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Research Article

Genetic analysis of excised leaf water loss and relative water content is strongly associated with drought tolerance in TNAU cotton cultures

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Abstract
Drought has a significant negative impact on cotton cultivation and production. Drought tolerance indices [Excised leaf water potential (ELWL), Relative water content (RWC), osmotic potential, water potential] were used to evaluate drought response in three TNAU pre release cultures *viz.,* TVH002, TVH003, TVH007 and a check variety KC3. The experiment had three treatments, T1: Control [100% of pot capacity (PC)], T2: Drought at square formation (Pots @ 45% of PC for 14 days), T3: Drought at flowering stage (Pots @ 45% of PC for 14 days). The study showed flowering stage in cotton was more susceptible to drought than squaring and among the genotypes, TVH002 was tolerant to drought. Genetic analysis revealed that ELWL and RWC had higher genotypic coefficients of variation, phenotypic coefficients of variance coupled with strong heritability. Thus, these can be used as drought indices to screen genotype for drought.

Keywords: Cotton, Drought, Drought indices, Genetic analysis

INTRODUCTION

Cotton, renowned as the supreme fiber, stands as a crucial and primary economic crop of the nation (Ananda Lekshmi *et al*., 2023). India possesses approximately 42% of the global cotton cultivation area, making it the second-largest cotton producer globally, contributing to around 26% of the world's cotton production (Cotton Corporation of India, 2021). It serves as the primary source of natural fiber, contributing nearly 35% of the global fiber consumption. Cotton's substantial revenue generation has led some countries to aptly refer to it as "white gold" (Ali *et al.,* 2014). Cotton, classified as a glycophyte, exhibits relatively higher tolerance to abiotic stresses compared to other major crops. However, the growth, productivity and quality of cotton's produce, particularly its fiber, can be adversely affected by extreme environmental conditions (Parida *et al.,* 2007). Among these extreme conditions, drought stands as the most significant one, as it severely restricts both plant diversity and agricultural productivity. Presently, drought causes a reduction of more than 50% in crop yields on a global scale (Baboev *et al.,* 2017). These challenges can be alleviated by developing breeding varieties that can produce stable yield under water deficit condition.

Various traits can be employed to categorise drought sensitivity and tolerance in cotton (Jaleel *et al.,* 2009). Physiological characteristics like leaf water potential (LWP), osmotic potential (OP), osmotic adjustment (OA), relative water content (RWC), excised leaf water loss (ELWL) is closely linked to the water status of cotton plants. Monitoring these traits can serve as valuable indicators for assessing water deficit stress in cotton germplasm (Asif *et al.,* 2015). Leaf water potential (LWP) represent the plant water status (Wang *et al.,* 2016) and maintenance of a high LWP is related to dehydration avoidance mechanisms (Sanjana, 2019).Osmotic potential is a physiological factor utilized to assess the stress level in plants (Kramer and Boyer, 1995).Osmotic adjustment is a water deficit coping mechanism which results in the accumulation of compatible solutes in the cytosol reducing osmotic potential (Mafakheri *et al.,* 2010). Cotton possesses the capability to undergo osmotic adjustment, enabling it to sustain a higher leaf turgor potential (Turner, 1986). An important element that determines a plant's resilience to drought is excised leaf water loss (ELWL) (Bayles *et al.,* 1937) and relative water content (RWC) (Malik 1995). ELWL is a moderately heritable trait. These characteristics are very simple to calculate, making them appropriate for application in huge populations (Clarke and Townley-Smith, 1986). Therefore, enhanced interpretation of drought-related traits and their genetic basis could potentially result in the utilization of these traits as selection criteria in the breeding process aimed at enhancing drought resistance. Hence the study was taken up to quantify the plant water status and to assess the constancy and heritability of excised leaf water loss (ELWL) and relative water content (RWC) to be used as drought indices, to evaluate the yield component and quality traits in cotton subjected to water – deficit stress and to correlate the plant water status with the yield under stress.

MATERIALS AND METHODS

Experiment 1: To assess the plant water status (RWC, ELWL, OP, WP) at squaring and flowering stage in cotton under drought stress: TNAU Cotton pre-release cultures *viz.,* TVH002, TVH003, TVH007 and a check variety for drought tolerance, KC3 were used for the study. Drought stress was imposed by using dry down method (Guha *et al*., 2012). The experiment had three treatments, T1: Control (100% of pot capacity), T2: Drought at square formation (Pots were maintained at 45% of pot capacity for 14 days), T3: Drought at flowering stage (Pots were maintained at 45% of pot capacity for 14 days). The work was carried out as a pot culture study during February-July, 2023 at the Department of Crop Physiology, TNAU, Coimbatore. Three seeds were sown per pot and only two healthy plants were retained per pot after thinning. Pot mixture was prepared by using black cotton soil, red soil and vermicompost in the ratio of 2:1:1. Uniform size pots filled with 22.5 kg of pot mixture were maintained. Standard measures for crop management and protection were implemented throughout the process. Factorial completely randomized design (FCRD) was adopted to set up the experiment with four replications. Physiological parameters were recorded both in the control and stress plants at 14 Days After Stress (DAS).

Relative water content (RWC): The relative water content (RWC) was determined using the formula proposed by Weatherley (1950)
RWC = [(Fresh weight – Dry weight)/ (Turgid weight – Dry weight)] x 100. The results were expressed as a percentage (%)

Excised leaf water loss (ELWL): Excised leaf water loss was calculated using the formula of Clarke and McCaig, (1982).
ELWL = [(Fresh weight –Wilted weight)/ (Fresh weight – Dry weight)] x100.

Osmotic potential (OP): The measurement of leaf osmotic potential was conducted using a vapour pressure osmometer (Vapro Model 5520, Wescor Inc., Logan, UT, USA). The osmotic potential (Ψs) was determined using the formula:Ψ_π = -CRT. Where, C = Concentration, R = Universal gas constant (0.0832), T = Temperature in degree Kelvin (310° K). Osmotic adjustment was determined by calculating the disparity between the turgid potential observed in the well-watered treatment and the stress treatment.

Water potential (WP): The leaf water potential was measured at various growth stages using a Pressure Chamber manufactured by Soil Moisture Equipment Corp., CA 93105, U.S.A and the values were expressed in units of megapascals (MPa).

Experiment 2: Evaluating the yield, yield components and quality traits of cotton genotypes under water – deficit stress : Observations on Number of bolls/plants (NB/plants): At the maturity stage, the number of bolls retained by each cotton plant was counted. Samples were collected from each replication of all the treatments, and the mean values were recorded. Boll weight (BW): The average boll weight, expressed in grams per boll (g/boll), was calculated from the weight of completely opened and developed bolls harvested from the plants. Seed cotton yield (SCY): The quantity of seed cotton obtained from each replication was recorded and reported as the yield, expressed in grams per plant (g/plant). Lint index (LI): Lint index, as defined by Santhanam (1976), denotes the quantity of lint obtained from 100 seeds after the ginning process. Seed index (SI): Hundred seeds were randomly selected, weighed, and assigned a seed index according to Santhanam's method.

Statistical analysis: The data were statistically analysed using the Statistical Tool SPSS, and a significance level of P<0.05. The genetic traits were analysed using TNAUSTAT statistical program. Correlation was performed by R Studio version 4.3.0.

RESULTS AND DISCUSSION

Plant water status at squaring and flowering stage in cotton under drought stress: An investigation was designed to study the plant water status and their association with drought tolerance in TNAU cotton cultures. Effective screening technique at different plant growth stages has been the prior need of the plant breeder to eliminate the unwanted lines and focus more on the promising lines (Rahman *et al.,* 2000). Relative water content (RWC) showed a gradual increasing trend from squaring to flowering under control condition and imposition of drought stress caused significant decline in RWC at squaring and flowering (**Fig. 1**). RWC varied significantly among the varieties under drought condition. Among the cultures highest RWC was observed in TVH002 (Squaring- 89.81%, flowering- 91.06%) which was on par with the check variety KC3 (Squaring- 89.09%, Flowering- 90.13%) under well irrigated condition. Under drought stress condition, TVH002 (squaring- 87.88%, flowering- 86.65%) showed the highest RWC and TVH007 (squaring- 81.75%, flowering- 80.52) had the lowest RWC (**Fig. 1**). The interaction between the treatment and variety was significant. Relative water content serves as an indicator of hydration level of plants, reflecting their metabolic vitality and functioning as a measure for their ability to tolerate dehydration (Lugojan and Ciulca, 2011). Genotypes that are well-suited for dryland conditions demonstrate a low rate of water loss from excised leaves, indicating that ELWL may play a role in preserving leaf water during drought periods (Basal and Unay, 2006). Irrespective of the varieties, ELWL was more at flowering compared to squaring stage. Under control condition, KC3 (squaring- 81.46%, flowering - 84.13%) showed significantly less value compared to other TNAU cotton cultures. Among the cultures TVH002 (squaring- 80.12%, flowering -82.19%) had less value and TVH007 (squaring- 90.75%, flowering – 93.42%) has the highest water loss. Under water deficit condition TVH002 (squaring- 78.18%, flowering - 81.25) recorded ELWL on par with KC3 (squaring- 79.32%, flowering - 80.2%) (**Fig. 1**).

Leaf water potential (LWP) is used as an important indicator of the water status of the entire plant (Wang *et al.,* 2016). LWP significantly decreased with increasing drought stress. Flowering stage showed higher reduction in LWP compared to squaring stage. LWP was decreased very much in TVH007 (squaring: -2.03MPa, flowering: -2.14MPa) than KC3(squaring: -1.85MPa, Flowering: -2.07MPa) under water deficit condition. In general, plants show more negative osmotic potential (OP) value under water stress condition than in irrigated condition. Higher negative OP was seen during flowering stage than squaring stage. At flowering stage, KC3 (-2.17 MPa) and TVH002 (-2.28MPa) showed less negative OP than TVH003 (-2.46 MPa) and TVH007 (-2.56 MPa) under stress condition. Among the varieties KC3 (0.51) and TVH002 (0.53) showed least osmotic adjustment. Higher genetic variability was recorded in RWC and ELWL which showed the constancy and heritability of ELWL and RWC to be used as drought indices (**Table 1 and Table 2**). ANOVA was used to test the suitability of the traits. Treatments, variety, and interaction had p-values less than 0.05 indicating statistical significance (**Table 3**). This indicates that ELWL and RWC can be used as efficient traits to assess the drought tolerance in TNAU cotton cultures.

Evaluating the yield, yield components and quality traits of cotton genotypes under water – deficit stress:Three TNAU cotton cultures and a check variety were subjected to drought stress at squaring and flowering stages. Significant reduction was recorded in the yield and yield component of cotton genotype subjected to water-deficit condition when compared to control condition (**Fig. 2**). Irrespective of varieties and treatments, seed cotton yield (SCY) had the highest percent reduction as compared to the boll weight. Stress at flowering caused higher percent reduction in SCY and boll weight compared to stress at squaring stage. The variety TVH007 showed more percent reduction at both stages of stress imposition (**Fig. 2**). Lowest number of bolls, seed index and lint index were observed in TVH007 followed by TVH003 (**Table 4**). Highest number of bolls (12), seed index (12.41 g) and quality parameters were found in KC3 under water deficit condition (**Table 4**). It had been observed that yield, yield components and quality traits are positively correlated to RWC and inversely correlated to ELWL (**Fig.3**).

Variability, heritability, and genetic advance: Phenotypic coefficient of variance (PCV) was found higher than genotypic coefficient of variance (GCV) for RWC and ELWL traits (**Table 1 and Table 2**). The reduced variance

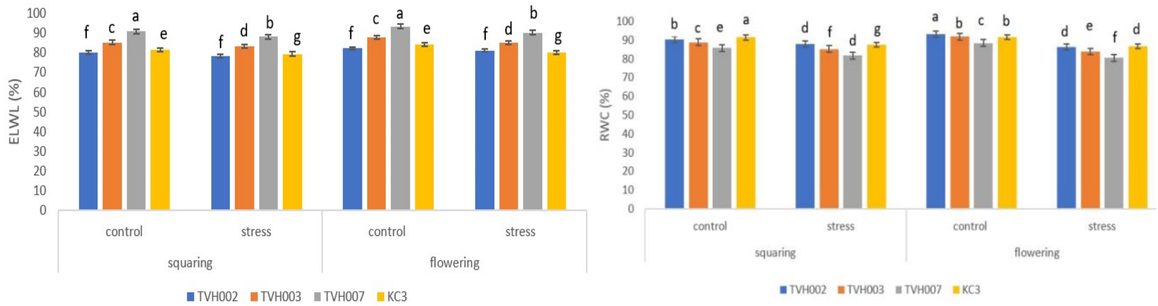

Fig.1. Drought induced genetic variability for relative water content and excised leaf water loss in cotton

Table 1. Genetic analysis of physiological and yield traits for drought stress tolerance in cotton at square formation

Traits	Variance			GCV (%)	PCV (%)	Heritability (%)	GAM (5%)
	Vg	Vp	Ve				
OP	0.060	0.062	0.002	-11.34	-11.52	96.80	-22.98
WP	0.062	0.066	0.003	-11.55	-11.87	94.73	-23.16
RWC	7.895	8.068	0.173	3.28	3.31	97.85	6.69
ELWL	19.333	19.404	0.070	5.34	5.35	99.63	10.98
SI	1.382	1.426	0.044	11.81	12.00	96.88	23.95
BW	0.388	0.395	0.007	13.95	14.08	98.18	28.48
LI	1.905	2.020	0.115	7.44	7.66	94.26	14.88
SCY	0.553	0.557	0.004	15.43	15.50	99.19	31.67

Table 2. Genetic analysis of physiological and yield traits for drought stress tolerance in cotton at flowering

Traits	Variance			GCV (%)	PCV (%)	Heritability (%)	GAM (5%)
	Vg	Vp	Ve				
OP	0.029	0.032	0.002	-7.29	-7.57	2.90	-14.49
WP	0.019	0.021	0.002	-6.98	-7.35	90.08	-13.65
RWC	7.895	8.068	0.173	3.33	3.36	97.85	6.78
ELWL	20.074	20.086	0.012	5.31	5.32	99.93	10.95
SI	1.817	1.878	0.060	13.49	13.71	96.75	27.33
BW	0.352	0.357	0.005	14.29	14.40	98.50	29.22
LI	3.459	3.576	0.116	10.61	10.79	96.73	21.50
SCY	0.823	0.828	0.005	19.67	19.73	99.31	40.38

Table 3. ANOVA for excised leaf water loss (ELWL) during flowering stage under control and drought stress conditions

Dependent Variable: ELWL					
Source	**Sum of Squares**	**DF**	**Mean Sum of Square**	**F**	**Sig.**
Corrected Model	574.195[a]	7	82.028	430.235	0.000
Intercept	177302.705	1	177302.705	9.300E5	0.000
Variety	449.956	3	149.985	786.672	0.000
Treatment	44.652	1	44.652	234.200	0.000
Variety*Treatment	5.829	3	1.943	10.190	0.000
Error	4.576	24	.191		
Total	235357.413	32			
Corrected Total	578.771	31			

a. R Squared = 0.992 (Adjusted R Squared = 0.990)

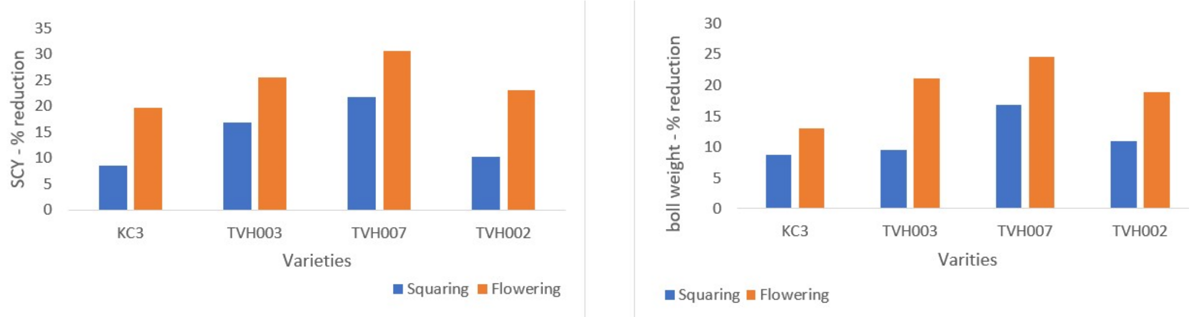

Fig.2. Percent reduction in seed cotton yield and boll weight in cotton varieties subjected to water – deficit stress

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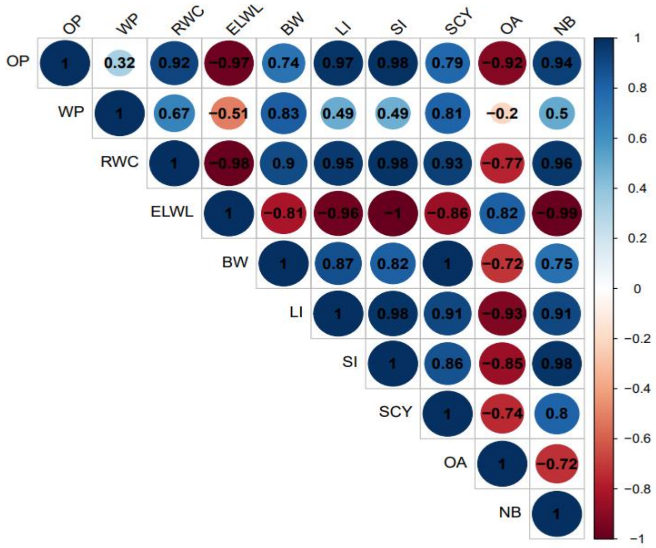

Fig 3. Correlating the plant water status and yield components with yield under stress in TNAU cotton cultures

Table 4. Yield and quality traits of TNAU cotton cultures exposed to drought stress

Varieties	No. of bolls/plants				Boll weight(g)				Seed cotton yield (g)			
	Squaring		Flowering		Squaring		Flowering		Squaring		Flowering	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress
TVH002	17	13	18	12	5.60	4.99	5.98	5.20	24.61	22.24	24.16	18.61
TVH003	15	11	16	11	4.49	4.06	4.99	3.94	22.25	18.5	22.88	17.05
TVH007	12	10	12	8	3.97	3.30	4.81	3.44	21.33	16.69	21.97	15.24
KC3	16	12	16	13	5.49	5.01	5.51	4.37	24.76	22.5	25.26	20.80
	SED	CD	SED	CD	SED	CD	SED	CD	SED	CD	SED	CD
V	0.088	0.150	0.176	0.301	0.041	0.070	0.044	0.075	0.143	0.244	0.162	0.277
T	0.062	0.106	0.125	0.213	0.029	0.049	0.031	0.053	0.101	0.172	0.114	0.195
V*T	0.124	0.212	0.250	0.427	0.059	0.100	0.083	0.142	0.202	0.345	0.229	0.496

Table 4. Continued..

Varieties	Seed index(g)				Lint index (g)			
	Squaring		Flowering		Squaring		Flowering	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress
TVH002	11.77	11.20	12.77	11.91	6.16	5.62	6.51	5.50
TVH003	11.24	10.74	11.69	10.50	5.71	5.43	5.93	4.52
TVH007	10.36	9.39	10.24	9.07	5.19	4.52	5.31	4.17
KC3	11.59	11.27	13.21	12.41	6.28	5.84	6.75	5.55
	SED	CD	SED	CD	SED	CD	SED	CD
V	0.070	0.119	0.072	0.123	0.038	0.065	0.065	0.111
T	0.050	0.085	0.51	0.087	0.027	0.046	0.046	0.078
V*T	0.100	0.171	0.111	0.189	0.054	0.092	0.092	0.157

between PCV and GCV across all traits could be attributed to less impact of the environmental factors. This implies that the variation in these traits is predominantly governed by genetic factors rather than environmental influences. Similar results were observed for seedling and seed quality traits in rice under anaerobic germination (Vinitha *et al.,* 2023). Least difference was observed in PCV and GCV were observed of RWC and ELWL (**Table 1 and**

Table 2). Hence, focusing on selecting and breeding for these specific traits could prove to be highly effective in achieving substantial genetic improvements. High heritability is an important selection criterion. High heritability was observed in ELWL (Squaring: 99.63%, flowering: 99.93%) and RWC (Squaring: 97.85%, flowering: 97.85%). The findings indicated that ELWL and RWC exhibited higher GCV and PCV values, along with a notable level of heritability. This suggests that for future breeding programs, focusing on the selection and enhancement of these traits would be particularly effective, especially considering that the primary cause of genetic variance is additive gene action (Mawblei *et al.,* 2022). Thus, these traits might be useful to assess the drought tolerant indices in cotton. RWC showed a significant positive correlation with yield and quality traits. Contradictory to RWC, ELWL recorded a significant negative correlation with yield and quality traits. This indicated that, lesser ELWL during drought situation enhances plant tolerance toward drought stress. OP and WP had a negative correlation with ELWL and OA. Positive correlation was recorded between ELWL and OA (**Fig.3**).

Different traits were analysed to assess the plant water status and their relation with the yield, yield components and quality traits under drought condition. Finally, the study concluded that flowering stage in cotton is more susceptible to drought than squaring stage. TVH002 was identified as a drought tolerant cotton culture. It recorded drought indices and yield on par with the check variety KC3.

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