segregates, whereas SCA is necessary for hybrid seed production and non-additive gene action is more significant as compared to additive gene action (Manan et al., 2022). GCA variation was greater for seed index and GOT% whereas SCA was greater for plant height, boll weight, bolls per plant and seed cotton yield (Zafar et al., 2022). The study helped to identify the suitable parents and their crosses after evaluating their combining ability (GCA and SCA) for yield and its attributing traits.

The objectives of the study were to develop single cross hybrids in upland cotton (*Gossypium hirsutum* L.) using line x tester mating design and evaluate them along with parents in a field trial. The study aimed to assess the gene action governing yield, yield components, and fiber quality traits in the breeding materials. Combining ability effects, including general combining ability (GCA) and specific combining ability (SCA), were estimated for all the traits to understand the relative importance of additive and non-additive gene actions. The overarching goal was to identify superior parents and cross combinations that could be further exploited to improve yield potential, yield components and fiber quality in cotton breeding programs tailored to current climatic conditions.

Materials and Methods

The research was performed at the experimental site of the Cotton Research Station (CRS), Ayub Agricultural Research Institute (AARI), Faisalabad (31°26'0"N 73°4'0"E). The five parental lines, FH-414, FH-415, FH-490, FH-492 and FH-ANMOL, and three testers, NIAB-SANAB-M, CIM-602 and EYE-111, all belonging to upland cotton (*G. hirsutum* L.) were collected from Cotton Research Station, AARI, Faisalabad. During October 2019-20, the parental genotypes were grown in earthen pots of size “30×30 cm^2” and placed in the glasshouse. At flowering, these lines crossed in line×tester fashion. The emasculation was done in the evening and the buds were covered with soda straw to avoid foreign contamination while the cotton flower was also tagged in the evening, which would be used as a male pollen parent the next day. On the next day, emasculated buds were pollinated using selected male flowers and covered again. Selfing was also done in some of the buds by using nail polish (sticky material) to prevent opening. To get sufficient seed, the maximum number of crosses were made between the lines and testers following the emasculation and pollination procedures described by Khan et al. (2018). According to Khan et al. (2018), making the maximum number of crosses with proper emasculation technique is crucial for obtaining enough F_1 hybrid seed for replicated field trials. The list of the parental genotypes along with their F_1 crosses is shown in Table 1.

The F_1 crossed seed of 15 hybrids and parental genotypes (5 lines, 3 testers) were planted in the field under randomized complete block design with three replications during the Kharif season of 2020-21. For recording the observations, three plants from each replication were randomly tagged. The distance between rows (R×R) and plants (P×P) was maintained as 75 and 45cm, respectively. The recommended agronomic/cultural practices e.g., fertilizers, weeding, chemicals and irrigations, etc. were followed as per recommendation and crop requirement of Punjab Agriculture Department [PAD] (2022), Government of Punjab throughout the growing season. The data were recorded for the parameters viz., plant height (cm), boll weight (g), days to maturity, days to 50% flowering, number of bolls per plant, monopodia per plant, sympodia per plant, seed-cotton-yield (kg/ha), ginning outturn percentage (GOT%) and various fiber related traits such as fiber fineness/micronaire value (µg/in), fiber length (mm) and fiber strength (g/tex) were measured using computerized high volume instrument developed by Uster Spinlab Company (model number HVI 900) that provides a comprehensive profile of raw data. The variance analysis was conducted by using Steel et al. (1997) approach to study the variability among the genotypes/crosses. GCA and SCA of the hybrids and parents were calculated through line-by-tester analysis developed by Kempthorne (1957). Further, the heterosis and heterobeltiosis were estimated through the formula suggested by Falconer and Mackay (1996). All statistical analysis was performed using R-Studio software (version 4.2.0) with the package ‘Agricolae’ for line×tester analysis.

Table 1 Parental (male and female) upland cotton genotypes and their F_1 hybrids

Lines (Female parent)	**Crosses (F_1 hybrids)**
1. FH-414	1. FH-414 × NIAB-SANAB-M
2. FH-415	2. FH-415 × NIAB-SANAB-M
3. FH-490	3. FH-490 × NIAB-SANAB-M
4. FH-492	4. FH-492 × NIAB-SANAB-M
5. FH-ANMOL	5. FH-ANMOL × NIAB-SANAB-M

Testers (Male parent)	6. FH-414 × CIM-602 7. FH-415 × CIM-602
1. NIAB-SANAB-M 2. CIM-602 3. EYE-111	8. FH-490 × CIM-602 9. FH-492 × CIM-602 10. FH-ANMOL × CIM-602 11. FH-414 × EYE-111 12. FH-415 × EYE-111 13. FH-490 × EYE-111 14. FH-492 × EYE-111 15. FH-ANMOL × EYE-111

Results and Discussion

Variance analysis and estimation of gene action

Analysis of variance (Line × Tester) depicted that genotypic differences for treatments (eight parents and fifteen crosses) were found significant ($P<0.01$) for all the traits under study (Table 3). Kempthorne's approach (1957) of line-tester was used for further data analyses. The results revealed that parents, parents vs. crosses and interaction (line × tester) were significant while non-significant differences were found in lines and testers. The significant parents and their crosses (F_1) indicated that the traits were governed by non-additive as well as additive gene action. The interaction between lines and testers also had a favorable effect on the differences in results. The findings revealed significant genetic variations between the lines and the testers. The results indicated that hybrid trait expression would likely to differ based on parental combinations (Farooq et al., 2022). The contribution of lines, testers and their crosses (F_1 hybrids) are given in Table 2. Estimation of GCA (σ^2gca) and SCA (σ^2sca) variance and expressing them as σ^2gca/σ^2sca may be used to evaluate the significance of GCA and SCA. If this ratio is around 1, it shows the significance of additive gene action (Mawblei et al., 2022). Tables 4 & 5 display GCA and SCA variations for yield and fiber-related characteristics, respectively. A higher magnitude of σ^2sca than σ^2gca was found, indicating non-additive gene action for plant height, monopodial branches, sympodial branches, days to 50% flowering, days to maturity, boll weight, bolls per plant, seed cotton yield, fiber strength and fiber length (Rani et al., 2020). When the GCA/SCA ratio is negative and smaller than one, it is indicative of "additive x dominant" gene interactions (Falconer & Mackay, 1996). The ratio between the GCA and SCA variance estimates for studied characteristics suggested that "additive dominance" gene interactions were present for plant height, GOT%, and fiber length. Previous studies have documented various gene influences on the characteristics studied. The dominant gene effects on ginning outturn fiber length and strength were reported (Nagarajan et al., 2022).

Bolls per plant is a reliable predictor of the overall production of seed cotton. Higher values for the SCA variance component suggested that the number of bolls produced per plant was the primary trait where non-additive gene activity was evident. Heterosis breeding is indicated to improve the number of flowers developed by each plant. Non-additive gene action was reported in bolls per plant (Fatima et al., 2022), monopodia per plant (Kanasagra et al., 2022) and sympodia per plant, SCY (Imran et al., 2012) and fiber strength (Balci et al., 2023). Characters should not be selected that are influenced by non-additive gene action until their genes have been well-established in subsequent generations (Aydin et al., 2019; Salman et al., 2019). Boll weight (Manan et al., 2022), fiber length and fiber fineness (Fatima et al, 2022) and GOT% (Manan et al., 2022) were governed by additive gene action.

Table 2 Contribution (%) of lines, testers and their crosses (line × tester**)**

	PH	MB	SB	DFF	DOM	NBP	BW	SCY	GOT	FF	FS	FL
Line	6.11	50.96	55.48	79.35	31.94	10.36	48.08	56.56	18.58	62.52	14.68	29.87
Tester	13.65	0.19	0.56	0.85	20.28	4.58	14.35	3.28	6.93	17.69	19.74	2.68
Line × tester	80.24	48.85	43.95	19.80	47.78	85.05	37.57	40.16	74.49	19.79	65.58	67.45

PH: Plant height; MB: Monopodial branches; SB: Sympodial branches; NBP: Number of bolls per plant; DFF: Days to fifty percent flowering; DOM: Days to maturity; BW: Boll weight; SCY: Seed cotton yield; GOT%: Ginning out turn; FF: Fiber fineness; FS: Fiber strength; FL: fiber length

Combining ability

Bolls per plant is a reliable predictor of the overall production of seed cotton. Higher values for the SCA variance component suggested that the number of bolls produced per plant was the primary trait where non-additive gene activity was evident. Heterosis breeding is indicated to improve the number of flowers developed by each plant. Non-additive gene action was reported in bolls per plant (Fatima et al., 2022), monopodia per plant (Kanasagra et al., 2022) and sympodia per plant, SCY (Imran et al., 2012) and fiber strength (Karademir et al., 2016). Characters should not be selected that are influenced by non-additive gene action until their genes have been well-established in subsequent generations (Salman et al., 2019; Aydin et al., 2019). Boll weight (Manan et al., 2022), fiber length and fiber fineness (Fatima et al, 2022) and GOT% (Manan et al., 2022) were governed by additive gene action.

Combining ability

The predominance of non-additive gene action observed in this study aligns with findings from several previous investigations on cotton (Kumar et al., 2017). Max et al. (2021) favored heterosis breeding over pure line selection due to the preponderance of dominance and epistasis effect and highlighted reciprocal recurrent selection as an efficient long-term breeding strategy to accumulate favorable epistatic interactions. Kumar et al. (2017) advised rigorous multi-environment testing and delayed selection in advanced generations to deal with unpredictable performance of segregating populations. Taken together, the complex non-additive genetic architecture necessitates tailored breeding schemes, advanced molecular tools and extensive field-testing to enhance selection efficiency and maximize genetic gains. GCA and SCA mean squares showed additive and non-additive gene action (Table 3). The general combining ability of parents (lines and testers) (Table 4) and SCA effects (Table 5) of F_1 hybrids for yield and fiber-related traits were also calculated.

General Combining ability of lines (GCA line)

Among lines, FH-414 depicted highly significant and positive GCA effects for days to maturity (3.0**), average boll weight (0.20**), fiber length (0.54**), fiber strength (0.65**) while maximum negative GCA effect showed for plant height (-2.98**), bolls per plant (-7.60**) and days to 50% flowering (-7.11**). FH-415 had the highest significant and negative GCA effects for days to maturity (2.00**), monopodial branches (-0.56**), number of sympodial branches (-6.49**), GOT% (-1.14**) and fiber fineness (-0.31**) while FH-ANMOL showed maximum positive and highly significant GCA effects number of bolls per plant (3.73**), number of sympodia (6.29**). FH-490 showed maximum positive GCA effects for plant height (5.36**), number of monopodia (0.89**), SCY (154.07**), GOT% (0.90**), fiber fineness (0.30**) while negative GCA recorded for average boll weight (-0.27**), fiber strength (-1.10**). Negative GCA is desirable for plant height, days to maturity and days to fifty percent flowering to develop short to medium-heightened, short-duration and early maturing genotypes (Munir et al., 2018). FH-414 from lines is a good general combiner for plant height, boll weight, days to 50% flowering, fiber fineness and fiber strength while FH-415 is the best general combiner for monopodia per plant. FH-490 for GOT% and FH-ANMOL for bolls per plant, sympodia per plant showed as a best general combiner.

General Combining ability of testers

Among the testers, CIM-602 showed positive GCA effects on plant height (5.96**), monopodial branches (0.02), sympodial branches (0.58**) and GOT% (0.30*) and fiber strength (0.95**) while negative GCA depicted for seed cotton yield (-35.67**). EYE-111 had negative significant GCA effects for plant height (-4.04**), monopodial branches (-0.04*), days to 50% flowering (-0.64**) and days to maturity (-1.6**) while depicting maximum positive GCA results for the number of bolls per plant (2.60), seed cotton yield (25.33**) and fiber fineness (0.16**). NIAB-SANAB-M depicted maximum positive significant results for days to 50% flowering (0.82**), days to maturity (1.80**), boll weight (0.01**) and monopodial branches (0.02) while negative GCA effect for fiber fineness (-0.10*). The same kind of results was reported earlier in the research of Kumar et al., (2017) and Thiyagu et al., (2019). EYE-111 as a tester was a good general combiner for plant height, monopodial branches, days to 50% flowering, days to maturity, number of bolls per plant, seed cotton yield and fiber fineness. The same kind of results were reported earlier in the research of Ahuja and Dhayal (2007). NIAB-SANAB-M is the best tester for boll weight. CIM-602 could be used as a good general combiner for the number of sympodia, GOT% and fiber strength. Jatoi et al. (2011) reported the same result. All three testers (NIAB-SANAB-M, CIM-602 and EYE-111) showed non-significant GCA effects for fiber length.

Specific combining ability

All the crosses showed the highly positive significance of SCA effects. Positive but significant SCA effects depicted by FH-490×CIM-602 for plant height (15.71**), FH-414×NIAB-SANAB-M for boll weight (0.24**), FH-490×EYE-111 for days to maturity (4.20**), FH-ANMOL×NIAB-SANAB-M for days to 50% flowering (7.51**), FH-ANMOL×CIM-602 for bolls per plant (15.27**), FH-492×NIAB-SANAB-M for seed cotton yield (203.0**) and fiber strength (2.72**), FH-ANMOL×EYE-111 for number of sympodial branches (6.58**), FH-415×EYE-111 for GOT% (2.49**) and FH-414×CIM-602 (1.09**) for fiber length. These results were matched with Zafar et al. (2022). While maximum negative SCA effect was revealed by FH-492×CIM-602 for plant height (-17.62**), FH-414×EYE-111 for boll weight (-0.23**) and fiber fineness (0.21*), FH-492×EYE-111 for days to maturity (-4.80**) and fiber strength (-1.98**), FH-ANMOL×EYE-111 for days to 50% flowering (-4.02**), FH-415×CIM-602 for monopodia per plant (-0.91**). These results were similar to Usharani et al. (2016). As per the requirement for days to maturity and days to 50% flowering, there is a need to find out if the cross has negative combining ability effects (Khokhar et al., 2018). Best Specific combiners are FH-492×CIM-602 for plant height, FH-492×EYE-111 for boll weight, FH-ANMOL×EYE-111 for days to 50% flowering and number of sympodial branches FH-ANMOL×CIM-602 for bolls per plant, FH-415×CIM-602 for number of monopodial branches, FH-492×NIAB-SANAB-M for fiber strength and could be used in further breeding programs. The same kind of results were reported earlier in the research of Munir et al. (2018).

Estimation of heterosis (%)

A broad range of heterosis from positive to negative for most of the characters was found in the crosses of upland cotton as shown in Table 6. Maximum but highly significant and positive heterosis was found in the crosses such as FH-490×CIM-602 for plant height (14.11%), FH-492×EYE-111 for boll weight (13.83%), FH-414×NIAB-SANAB-M for days to maturity (4.02%), FH-492×NIAB-SANAB-M for days to 50 percent flowering (10.84%), FH-ANMOL×CIM-602 for bolls/plant (108.26%) and fiber strength (13.98%), FH-ANMOL×EYE-111 for sympodial branches (104.11%) and seed cotton yield (25.83%), FH-414×CIM-602 for GOT% (8.32%) and fiber length (84.33%) and FH-490×EYE-111 for fiber fineness (3.69%). Maximum negative and significant heterosis was shown by FH-490×EYE-111 for plant height (-10.33%), FH-414×EYE-111 for boll weight (-5.46%), FH-492×EYE-111 for days to maturity (-2.82%), ANMOL×EYE-111 for days to 50 percent flowering (-8.89%), FH-490×NIAB-SANAB-M for bolls/plant (-20.87%) for bolls per plant, FH-ANMOL×CIM-602 for the number of monopodial branches (100.00%), FH-415×CIM-602 for the number of monopodial branches (-33.33%), FH-492×CIM-602 for seed cotton yield (-3.77%), FH-415×NIAB-SANAB-M (-6.77%) for GOT%, FH-414×CIM-602 for fiber fineness (-74.29%), FH-492×EYE-111 fiber strength (-8.23%) and FH-ANMOL×NIAB-SANAB-M for fiber length (-9.44%). The most important aim of the study is to find hybrids that have short to medium-heightened plants that prevent lodging losses and as a result yield increases. The crosses having minimum plant height (Zhang et al., 2017), days to maturity and days to 50% flowering (Khokhar et al., 2018) would be used as selection criteria for early maturity, short duration, lodging resistant genotypes while positive heterosis is required for bolls per plant, bolls per plant, and sympodia per plant (Zapadiya et al., 2021). Monopodial branches bear indirect fruits, so a smaller number of monopodia was required. Similar findings were also reported by Khokhar et al. (2018) for negative heterosis of monopodia per plant while positive heterosis is required for bolls per plant (Zafar et al., 2022), fiber length (Thiyagu et al., 2019) fiber fineness and fiber strength (Yehia & El-Hashash, 2019). Thiyagu et al. (2019) reported similar results of positive heterosis for seed cotton yield while Ahuja (2018) reported GOT%. Maximum desirable heterosis was found by crosses like FH-490×EYE-111 for plant height, FH-492×EYE-111 for days to maturity and boll weight, FH-ANMOL×EYE-111 for number of sympodial branches, FH-415×CIM-602 for number of monopodia, FH-490×NIAB-SANAB-M for bolls per plant, FH-415×EYE-111 for seed cotton yield and FH-414×CIM-602 for GOT% and fiber length could be used in the future for breeding purposes.

Estimation of heterobeltiosis (%)

Heterobeltiosis (better parent heterosis) for various yields and fiber-related character was given in Table 7. Maximum positive heterobeltiosis was depicted by the crosses, FH-490×CIM-602 for plant height (13.06%), FH-492×EYE-111 for boll weight (13.28%) and monopodial branches (91.03%), FH-414×NIAB-SANAB-M for days to maturity (3.84%), FH-492×NIAB- SANAB-M for days to 50% flowering (8.14%), FH-ANMOL×CIM-602 bolls per plant (92.37%), sympodia per plant (83.33%) and fiber strength (13.28%), FH-415×EYE-111 for seed cotton yield (32.94%), FH-ANMOL×EYE-111 for GOT% (7.01%), FH-490×EYE-111 for fiber fineness (1.75%) and FH-414×CIM-602 for fiber length (6.98%). While maximum negative but significant heterobeltiosis was shown by FH-490×EYE-111 for plant height (-11.43%), FH-415×EYE-111 for boll weight (-14.30%), FH-492×EYE-111 for sympodia per plant, days to maturity and seed cotton yield (-16.67%, -3.07%, -10.26%, respectively), FH-ANMOL×EYE-111 for days to 50% flowering (-12.42%), FH-492×NIAB-SANAB-M for GOT%. (-7.49%) and FH-414×CIM-602 for fiber fineness (-85.04%). Negative heterobeltiosis is desirable for plant height (Fetahu et al., 2015), the number of monopodia (Monicashree et al., 2017; Riaz et al., 2023). The crosses having minimum values for days to 50% flowering would be used as selection criteria for early maturity. Similar results of positive heterobeltiosis were reported for boll weight (Rani et al., 2020), sympodia per plant, SCY (Munir et al., 2018), GOT% (Islam et al., 2021), fiber length and fiber fineness (Vadodariya et al., 2022), fiber strength (Hamed and Said, 2021). The crosses having maximum values for the number of bolls per plant was the best hybrid and could be used in the future for breeding purpose. Similar outcomes for positive heterobeltiosis for bolls per plant (Keerthivarman et al., 2022). The best desirable hetrobeltoisis showed by hybrids such as FH-490×EYE-111 for plant height and fiber fineness, FH-492×EYE-111 for boll weight and days to maturity, FH-ANMOL×EYE-111 for days to 50% flowering and GOT%, FH-ANMOL×CIM-602 for sympodial branches, bolls per plant and fiber strength, FH-414×CIM-602 for fiber length and FH-415×EYE-111 for monopodial branches and seed cotton yield.

Conclusion

Estimation of combining ability effects plays a key role in determining yield and related traits. The SCA effect showed non-additive gene influences in all characteristics studied. To prevent losing better genotypes, selection might be delayed for future generations. F_1 crosses contributed more than lines or testers in terms of plant height, bolls per plant, days to maturity, GOT%, fiber strength and fiber length, but lines consistently performed better in terms of monopodial and sympodial branches, days to fifty percent flowering, boll weight, seed cotton yield and fiber fineness. The tester is positioned between lines and crosses for all attributes. One of the lines, FH-414 had the highest GCA for plant height, monopodial branches, days to 50% flowering, boll weight, fiber strength and fiber length whereas FH-490 displayed the highest GCA for fiber fineness, seed cotton yield, GOT% and days to maturity. EYE-11 had the best GCA effect for plant height, monopodial branches, days to 50% blooming, and seed cotton among the tested varieties. The study has some potential limitations that need to be considered. non-additive gene action governed most traits, indicating performance may fluctuate in subsequent generations. Specific combining ability was also significant, highlighting the influence of specific parental combinations. Future multi-location and multi-year trials across contrasting environments are recommended to validate the present findings. Expanding the genetic base by including more lines and testers could help test a wider array of combinations. Advanced generation progeny tests would be useful before varietal release to stabilize hybrid performance. The best hybrids identified here, like FH-492 × NIAB-SANAB-M and FH-414 × CIM-602, need further evaluation across environments and assessment for yield stability before commercial exploitation. The best general combiners, namely FH-414, FH-490 and EYE-111, should be extensively utilized in cotton breeding tailored to current climatic conditions. The insights gained from this study regarding gene action, general combining ability, specific combining ability and heterosis will be valuable for selecting ideal parents and crosses to develop high-yielding cotton varieties adapted to changing climate.

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Table 3 Variance analysis of combining ability for fiber quality and yield contributing traits in upland cotton

SOV	DF	PH	MB	SB	DFF	DOM	NBP	BW	GOT%	SCY	FF	FS	FL
Replications	2	235.406**	0.406	33.783	86.623	81.565**	115.928*	0.010**	0.060	1988.484	0.0138	0.016	1.328 *
Treatments	22	400.026**	1.477**	166.979**	113.286**	24.300**	569.825**	0.227**	7.717**	7547.998**	72.615**	7.349**	81.456 **
Parents (P)	7	361.714**	0.262	15.851**	72.857**	12.232**	47.143**	0.316**	5.385**	609.963**	206.343**	4.419**	243.963**
P vs. C	1	171.602**	7.548**	1847.33**	5.845**	16.984**	5222.68**	0.010**	24.917**	73662.143**	149.973**	2.365**	60.544**
Crosses (C)	14	435.498**	1.651**	122.517**	141.175**	30.857**	498.819**	0.199**	7.654**	6294.577**	0.225**	9.167**	1.695**
Lines (L)	4	93.189	2.944	237.922	392.056**	34.500	180.922	0.334	4.978	1103.884	0.493*	4.710	1.772
Testers (T)	2	416.089	0.022	4.822	8.422	43.800	160.067	0.200	3.711	1435.193	0.279	12.665	0.318
L × T	1	611.506**	1.411**	94.239**	48.922**	25.800**	742.456**	0.131**	9.978	10104.77**	0.078*	10.520**	2.001 **
Error	44	0.588	0.224	0.722	0.108	0.202	0.200	0.001	0.243	3.143	0.027	0.226	0.266
σ^2gca		-6.22	0.01	1.00	3.26	0.18	-8.61	0.00	-0.08	693.09	0.01	-0.05	-0.01
σ^2sca		203.74	0.39	31.31	16.28	8.58	247.45	0.04	3.24	15440.64	0.01	3.40	0.61
σ^2gca/σ^2sca		-0.03	0.02	0.03	0.20	0.02	-0.03	0.06	-0.03	0.04	0.36	-0.01	-0.02

* = Significant (P≤0.05), ** = Highly significant (P≤0.01) ns = Non-significant; SOV: Sources of variance; DF: Degree of freedom; PH: Plant height; MB: Monopodial branches; SB: Sympodial branches; NBP: Number of bolls per plant; DFF: Days to fifty percent flowering; DOM: Days to maturity; BW: Boll weight; SCY: Seed cotton yield; GOT%: Ginning out turn; FF: Fiber fineness; FS: Fiber strength; FL: fiber length; σ^2gca: general combining ability variance; σ^2sca: specific combining ability variance

Table 4 Predicted general combining abilities (GCA) effects for lines and testers concerning yield, yield components and fiber traits

Genotypes	PH	MB	SB	NBP	DFF	DOM	BW	SCY	GOT%	FF	FS	FL
						Lines						
FH-414	-2.98**	-0.33*	-3.82**	-7.60**	-7.11**	3.00**	0.20**	31.73**	0.18	0.06	0.65**	0.54**
FH-415	0.36	-0.56**	-6.49**	0.73**	-4.44**	-2.00**	0.08**	58.07**	-1.14**	-0.31**	-0.15	0.23
FH-490	5.36 **	0.89**	1.18**	2.84**	3.56**	-0.33**	-0.27**	154.07**	0.90**	0.30**	-1.10**	-0.45**
FH-492	-1.31**	-0.22	2.84**	0.29*	9.44**	0.67**	0.12**	-112.60**	-0.17	0.10	-0.06	-0.47**
FH-ANMOL	-1.42**	0.22	6.29**	3.73**	-1.44**	-1.33**	-0.13**	-31.27**	0.23	-0.14*	0.65**	0.15
SE	0.26	0.16	0.28	0.15	0.11	0.15	0.00	0.26	0.16	0.05	0.16	0.17
						Tester						
NIAB-SANAB-M	-1.91**	0.02	-0.56**	-3.67**	0.82**	1.80**	0.01**	10.33**	-0.57**	-0.10*	-0.07	-0.05
CIM-602	5.96**	0.02	0.58**	1.07**	-0.18*	-1.60**	0.11**	-35.67**	0.30*	-0.06	0.95**	0.16
EYE-111	-4.04**	-0.04	-0.02	2.60**	-0.64**	-0.20**	-0.12**	25.33**	0.27*	0.16**	-0.88**	-0.11
SE	0.20	0.12	0.22	0.12	0.08	0.12	0.00	0.20	0.13	0.04	0.12	0.13

PH: Plant height; MB: Monopodial branches; SB: Sympodial branches; NBP: Number of bolls per plant; DFF: Days to fifty percent flowering; DOM: Days to maturity; BW: Boll weight; SCY: Seed cotton yield; GOT%: Ginning out turn; FF: Fiber fineness; FS: Fiber strength; FL: fiber length

Table 5 Predicted specific combining abilities (SCA) effects of crosses for yield, yield components and fiber traits

Genotypes	PH	MB	SB	NBP	DFF	DOM	BW	SCY	GOT%	FF	FS	FL
FH-414 × NSM	6.91**	0.20	3.89**	10.00**	-3.16**	-0.13	0.24**	-36.33**	0.30	-0.05	-1.59**	-1.25**
FH-414 × CIM-602	-15.96**	-0.47	-3.24**	-8.73**	-0.16	-0.73**	-0.01**	-101.33**	1.11**	-0.16	-0.69*	1.09**
FH-414 × EYE-111	9.04**	0.27	-0.64	-1.27**	3.31**	0.87**	-0.23**	137.67**	-1.41**	0.21*	2.28**	0.16
FH-415 × NSM	-6.42**	0.42	5.56**	-0.33	-0.82**	0.87**	-0.14**	-62.67**	-1.92**	-0.14	-0.29	0.44
FH-415 × CIM-602	5.71**	-0.91**	-1.58**	-6.07**	1.18**	-0.73**	0.21**	5.33	-0.56	0.01	-0.19	-0.07
FH-415 × EYE-111	0.71**	0.49	-3.98**	6.40**	-0.36*	-0.13	-0.06**	57.33**	2.49**	0.13	0.48	-0.37
FH-490 × NSM	-1.42**	-0.02	-0.11	-24.44**	-3.82**	-2.80**	-0.10**	-39.67**	1.22**	0.13	0.96**	0.44
FH-490 × CIM-602	15.71**	-0.02	4.76**	10.49**	2.18**	-1.40**	-0.09**	85.33**	-0.11	0.03	0.14	-0.63**
FH-490 × EYE-111	-14.29**	0.04	-4.64**	13.96**	1.64**	4.20**	0.19**	-45.67**	-1.11**	-0.15	-1.10**	0.18
FH-492 × NSM	5.24**	0.09	-0.78*	12.78**	0.29	1.20**	-0.17**	203.00**	-1.50**	-0.07	2.72**	0.88**
FH-492 × CIM-602	-17.62**	0.76*	-1.91**	-10.96**	0.29	3.60**	-0.03**	-51.00**	1.26**	0.13	-0.74*	-0.63**
FH-492 × EYE-111	12.38**	-0.84**	2.69**	-1.82**	-0.58**	-4.80**	0.20**	-152.0**	0.24	-0.06	-1.98**	-0.25
FHA × NSM	-4.31**	-0.69*	-8.56**	2.00**	7.51**	0.87**	0.17**	-64.33**	1.91**	0.13	-1.79**	-0.51*
FHA× CIM-602	12.16**	0.64*	1.98**	15.27**	-3.49**	-0.73**	-0.08**	61.67**	-1.69**	0.00	1.48**	0.24
FHA× EYE-111	-7.84**	0.04	6.58**	-17.27**	-4.02**	-0.13	-0.10**	2.67	-0.22	-0.13	0.31	0.27
SE	0.44	0.27	0.49	0.26**	0.19	0.26	0.00	0.44	0.28	0.11	0.27	0.30

NSM: NIAB-SANAB-M; FHA: FH-ANMOL; PH: Plant height; MB: Monopodial branches; SB: Sympodial branches; NBP: Number of bolls per plant; DFF: Days to fifty percent flowering; DOM: Days to maturity; BW: Boll weight; SCY: Seed cotton yield; GOT%: Ginning out turn; FF: Fiber fineness; FS: Fiber strength; FL: fiber length

Table 6 Heterosis for yield and fiber-related traits in upland cotton

Crosses	PH	MB	SB	NBPP	DFF	DOM	BW	SCY	GOT%	FF	FS	FL
FH-414 × NSM	3.79**	23.08	39.74**	58.25**	5.43**	4.02**	10.53**	73.13**	1.36	-73.97**	-6.16**	60.22**
FH-414 × CIM-602	-8.05**	0.00	17.42**	14.15**	-7.49**	1.39**	1.14**	19.07*	8.32**	-74.29**	0.54	84.33**
FH-414 × EYE-111	1.81 **	33.33	20.50**	45.81**	-5.90**	2.42**	-5.46**	34.44**	1.13	-70.50**	4.22**	76.32**
FH-415 × NSM	-5.77**	23.08	30.86**	40.18**	-1.54**	2.19**	-4.08**	33.98*	-6.78**	-17.20**	1.46	-0.01
FH-415 × CIM-602	4.52**	-33.33	9.32**	33.91**	-1.53**	-0.96**	2.37**	33.06*	1.40	-11.84**	5.74**	2.07
FH-415 × EYE-111	-4.43**	33.33	-5.39*	77.38**	-5.02**	-0.43*	-5.39**	48.83**	8.28**	-2.49	1.44	-0.18
FH-490 × NSM	0.84*	46.67**	49.33**	-20.87**	1.00**	0.00	-1.91**	-3.87	6.13**	0.68	2.53*	-6.24**
FH-490 × CIM-602	14.11**	57.14**	74.50**	77.97**	4.97**	-1.55**	-4.48**	72.24**	7.81**	0.66	3.49**	-6.32**
FH-490 × EYE-111	-10.33**	57.14**	29.03**	98.24**	2.45**	1.54**	2.59**	88.64**	4.42**	3.69	-7.58**	-4.74**
FH-492 × NSM	12.57**	23.08	47.44**	59.18**	10.48**	2.33**	6.62**	68.88**	-5.31**	-10.02**	11.01**	-4.31**
FH-492 × CIM-602	-0.46	66.67**	48.39**	9.96**	8.40**	1.29**	7.54**	30.42	6.21**	-3.84	2.69*	-5.79**
FH-492 × EYE-111	13.69**	-16.67	57.76**	40.50**	5.52**	-2.82**	13.83**	67.13**	2.86**	-1.13	-8.23**	-5.68**
FH-A × NSM	-0.88*	16.67	44.68**	63.21**	6.29**	1.65**	6.84**	71.17**	8.32**	-3.39	-1.52	-9.44**
FH-A × CIM-602	12.59**	100.00**	95.71**	108.26**	-6.56**	-1.47**	-2.97**	100.79**	4.21**	-4.24	13.98**	-3.59**
FH-A × EYE-111	-6.48**	63.64**	104.11**	28.23**	-8.89**	-0.95*	-4.56**	42.31**	7.14**	0.04	3.19*	-4.60**

NSM: NIAB-SANAB-SANAB; FH-A: FH-ANMOL; PH: Plant height; MB: Monopodial branches; SB: Sympodial branches; NBPP: Bolls per plant; DFF: Days to fifty percent flowering; DOM: Days of maturity; BW: boll weight; SCY: Seed cotton yield; GOT%; Ginning out turn percentage; FF: Fiber fineness; FS: fiber strength; FL: Fiber length

Table 7 Heterobeltiosis for yield and fiber-related traits in upland cotton

Crosses	PH	MB	SB	NBPP	DFF	DOM	BW	SCY	GOT%	FF	FS	FL
FH-414 × NSM	3.46 **	14.29	31.32**	45.54**	-11.40**	3.84**	1.22**	66.75**	0.46	-84.80**	-11.26**	-7.77**
FH-414 × CIM-602	-9.77 **	0.00	9.64**	2.54**	-11.18**	1.04**	-2.44**	15.34*	4.57**	-85.04**	-5.13**	6.98**
FH-414 × EYE-111	0.21	33.33	16.87**	35.78**	-10.87**	1.54**	-13.43**	28.85**	-1.64	-82.94**	-1.54	2.27
FH-415 × NSM	-8.92 **	14.29	19.10**	40.18**	-6.51**	2.10**	-13.11**	19.25**	-7.04**	-19.49**	1.35	-3.20*
FH-415 × CIM-602	3.25 **	-33.33	-1.12	30.51**	-7.35**	-1.56**	-2.38**	26.31**	-1.52	-13.28**	5.61**	1.81
FH-415 × EYE-111	-5.88**	33.33	-11.27**	75.00**	-11.8**	-1.54**	-14.30**	31.88**	5.96**	-3.08	1.44	-0.67
FH-490 × NSM	-2.24 **	37.50*	45.45**	-22.88**	-1.63**	-1.03**	-2.05**	-5.88	5.90**	-0.85	2.42	-7.04**
FH-490 × CIM-602	13.06**	37.50*	68.83**	77.97**	1.28**	-2.06**	-9.37**	64.15**	4.78**	0.29	3.36*	-9.83**
FH-490 × EYE-111	-11.43**	37.50*	28.21**	90.68**	-2.48**	1.54**	2.44**	83.78**	2.26*	1.75	-7.59**	-8.11**
FH-492 × NSM	4.13**	14.29	38.55**	46.62**	8.14**	1.02**	6.11**	56.34**	-7.49**	-10.74**	9.66**	-4.55**
FH-492 × CIM-602	-9.77**	66.67**	38.55**	3.76**	5.11**	0.51**	2.36**	29.15*	1.12	-5.72	1.22	-8.79**
FH-492 × EYE-111	3.35**	-16.67	53.01**	27.82**	0.93**	-3.07**	13.28**	53.99**	-1.34	-5.16	-9.43**	-8.47**
FH-A × NSM	-2.17**	0.00	39.73**	54.46**	4.56**	0.86**	3.88**	66.02**	6.42**	-9.99**	-1.89	-12.10**
FH-A × CIM-602	8.73**	83.33**	90.28**	92.37**	-8.95**	-1.72**	-5.52**	82.34**	3.28**	-9.80**	13.28**	-9.10**
FH-A × EYE-111	-9.41**	50.00*	91.03**	22.94**	-12.42**	-1.20**	-7.21**	38.73*	7.01**	-3.72	2.69	-9.86**

NSM: NIAB-SANAB-M; FH-A: FH-ANMOL; PH: Plant height; MB: Monopodial branches; SB: Sympodial branches; NBPP: Number of bolls per0plant; DFF: days to fifty percent0flowering; DOM: Days to maturity; BW: boll weight; SCY: Seed cotton yield; GOT%; Ginning out turn percentage; FF: Fiber fineness; FS: fiber strength; FL: fiber length.

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