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Genetic Variability and Trait Association Analysis in Upland Cotton (Gossypium Hirsutum L) for Yield, Fiber Quality, and Physiological Attributes

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Abstract

Cotton is a major cash crop globally, with both yield and the fiber quality parameters highly influenced by both genetic and environmental factors. The present study evaluated 30 cotton (Gossypium hirsutum L.) genotypes for morphological, physiological, and fiber quality traits to assess genetic variability, heritability, and trait associations under field conditions. The experiment was laid out in a randomized complete block design (RCBD) with three replications. Significant genotypic variation ($p < 0.001$) was observed across all studied traits, including plant height, boll weight, number of bolls, seed cotton yield per plant, ginning outturn, and fiber parameters such as span length, micronaire, and strength. Physiological traits such as photosynthetically active radiation, leaf temperature, net photosynthesis, and transpiration rate also showed considerable variation. High genotypic and phenotypic coefficients of variation (GCV and PCV) were recorded for yield per plant, number of bolls, and physiological traits, while fiber traits exhibited moderate to low variation. Broad-sense heritability measures were high (>80%) for maximum traits, with substantial genetic advance, indicating predominance of additive gene action and the potential for improvement through simple selection. Positive and significant correlations were observed between yield per plant and physiological traits, particularly photosynthesis ($r = 0.91$), stomatal conductance ($r = 0.72$), and transpiration rate ($r = 0.60$). Multiple regression analysis confirmed that photosynthesis, transpiration rate, plant height, and boll weight significantly contributed to yield variability. Principal component analysis (PCA) revealed that five principal components accounted for 78.5% of the total variation, with PC1 alone explaining 33.1%. Genotypes such as Bahar-7, CIM-599, FH-901, and CKC-28 were identified as genetically diverse and high-yielding, making them suitable candidates for future breeding programs.

Introduction

Cotton belongs to genus Gossypium and it has four cultivated species namely Gossypium hirsuitum, Gossypium herbacium, Gossypium barbadence and Gossypium arboreum (Singh, 2004). The genus Gossypium comprises of 50 species including 45 diploid species and 5 species are allotetraploid. The cultivated area of cotton during 2020-21 is 2,079 thousand hectares and production is 7.064 million bales. The area of cotton crop for the year 2020-21 showed a decrease of 17.4% over the last year and production showed a decrease of 22.8 % over the last year (Economic Survey of Pakistan, 2020-21). The cotton production was decreases in Pakistan mainly due to unfavorable weather conditions includes extensive dry and heat spells. Other reasons are the attack of different insects/pests like whitefly, pink bollworm, etc (Iqbal et al., 2022; Rehman et al., 2023).

To mitigate these challenges and ensure long-term improvement in cotton productivity, it is essential to explore and utilize the genetic diversity available within cotton germplasm (Farooq et al., 2023). Breeding efforts aimed at developing high-yielding and stress-tolerant cultivars require a thorough understanding of the genetic variability, heritability, and trait interrelationships present in the breeding material (Majeed et al., 2024). This genetic information enables breeders to identify promising genotypes with desirable agronomic, physiological, and fiber quality traits, and to design effective selection and hybridization strategies accordingly (Wang et al., 2023; Maqbool et al., 2022).

Traditional breeding has primarily focused on morphological traits such as plant height, boll number, boll weight, and lint yield (Wendel et al., 2020). However, these traits are often complex and influenced by environmental factors, making selection based on phenotype alone less effective. Recent advancements in physiological trait analysis, such as net photosynthesis rate, stomatal conductance, transpiration rate, and photosynthetically active radiation (PAR), provide new avenues to understand plant responses under stress and their contribution to yield (Yang et al., 2023). These physiological parameters, measured through instruments like the Infrared Gas Analyzer (IRGA), offer insight into the plant's internal efficiency and adaptability to external stressors. Moreover, fiber traits including span length, micronaire, and fiber strength are critical quality indicators for the textile industry and must be concurrently improved along with yield traits (Shang et al., 2022).

Genotypic and phenotypic coefficients of variation (GCV and PCV), genetic advance (GA) and broad sense heritability are important in determining the effectiveness of selection (Johnson et al., 1955; Rauf et al., 2023). Traits with maximum value of heritability and GA are supposed to be under the influence of additive gene action and can be improved through direct selection. In contrast, traits governed by non-additive gene action may require more complex breeding approaches such as hybrid development (Khan et al., 2023; Shakeel et al., 2023). Furthermore, correlation and regression analyses help in identifying the strength and direction of relationships among traits, providing guidance for indirect selection strategies. Principal component analysis (PCA) is another powerful multivariate tool used to assess genetic divergence and classify genotypes based on multiple traits simultaneously, thus enhancing the efficiency of genotype selection in breeding programs. The main objectives of this study was to evaluate the genetic variability among 30 cotton **(**Gossypium hirsutum **L.)** genotypes based on morphological, physiological, and fiber quality parameters under field environments. Another objective was to assess the interrelationships among morphological, physiological, and fiber traits using correlation and multiple regression analyses to identify key contributors to seed cotton yield.

Given the urgent need to boost cotton productivity and resilience, it becomes imperative to identify genotypes with superior performance across a range of morphological, physiological, and fiber quality traits. Therefore, the current study was commenced to assess 30 diverse G. hirsutum genotypes under field conditions to estimate genetic variability, genetic advance, heritability, and trait relation. The aim was to identify high-yielding and genetically diverse cotton genotypes suitable for use in imminent breeding programs to improve yield and fiber quality under the specific agro-climatic conditions of southern Punjab, Pakistan.

Material and Methods

Experimental Site and Plant Material

The present study was conducted at the Cotton Research Institute, Multan, located in the cotton belt of southern Punjab, Pakistan. The experimental site is characterized by an arid to semi-arid climate with high summer temperatures and low annual rainfall. The soil type was clay loam with moderate fertility.

A total of 30 cotton genotypes (Table 1) were selected for evaluation, including both commercial cultivars and advanced breeding lines, representing a wide range of genetic backgrounds. These genotypes were sourced from various research institutes and breeding programs across Pakistan.

Experimental Design and Layout

The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications to account for environmental variation. Each genotype was sown with row-to-row spacing of 75 cm and plant-to-plant spacing of 30 cm. Standard agronomic practices for cotton cultivation were followed throughout the growing season, including irrigation, pest management, and fertilization, to ensure uniform crop growth and development.

Data Collection

Data were recorded on a range of morphological, physiological, and fiber quality traits. For each genotype, five plants were arbitrarily selected and labelled in each replication**,** and measurements were taken on these plants to ensure accuracy and consistency.

1. Morphological Traits

Field observations and measurements were taken at crop maturity for the following agronomic traits:

- **Plant Height (cm):** Plant height (PH) was measured from ground level and up to the main stem.
- **Boll Weight (g):** The average boll weight (BW) of five open bolls per plant selected on random basis.
- **Seed Cotton Yield/ Plant (g):** Seed cotton yield (SCY) was measured form total seed cotton weight harvested from individual tagged plants.
- **Ginning Outturn (GOT%):** Calculated as percentage of lint collected from seed cotton after the ginning.

2. Fiber Quality Traits

Fiber quality parameters were measured using a **High Volume Instrument (HVI-900)** at the Cotton Research Institute fiber testing laboratory. The following fiber traits were assessed:

- **2.5% Span Length (mm):** Indicates fiber length.
- **Micronaire (µg/inch):** Reflects fiber fineness and maturity.
- **Fiber Strength (g/tex):** Resistance of fiber to breakage.

3. Physiological Traits

Physiological parameters were recorded during the flowering stage using an **Infra-red Gas Analyzer (IRGA), Model CI-340** (CID Bio-Sciences, USA). Measurements were taken between 9:00 AM to 11:00 AM on clear days to minimize environmental variability. The following parameters were recorded:

- **Photosynthetically Active Radiation (PAR) (µmol m^{-2} s^{-1})**
- **Leaf Temperature (°C)**
- **Net Photosynthetic Rate (Pn) (µmol CO_2 m^{-2} s^{-1})**
- **Transpiration Rate (Tr) (mmol H_2O m^{-2} s^{-1})**

Statistical analysis

The analysis of variance was calculated by method steel et al., (1997), for the comparison of variability coefficient of variance was also calculated for all the characters. Genotypic, phenotypic, and environmental coefficient of variation was divided by the means of respective trait. Broad Sense heritability was measured through dividing the variance (genotypic) over variance (phenotypic). The genetic advance was measured at 20% selection intensity. The simple correlation was calculated according to Pearson method and the divergence were measured through principal component analyses in r-software (R development core team 2015).

- The coefficient of variance (Genotypic and Phenotypic) was calculated from Burton's method (1952) and further classified based on the criteria outlined by Sivasubramanian and Madhavamenon (1973). According to this classification, values were categorized as low (<10%), further in medium (10-25%), and high (>25%).

- Broad sense heritability estimates were classified following the guidelines set by Robinson (1965). Categories included high, moderate and low (>30%, 10-30%<10% respectively).
- The genetic advance as mean percent was categorized according to the recommendations by Johnson et al. (1955a). This classification included high (>20%), moderate (10-20%), and low (<10%) ranges.

Table 1: List of cotton genotypes evaluated for morpho-physiological, fiber and yield related traits

S. No.	**Genotypes**	**S. No.**	**Genotypes**
1	Bahar-7	16	CIM-506
2	IR-10	17	CIM-446
3	SLH-19	18	Suncrop-5
4	NIAB-135	19	CIM-534
5	CKC-25	20	CIM-343
6	CIM-599	21	BZU-20
7	Cyto-510	22	CKC-9
8	SS-32	23	CKC-10
9	CEMB-100	24	CKC-28
10	FH.901	25	CKC-24
11	MNH-1050	26	CKC-26
12	Suncrop-11	27	CIM-473
13	GH-Hamaliya	28	FH-2017
14	CKC-12	29	CIM-613
15	SA-63/2	30	VH-402

SOV	**DF**	**CLCuD%**	**PH**	**NB**	**BW**	**Y/P**	**GOT**	**SL**	**Mic.**	**SS**
Reps	2.0	24.0	5.5	47.4	0.1	34.5	14.8	0.2	0.5	55.02
Genotypes	29.0	311.0**	950.2**	405.4**	0.8***	2710.7**	115.5 **	18.1**	1.3**	28.2 **
Error	58.0	3.4	5.2	3.6	0.0	3.9	5.4	5.0	0.1	2.6

Table 2: Mean squares of Morphological and Fiber traits

SOV: Source of Variation; **DF**: Degrees of Freedom; **CLCuD%**: Cotton Leaf Curl Disease Incidence (%); **PH**: Plant Height; **NB**: Number of Bolls; **BW**: Boll Weight; **Y/P**: Yield per Plant; **GOT**: Ginning Outturn (%); **SL**: Staple Length; **Mic.**: Micronaire; **SS**: Staple strength *, **, ***: Significant at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

Results

Additionally, a significant variation among genotypes was also evident in physiological characteristics, including photosynthetically active radiation, leaf temperature, photosynthesis, transpiration rate, and stomatal conductance, as detailed in (Tables 2 and 3). Notably, the phenotypic variance was consistently higher than the genotypic variance across all examined traits, indicating a substantial influence of environmental factors on these traits. This underscores the significance of environmental conditions in shaping the observed variations. In the context of future breeding programs, the success of these initiatives relies heavily on understanding and harnessing the variation present within the germplasm. When the phenotypic coefficient of variation exceeds the genotypic counterpart, it suggests that the variability observed is a result of a combination of genotypic differences, inherent traits, and environmental factors (Table 4). This emphasizes the intricate interplay between genetic and environmental elements, highlighting the need for a comprehensive approach in breeding programs to account for both these influences on trait variation.

Table 3: Mean square values of physiological traits in cotton.

SOV	DF	PAR	LT	P	TR	SC
Reps	2	11292.0	1.2538	12.6	2.2547	5570
Genotypes	29	454463**	14.4243**	201.37**	10.2304**	86716**
Error	58	12050.0	0.1	6.1	0.3	1000.0

Where SOV: Source of Variation; **DF**: Degrees of Freedom; **PAR**: Photosynthetically Active Radiation; **LT**: Leaf Temperature; **P**: Photosynthesis; **TR**: Transpiration Rate; **SC**: Stomatal Conductance; **: Significant at $p < 0.01$.

High genetic coefficient of variation (GCV) was observed in traits such as Cotton Leaf Curl Virus disease (27.32), number of bolls (51.75), yield per plant (56.81), photosynthetically active radiation (35.55), photosynthesis (60.09), transpiration rate (26.25), and stomatal conductance (54.14). Conversely, span length (7.97), fiber strength (9.99), and leaf temperature (5.24) exhibited lower GCV, while other traits fell within a medium range of GCV. Additionally, substantial phenotypic coefficient of variation (PCV) was noted in Cotton Leaf Curl Virus disease (27.45), number of bolls (52.44), yield per plant (56.97), photosynthetically active radiation (36.97), photosynthesis (62.85), transpiration rate (27.54), and stomatal conductance (55.08) (see Table 4).

Table 4 **Estimates of Variability, Heritability, and Genetic Advance for Morphological, Physiological, and Fiber Traits in 30 Cotton Genotypes**

Variables	EV	GV	PV	ECV	GCV	PCV	H2b%	GA	GAM
CLCuD%	3.40	351.25	354.65	2.69	27.32	27.45	0.99	38.42	56.00
PH	5.21	314.98	320.19	1.93	15.00	15.12	0.98	36.26	30.64
NB	3.60	133.92	137.52	8.49	51.75	52.44	0.97	23.52	105.20
BW	0.04	0.26	0.29	8.97	22.97	24.65	0.87	0.97	44.07
Y/P	3.87	902.26	906.13	3.72	56.81	56.93	0.99	61.75	116.77
GOT	5.39	36.69	42.08	5.85	15.25	16.34	0.87	11.65	29.34
SL	4.98	4.39	9.37	8.49	7.97	11.64	0.47	2.95	11.24
Mike	0.15	0.38	0.53	8.32	13.20	15.61	0.72	1.07	23.01
SS	2.59	8.53	11.11	5.50	9.99	11.41	0.77	5.27	18.03
PAR	12049.70	147470.99	159520.68	10.16	35.55	36.97	0.92	760.62	70.41
LT	0.10	4.77	4.88	0.76	5.24	5.30	0.98	4.45	10.69
P	6.12	65.08	71.20	18.43	60.09	62.85	0.91	15.89	118.34
TR	0.33	3.30	3.63	8.34	26.25	27.54	0.91	3.57	51.54

SC	1000.39	28571.93	29572.32	10.13	54.14	55.08	0.97	342.27	109.63

Where EV = Environmental Variance; GV = Genotypic Variance; PV = Phenotypic Variance; ECV = Environmental Coefficient of Variation; GCV = Genotypic Coefficient of Variation; PCV = Phenotypic Coefficient of Variation; $H^2b\%$ = Broad-Sense Heritability (%); GA = Genetic Advance; GAM = Genetic Advance as Percent of Mean.

High levels of genetic advance as mean percentage (GAM%) were detected in all examined traits, except for span length, which exhibited a moderate (GAM%). This observation suggests that the expression of all traits is influenced by additive genetic factors. However, in the case of span length, non-additive gene action was identified. These results relate with finding of Kumar et al. (2019) and Rasheed et al. (2009).

According to Johnson et al. (1955), the estimation of heritability, along with a high genetic advance, plays a crucial role in the selection process for specific traits. Traits such as plant height, numbers of bolls, boll weight, yield per plant, photosynthesis, transpiration rate, and SC revealed high heritability coupled with a high GA percentage. This suggests that these traits can be improved relatively quickly through simple selection methods. On the other hand, traits like ginning outturn percentage, span length, micronaire, and fiber strength show high heritability coupled with low to moderate genetic advance percentages. This implies the involvement of non-additive gene action in these traits. Simple selection methods are insufficient for improving these traits; hybrid breeding methods are more suitable for exploiting the genetic mechanisms behind these characteristics.

Trait association

The seed cotton yield is a resulting product of various component characters that are not controlled by a single gene. Through correlation analysis, it has been determined that the yield per plant shows a highly significant positive relationship with photosynthesis (0.91), stomatal conductance (0.72), and transpiration rate (0.60). Additionally, the transpiration rate is positively and significantly associated with photosynthesis (0.61), while stomatal conductance also showed significant positive relationship with photosynthesis (0.79). Several components exhibit a positive association with photosynthesis, and photosynthesis itself has a positive and highly significant association with these components. The number of bolls has a positive and significant association with yield per plant (0.66), and boll weight also shows a significant and moderately positive relationship with yield per plant (0.47). Micronaire and span length exhibit a moderate negative association with plant height, although this relationship is not statistically significant. The presence of Cotton Leaf Curl Virus disease has a moderate negative while significant association with the NB (-0.47), and it displays a weak negative but non-significant relationship with both photosynthesis and yield per plant. Furthermore, leaf temperature is highly negatively correlated with stomatal conductance (-0.61) and photosynthesis (-0.50), and these relationships are highly significant. Photosynthetically active radiation demonstrates a highly significant positive relationship with leaf temperature (0.55) present result align with (Shakeel et al., 2018; Nazir et al., 2013; Chapepa et al., 2020).

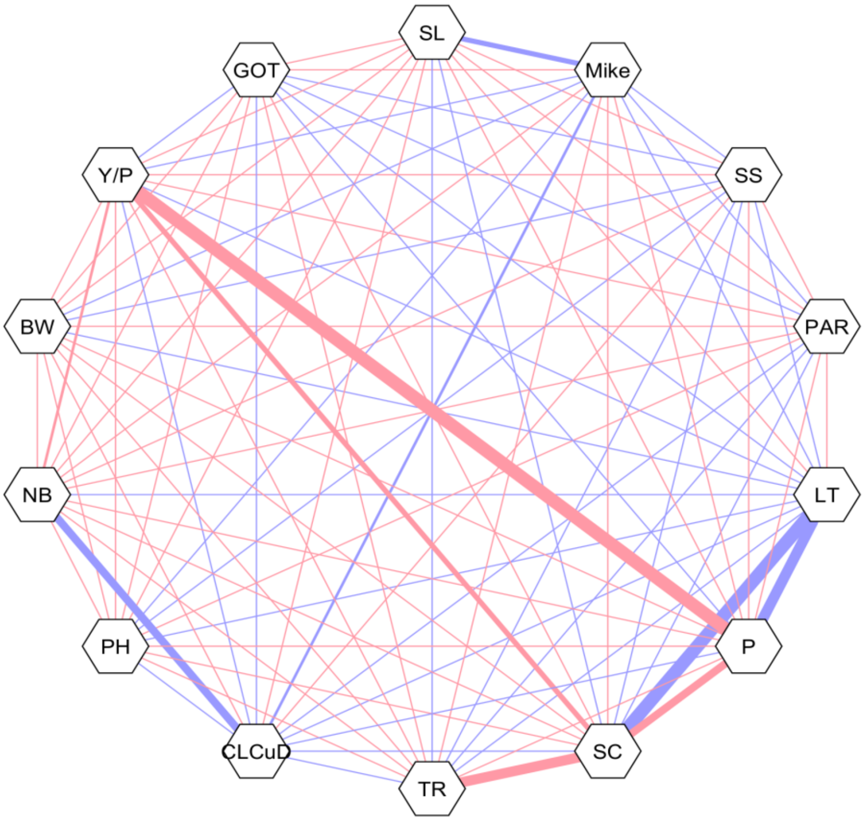

Fig 1: Y/P (yield per plant), BW (boll weight), NB (numbers of bolls), PH (plant height cm), CLCuD% (cotton leaf curl virus disease percentage), TR (transpiration rate), SC (stomatal conductance), P (photosynthesis), LT (leaf temperature), PAR (photosynthetically active radiation), SS (staple strength), Mike (micronaire), SL (span length). The positive association between trait showed in pink color while negative association represent in purple color while the magnitude of association depends on thickness of lines between components.

Multiple Linear regression

The linear regression was performed to check the effect of yield related component on the final product i.e., seed cotton yield per plant in this context the following trait has positive and highly significant ($p < 0.001$) impact on yield per plant which is out dependent variables namely plant height the coefficient of determination is (r^2=0.32) revealed 32% the total variation in yield per plant is due to its relationship with number of plant height. While slope is 0.99, which indicates a unit increase in plant height can increase the yield per plant of 0.99g, Doggett (1988) also reported height influence number of nodes and sympodial branches which equates with number of leaves produced resultant increase an increase in photosynthesis. For instance, photosynthesis make a perfect regression line with yield per plant but statistically not significant while the coefficient of determination found is (r^2 = 0.81) which explain the variation is 81% with yield per plant due to its association, and the slope is found (b = 3.33) and aunit increase in photosynthesis can increase the 3.33 g in yield per plant. The transpiration rate which has statistically significant impact on yield per plant the coefficient of determination is (r^2=0.33) which explain 33% of the total variation in yield per plant due to its relationship with transpiration rate the regression coefficient (b=9.7) which showed a unit increase in transpiration rate can increase a 9.7 g of yield per plant. Similarly, boll weight (g) had coefficient of determination (r^2 = 0.19), variation of 19% is due to its relationship with yield per plant and regression coefficient (b=27.2) which explain a single unit of boll weight can increase 27.2 g of yield per plant these finding report by (Worley et al., 1974: Salahuddin et al., 2010;; Baloch et al., 2014) the result might be different a bit due to genetic difference of cotton genotypes. Furthermore, Cotton leaf curl virus disease percentage showed coefficient of determination ($r^2 = 0.046$) which explain 4.6% variation of CLCuD% is due to its relation with yield per plant which is quite a low and the regression coefficient is (b= -0.45) which depict increase in a percentage of disease can decrease the yield per plant by -0.45 g but statistically non-significant its because the disease directly effect on yield per plant is very on the other hand it has no direct effect on yield it cause effect on other trait which contributed to yield which ultimately decrease the yield. The presence of outlier in analysis which indicates not the single independent variable have effect on depended on variable some other factor may influence.

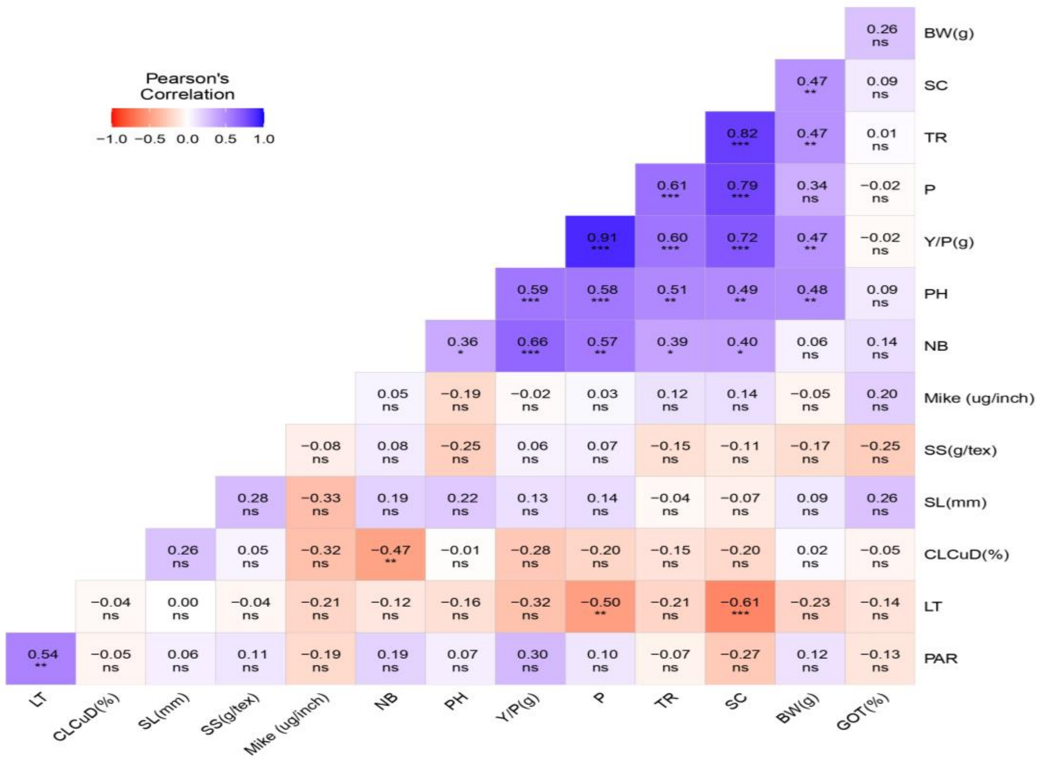

Fig 2. Pearson correlation of different morphological, physiological and traits.

Principal component analysis

Table 5: Eigenvalues, proportion of variance, and cumulative variance explained by principal components for morphological, physiological, and fiber traits in cotton genotypes

	PC 1	**PC 2**	**PC 3**	**PC 4**	**PC 5**	**PC 6**	**PC 7**
Standard Deviation	2.151	1.414	1.295	1.192	1.121	0.894	0.754
Proportion of Variance	0.331	0.143	0.119	0.102	0.089	0.057	0.041
Cumulative Proportion	0.331	0.474	0.593	0.695	0.785	0.842	0.882
	PC 8	**PC 9**	**PC 10**	**PC 11**	**PC 12**	**PC 13**	**PC 14**
Standard Deviation	0.733	0.644	0.534	0.491	0.318	0.212	0.149
Proportion of Variance	0.038	0.029	0.0203	0.017	0.007	0.003	0.002
Cumulative Proportion	0.921	0.950	0.971	0.987	0.995	0.998	1

Table 6: Factor loadings of morphological, physiological, and fiber traits for the first five PC in cotton genotypes.

	PC1	**PC2**	**PC3**	**PC4**	**PC5**
CLCuD (%)	-0.130	-0.132	**-0.574**	-0.088	-0.228
PH	**0.324**	-0.175	-0.199	0.228	-0.076
NB	0.293	-0.168	**0.321**	-0.084	**0.344**
BW(g)	0.264	-0.039	-0.274	**0.330**	-0.057
Y/P(g)	**0.419**	-0.190	0.135	-0.053	-0.035
GOT (%)	0.064	0.153	-0.229	**0.300**	0.694
SL (mm)	0.051	-0.378	-0.360	-0.200	**0.464**
Mike (ug/inch)	0.037	**0.455**	0.302	0.024	0.219
SS(g/tex)	-0.042	-0.245	0.093	-0.644	0.080
PAR	0.001	-0.518	0.291	0.263	-0.045
LT	-0.229	-0.362	0.246	**0.391**	-0.034
P	**0.420**	-0.080	0.051	-0.186	-0.049
TR	**0.367**	0.071	-0.001	0.110	-0.206
SC	**0.417**	0.206	-0.065	-0.086	-0.145

Where CLCuD (%): Cotton Leaf Curl Disease Incidence (%); **PH**: Plant Height; **NB**: Number of Branches; **BW (g)**: Boll Weight (g); **Y/P (g)**: Yield per Plant (g); **GOT (%)**: Ginning Outturn (%); **SL (mm)**: Staple Length (mm); **Mike (µg/inch)**: Micronaire (µg/inch); **SS (g/tex)**: Staple strength (g/tex); **PAR**: Photosynthetically Active Radiation; **LT**: Leaf Temperature; **P**: Photosynthesis; **TR**: Transpiration Rate; **SC**: Stomatal Conductance.

The PC's whom eigen value greater than 1 can effectively represent themselves. So, we select the PC showed eigen value greater than 1 out of forty PC's five PCs showed >1 eigenvalue. The five PCs shared a cumulative percentage of variation of 78.5%. The PC I had variation of (33.1%) and eigen value 4.6, PC II had variance of (14.3%) eigenvalue 1.9, PC III had variation of (12%) eigen value of 1.6, PC IV had variation of (1.4) lastly the PC V had variation of (9%) and eigen value 1.2 (see Table 5 & Fig 5).

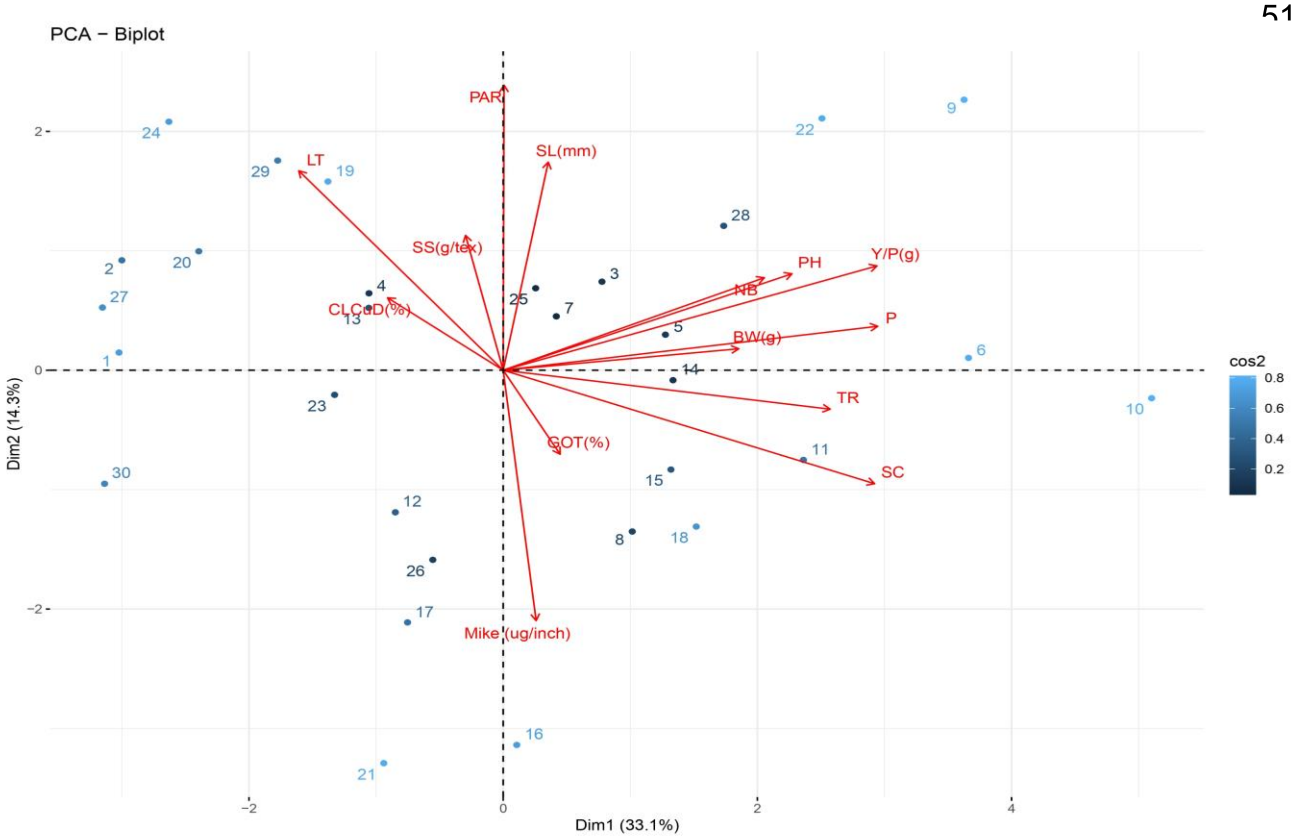

Fig 3: PCA Biplot, Y/P yield per plant (g), PH (Plant Height), BW(g), NB (Number of bolls), TR (transpiration rate), SC (stomatal conductance), GOT% (Ginning out turn %), SS (Staple strength), SL (Staple length), LT (leaf temperature), CLCuD% (cotton leaf curl virus disease %).

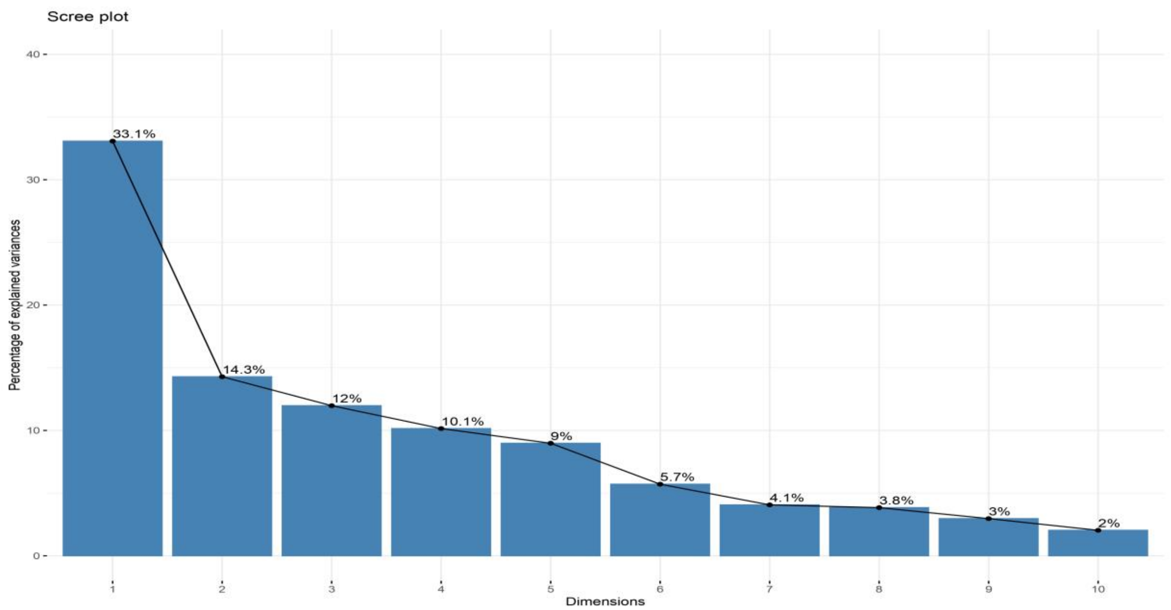

Fig 4: Scree plot showing percentage of explained variance by PCs.

The character including plant height, yield per plant (g), photosynthesis, transpiration rate and stomatal conductance has displayed positive relation of factor loading on PC-I, while CLCuD, fiber strength and leaf temperature had significantly negative factor loading (Table 6). The PC-II was described by micronaire with positive factor loading meanwhile other traits found negative factor loading obtained in that PC. PC-III was compiled with NB with positive factor loading while cotton leaf curl virus got high negative factor loading in that PC. The PC-IV was explained by boll weight (g), ginning out turn % and leaf temperature with positive factor loading meanwhile fiber strength had negative factor loading over this PC. Lastly the PC-V was explained through variance of NB and span length with positive factor loading.

Scatter Plot

Scatter plot depicted that genotypes which form grouped together have less PCA score and low yielder while examined against fourteen characters. The following genotypes showed high PCA scores 1 (Bahar-7), 6 (CIM-599), 9 (CEMB-100), 10 (FH-901), 16 (CIM-506), 18(Suncrop-5), 19 (CIM-534), 21(BZU-20), 22(CKC-9), 24(CKC-28). These genotypes are diverse and recommended for further breeding programme concerning fourteen characters similar approach made by Nadeem et al., 2022 and Shakeel et al., 2015 in selection of genotypes (Fig 4).

PCA, Biplot

Principal component Bi-plot presented the variables in super imposed and form a plot as factor. The relative length of each variables showed its proportion of variation. Yield per plant (g), photosynthesis, transpiration rate, stomatal conductance, micronaire, leaf temperature and photosynthetically active radiation showed more variation while ginning out turn and cotton leaf curl virus disease % showed less variation as their vector were smaller as compared to other (Fig 3).

Discussion

This study evaluated 30 diverse Gossypium hirsutum genotypes to investigate genetic variability, trait associations and heritability. The comprehensive analysis of morphological, physiological, and fiber quality traits under field conditions provides critical insights into the genetic architecture of important agronomic characteristics and highlights key traits influencing cotton yield performance.The significant genotypic differences ($p < 0.001$) observed across all traits suggest a substantial reservoir of genetic variability among the evaluated genotypes. Traits such as NB, SCY per plant, net photosynthesis, and stomatal conductance exhibited high genotypic (GCV) and coefficients of variation on phenotypic basis (PCV), indicating a strong genomic basis intended for these traits (Johnson et al., 1955). These results are same as mentioned in earlier reports by Kumar et al. (2019) and Rauf et al. (2023).

High broad-sense heritability estimates (>80%) for most traits, coupled with high genetic advance as a percent of the mean (GAM). This indicates that direct phenotypic selection might be helpful for the selection based on these traits (Johnson et al., 1955; Majeed et al., 2024). Notably, traits such as PH, NB, photosynthesis, transpiration rate, and stomatal conductance showed both high heritability and high GAM, making them ideal targets for selection-based breeding. However, traits like span length, micronaire, and fiber strength displayed high heritability but only low genetic advance, indicating the involvement of non-additive gene action. For these traits, hybrid breeding or recurrent selection strategies may be more appropriate (Khan et al., 2023; Rasheed et al., 2009). Correlation analysis revealed that yield per plant was significantly and positively correlated with photosynthesis ($r = 0.91$), stomatal conductance ($r = 0.72$), and transpiration rate ($r = 0.60$). These findings suggest that physiological efficiency, particularly carbon assimilation and water regulation, directly impacts biomass accumulation and reproductive success. This supports the answers of Shakeel et al. (2018) and also Chapepa et al. (2020), who emphasized the physiological basis of yield variation in cotton under stress conditions. The NB also exhibited strong positive correlation with yield ($r = 0.66$), reaffirming its role as a primary yield component (Salahuddin et al., 2010). Boll weight contributed moderately ($r = 0.47$), indicating its partial influence on total yield. The negative association of leaf temperature with photosynthesis and stomatal conductance ($r = -0.50$ and -0.61, respectively) suggests that thermal stress impairs physiological function, which may reduce yield an increasingly relevant concern under climate change scenarios (Ali et al., 2023). Regression analysis reinforced the critical role of physiological and morphological traits in determining yield. Among the independent variables, photosynthesis accounted for 81% of the variation in seed cotton yield ($R^2 = 0.81$), confirming it as the single most influential factor in yield prediction. Similarly, plant height ($R^2 = 0.32$), transpiration rate ($R^2 = 0.33$), and boll weight ($R^2 = 0.19$) were also found to significantly affect yield per plant. Interestingly, Cotton Leaf Curl Virus (CLCuV) had a negative, though statistically non-significant, impact on yield (b = -0.45), with a very low coefficient of determination ($R^2 = 0.046$). This implies that while CLCuV indirectly affects yield by influencing traits such as boll number and photosynthesis (Nazir et al., 2013), its direct effect on yield is limited, possibly due to varietal resistance in the studied genotypes.

These results align with previous findings that emphasize the integrative role of plant physiological capacity and morphology in driving yield performance (Baloch et al., 2014; Doggett, 1988). The PCA revealed that five principal components accounted for 78.5% of the total variation among genotypes, with PC1 alone contributing 33.1%. The high factor loadings of yield per plant, photosynthesis, stomatal conductance, and transpiration rate on PC1 indicate these are the most informative traits for genotype discrimination and selection (Khan et al., 2023; Nadeem et al., 2022). Conversely, traits like fiber strength and leaf temperature had negative loadings on PC1, suggesting an inverse relationship with productivity under field conditions.

Genotypes such as Bahar-7, CIM-599, FH-901, and CKC-28 were identified as genetically diverse and high-performing across key traits. These findings support their potential use as parental lines in breeding programs aimed at enhancing yield and fiber quality under stress-prone environments (Farooq et al., 2023; Shakeel et al., 2015).

Conclusion

The study underscores the value of integrating morphological, physiological, and fiber quality parameters in selecting superior cotton genotypes. High heritability and genetic advance in key traits suggest that additive gene action governs many yield and physiological parameters, making them amenable to improvement through selection. Physiological traits such as photosynthesis, stomatal conductance, and transpiration rate emerged as major contributors to yield, emphasizing their relevance in breeding programs, especially under environmental stress.

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