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SEQUENCED BASED ANALYSIS OF *GhqOil3* GENE IN INDIGENOUS UPLAND COTTON GERMPLASM FOR OIL CONTENT

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ABSTRACT

Cotton (Gossypium hirsutum L.) has long held economic significance in agriculture and the textile industry, earning the moniker "White gold" due to its vital role as a cash crop. Cottonseed oil extracted from it, has been a major contributor in farmer income as well as the GDP of Pakistan. The GhqOil3 gene has been identified in regulating the seed oil content of cotton. Previous studies had primarily focused on commercially available cotton varieties, without understanding the genetic diversity among native upland cotton cultivars. Thus, this research focused to fill this gap by developing a SNP-based marker for the GhqOil3 gene in upland cotton. Forty cotton lines were sown in fields using a Randomized Complete Block Design (RCBD) with three replications for morpho-physiological characterization. Genome sequencing, alignment, SNP-based primer designing, and dCAPs marker development were employed to unravel the genetic makeup of various cotton varieties. The research involved comparing their genetic sequences to a reference genome to identify genetic changes, particularly single nucleotide polymorphisms (SNPs) within the GhqOil3 gene. The research elaborated the genetic basis of high oil content in indigenous upland cotton by identifying specific genotypes associated with oil-related traits such as boll weight and seed oil content. The research aimed to facilitate the identification of cotton cultivars with high oil content for utilization in future cotton breeding programs

INTRODUCTION

Upland cotton (*Gossypium hirsutum* L.) is one of the most significant crops globally, and it is a significant natural fiber in many countries, including Pakistan. It belongs to the mallow family, Malvaceae, with a chromosome number of 2n=4x=52 and is tetraploid. Cotton is an essential cash crop for the country, providing livelihoods for millions of farmers and contributing significantly to the economy.

The American Cotton (*G. hirsutum* L.) is well-known for its yield and long staple (Rauf *et al.*, 2019). Cotton has been considered a good crop because it can be grown in tropical and subtropical climates (Yan *et al.*, 2021). Cotton is sometimes referred to as white gold due to its commercially important strong white fiber (Iqbal *et al.*, 2013). Cotton was grown on over 31.98 million hectares globally in 2023-2024. With the massive growth of population globally, cotton consumption is rising. Cotton has played a major economic role by contributing 1.34% percent to the value in agriculture GDP and 0.32 percent to the overall GDP (Pakistan economic Survey 2023-24).

The cotton industry faces numerous challenges that threaten its sustainability. Climate change is one of the most pressing issues, causing unpredictable weather patterns that affect crop yields (Ahmad *et al.*, 2023). Additionally, biotic stresses, such as pest infestations and diseases, pose significant threats to cotton crops. Economic pressures, including fluctuating global market prices and competition with synthetic fibers, further complicate the situation. Addressing these challenges requires a multifaceted approach that includes technological innovations, policy support, and sustainable farming practices. During 2018–2019, a decrease of 17.5% in cotton was seen with an overall production of 9,861 million bales versus 11,946 million bales (Razzaq *et al.*, 2021). This decrease in cotton production was due to lack of knowledge in farmers and also by the shrinkage in cultivation area from 2700 thousand to 2373 thousand hectares (Sohail *et al.*, 2011).

At present, Pakistan is the 5th largest producer of cotton and 4th largest consumer /producer of cotton yarn in the world. About 1.3 million out of 5 million farmers cultivate cotton on an area of 6.0 million acres, covering 15% of cultivated area in the country. Cotton has 51% share in total foreign exchange earnings of the country. The remarkable growth in cotton production facilitated the emergence of a large and vibrant textile industry (Radhakrishnan, 2017) with over 1000 ginning factories, 400 textile mills, 7 million spindles, 27000 looms in the mill sector, over 25000 looms in the non-mill sector, 700 knitwear units, 4000 garment units, nearly1000 ginners and 5000 oil expellers making cotton industry the most important sector of the economy of Pakistan (Ali *et al.*, 2022).

After soybeans, cotton *(G. Hirsutum* L) is the second-most important oilseed crop (Wu *et al.*, 2022) and a significant source of fiber (Liu *et al.*, 2012). Similar to output, cotton fiber quality is extremely important and affects producers, ginners, and the textile industry at the same time (Meredith Jr, 2000). When compared to the average productivity of other cotton-growing nations, Pakistan's cotton yield is low. This is because there aren't enough cultivars that can withstand biotic and abiotic challenges such pest attacks, high temperatures, diseases caused by the cotton leaf curl virus (CLCV), and inadequate production techniques. Some methods to avoid this issue include genetically altering elite cultivars, applying herbicides, and increasing inputs. In future, sustainable cotton cultivars with high yields, excellent seed cotton quality, and resilience to biotic and abiotic stresses will be developed.

Pakistan’s economy is primarily reliant on cotton and its byproducts. Ten percent of the country’s GDP and more than half of its foreign exchange profits are derived from cotton and its products. Compared to 2018 (7600) and 2017 (8200) Pakistan’s cotton production in 2019 was 6600 million bales (480 lb. bales) (Khan *et al.*, 2022). Despite the best input conditions efforts to develop new cotton varieties with the highest yield potential are typically unsuccessful due to a lack of genetic diversity. Because it hinders the development of breeding practices for cotton breeding and biotechnology cotton breeders generally steer clear of using undomesticated germplasm genetic tools.

Material and Methods

The present study was conducted at Cotton Research Institute, Multan. 30 cotton genotypes were sown in randomize complete block design (RCBD) in triplicate manner. The data was recorded for morphological traits and physiological traits on randomily selected five plant from each genotype in each replication and tagged later on.

The Morphological traits

Following agronomical traits were measured in field i.e., plant height, Boll weight, Yield per plant, ginning out turn (GOT%),

1. Fiber Traits

The fiber traits measured in HVI-900, 2.5% Span length, Micronaire, Fiber strength,

2. Physiological Traits

The physiological data were recorded by Infra-red gas analyzer (IRGA), Model CI-340 by CID-Bio sciences in each replication. The following data IRGA measured, photosynthetically active radiation (PAR), Leaf temperature (LT), Net photosynthesis (Pn) and Transpiration rate (TR).

Statiscal 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. Heritability (Broad Sense) was calculated by divide genotypic variance over phenotypic variance. 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).

1. The genotypic and phenotypic coefficient of variance was calculated using Burton's methods of calculation (1952) and classified based on the criteria outlined by Sivasubramanian and Madhavamenon (1973). According to this classification, values were categorized as low (<10%), medium (10-25%), and high (>25%).
2. Broad heritability estimates were classified following the guidelines set by Robinson (1965). Categories included high (>30%), moderate (10-30%), and low (<10%).
3. 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.

Result

Table 2: Mean squares of Morphological and Fiber traits

The analysis of variance conducted for morphological and fiber traits revealed significant differences ($p < 0.001$) among the various genotypes. These differences were observed in traits such as plant height, number of bolls, boll weight, yield per plant, ginning outturn, span length, micronaire, and fiber strength. 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.

Moreover, the phenotypic coefficient of variation differed slightly from the genotypic coefficient of variation in terms of their values. 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 of Physiological traits in cotton.

	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

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),

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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

photosynthesis (62.85), transpiration rate (27.54), and stomatal conductance (55.08) (see Table 4).

Table 4

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 findings are consistent with previous studies by 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 stomatal conductance exhibit high heritability coupled with a high genetic advance percentage, indicating the presence of additive genes in their expression. 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 shows a positive and significant 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 but significant association with the number of bolls (-0.47), and it displays a weak negative but non-significant relationship with both photosynthesis and yield per plant. Furthermore,

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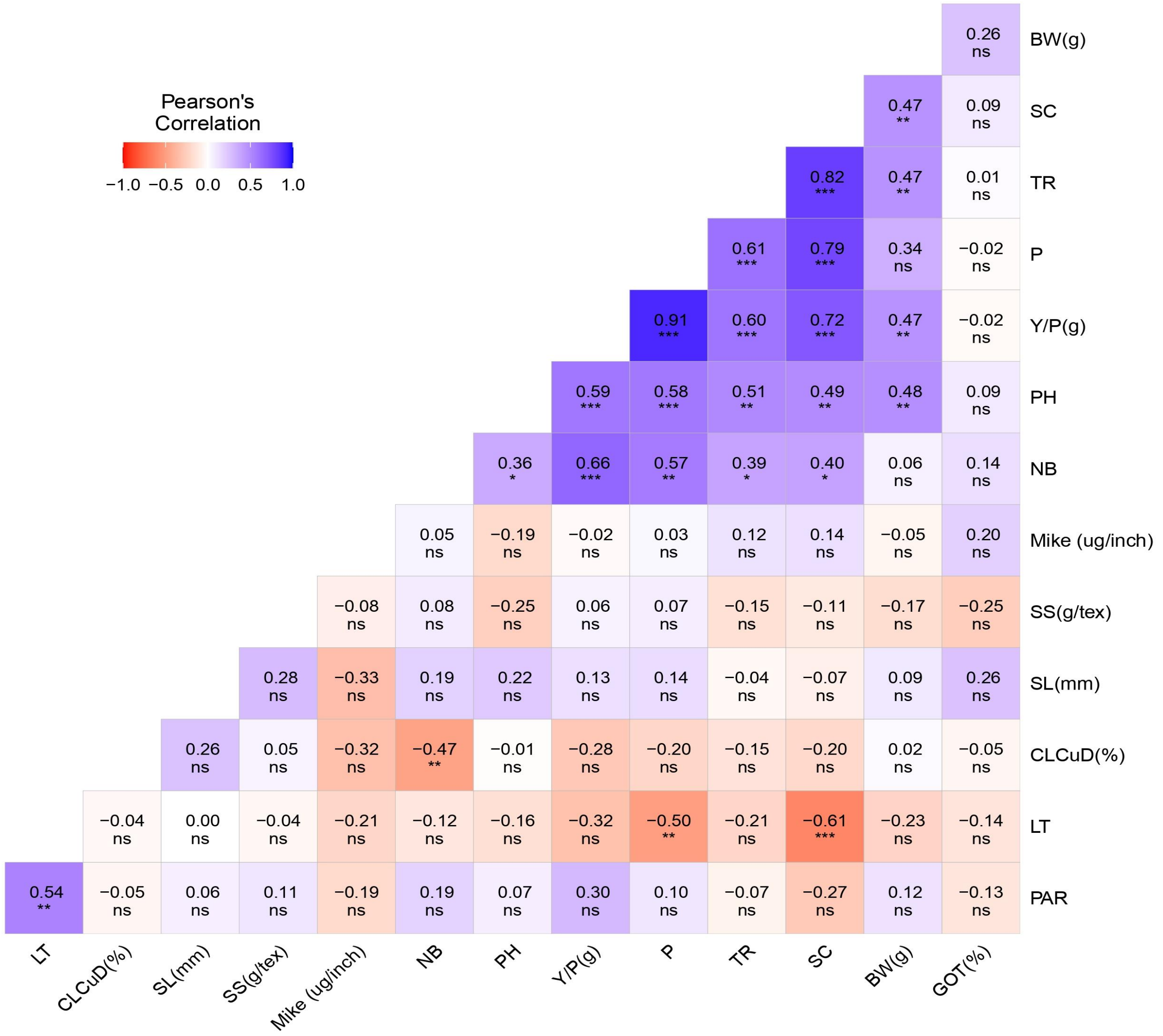
leaf temperature is highly negatively correlated with stomatal conductance (-0.61) and photosynthesis (-0.50), and these relationships are highly significant. Photosynthetically active radiation demonstrates a highly significant positive relationship with leaf temperature (0.55) present result align with (Shakeel et al., 2018; Nazir et al., 2013; Chapepa et al., 2020).

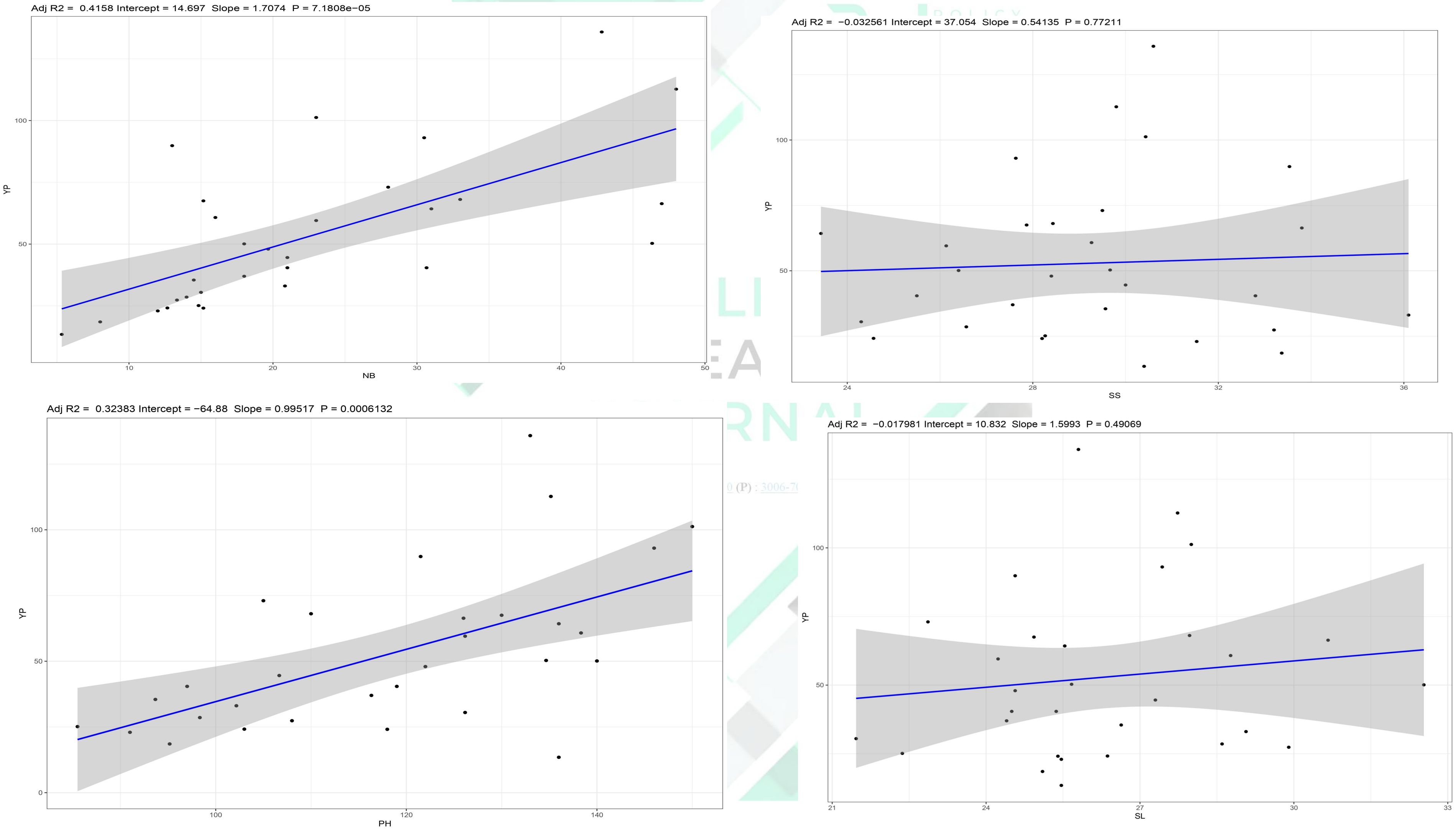
Multiple Linear regression

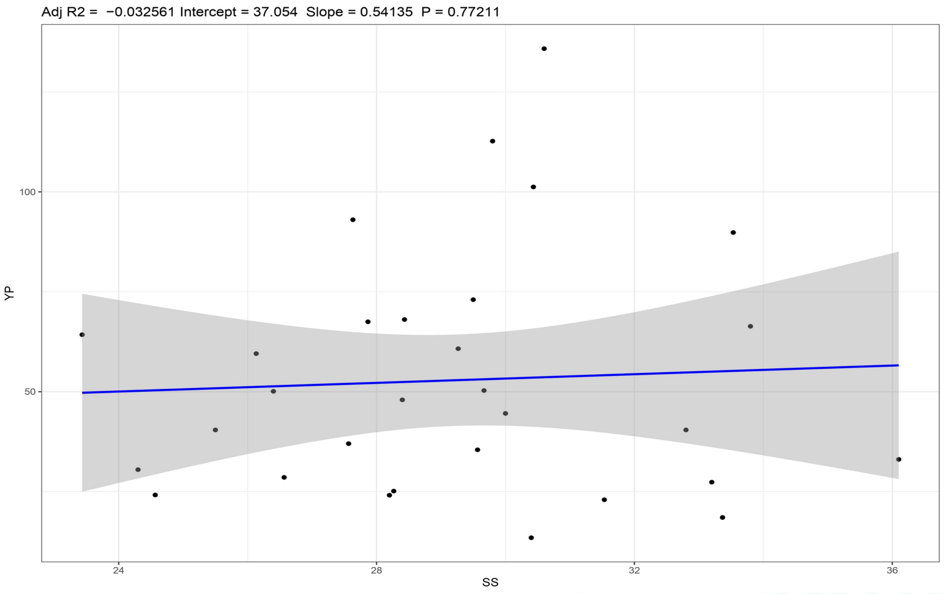
The linear regression was performed to check the effect of yield related component on the final product i.e., seed cotton yield per plant in this context the following trait has positive and highly significant ($p < 0.001$) impact on yield per plant which is out dependent variables namely plant height 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 (Salahuddin et al., 2010; Worley et al., 1974; 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.

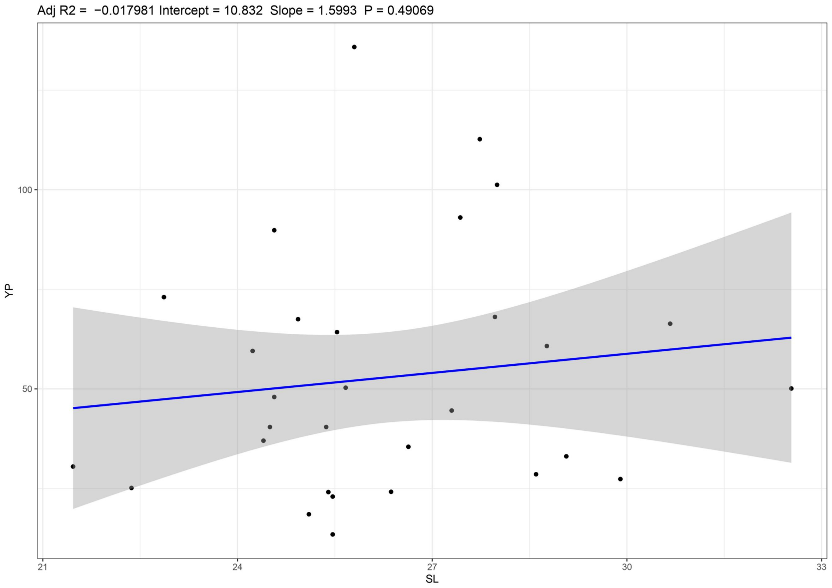


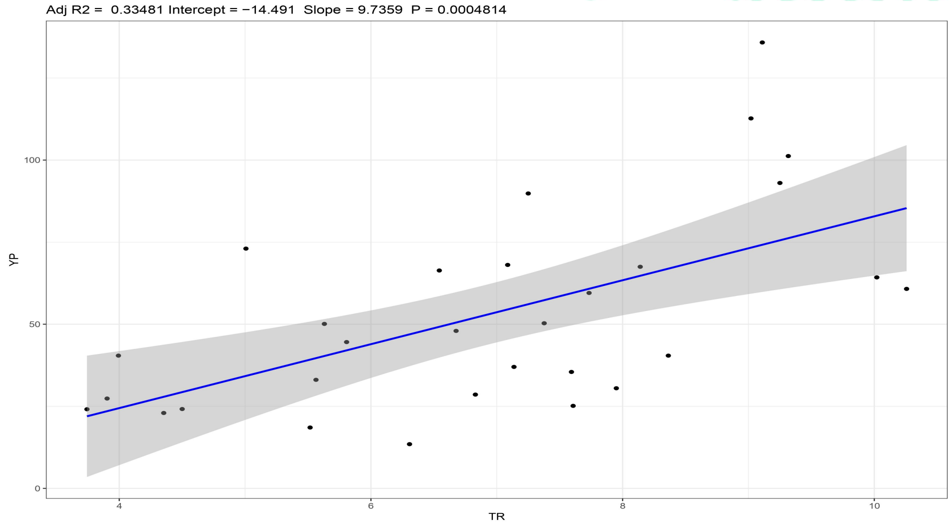
	LT	CLCuD(%)	SL(mm)	SS(g/tex)	Mike (ug/inch)	NB	PH	Y/P(g)	P	TR	SC	BW(g)	GOT(%)
BW(g)													0.26 ns
SC												0.47 **	0.09 ns
TR											0.82 ***	0.47 **	0.01 ns
P										0.61 ***	0.79 ***	0.34 ns	−0.02 ns
Y/P(g)									0.91 ***	0.60 ***	0.72 ***	0.47 **	−0.02 ns
PH								0.59 ***	0.58 ***	0.51 **	0.49 **	0.48 **	0.09 ns
NB							0.36 *	0.66 ***	0.57 **	0.39 *	0.40 *	0.06 ns	0.14 ns
Mike (ug/inch)						0.05 ns	−0.19 ns	−0.02 ns	0.03 ns	0.12 ns	0.14 ns	−0.05 ns	0.20 ns
SS(g/tex)					−0.08 ns	0.08 ns	−0.25 ns	0.06 ns	0.07 ns	−0.15 ns	−0.11 ns	−0.17 ns	−0.25 ns
SL(mm)				0.28 ns	−0.33 ns	0.19 ns	0.22 ns	0.13 ns	0.14 ns	−0.04 ns	−0.07 ns	0.09 ns	0.26 ns
CLCuD(%)			0.26 ns	0.05 ns	−0.32 ns	−0.47 **	−0.01 ns	−0.28 ns	−0.20 ns	−0.15 ns	−0.20 ns	0.02 ns	−0.05 ns
LT		−0.04 ns	0.00 ns	−0.04 ns	−0.21 ns	−0.12 ns	−0.16 ns	−0.32 ns	−0.50 **	−0.21 ns	−0.61 ***	−0.23 ns	−0.14 ns
PAR	0.54 **	−0.05 ns	0.06 ns	0.11 ns	−0.19 ns	0.19 ns	0.07 ns	0.30 ns	0.10 ns	−0.07 ns	−0.27 ns	0.12 ns	−0.13 ns

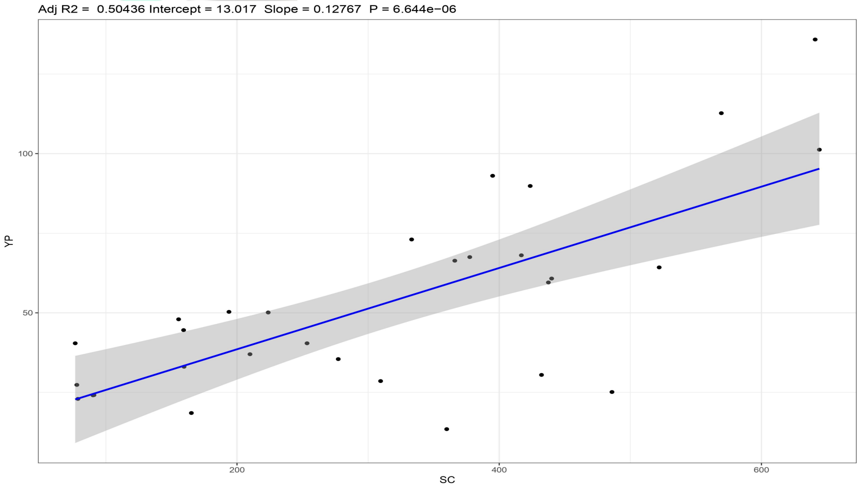
Fig 3 Linear Regression




Adj R2 = −0.032561 Intercept = 37.054 Slope = 0.54135 P = 0.77211


Adj R2 = −0.017981 Intercept = 10.832 Slope = 1.5993 P = 0.49069


Adj R2 = 0.33481 Intercept = −14.491 Slope = 9.7359 P = 0.0004814


Adj R2 = 0.50436 Intercept = 13.017 Slope = 0.12767 P = 6.644e−06

Principal component analysis

Table 5

	PC1	PC2	PC3	PC4	PC5	PC6	PC7
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
	PC8	**PC9**	**PC10**	**PC11**	**PC12**	**PC13**	**PC14**
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 loading.

	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

Principal component analysis

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).

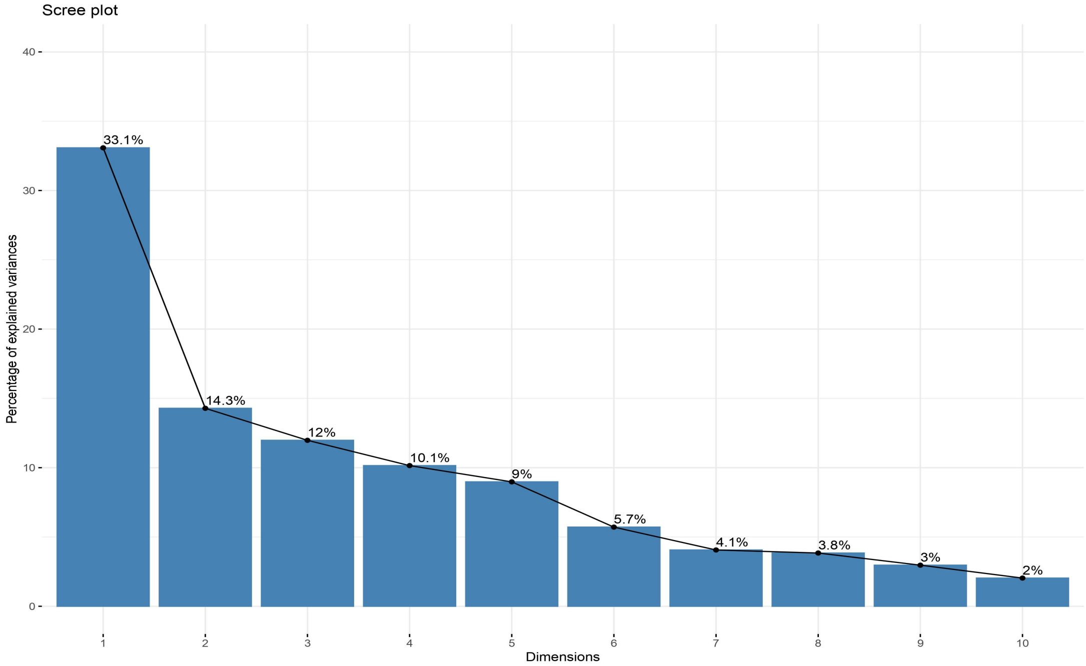


Fig 4: PCA Biplot, Y/P yield per plant (g), PH (Plant Height), NB (Number of bolls), BW(g), TR (transpiration rate), SC (stomatal conductance), GOT% (Ginning out turn %), SS (Fiber strength), SL (span length), LT (leaf temperature), CLCuD% (cotton leaf curl virus disease %)

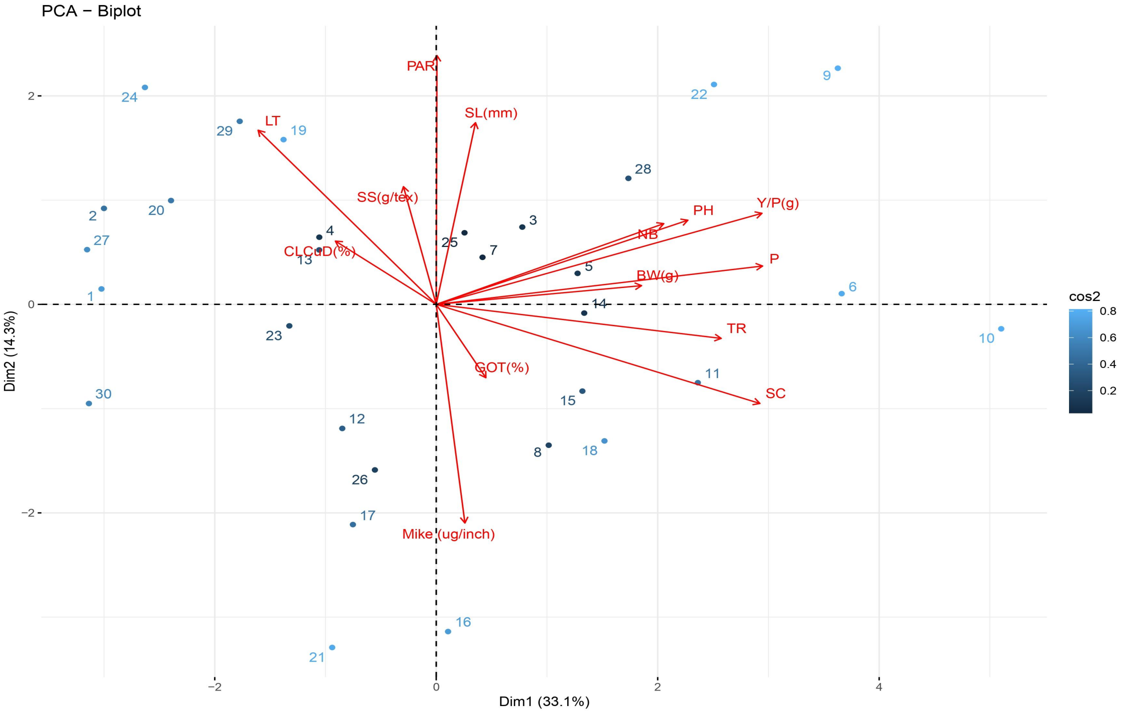


Fig 5: Scree plot showing percentage of explained variance by PCs

The character including plant height, yield per plant (g), photosynthesis, transpiration rate and stomatal conductance has displayed positive correlation of factor loading on PC-I, while Cotton leaf curl virus disease, 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 number of bolls 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 by the variance of number of bolls and span length with positive factor loading.

Scatter plot

The scatter plot depicted that the 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.

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 4)

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