

Assessment of Yield Potential of Newly Developed Cotton (*Gossypium Hirsutum* L.) Advance Lines Under Normal Sowing Field Conditions and Their DNA Fingerprinting

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Abstract

Cotton (*Gossypium hirsutum* L.) is the backbone of Pakistan's economy and the primary source of natural fiber worldwide. Despite its significance, cotton productivity remains suboptimal due to a narrow genetic base, biotic and abiotic stresses, and declining soil fertility. Breeding programs therefore focus on developing new, high-yielding, and stress-tolerant advance lines that can outperform existing commercial cultivars. In this study, field evaluation and molecular profiling were conducted on a set of advance lines derived from *FH-490* and compared with standard commercial checks (e.g., *FH-942*, *SLH-2010*, *CIM-70*). The experiment was laid out in a randomized complete block design with three replications under normal sowing conditions. Agronomic traits including plant height, monopodial and sympodial branches, number of bolls, boll weight, and seed cotton yield were recorded alongside fiber quality and physiological parameters. Significant variation was observed among the genotypes, with L-1 and L-4 recording the highest yields, while FH-942 and SLH-2010 performed poorly. Molecular analysis using SSR markers revealed polymorphism that differentiated the advance lines, with SSR4-170 notably associated with tolerance under limited water conditions. The integration of field performance with DNA fingerprinting allowed a clearer understanding of both genetic diversity and adaptive potential. This study identifies promising lines (L-1, L-2, L-4, L-5) with superior yield and fiber quality, suggesting their suitability for inclusion in breeding pipelines. Findings emphasize the importance of combining morphological evaluation with molecular tools to accelerate the development of resilient cotton varieties capable of sustaining production under evolving climatic and resource constraints.

Keywords: Upland cotton, advance lines, yield, fiber quality, SSR markers, molecular diversity.

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INTRODUCTION

One of the most significant fiber crops in the world is cotton, which provides approximately one-third of the world natural fiber consumption. As the fourth biggest cotton producer, Pakistan is a nation that depends on its textile sector and export economy. However, in recent years, cotton production has stagnated as a result of several factors such as the pressure of pests, lack of water, climatic inconsistency, or absence of new high yielding cultivars. The low genetic diversity of the current commercial varieties has also restricted the possibilities of sustainable enhancement. To counter these difficult times, cotton breeders are continually developing new advance lines, which are highly assessed prior to their entry in the market (Ali *et al.*, 2025). These

lines should exhibit excellent yield, agronomic and adaptability to the changing environment. In addition to field characteristics, their commercial acceptability is based on their quality parameters which are fiber length, fineness, and strength. In addition, as water scarcity becomes a serious bottleneck, physiological and biochemical screening further introduces a new layer of varietal selection. Simple sequence repeats (SSRs) are molecular markers that have become invaluable in the study of genetic diversity as well as the association of characteristics (Ur Rahman *et al.*, 2020). The SSRs offer codominant, reproducible and highly polymorphic profiles that enable breeders to detect agronomically significant variation and associate markers with genotypes. This kind of combination of both molecular information and field performance, facilitates faster

selection in breeding using markers, and prevents excessive overlap in breeding material. The current experiment will compare the performances of several cotton advance lines, sown under a normal sowing environment, in yield, fiber quality, and physiological performances with commercial checks (Revanasiddayya *et al.*, 2023). Field evaluation is complemented by molecular characterization with SSRs to detect lines that have potential breeding in the future. Our hypothesis was that some of the lines of advancement would perform better than checks in terms of yield and flexibility, and that the molecular data would show clear patterns of diversity that could be used to select. The research helps fill the gap between traditional breeding and molecular breeding in cotton breeding.

MATERIALS AND METHODS

Experimental Site and Design

The research was carried out in the research farm of Government College University Faisalabad under normal conditions of sowing (Altaf *et al.*, 2024). A randomized complete block design (RCBD) was used in this experiment to reduce environmental variation by use of three replications. Plots were 10 m x 4.5 m and agronomic practices were recommended during the entire growing period.

Plant Material

The primary material included advance lines developed from *FH-490*, hereafter referred to as L-1, L-2, L-3, L-4, and L-5 (Varshney *et al.*, 2021). Commercial varieties (*FH-942*, *SLH-2010*, *CIM-70*, *CIM-496*, *CIM-506*, *COKAR-31*) were included as checks to benchmark performance. Seeds were surface sterilized before sowing to minimize seed-borne contamination.

Crop Management

The crop was sown in early spring using a plant-to-plant spacing of 30 cm and row-to-row spacing of 75 cm. Standard fertilization and weed management practices were followed. To test resilience under

moderate water stress, irrigation was restricted to three applications at 60, 90, and 120 days after sowing. Plants were thinned at the two-leaf stage to maintain uniform stands. Standard herbicide and pest management protocols were applied (Ahmed *et al.*, 2020).

Data Collection

Agronomic traits included plant height, number of monopodial and sympodial branches, bolls per plant, boll weight, and seed cotton yield (Patil *et al.*, 2024). Physiological and biochemical traits assessed were chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, total soluble proteins, phenolics, hydrogen peroxide, malondialdehyde (MDA), catalase (CAT), and peroxidase (POD). Fiber quality traits including staple length, fineness, and strength were measured at the AARI/CRI Fiber Testing Lab, Faisalabad.

Molecular Profiling

Leaf samples were collected for DNA extraction using the CTAB method. PCR amplification was performed with selected SSR primers known for their informativeness in cotton. Amplified products were resolved on polyacrylamide gels alongside a 1,000 bp ladder. Banding patterns were scored to generate binary data matrices (Shahzad *et al.*, 2022). Software including GenAlEx, STRUCTURE, and TASSEL were used for genetic diversity and association analyses.

Statistical Analysis

Field data were subjected to analysis of variance (ANOVA) using RCBD models. Mean comparisons were conducted using least significant difference (LSD) tests at 5% probability. Correlation analyses among agronomic and biochemical traits were performed. Molecular data were analyzed for polymorphism information content (PIC), cluster formation, and population structure.

RESULTS

Table 1: Antioxidant Enzyme Activity ANOVA Summary

Trait	F (Varieties)	p-value	Significance	R ²	CV (%)
Catalase (CAT)	68.68731	0	***	0.972234	4.682138
Peroxidase (POD)	32.97014	0	***	0.942972	13.54488

The analysis of variance revealed highly significant differences among cotton genotypes for antioxidant enzyme activities, namely catalase (CAT) and peroxidase (POD). Catalase exhibited a remarkably high F-value (68.68, $p < 0.001$), with a strong coefficient of determination ($R^2 = 0.97$) and low variability (CV = 4.68%), indicating consistent and reliable variation

across genotypes. Similarly, peroxidase activity showed significant variation ($F = 32.97$, $p < 0.001$), supported by a high R^2 value (0.94). These findings suggest that antioxidant defense mechanisms are strongly genotype-dependent, highlighting CAT and POD as critical biochemical indicators for stress tolerance and yield stability in cotton breeding.

Table 2: Biochemical Traits ANOVA Summary

Trait	F (Varieties)	p-value	Significance	R ²	CV (%)
Chlorophyll a	16.60976	0	***	0.900341	8.425196
Chlorophyll b	6.255134	0	***	0.829283	3.271183

Trait	F (Varieties)	p-value	Significance	RA ²	CV (%)
Chlorophyll (a+b)	3.842221	0.0003	***	0.723608	6.920081
Chlorophyll a/b	3.513817	0.0006	***	0.656607	30.02708
Carotenoids	29.79343	0	***	0.937385	3.443363
Total soluble protein (TSP)	3.807388	0.0003	***	0.657969	10.92759
Phenolics	5.319516	0	***	0.73154	11.46041
Hydrogen peroxide (H2O2)	4.703866	0	***	0.733503	11.27672
Malondialdehyde (MDA)	18.72948	0	***	0.909892	8.096766

The physiological and biochemical traits exhibited significant genotypic variation, reflecting their importance in cotton adaptability and performance. Chlorophyll a ($F = 16.61$, $R^2 = 0.90$) and carotenoids ($F = 29.79$, $R^2 = 0.94$) showed strong genotype effects with high explanatory power and low coefficients of variation, indicating stable photosynthetic efficiency among selected lines. Large values of chlorophyll b, total chlorophyll, and chlorophyll a/b ratio were also reported,

but with medium R^2 , implying that these components vary in their contribution to the light-harvesting ability. Stress-related metabolites like hydrogen peroxide and malondialdehyde were not similar, meaning there was a genotypic response disparity to oxidative stress. Likewise, TSP and phenolics were quite diverse, as well, which highlights their contribution to metabolic resilience.

Table 3: Agronomic & Yield Traits ANOVA Summary

Trait	F (Varieties)	p-value	Significance	RA ²	CV (%)
Monopodial branches	8.470046	0	***	0.809231	35.08776
Sympodial branches	3.337157	0.0009	***	0.639201	22.994
Number of nodes	0.914657	0.5674	ns	0.334705	26.28515
Number of bolls	11.57552	0	***	0.852789	42.98794
Boll weight (g)	6.04011	0	***	0.751402	29.73583
Plant height (cm)	15.22889	0	***	0.885006	11.31364
Chlorophyll a	16.60976	0	***	0.900341	8.425196
Seed cotton yield (g/plant)	13.86448	0	***	0.874491	41.50797

The agronomic and yield characteristics also indicated a great deal of genetic variation amongst the cotton genotypes. Significant effects were found on monopodial branches, number of bolls, boll weight, height of plant, chlorophyll a, and seed cotton yield with R^2 values between 0.75 and 0.90, which have strong model fit and good trait expression. Especially

significant was plant height ($F = 15.23$) and seed cotton yield ($F = 13.86$), which are also critical factors affecting productivity. The number of monopodial branches and bolls was also a major contributor and this associated vegetative growth with reproductive performance. However, the node count was not significant, indicating low discriminatory capacity to select.

Table 4: Correlation

Traits	N.B	SCY	B.W	P.H	S.P	M.N	N.N	TSP	Chl a	Chl b	Chl a+b	chl a/b	carot e	TCA(MDA)	TCA(H2O2)	Phenolic	CAT	POD
N.B	1																	
SCY	0.87***	1																
B.W	0.004 ns	0.38**	1															
P.H	-0.27*	-0.48***	-0.55*	1														

POD	CAT	Phenolic	TCAH	TCA(MDA)	carote	chl a/b	chl a+b	chl b	chl a	TSP	N.N	M.N	S.P
-0.24ns	-0.06***	-0.005 ns	-0.36**	-0.44***	-0.08ns	0.46***	0.461***	-0.05ns	0.65***	0.03ns	-0.14ns	0.19ns	0.52***
-0.23 ns	-0.5***	0.028ns	-0.23 ns	-0.27*	-0.07 ns	0.52***	0.39**	0.01 ns	0.48***	0.10 ns	-0.22 ns	0.44***	0.56***
-0.12 ns	-0.02**	-0.004*	0.18 *	0.17 ns	0.11 ns	0.18 *	0.13 ns	0.23 ns	-0.01***	0.10 ns	-0.34 ns	0.38**	0.23 ns
0.31 ns	0.30 ns	-0.2**	-0.07*	-0.02 ns	-0.1***	-0.2**	-0.11 ns	-0.06**	-0.15 ns	-0.31*	0.24 ns	-0.6***	-0.05**
-0.07 ns	-0.27**	-0.08*	-0.23 ns	-0.27**	0.15 ns	0.28 ns	0.26**	0.02 *	0.29 ns	-0.09**	-0.11**	0.12 ns	1
-0.13*	-0.1ns	0.16ns	0.16ns	0.13*	0.22ns	0.25* *	-0.0009ns	0.1ns	-0.07**	0.17ns	-0.20**	1	
0.23 ns	-0.05**	0.12 ns	-0.008*	0.11 ns	-0.03***	0.02 ns	0.04 ns	0.03 ns	-0.05***	-0.05***	1		
0.16 ns	-0.07**	0.06 ns	0.17 ns	0.12 ns	-0.11*	0.05 ns	0.04 ns	0.10 ns	0.023ns	1			
-0.10**	-0.70 ns	-0.03***	-0.48**	-0.58**	0.027ns	0.51 ns	0.72 ns	-0.08**	1				
0.02 ns	0.09 ns	-0.16*	0.32**	0.37 ns	0.095ns	-0.002*	0.24 ns	1					
0.02 ns	-0.52 ns	-0.13***	-0.15 ns	-0.39**	0.11 8	0.42 ns	1						
0.11 ns	-0.29*	0.37 ns	-0.10**	-0.14&	-0.005**	1							
0.09 ns	-0.17***	-0.20**	0.001*	0.21 ns	1								
0.20ns	0.47ns	0.33*	0.36ns	1									
0.046n s	0.56ns	-0.062**	1										
0.091ns	0.023*	1											
0.10ns	1												

The correlation analysis identified the main relationships between agronomic, physiological, and biochemical characteristics that have a joint role in the determination of yield potential. Seed cotton yield (SCY) was positively correlated with monopodial branches ($r = 0.87^{***}$), sympodial branches, and boll weight, which supports the value of reproductive structures in productivity. In contrast, plant height exhibited significant negative associations with SCY and related traits, suggesting that excessive vegetative growth may reduce yield efficiency. Positive correlations of SCY with chlorophyll a and phenolics further emphasized the role of photosynthetic capacity and antioxidant metabolism in yield stability. Conversely, stress markers such as MDA and H_2O_2 correlated negatively with SCY, reflecting the adverse impact of oxidative stress on cotton performance.

DISCUSSION

The present study demonstrated substantial genotypic variability among upland cotton (*Gossypium hirsutum* L.) advance lines and commercial checks, highlighting the scope for genetic improvement under normal sowing conditions. Agronomic traits such as monopodial and sympodial branches, number of bolls, boll weight, and seed cotton yield exhibited highly significant differences across genotypes, with high coefficients of determination (R^2 values >0.80 in several traits), suggesting reliable expression of genetic potential. Yield-related attributes, particularly boll number and boll weight, emerged as critical determinants of seed cotton yield, consistent with earlier reports that reproductive allocation strongly predicts productivity in cotton (Sarwar *et al.*, 2025). The superior performance of advance lines L-1 and L-4, relative to commercial checks, indicates their potential as promising candidates for future varietal release or use in hybridization programs. Physiological and biochemical analyses provided further insights into genotypic adaptability. Chlorophyll an and carotenoids displayed strong genotype effects with high explanatory power, reinforcing their role in photosynthetic efficiency and biomass accumulation. While chlorophyll b and the a/b ratio showed moderate significance, these traits still contributed to differential light-harvesting strategies among lines. Total soluble proteins and phenolic content also varied significantly, reflecting distinct metabolic capacities that can enhance tolerance to stress environments. The detection of significant variation in stress markers such as hydrogen peroxide and malondialdehyde further underlines the inherent diversity in oxidative stress responses, where low accumulations in high-yielding lines suggest improved cellular homeostasis (Saeed *et al.*, 2021). Enzyme assays confirmed the importance of antioxidant defense mechanisms. Both catalase (CAT) and peroxidase (POD) showed exceptionally high F-values and strong R^2 values (>0.94), emphasizing their robustness as biochemical indicators. Elevated CAT and POD activities in superior lines suggest that efficient detoxification of reactive

oxygen species contributes to better growth and yield stability. These findings corroborate prior studies linking antioxidant capacity with improved cotton resilience under suboptimal conditions. Correlation analysis provided integrative evidence of trait interdependencies. Seed cotton yield was strongly and positively correlated with monopodial branches, sympodial branches, and boll number, reaffirming the centrality of reproductive structures in yield formation. In contrast, plant height exhibited significant negative associations with yield, indicating that excessive vegetative growth can divert assimilates away from reproductive sinks (Iqbal *et al.*, 2023). The positive association of yield with chlorophyll a and phenolics further emphasizes the contribution of efficient photosynthesis and antioxidant metabolism to overall productivity. The adverse associations between yield and malondialdehyde/hydrogen peroxide, on the other hand, indicate that the effect of oxidative stress is deleterious, and supports the usefulness of biochemical screening in cotton enhancement. The combination of agronomic, biochemical and enzymatic data will give an overall picture of cotton performance. One way to explain their breeding potential is that the most desirable lines (L-1, L-2, L-4, L-5) are described based on high yield and other beneficial physiological qualities (Kumar *et al.*, 2021). It is interesting to note that these results affirm the utility of combining classic field evaluation with molecular and biochemical testing in accelerating the development of strong cotton hybrids capable of surviving the modified agricultural conditions in Pakistan.

Besides, to preserve the genetic diversity and to accelerate the selection process, it is important to maintain the use of the data concerning the molecular markers in the field assessment.

This test revealed that there was a lot of genetic variation among upland cotton (*Gossypium hirsutum* L.) advance lines and commercial checks when they were sown under normal conditions. Agronomic characteristics that included boll number, boll weight, plant height and seed cotton yield were closely related to productivity and biochemical and enzymatic studies indicated the role of chlorophyll content, antioxidant activity, and metabolites related to stress in being useful to define performance. The high yield and physiological stability of L-1, L-2, L-4, and L-5 lines make them good candidates to be included in breeding initiatives to improve yield potential and stress resistance. These lines are advised to be tested in multi-location, in order to establish their flexibility in different environments. Besides, to obtain the genetic diversity and to accelerate the selection efficiency, the introduction of the data regarding the types of molecular markers into the field evaluation will need to be maintained. A combination of agronomic, biochemical and molecular methods provides a strong avenue to producing resilient cotton plant varieties to support future textile requirements in Pakistan.

Abbreviations

AARI — Ayub Agricultural Research Institute
 ANOVA — Analysis of Variance
 CAT — Catalase
 CIM — Central Institute of Cotton (e.g., CIM-70, CIM-496, CIM-506)
 CV — Coefficient of Variation
 DAS — Days After Sowing
 DNA — Deoxyribonucleic Acid
 EST-SSR — Expressed Sequence Tag–Simple Sequence Repeat
 FH — Faisalabad Hybrid (e.g., FH-490, FH-942)
 H₂O₂ — Hydrogen Peroxide
 ISSR — Inter Simple Sequence Repeat
 LSD — Least Significant Difference
 MDA — Malondialdehyde
 PAGE — Polyacrylamide Gel Electrophoresis
 PCR — Polymerase Chain Reaction
 PIC — Polymorphism Information Content
 POD — Peroxidase
 QTL — Quantitative Trait Loci
 RCBD — Randomized Complete Block Design
 SCY — Seed Cotton Yield
 SE — Standard Error
 SLH — Sahiwal Hybrid (e.g., SLH-2010)
 SP — Sympodial Branches
 SSR — Simple Sequence Repeat
 TSP — Total Soluble Protein

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