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## Effect of Drought on Trichome Density and Length in Cotton (*Gossypium Hirsutum*)

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### Cover Page Footnote

We acknowledge the technical support of Dr. Wajid Nazir, CRI, Multan during this study.

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## EFFECT OF DROUGHT ON TRICHOME DENSITY AND LENGTH IN COTTON (*GOSSYPIUM HIRSUTUM*)

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### ABSTRACT

Cotton is a major cash crop and backbone of the textile industry in Pakistan which is badly affected by sucking insects. Drought is an important abiotic factor in trichome development. The objective of the study was to determine the effects of drought on trichome density and length. Trichome density was measured in two ways, one through the scaling method and the other through counting the trichome density manually. The scaling method is qualitative grading while quantitative grading includes trichome-count in a card of optimized length. Three scales were finalized to classify leaves on the basis of trichomes which were counted in a specific area (0.25cm<sup>2</sup>) on abaxial side of the leaf. In drought stress, trichomes density and length were measured and compared to that in normal conditions. Trichome density varies from 12 to 56 in 0.25cm<sup>2</sup> under drought stress. On the basis of correlation of trichome density with stomatal conductance, photosynthetic rate, PAR and transpiration ratio under drought and normal conditions, it was concluded that trichome density increased as a result of drought stress.

**Keywords:** Cotton, trichome, drought stress, gene expression, sucking insects.

### INTRODUCTION

Trichomes are thorn-like outgrowth on aerial parts of the plant, involved in plant protection against insect attack. Cotton fibers are seed trichomes, which are most abundantly used fiber in the textile industry (Larkin et al., 2003; Wilkins et al., 2000). The degree of hairiness describes the chances of attack of sucking insects. Leaves with more hairiness offer more resistance toward jassids attack and are less susceptible to damage (Sikka et al., 1966). There may be different types of leaves with respect to hairiness. Leaves with less hairy character are glabrous. Hirsute leaves are with

moderate pubescence and pilose leaves with maximum hairiness (Bourland et al., 2003).

It was observed that the average number of trichomes varies from 2 to 205 per cm<sup>2</sup>. A smooth leaf has 5 trichomes cm<sup>2</sup> (Smith, 1964). It was clear that plant leaves on the upper position exhibit more hairiness as compared to the lower and middle positions. This study depended on visual observation of trichomes and leaves with thick trichome density offer more resistance toward insect attack (Bourland et al., 2003).

It was reported that the smooth-leaf trait is helpful in showing resistant towards sucking insects (Jenkins et al., 1996). A

three-scale system was proposed later to categorize leaves on the basis of trichomes (Nawab et al., 2014). These classes were smooth, moderately hairy and hairy (Rayburn Jr and Libous, 1983). Three phenotypes are common in cotton, Glabrous, hirsute, and pilose (Peter et al., 1995).

In this article, a leaf pubescence rating system based on visual examination of the relative density, distribution, and length of leaf trichomes on the abaxial leaf surface is introduced and tried to validate through comparisons with trichome densities and consistencies into different genotypes under stress.

Molecular mechanisms and genetics involved trichome development have been revealed by many researchers. It was observed that *GLABRA1 (GL1)* and *GL3* (transcription factors) are involved in trichome development as positive regulators. *R2-R3 MYB* protein encoded by these transcription factors is a basic helix-loop-helix-related protein used in regulation of trichome development in cotton (Oppenheimer et al., 1991). Trichomes density and length is affected by abiotic stresses. Drought stress increases trichome density. In low moisture availability, plant trichomes increase in number and length to cover most of the plant surface to conserve the water. Trichomes density and length vary with the position of the leaf on the plant Top leaves have more trichome density as compared to medium leaves while medium leaves have more density of trichomes as compared to leaves on the bottom (Bourland et al., 2003). Water deficiency to the extreme limit is a single limiting factor of photosynthesis which increases trichome density to conserve moisture (Lawlor and Tezara, 2009; Medrano et al., 2002).

Cotton genotypes were screened on the basis of various parameters like low transpiration (Bréda et al., 1993) deep roots and increased tolerance to water

deficiency. Molecular markers like SNP, SSR are also important in recognition of

genotypes resistant to drought and to detect polymorphism (Khandagale et al., 2007). Elevated temperature and drought stress are inversely related in drought conditions (Ackerson, 1980) fall due to decrease of stomatal conductance. The decrease in stomatal conductance is due to stomatal closure in drought stress while high temperature causes stomatal opening (Lu et al., 1994) and hence photosynthesis and transpiration rate increased (Genty et al., 1987) but up to a certain limit, temperature above 35<sup>0</sup>C will stop the process of photosynthesis.

The study was mounted with the objective of evaluating trichome densities associated with cotton plant for insect resistance and its rating system under drought stress conditions.

## MATERIAL AND METHODS

### *Plant Materials and Growing Conditions*

Ten different genotypes from different sources were grown in the field with plant spacing 25cm and row to row spacing 75 cm. (Table 1) Drought applications was started at seedling stage which continued to flowering stage. A replicate of these genotypes was also grown in Glasshouse to confirm the effect of drought on trichomes in controlled conditions. Moisture level was measured through a moisture meter.

### *Quantitative Grading of Trichomes*

Plants leaves were collected after four drought applications( these applications were given after intervals of 5 days) and observed through a scanning electron microscope. A card of 0.25cm<sup>2</sup> was used to count trichome density in both drought and normal conditions. Three scales were optimized to categorize plant leaves on the basis of trichomes density and length.

**Table 1: List of genotypes and Name of the institute.**

Sr No.	Genotype	Name Of Institute
1	MNH-875	CRI Multan
2	MNH-872	CRI Multan
3	MNH-886	CRI Multan
4	IUB-13	Islamia University of Bahawalpur
5	CEMB-33	Punjab University Lahore
6	FH-444	CRS Faisalabad
7	MNH-1050	CRI Multan
8	MNH-1027	CRI Multan
9	CIM-602	CCRI Multan
10	FH-2017	CRS Faisalabad

**Table 2: Trichomes scaling.**

Sr No.	phenotype	Leaf description
1	Pilose	Plant leaves with maximum trichome density
2	Hirsute	Plant leaves with medium trichomes density
3	Glabrous	Plant leaves with minimum trichomes density



**Figure 1: Microscopic view of glabrous leaf. These types of leaves have minimum trichomes on midrib and susceptible towards attack of sucking insects.**



**Figure 2: Microscopic view of hirsute leaf. This type of leaf shows a medium density of trichomes.**



**Figure 3: Microscopic view of a pilose leaf. This type of leaf with maximum trichome density and most often midrib is covered by thick trichomes and such type of leaves show more resistance towards sucking insect attack.**

### **Qualitative Grading of Trichomes**

Trichomes were classified into three main categories depending on trichome density and length (Table 2).

### **Plant Physiological Parameter**

The following parameters were recorded with the help of IRGA in both drought and normal conditions.

- Stomatal conductance (c)
- Photosynthetic rate (Pn)
- Transpiration rate (e)
- PAR (Photosynthetic Active Radiations)

#### **i. Stomatal Conductance**

Stomatal conductance was measured by using IRGA (Infrared gas analyzer). Stomatal conductance varies with irrigation rate. Fully expanded leaves were used to measure stomatal conductance.

#### **ii. Transpiration Rate**

Transpiration rate was also measured with the help of IRGA. 3 fully expanded leaves were selected to measure transpiration data. Variation in transpiration rate was observed among different genotypes

#### **iii. Photosynthetic Rate**

Photosynthesis also changes with moisture availability. Drought stress also affects the photosynthetic rate. This rate can be measured with the help of IRGA.

#### **iv. Photosynthetic Active Radiation (PAR)**

PAR was also measured with the help of IRGA. Three fully expanded leaves were taken for the measurement of PAR.

## **RESULTS**

### **Trichome Density and Length**

Trichome density and length were measured in both drought and normal conditions. It was observed that trichomes density increased in drought stress (Table 3). Trichomes density and length showed variation in different genotypes and an increase in Trichome density was observed in drought stress.

Trichome density on different positions on the same plant was recorded (Table 4) and observed clear variation. We compared trichome density in drought stress to normal conditions. Trichome density and length showed variation in drought stress (Table 5).

**Table 3: ANOVA of trichome-density in drought and normal condition.**

<b>Sov</b>	<b>DF</b>	<b>SS</b>	<b>MS</b>	<b>Value</b>	<b>Pr&gt;F-Vlaue</b>
Rep	2	1134.3	567.15	4.2311	0.02193*
Env	1	470.4	470.40	3.5093	0.06873
Genotype	9	13665.7	1518.41	11.3277	2.334***
Env. Genotypes	9	166.3	18.47	0.1378	0.99822

Significant=\*, Highly significant=\*\*\*

**Table 4: Trichome count on different positions.**

Sr. No.	Genotypes	Trichomes	Normal	Drought
1	MNH-875	Top	70	75
		Medium	55	70
		Bottom	32	40
2	MNH-872	Top	26	36
		Medium	20	30
		Bottom	12	20
3	MNH-886	Top	40	53
		Medium	30	35
		Bottom	17	19
4	IUB-13	Top	55	60
		Medium	35	36
		Bottom	10	16
5	CEMB-33	Top	32	33
		Medium	30	20
		Bottom	12	14
6	FH-444	Top	24	29
		Medium	24	20
		Bottom	24	18
7	MNH-1050	Top	70	78
		Medium	53	61
		Bottom	45	55
8	MNH-1027	Top	19	23
		Medium	10	16
		Bottom	9	13
9	CIM-602	Top	38	41
		Medium	30	32
		Bottom	17	27
10	FH-2017	Top	15	18
		Medium	12	16
		Bottom	8	12

**Table 5: Trichome density in drought and normal conditions.**

Sr No.	Genotype	Trichome rating	Trichome count	
			Normal	Drought
1	MNH-875	3	53	185
2	MNH-872	2	20	86
3	MNH-886	2	87	107
4	IUB-13	2	34	112
5	CEMB-33	2	25	67
6	FH-444	1	24	67
7	MNH-1050	3	56	194
8	MNH-1027	1	13	52
9	CIM-602	2	28	100
10	FH-2017	1	12	56

Trichome density increased in drought stress conditions. It was clear from the table that trichome density was more in drought conditions than in normal.

### **Transpiration Rate**

Transpiration is affected greatly by the availability of moisture. In low availability of water, plants try to conserve moisture hence transpiration falls in drought conditions. Our results indicated that the transpiration rate was higher in normal irrigation than in drought stress (Table 6) But the result was not highly significant as there is not very much difference in transpiration rate in drought and transpiration rate in normal irrigation.

### **Stomatal Conductance**

Stomatal conductance is a parameter which is affected by application of drought stress. Stomata are closed during drought stress to conserve moisture and gases. Few genotypes showed more difference in stomatal conductance in normal and drought stress, although the variance among genotypes is not significant (Table 7). CEMB-33 and FH-444 showed increase in stomatal conductance in drought stress while other genotypes like IUB-13, CIM-602 showed decrease in stomatal conductance in drought stress.

**Table 6: ANOVA for transpiration rate.**

SoV	Df	SS	MS	F Value	Pr>f value
Rep	2	66631	33316	2.3911	0.1052
Env	1	4932	4932	0.3540	0.5554
Genotype	9	173894	19322	1.3867	0.2283
Env. Genotype	9	151609	16845	1.2090	0.3182

**Table 7: ANOVA for stomatal conductance.**

SoV	DF	SS	MS	Fvalue	Pr>Fvalue
Rep	2	5.358	2.6790	1.2017	0.3118629
Env	1	0.753	0.7526	0.3376	0.5646499
Genotype	9	92.186	10.2429	4.5945	0.0003837



**Photosynthetic Active Radiation (PAR)**

The value of PAR decreased in drought stress and it was clearly evident from the data given below. Data showed that PAR value is down in low availability of moisture. Our results showed that PAR in drought is less as compared to that in normal irrigations. Analysis of variance is provided in (Table 8).

**Pn (Net Photosynthetic Rate)**

Photosynthesis is vital for the life of plants. Photosynthesis Rate decreased in drought stress. Data indicate that the value of PAR was less in drought stress than in normal irrigation. as photosynthesis depends on CO<sub>2</sub> in and out and other factors which are involved in photosynthesis and due to reduction in H<sub>2</sub>O and CO<sub>2</sub>, photosynthesis also decreased resulting in reduced net rate fall. Analysis of variance is provided in (Table 9).

**Table 8: ANOVA for photosynthetic active radiation (PAR).**

SoV	DF	SS	MS	F-value	Pr>f -value
Rep	2	136142	68071	2.7247	0.07838
Env	1	405	4055	0.1623	0.68931
Genotype	9	1545816	171757	6.8749	8.406
Env. Genotype	9	471008	52334	2.0948	0.05464

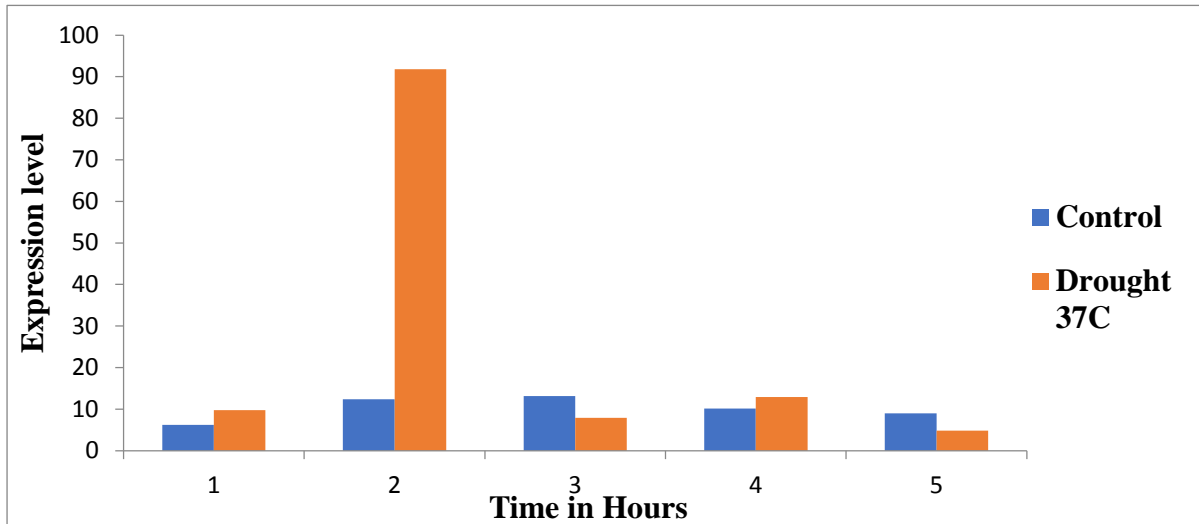
**Table 9: ANOVA for net photosynthesis rate.**

SoV	DF	SS	MS	F-value	Pr>F
Rep	2	64.77	32.387	0.6612	0.5220
Env	1	6.53	6.527	0.1333	0.7171
Genotype	9	468.50	52.056	1.0628	0.4113
Env. Genotype	9	262.06	29.118	0.5945	0.7933

**Expression of GL2 in Cotton**

Drought condition was applied on cotton genotypes and expression analysis was recorded after different intervals. *GL2* showed more expression in drought condition generally but showed maximum trend after 12 hour and expression

decreased after 24 hours but still more than normal condition. Expression of *GL2* after 24 hours was less in drought than in normal conditions (Figure 4). Expression of *GL2* showed exceptional behavior after 24 hours as it is more in normal condition than drought condition.



**Figure 4: Chart of expression analysis of *GL2* in cotton. Expression analysis of *GL2* in cotton leaves samples in drought and normal condition after 12, 24, 36, 48 and 60 hours. Time in hour (scale 1=12 hours) is on x-axis while expression of gene is on y-axis. From above it can be seen that expression of *GL2* is maximum after 12 hours.**

### *Expression Analysis in Arabidopsis*

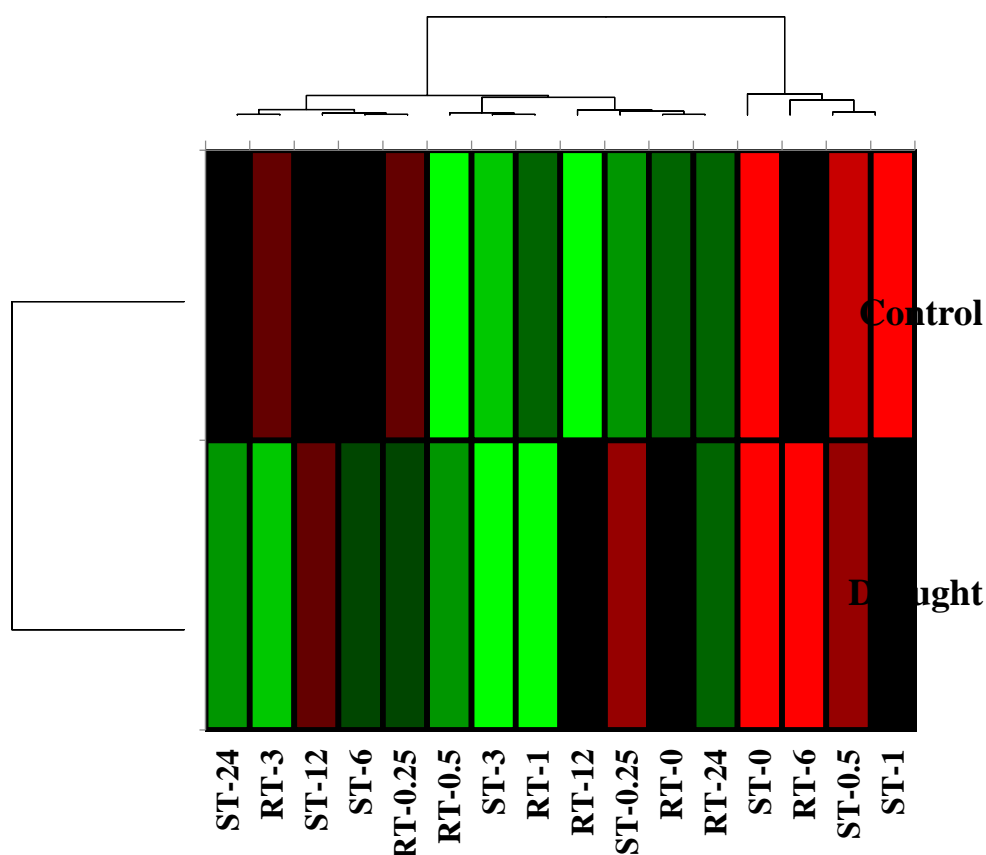
Expression analysis of *GL2* was observed in Arabidopsis in normal and drought conditions. Drought was applied after different intervals and expression of the gene was recorded. It is clear from the result in Figure 3.2 that genetic expression increases after drought application but up to a certain limit. ST-3 shows maximum expression of the gene after 3 hours of drought then the expression of *GL2* started decreasing but still more than normal condition. Again, a sudden increase in expression of *GL2* was observed after 24 hours.

Expression of *GL2* in roots was also observed in drought and normal conditions and from Figure 5 it can clearly be seen that *GL2* expression is more in drought condition as compared to normal conditions. Expression of *GL2* is maximum after one hour and then it decreased but still more than normal condition. *GL2* expression is better after 3 hours of drought as compared to 12 or 24 hours after drought application.

### *Correlation Analysis*

Strong correlation exists between net photosynthetic rate and stomatal conductance while negative correlation exists between PAR and stomatal conductance. Dark blue color indicates a strong correlation. Statistical data also denote strong relationship between stomatal conductance and transpiration rate while no correlation was found between PAR and net photosynthetic rate. Graphical representation of data indicates that photosynthesis active radiation and net photosynthetic rate have no relation at all (Figure 6).

There is a positive correlation between transpiration rate and net photosynthesis rate. Graph indicates that there is a positive correlation between these two parameters. A strong correlation exists between transpiration rate and PAR. Photosynthetic active radiation was positively correlated with transpiration rate (Figure 7).



**Figure 5:** Heat map of expression analysis of *GL2*. *Expression of GL2* in Arabidopsis in drought and normal conditions. Red color shows maximum expression while green color is for medium expression of the gene and black color denotes no expression. ST shows shoot expression while RT shows root expression.

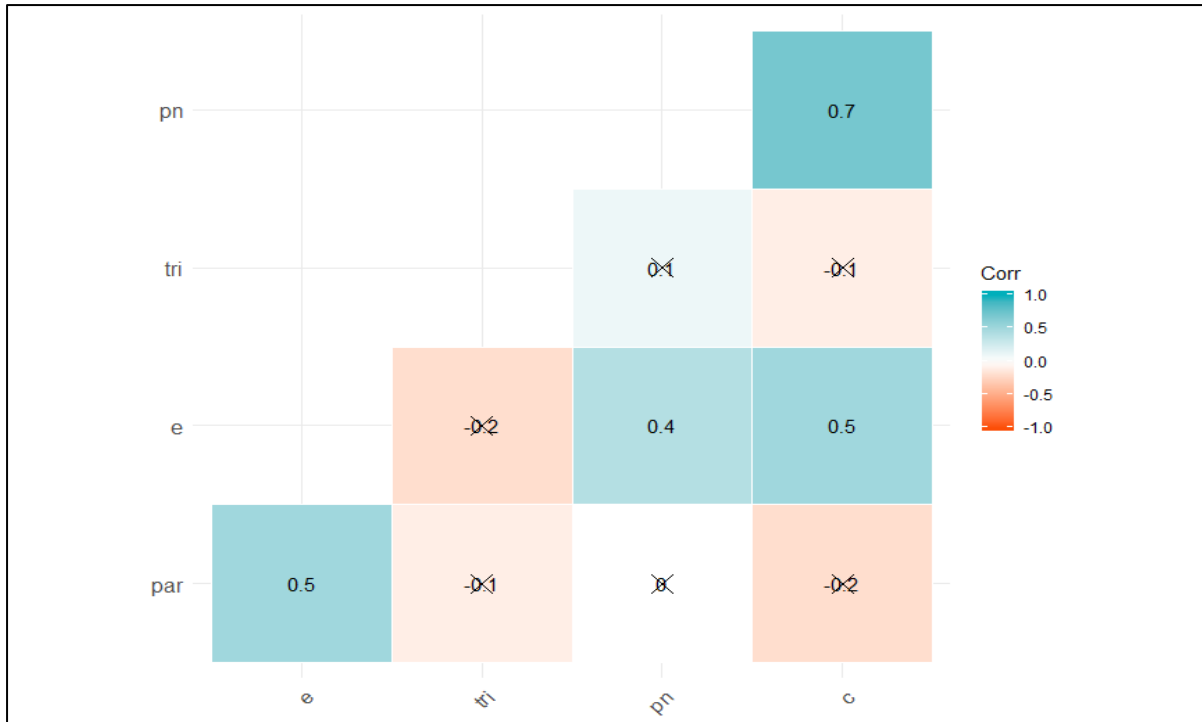
Trichomes correlation with other parameters was also checked and no significant correlation was found with other parameters. A very little variation was found in genotypes regarding trichome density and length.

### ***Biplot Analysis***

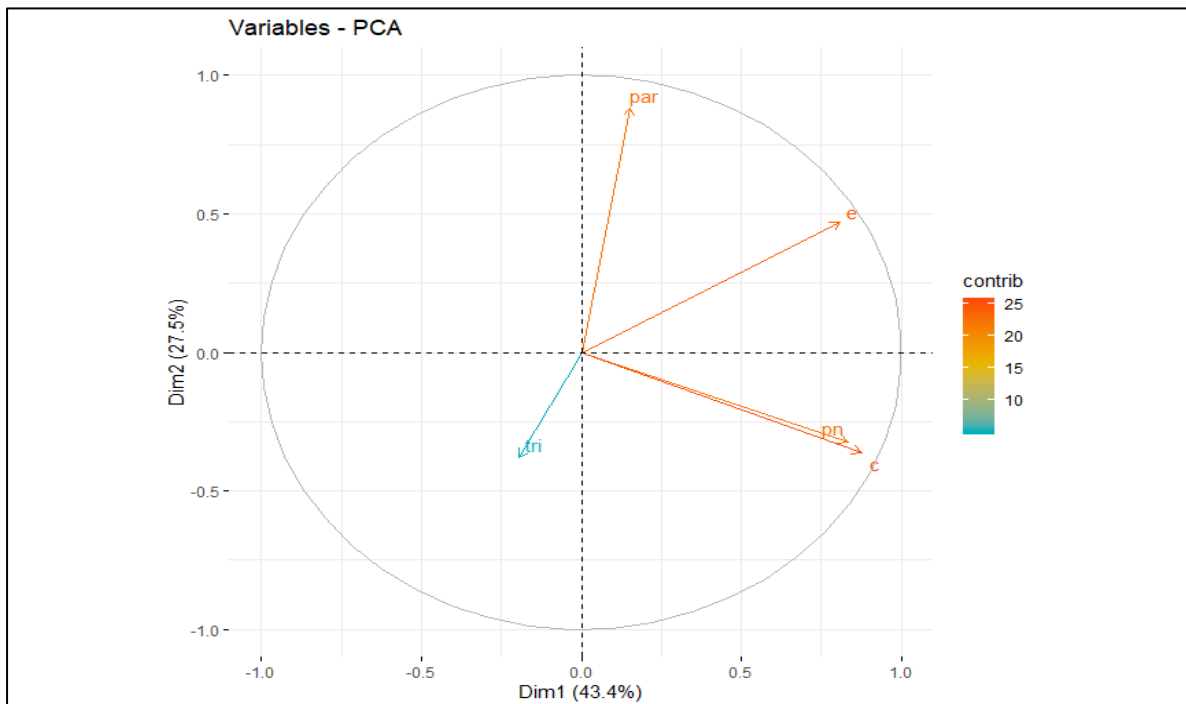
Bi plot was also done to analyze the data for all genotypes. Variation was observed in trichomes, variation among genotypes was much lower than other traits. Values of transpiration ratio and stomatal conductance were very close for most of the genotypes (Figure 8). Bi plot analysis of 5 traits showed close relation

between transpiration ratio and stomatal conductance (Figure 9).

It was obvious from the analysis that PAR was negatively related with trichomes density. As we know first ten environment shows drought so we can see that trichomes are more related to drought than any other trait. No relationship was found between PAR and transpiration ratio. Transpiration ratio was more in the normal irrigation than the drought.



**Figure 6: Correlation analyses among PAR, net photosynthetic rate, stomatal conductance, transpiration ratio and trichomes density.**



**Figure 7: Principal component analyses between transpiration ratio (e), PAR, Pn, Stomatal conductance(c) and trichomes density. It has been clear that strong relationship between Pn and c and there is almost negative relationship between PAR and trichome density**

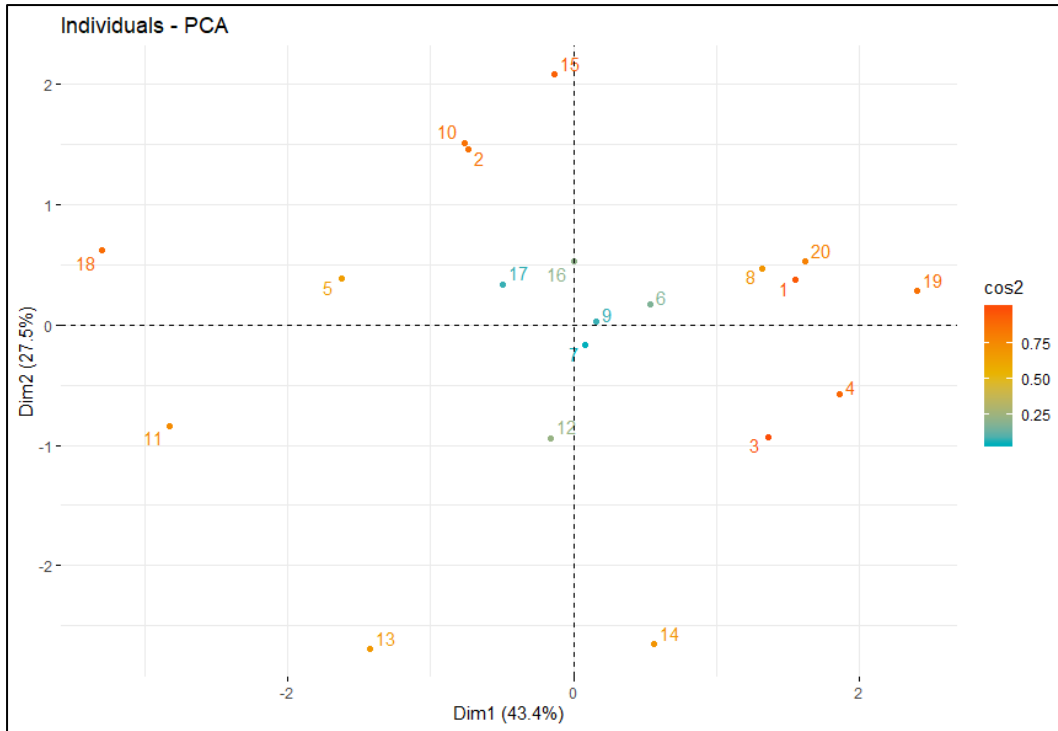


Figure 8: Traits were analyzed in two different environments individually; first 10 environments were drought while next 10 environments were normal irrigation.

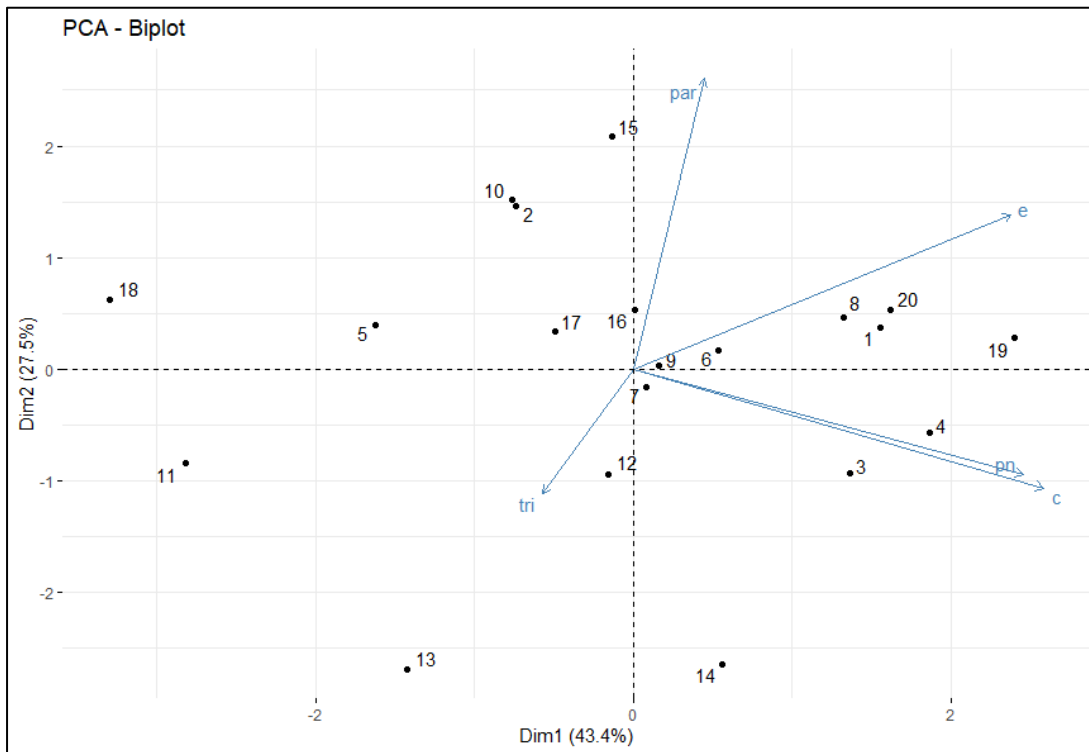


Figure 9: Biplot analysis of PAR, Pn, stomatal conductance and transpiration ratio and trichomes density in two different environments, drought and normal irrigation. First 10 environments represent drought stress while rest of the 10 environment shows normal irrigation.

## DISCUSSION

The conclusion of our research was an increase in trichome density in drought as trichome count was more in leaves with drought than those with normal irrigation. Three scales were finalized to measure trichome density and length. These scales were given on the bases of both trichome density and length. Hirsute leaves possess maximum trichome density, pilose leaves have medium trichome density while glabrous leaves have minimum or no trichomes. (Bourland et al., 2003) used 7 classes to measure trichome density and length. Cotton leaves were categorized into seven classes. For measurement of trichome a card of 0.33cm<sup>2</sup> were used and trichome vary from 2 to 50 per cm in our research a card hole of about 0.25cm<sup>2</sup> was used and trichome ranging from 10 to 70 were measured.

Measurement of parameters, like stomatal conductance, photosynthetic rate, PAR and transpiration ratio, in normal and drought stress clearly indicate the effect of moisture limitation on plant growth and survival. Those varieties which had potential to survive in drought stress survived like MNH-886 while there are some genotypes who showed increase in stomatal conductance in drought stress like CEMB-33 were very susceptible towards drought applications and very few plants survive after 4 successive drought applications.

Trichome density were more in leaves with drought stress as compared to leaves with normal irrigation. Trichome density varies from 12 to 56 in 0.25cm<sup>2</sup> (Bourland et al., 2003).

PAR value was less in drought treatment MNH-875, MNH-872, IUB-13 showed greater changes in PAR value while MNH-1050 and MNH-1027 showed less difference as compared to other genotypes. Our result about stomatal conductance was in favor of the research which showed that plant decrease their

stomatal conductance in water shortage (Saleem et al., 2015).

Effect of low moisture on photosynthesis was observed and it was clear from the results that net photosynthetic ratio was low in drought situation. Transpiration ratio is also a parameter which depends on water availability and it was concluded in our research that in low water or drought condition transpiration ratio decreased (Bréda et al., 1993).

Findings of research showed that stomatal conductance vary with genotypes in some genotypes values of stomata increase with drought application but in most of genotypes its value fall in drought stress. CEMB-33, FH-444 and MNH-875 showed increase in value of stomatal conductance while in all other genotypes stomatal conductance decrease in drought stress (Haworth et al., 2017).

Findings showed that the transpiration rate was increased in CEMB-33 and FH-444 in drought conditions as compared to normal irrigation while other genotypes marked a decrease in transpiration rate.

Expression analysis of *GL2* (universal gene for trichome development) in *Arabidopsis* indicated that drought stress increased expression of *GL2* as compared to normal conditions but up to certain limit and shoot samples showed maximum expression after 3 hours of drought treatment.

The level of expression of *GL2* was changing after different intervals but mostly *GL2* expression was more in drought samples. In cotton, *GL2* expression in drought is maximum after 1 hour of drought treatment then it started decreasing. After 24 hours expression of *GL2* showed reverse behavior as expression level of *GL2* in drought was less than expression at normal condition.

## AUTHOR CONTRIBUTIONS

All authors contributed equally.

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## CONFLICTS OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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