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RESEARCH ARTICLE

Genetic evaluation of plant water status, physiological and biochemical traits for abiotic stress tolerance in Tamil Nadu Agricultural University in cotton cultures

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Abstract

The frequency of drought and heat stress events has increased due to global climate change, posing a significant threat to current and future cotton production. Understanding the fundamental mechanisms of adaptation to heat and drought stress is essential for improving cotton resilience. Three TNAU pre-release cotton cultures (TVH002, TVH003 and TVH007) and a check variety (KC3) were subjected to drought and heat stress at two growth stages: squaring and flowering. Plants were exposed to 45 % Pot Capacity (PC) under drought stress conditions and Ambient temperature + 5 ˚C under heat stress condition respectively. TVH002 exhibited the highest drought tolerance, while the flowering stage was more susceptible to drought than the squaring stage. Genetic analysis revealed that Excised Leaf Water Loss (ELWL) and Relative Water Content (RWC) exhibited high heritability, along with large genotypic and phenotypic coefficients of variation. These traits can serve as reliable indicators for drought screening in cotton breeding programs. Proline accumulation showed the highest heritability (0.81), followed by antioxidant enzyme activity (0.77) and ELWL (0.72), indicating strong genetic control. Traits with high heritability and low GCV-PCV differences, such as proline accumulation and relative water content, are ideal for selection in breeding programs for drought and heat stress tolerance.

Keywords: cotton, drought, drought indices, genetic analysis

Introduction

Climate change poses a serious threat to agriculture and environmental sustainability, leading to rising temperatures, erratic rainfall and disruptions in seasonal cycles (1) According to Intergovernmental Panel on Climate Change (IPCC), atmospheric carbon dioxide levels are projected to rise to 500-970 ppm by the end of the century, leading to a global temperature increase of 1-5.5 ˚C. Changes in temperature and rainfall patterns significantly impact agriculture by exposing plants to multiple abiotic stressors, thereby reducing economic output. Climate change has an impact on cotton's growth and development, which decreases production and fibre quality mostly due to the combined effects of rising temperatures, decreased water availability and increased moisture loss from soil and plants (2, 3).

Cotton (*Gossypium* spp.), one of the oldest cultivated commercial crops, holds significant economic importance as a primary source of fiber for the textile industry. Cotton is primarily cultivated for fiber extraction, which is used in clothing production. Global cotton demand has steadily increased, with approximately 35 million hectares under cultivation since the 1950s. The leading consumers of cotton include China, India, Bangladesh, Pakistan and Turkey, which collectively account for most of the global cotton demand due to their extensive textile and apparel industries. As a result, cotton ranks among the top 20 most valuable global commodities (4).

India contributes 25 % of global cotton production and plays a crucial role in supporting the livelihoods of approximately 6 million cotton farmers and 40-50 million individuals employed in related industries. Because of its economic significance, cotton is known as ‘White Gold’ or ‘King of Fiber.’ According to the Committee on Cotton Production and Consumption (COCPC, 2022), India ranks first in cotton acreage, covering 120.55 lakh hectares. However, in terms of productivity, it ranks 40th, with a yield of 445 kg per hectare. It is also one of the largest consumers with an approx. consumption of 326 lakhs bales.

Cotton is primarily grown in arid and semi-arid regions, either as rainfed or under irrigated condition. India's cotton production is concentrated in ten major states, categorized into three agro-ecological zones: Northern, Central and Southern. In the Southern zone, Telangana andhra Pradesh, Karnataka and Tamil Nadu collectively contribute 30 % of India's total cotton production (COCPC, 2021). In Tamil Nadu, cotton is cultivated on 1.25 lakh hectares, producing 2.96 lakh bales, with an average productivity of approximately 403 kg per hectare (5).

Drought stress from emergence to first square has minimal impact on yield, but stress between first square to peak bloom is the most critical stage, affecting fruiting site development, causing fruit abortion and ultimately reducing yield (6). According to (7) increased evaporation through plant surfaces exacerbates water stress in cotton under high temperatures. Drought stress has a major impact on cotton growth and development, which mainly restricts the yield and fiber quality (8). Cotton mainly requires water during flowering and boll formation.

Osmotic changes were observed in response to heat shock proteins in cotton. Various chemical compounds, including amino acids, accumulate in plants under water stress and play a crucial role in maintaining osmotic balance (9). Significant genotypic variations were observed in RWC, leaf osmotic potential and ion leakage, likely due to differences in genetic background and water stress response.

Antioxidant enzyme activity varies among genotypes and is linked to genetic traits associated with drought stress resistance (10). Superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) are key antioxidant enzymes involved in scavenging reactive oxygen species (ROS). Biochemical traits, such as glycine betaine and proline show a significant positive correlation with seed cotton yield under drought conditions (11). Cotton experiences significant yield reductions when exposed to drought stress between flowering and fructification (12) and high temperatures during flowering to boll development stage (13). This research aims to enhance the ability of cotton plants to withstand water scarcity and high-temperature stress. Therefore, the present study was designed to evaluate the physiological responses of TNAU cotton cultures to abiotic stress tolerance.

Material and Methods

Screening of drought and heat stress tolerance in cotton cultures/varieties

This experiment aimed to identify drought- and heat-tolerant pre-release TNAU cotton cultures and to understand the physiological mechanisms contributing to their stress tolerance. The pot mixture was prepared using black cotton soil, red soil and vermicompost in the ratio of 2:1:1. Uniform-sized pots (37 × 35 cm) were filled with 22.5 kg of pot mixture, saturated with water and the field capacity of the soil was recorded. Three seeds were sown per pot and only two healthy plants were maintained per pot after thinning. Standard measures for pot culture protection were implemented throughout the process. During the study period, the climatic conditions were characterized by high temperatures (ranging from X to Y ˚C) and reduced relative humidity (Z %), simulating drought and heat stress conditions to evaluate plant responses effectively.

Plant material

The pre-release TNAU cotton cultures viz., TVH002, TVH003 and TVH007 were selected as test varieties for their promising drought and heat tolerance traits, including enhanced water-use efficiency, high proline accumulation and improved antioxidant enzyme activity. The widely cultivated variety KC3 was included as a check (control) variety to compare the performance of the test cultures under stress conditions.

Drought stress treatment

Drought stress was imposed by dry down method (14). Pots were maintained at 45 % pot capacity for 14 days at two different growth stages: T1 (Control, well-watered), T2 (Drought at square formation) and T3 (Drought at flowering). The selection of 45 % pot capacity as the drought threshold was based on prior studies that identified this level as the critical point where water limitation significantly affects physiological processes without causing irreversible plant damage. Soil water content was measured using a Theta Probe, which provides accurate soil moisture readings. The probe was calibrated using soil-specific calibration curves to ensure precise measurements at different soil moisture levels. The recorded soil water content at 100 % pot capacity was 59.89 %, while at 45 % pot capacity, it was 32.45 %. To maintain the desired drought stress conditions, the soil moisture level was gradually reduced and water was added only when necessary to ensure that the imposed stress levels were sustained. The plants were subjected to drought stress for 14 days at both the squaring and flowering stages. Physiological and biochemical parameters were recorded before the imposition of drought stress and after 14 days of stress for both growth stages. Stomatal traits were specifically observed during the flowering stage after the drought stress period. Yield and yield-related parameters were recorded at the maturity stage to assess the overall impact of drought stress on cotton performance.

High temperature stress and growth chambers conditions

The experiment was conducted at the Open Top Chamber (OTC) facility within the Department of Crop Physiology in TNAU, during February 2023. The OTC used for this experiment had dimension of 4x4x4 m and were constructed using polycarbonate sheets. These chambers were equipped with advanced temperature, humidity monitoring, control systems and signal transmission capabilities using Supervisory Control and Data Acquisition (SCADA) technology. Two OTC chamber were utilized for this experiment. One chamber was used as ambient chamber which maintain conditions like the surrounding environment, while the other was the elevated chamber. In the elevated chamber, temperature was increased by 5 ˚C

above ambient levels for 14 days at squaring stage and flowering stage (Fig. 1). Plants imposed for heat stress were divided into four sets with four replications. Heat stress was imposed at three different levels including T1: Ambient condition (AC), T2: At squaring stage (AC+ 5 ˚C) and T3: At flowering stage (AC +5 ˚C). All the physiological and biochemical parameters were recorded before the onset of high temperature stress and 14 days after the initiation of heat stress at both squaring and flowering stage respectively.

Physiological parameters for screening cotton cultures/ varieties

Leaf water potential (-MPa)

The leaf water potential was measured using a pressure chamber manufactured by Soil Moisture Equipment Corp., located in CA 93105, U.S.A. the values were expressed in units of megapascals (MPa).

Leaf osmotic potential (-MPa)

Leaf osmotic potential was measured by using a vapour pressure osmometer (Vapro Model 5520 Wescor Inc., Logan, UT, USA). Osmotic potential (Ψ_s) was calculated as

$$\Psi_\pi = -CRT$$

Where, C = Concentration, R = Universal gas constant (0.0832), T = Temperature in degree Kelvin (310 °K)

The difference between the turgid potential in the well-watered treatment and the stress treatment was used to compute osmotic adjustment.

Relative Water Content (RWC %)

RWC was determined using the formula provided by (15). The results were expressed as a percentage (%).

$$RWC\ (\%) = \frac{\text{Fresh weight - Dry weight}}{\text{Turgid weight - Dry weight}} \times 100 \quad \text{(Eqn. 1)}$$

Excised Leaf Water Loss (ELWL %)

ELWL was calculated using the formula (16).

$$ELWL\ (\%) = \frac{\text{Fresh weight -Wilted weight}}{\text{Fresh weight - Dry weight}} \times 100 \quad \text{(Eqn. 2)}$$

Chlorophyll fluorescence

Leaf chlorophyll fluorescence was measured using a portable fluorometer (OS1p040111 Advanced, Opti-Sciences, USA) to assess the efficiency of the photosynthetic system under both normal and high-temperature stress conditions. The fluorescence parameters recorded included initial fluorescence (Fo), which represents the minimum level of fluorescence emitted when all photosystem II (PSII) reaction centers are open and maximum fluorescence (Fm), which is the peak fluorescence when all reaction centers are closed following exposure to a saturating light pulse. Variable fluorescence (Fv), calculated as the difference between maximum and initial fluorescence (Fv = Fm - Fo), indicates the ability of PSII to capture and utilize light energy. The ratio of Fv/Fm was recorded as it reflects the maximum quantum efficiency of PSII and is commonly used as an indicator of plant stress tolerance and photosynthetic performance. The ratio of Fo/Fm was also measured to evaluate the structural integrity and functional status of PSII. To ensure accurate readings, leaves were kept in complete darkness for 30 minutes, allowing the PSII reaction centers to fully open before measurements were taken. Observations were conducted between 09:00 and 12:00 IST to maintain consistency in environmental conditions and avoid fluctuations due to changes in natural light intensity.

Measurement of stomatal size and frequency

The stomatal size and stomatal frequency for each film strip were promptly assessed using a phase contrast microscope (Leica LM 2000 LD) equipped with a computer attachment, at a magnification level of 100x under bright field conditions. Measurement of stomatal length and width was taken and expressed in micrometer (µm). The stomatal size was defined as the distance between the junctions of the guard cells of the stoma. This measurement does not represent the actual size of the stomatal pore opening but rather the maximum potential opening of the stomata. This interpretation aligns with previous results (17 - 19).

Stomatal frequency is defined as number of stomata per unit leaf area. It was calculated using the formula (20) and expressed as number of stomata/ mm^2.

$$\text{Stomatal frequency} = \frac{\text{Number of stomata}}{\text{Area of microscopic field}} \times mm^2 \quad \text{(Eqn. 3)}$$

Enzyme activity and biochemical parameters

Nitrate reductase activity

The estimation of nitrate reductase activity was performed (21). Fresh leaf samples (500 mg) were collected and finely chopped. The leaf fragments were transferred into test tubes and 10 mL of assay medium was added. The test tubes were placed in a desiccator for 5 minutes to facilitate enzyme extraction, followed by incubation in the dark for 1 hr. After incubation, a 2 mL aliquot of the reaction mixture was taken into a test tube, to which 1 mL of zinc acetate and 2 mL of ethanol were added. The resulting solution was filtered using Whatman filter paper. Subsequently, 1 mL of 1 % sulfanilamide and 1 mL of 0.02 % N-(1-naphthyl) ethylenediamine dihydrochloride (NEDH) were added to the filtered solution. The optical density (OD) of the final solution was measured at 540 nm using a spectrophotometer. Nitrate reductase activity was expressed in units of $NO_2\ g^{-1}\ h^{-1}$.

Proline content

The proline content in leaves was determined (22). 500 milligrams of fresh leaf was homogenized in 4 mL of 3.0 % Sulphosalicylic acid. The homogenate was centrifuged for 10 min @1000 rpm. 1ml supernatant was collected from the homogenized solution then 2 mL acid ninhydrin reagent and 2 mL lacial acetic acid were added to it. The mixture was subjected to incubation in a water bath set at 100 ˚C for

60 minutes. Subsequently, the mixture was rapidly cooled by immersing it in an ice bath. After cooling, 4 mL of toluene was added into the solution and vortexed. The toluene layer, which contains the chromophore, was carefully transferred to a new test tube. To determine the concentration of proline, the absorbance of the solution was measured at a wavelength of 520 nm using a spectrophotometer, with toluene serving as the blank. The concentration of proline was then calculated using a standard curve and expressed mg g^{-1} fresh weight.

Chlorophyll Stability Index (CSI)

The Chlorophyll stability index was calculated using the method (23) and was expressed as a percentage using the following formula.

$$CSI = \frac{\text{Total chlorophyll content (treated)}}{\text{Total chlorophyll content (control)}} \times 100 \quad \text{(Eqn. 4)}$$

Statistical analysis

A Complete Randomised Design (CRD) was used for analysing data before imposition of stress and a Factorial Completely Randomised Design (FCRD) was used for data analysis for the experiments conducted in cotton exposed to drought and heat stress. Various physiological, biochemical and yield and yield components were studied for analysis of variance. To know the significance difference among the cultures/varieties and between the treatment ANOVA was performed using SPSS (Statistical Package for Social Science) software (SPSS version 26). Critical differences were carried out at five percent probability level. Correlation was performed by R Studio version 4.3.0.

Results and Discussion

Three pre-released TNAU cultures namely TVH002, TVH003 and TVH007, along with the check variety KC3, were evaluated for drought and heat stress tolerance. Imposition of drought and heat stress was given at two stages (i) squaring stage (ii) flowering stage. Observations were recorded on various physiological and biochemical traits before and after stress period. The results of various physiological, biochemical and yield parameters are presented in this section.

Physiological and biochemical parameters prior to drought and heat stress imposition

Physiological and biochemical traits of cotton cultures/ varieties prior to drought and heat stress imposition are presented (Table 1). Among the physiological parameters, namely, photosynthetic rate, transpiration rate, stomatal conductance, intrinsic water use efficiency, osmotic potential, relative water content, excised leaf water loss and chlorophyll fluorescence, only photosynthetic rate, stomatal conductance, osmotic potential and chlorophyll fluorescence showed significant variation among the cultures and varieties. TVH002 and KC3 showed higher and on par relation with all the physiological traits. Among the biochemical parameters, only malondialdehyde (MDA) showed significant variation, whereas chlorophyll stability index, cell membrane leakage, membrane stability index, superoxide dismutase, nitrate reductase, catalase and proline showed no significant variation among the cultures/ varieties prior to stress imposition.

Analyzing the physiological traits for drought tolerance in cotton

Leaf water potential (LWP, -Mpa)

LWP significantly decreased with increasing drought stress (Table 2). The reduction in LWP was more pronounced during the flowering stage when compared to the squaring stage. Less negative LWP was recorded under well water condition. TVH002 (Squaring: -1.72 MPa, Flowering: - 1.66 MPa) recorded less negative value followed by KC3 (Squaring: -1.76 MPa, Flowering: - 1.72 MPa), TVH003 (Squaring: -1.86 MPa, Flowering: - 1.78 MPa) and TVH007 (Squaring: -1.98 MPa, Flowering: - 1.85 MPa). Higher reduction was observed in TVH007 at both squaring (-2.03 MPa) and flowering (-2.14MPa) stage followed by TVH003 (squaring: -2.02 MPa, flowering: -2.17). Whereas TVH002 (squaring: -1.99 MPa, flowering: -2.09 MPa) recorded on par with check variety KC3 (squaring: -1.85 MPa, flowering: -2.07 MPa).

4.2.2. Osmotic potential (-MPa) and Osmotic adjustment (MPa)

Plants tend to exhibit more negative osmotic potential (OP) values under water stress conditions compared to well-irrigated conditions (Table 2). At squaring stage under well-watered conditions, TVH002 exhibited the least negative OP value (-1.60 MPa) among all cultures, followed by TVH003 (-1.89), TVH007 (-2.03 MPa) and the check variety KC3 (-1.65 MPa). Under water stress conditions, TVH007 exhibited the most negative OP value (-2.24 MPa), followed by TVH003 (-2.19 MPa), TVH002 (-1.98 MPa) and KC3 (-1.99 MPa). Among the cultures/varieties highest osmotic adjustment (OA) was made by TVH002 (0.38MPa) which was on par with KC3 (0.34 MPa). Significantly higher reduction in OP was recorded under water deficit condition at flowering stage. Among all the cultures/varieties KC3 (-2.17 MPa) exhibited a comparatively less negative followed by TVH002 (-2.28 MPa), TVH003(-2.46 MPa) and TVH007(-2.56 MPa). Higher OA was observed in TVH002 (0.53MPa) followed by KC3 (0.51 MPa), TVH003 (0.44 MPa) and TVH007 (0.44 MPa).

Relative Water Content (%)

RWC exhibited a gradual increase from the squaring stage to the flowering stage under well-watered conditions, however imposition of drought stress results in significant decrease in RWC during both squaring and flowering stages. RWC showed notable variations among cultures/varieties under drought conditions (Fig. 1). Among the cultures, TVH002 had the highest RWC (89.81 % at squaring and 91.06 % at flowering), similar to the check variety KC3 (89.09 % at squaring and 90.13 % at flowering) under well-watered conditions. However, in drought stress, TVH002 maintained the highest RWC (87.88 % at squaring and 86.65 % at flowering), while TVH007 had the lowest RWC (81.75 % at squaring and 80.52 % at flowering) among the cultures.

Table 1. Genetic variability in physiological traits of cotton before imposition drought and heat stress

Parameters	TVH002	TVH003	TVH007	KC3	S.Ed	CD
Photosynthetic rate (µmol of CO2 m-2 s-1)	27.33	24.25	26.70	28.82	1.086	1.932
Transpiration (mmol of H2O m-2 s-1)	13.22	11.95	12.7	13.32	NS	NS
Conductance (mol of H2O m-2 s-1)	0.26	0.22	0.25	0.28	0.010	0.017
Intrinsic water use efficiency (mmolCO2 mol-1 H2O)	2.06	2.02	2.10	2.16	NS	NS
Osmotic potential (-Mpa)	-1.51	-1.19	-1.26	-1.27	0.052	0.092
Relative Water Content %)	92.12	89.67	87.89	93.01	NS	NS
Excised leaf water loss (%)	77.78	81.12	80.45	78.98	NS	NS
Chlorophyll fluorescence	0.74	0.71	0.67	0.78	0.029	0.051
Chlorophyll stability index (%)	81.23	78.67	74.89	83.76	NS	NS
Cell membrane leakage (%)	56.78	58.50	61.12	55.98	NS	NS
MDA (µmol g-1 of fresh weight)	9.83	11.32	11.89	10.49	0.431	0.768
Membrane stability index (%)	83.89	81.58	80.67	86.53	NS	NS
SOD (mg protein-1 min-1)	8.10	7.89	7.54	8.05	NS	NS
Nitrate reductase activity (µg of NO2 g-1 h-1)	132.67	130.54	129.89	130.98	NS	NS
Catalase (µgram H2O2 min -1 g-1)	5.98	5.46	5.67	6.01	NS	NS
Proline (mg g-1 fresh weight)	7.65	7.71	7.47	8.12	NS	NS

Table 2. Effect of drought stress on plant water status in cotton cultures

Cultures/ Varieties	Osmotic potential (MPa)				Water potential (MPa)			
	Squaring		Flowering		Squaring		Flowering	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress
TVH002	-1.60	-1.98	-1.75	-2.28	-1.72	-1.99	-1.66	-2.09
TVH003	-1.89	-2.19	-2.02	-2.46	-1.86	-2.02	-1.78	-2.17
TVH007	-2.03	-2.24	-2.12	-2.56	-1.98	-2.03	-1.85	-2.14
KC3	-1.65	-1.99	-1.66	-2.17	-1.76	-1.85	-1.72	-2.07
	S.Ed	CD	S.Ed	CD	S.Ed	CD	S.Ed	CD
V	0.026	0.053	0.018	0.038	0.022	0.046	0.016	0.033
T	0.018	0.038	0.013	0.027	0.016	0.032	0.011	0.023
V*T	0.036	0.075	0.026	0.053	0.031	0.064	0.023	0.047

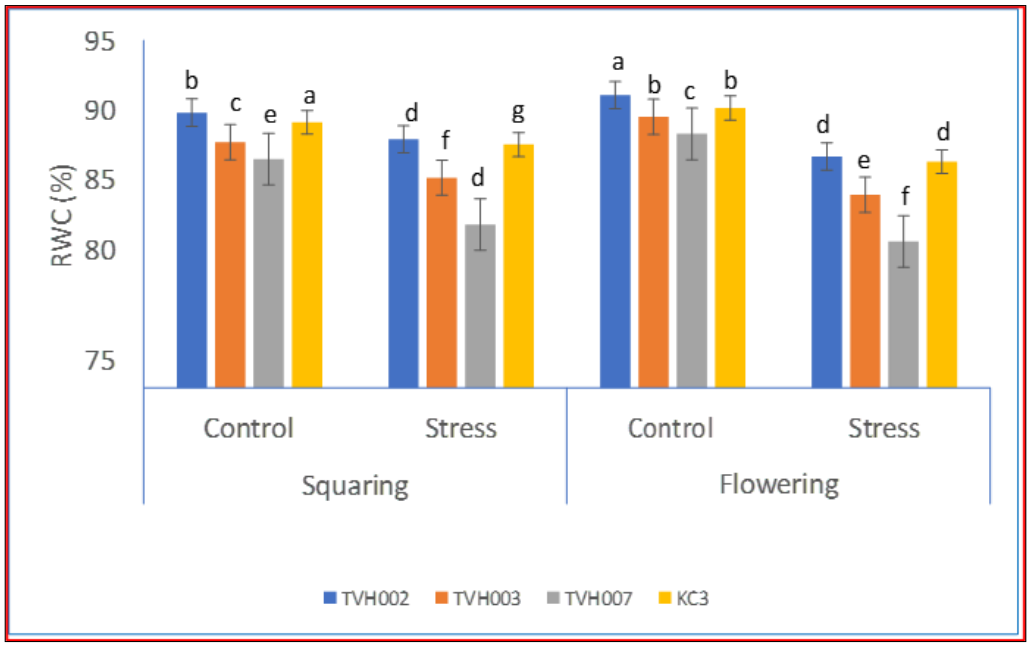

Fig. 1. Drought induced genetic variability for relative water content in cotton.

Excised Leaf Water Loss (%)

Irrespective of the cultures/varieties ELWL was more at flowering compared to squaring stage (Fig. 2). Under normal conditions, KC3 exhibited a lower ELWL at both squaring (81.46 %) and flowering (84.13 %) compared to cotton cultures. Among the different cultures, TVH002 had a lower ELWL at squaring (80.12 %) and flowering (82.19 %), while TVH007 had more water loss (Squaring: 90.75 %, Flowering: 93.42 %) under well-watered conditions. Drought conditions, TVH002 (Squaring: 78.18 %, Flowering: 81.25 %) recorded ELWL values like KC3 (Squaring: 79.32 %, Flowering: 80.2 %).

Leaf area (cm^2)

Leaf area significantly varies among the cultures/varieties. Leaf area ranged from 388.32 to 556.58 cm^2 in control conditions and 296.54 to 425.93 cm^2 in drought conditions. Under well-watered conditions KC3 had highest leaf area of 418.24 cm^2 and 556.58 cm^2 at squaring and flowering stage respectively. Drought stress induced observed variations in leaf area among the cultures/varieties and between the treatment. Significant decrease was recorded in leaf area at squaring and flowering stage. KC3 recorded highest leaf area of 358.86 cm^2 and 488.92 cm^2 at both the stages, squaring and flowering respectively followed by TVH002 (squaring: 341.3 cm^2 and flowering: 470.03 cm^2). Lower leaf area was observed in TVH007 under well-watered and drought stress conditions.

Stomatal traits

Stomatal length (µm)

Significant variation was found in the stomatal size among the cultures/varieties under both control and drought condition. Stomatal length ranged from 43.52 µm to 58.87 µm and 40.22 µm to 56.77 µm at well-watered and drought stress conditions respectively. Significant reduction in stomatal length was recorded under drought conditions (Table 3). Tolerant cultures/varieties TVH002 (40.22 µm) exhibited maximum decrease in length followed by KC3 (43.52 µm). TVH003 (53.9 µm) and TVH007 (56.77 µm) recorded comparatively higher length of stomata under water stress conditions.

Stomatal width (µm)

A substantial decrease in stomatal width was recorded in drought stress conditions (Table 3). Significant differences were observed in the size of stomata in various cotton cultures or varieties in both controlled and drought conditions. Stomatal width varied from 18.03 µm to 28.34 µm in control conditions. TVH003 and TVH007 recorded higher stomatal width of 20.56 µm and 26.09 µm respectively compared to TVH002 (17.70 µm) and KC3 (18.03 µm) under water stress condition.

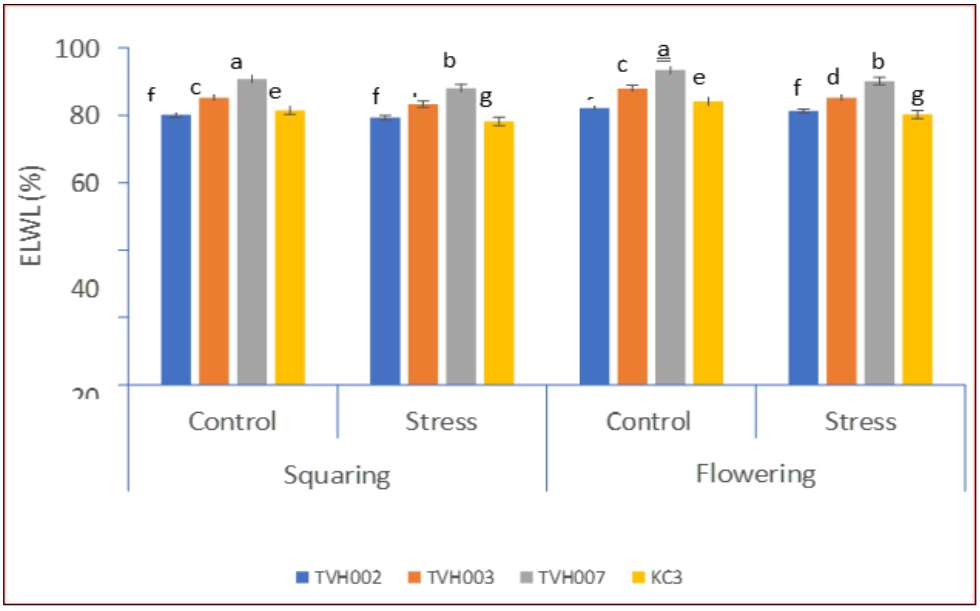

Fig. 2. Drought induced genetic variability for ELWL in cotton.

Table 3. Alteration in size and frequency of stomata in cotton grown at water deficit condition

Cultures/ varieties	Drought stress	Length of stomata (µm)		Width of stomata (µm)		Stomatal size (µm2)		Stomatal frequency (number/ mm2)	
	100 % PC	43.52		19.63		854.29		600	
TVH002	45 % PC	40.22		17.70		711.89		775	
	100 % PC	55.34		22.87		1265.62		530	
TVH003	45 % PC	53.9		20.56		1108.18		685	
	100 % PC	58.87		28.34		1668.37		410	
TVH007	45 % PC	56.77		26.09		1481.12		670	
	100 % PC	45.46		20.56		934.65		750	
KC3	45 % PC	43.52		18.03		784.66		810	
		S.Ed	CD	S.Ed	CD	S.Ed	CD	S.Ed	CD
V		0.074	0.152	0.058	0.119	0.766	1.582	1.990	4.106
T		0.052	0.107	0.041	0.084	0.542	1.11	1.407	2.904
V*T		0.104	0.215	0.082	0.169	1.08	2.23	2.814	5.807

Stomatal area (µm²)

Reduction in stomatal length and stomatal width resulted in decreased stomatal area in drought conditions (Table 3). Significant variation was recorded among the cultures/ varieties and between the treatment. It ranged from 854.29 µm² to 1668.37 µm² under well water condition and from 711.89 µm² to 1481.12 µm² under drought conditions. Reduction in stomatal area was higher in KC3 (784.66 µm²) and TVH002 (711.89 µm²) during water stress condition indicating its tolerance to drought stress. TVH003 and TVH007 recorded stomatal area of 1108.18 µm² and 1481.12 µm² respectively

Stomatal frequency (number/mm²)

Stomatal frequency is inversely related to stomatal length, width and area. It had been observed that stomatal frequency increased under drought stress due to decrease in stomatal size under stress conditions (Table 3). It ranged from 410 to 750 number/mm² well-watered conditions. KC3 (810 number/mm²) recorded highest stomatal frequency under drought conditions followed by TVH002 (775 number/ mm²) and TVH003 (685 number/mm²) TVH007 (670 number/ mm²) recorded least number of stomata under drought condition.

Analysing the biochemical traits in cotton for drought tolerance

Proline (mg g^{-1} fresh weight)

Distinct alterations in proline content were recorded in cultures/varieties under both control and stress conditions. Drought stress results in higher accumulation of proline than control conditions. Proline content varied from 7.54 mg g^{-1} fresh weight to 13.50 mg g^{-1} fresh weight and 10.07 mg g^{-1} fresh weight to 15.32 mg g^{-1} fresh weight both at squaring and flowering stage. Highest proline content of 8.33 mg g^{-1} fresh weight and 13.50 mg g^{-1} fresh weight in control and stressed plant was exhibited by KC3 at squaring stage followed by TVH002. TVH007 recorded lowest proline content at both squaring (control: 7.54 mg g^{-1} fresh weight, stress: 11.05 mg g^{-1} fresh weight) and flowering stage (control: 10.07 mg g^{-1} fresh weight, stressed: 13.08 mg g^{-1} fresh weight). However, Plants experiencing stress during the flowering stage displayed an extended range of proline accumulation. KC3 (15.32 mg g^{-1} fresh weight) recorded maximum proline content compared to TVH002 (15.03 mg g^{-1} fresh weight), TVH003 (13.64 mg g^{-1} fresh weight) and TVH007 (13.08 mg g^{-1} fresh weight).

Chlorophyll Stability Index (CSI %)

The CSI exhibited significant variation among the varieties. Well water plants showed high CSI (KC3- Squaring: 83.31 %, Flowering: 85.98 %, TVH002- Squaring: 80.28 %, Flowering: 82.62 %, TVH003- Squaring: 77.33 %, Flowering: 79.78 %, TVH007- Squaring: 74.69 %, Flowering: 77.03 %). The reduction in CSI was more pronounced during flowering stage compared to squaring stage under drought condition. KC3 (Squaring: 80.76 %, Flowering: 82.43 %) recorded minimum reduction followed by TVH002- Squaring: 77.97 %, Flowering: 79.77 %), TVH003 (Squaring: 73.66 %, Flowering:75.22 %, TVH007- Squaring: 70.15 %, Flowering: 71.56 %).

Enzyme activity under drought stress

Nitrate reductase (µg of NO_2 g^{-1} h^{-1})

Nitrate reductase (NRase) activity decreased in response to drought-induced stress at both squaring and flowering stage. Maximum decline in NRase was observed at flowering stage compared to squaring stage (Table 4). Nrase ranged from 128.94 µg of NO_2 g^{-1} h^{-1} to 134.93 µg of NO_2 g^{-1} h^{-1} and 130.93 µg of NO_2 g^{-1} h^{-1} to 114 µg of NO_2 g^{-1} h^{-1} under well water condition at squaring and flowering stage respectively. Among the cultures/varieties maximum reduction value was found in TVH007 (squaring: 10.86 %, flowering: 15.62 %) followed by TVH003 and TVH002. Least reduction in NRase activity was recorded in KC3 (squaring: 5.18 %, flowering: 9.81 %).

Discussion

Cotton (*Gossypium hirsutum*) is one of the major crops worldwide, ranking high in both area coverage and agricultural value (24). Cotton classified as a glycophyte, demonstrates greater resilience to abiotic stresses compared to many other prominent crops. However, adverse climatic circumstances like drought and elevated temperatures are recognized as primary abiotic stressors that have detrimental effects on the phenological stages, growth patterns, fiber yield and cotton quality on a global scale. The issue is anticipated to exacerbate in future climate change scenarios due to the increased prevalence of elevated temperatures and water scarcity. A primary focus for cotton breeders is developing high-yield cotton cultures/ varieties that exhibit resilience to both drought and heat stress. Hence, it is crucial to assess the performance under conditions of heat and drought stress. Additionally, gaining

Table 4. Osmolyte accumulation and CSI in cotton grown at water deficit condition

Cultures/ Varieties	Proline (mg g^{-1} fresh weight)				CSI (%)				Nitrate reductase (µg of NO2 g^{-1} h^{-1})			
	Squaring		Flowering		Squaring		Flowering		Squaring		Flowering	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress
TVH002	7.82	12.30	11.05	15.03	80.28	77.97	82.62	79.77	134.93	121.78	137.43	119.22
TVH003	7.70	11.44	10.32	13.64	77.33	73.66	79.78	75.22	130.67	118.44	132.90	114.0
TVH007	7.54	11.05	10.07	13.08	74.69	70.15	77.03	71.56	128.94	114.93	130.93	109.69
KC3	8.33	13.50	11.00	15.32	83.31	80.76	85.98	82.43	132.97	126.08	135.38	122.07
	S.Ed	CD	S.Ed	CD	S.Ed	CD	S.Ed	CD	S.Ed	CD	S.Ed	CD
V	0.074	0.153	0.061	0.126	0.239	0.493	0.239	0.493	0.194	0.401	0.169	0.350
T	0.053	0.121	0.043	0.089	0.169	0.349	0.169	0.349	0.137	0.283	0.120	0.247
V*T	0.105	0.217	0.086	0.178	0.338	0.698	0.338	0.698	0.275	0.567	0.240	0.494

insights into the physiological and biochemical responses of these cultures/varieties their agronomic performance under stress conditions, holds significant importance (25). Considering these, the current study has been undertaken to examine physiological evaluation of TNAU cotton cultures for abiotic stress tolerance.

5.1. Effect of drought stress on plant water status and leaf area in cotton cultures/varieties

Drought stress has detrimental impacts on the osmotic equilibrium in plants. To cope with the drought stress, plants often accumulate various organic and inorganic substances as part of their adaptive mechanisms to reduce the osmotic potential. This helps them maintain proper hydration and turgor pressure in their cells (26). In the present study flowering stage of cotton recorded higher percent increase in osmotic potential than squaring stage. At squaring stage among all the cultures/varieties TVH002 recorded maximum increase of 23.75 % and TVH007 recorded minimum increase of 10.34 % in osmotic potential over control, which signifies TVH002 thrived to drought stress. During flowering stage KC3 (30.72 %) recorded higher percent increase of osmotic potential followed by TVH002 (30.28 %) which shows tolerance to water deficit condition at critical stage (Table 3, Fig. 3). Higher percent increase in osmotic potential indicates more accumulation of compatible osmolytes (amino acids, amines, sugar, etc) for osmotic adjustment. These solutes assist in protecting proteins and membranes from the damage due to high concentrations of inorganic ions and oxidative damage under drought stress (27). Higher osmotic adjustment was recorded in KC3 and TVH002 at both squaring and flowering stages indicating its tolerance to drought stress (Fig. 2). LWP is an important indicator of the overall water status of the plant (28). High LWP is associated with dehydration avoidance mechanism (29). Drought stress leads to substantial decrease in LWP, this decrease in LWP highlights the reduced ability of the plant to retain water during drought periods (30). Similar result had been observed in this study. LWP was highly decreased in TVH007 under drought condition at both squaring (-2.03) and flowering stage (-2.17) indicating its more chance to get susceptible under stress conditions (Table 2). Decrease in LWP in cotton was due to root zone dehydration stress (31).

Leaf RWC serves as an important measure of a leaf's hydration level and is a valuable gauge of a plant's ability to withstand drought conditions (32). This parameter is strongly correlated with a plant's water potential (33). Drought stress led to a significant reduction in leaf RWC (TVH002) showed relative tolerance to drought tolerance compared to other TNAU cotton cultures. It recorded RWC on par with the check variety KC3 under both squaring and flowering stage (Fig. 3). Maximum reduction in RWC was observed in TVH007 (5.44 %. flowering: 8.76 %) and TVH003 (squaring: 2.93 %, flowering: 6.25 %) under stress condition.

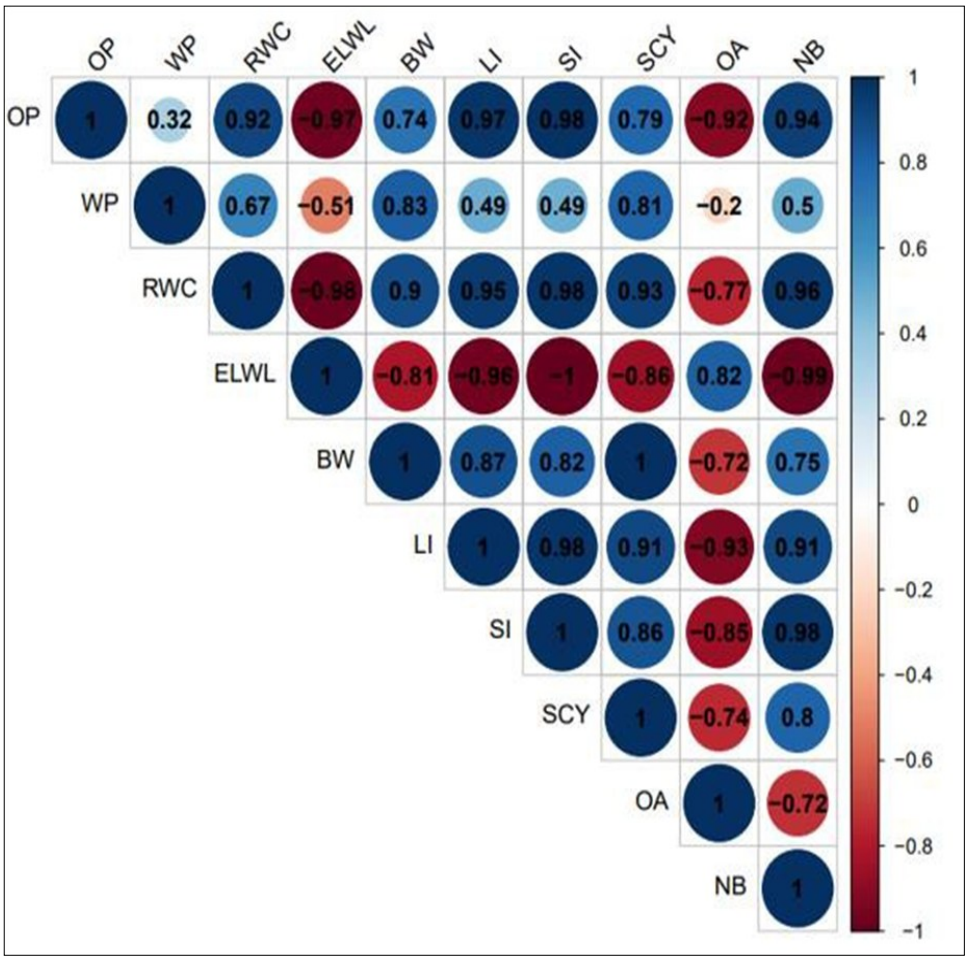

Fig. 3. Correlating plant water status and yield components with yield under stress in TNAU cotton cultures.

OP: Osmotic Potential, WP: Water Potential, RWC: Relative Water Content, ELWL: Excised Leaf Water Loss, BW: Boll Weight, LI: Lint Index, SI: Seed Index, SCY: Seed Cotton Yield, OA: Osmotic Adjustment, NB: Number of Bolls

The above findings are supported by the results of early works where RWC got reduced under drought stress condition in mulberry genotype. Leaf water stress is often measured by ELWL (34). ELWL serves as an indicator of cuticle thickness, as it measures the rate of water loss from leaves after they have been removed from the plant. Therefore, less water loss from excised leaves is a crucial factor for selection of crop plants against drought stress (35). The rate of water loss from excised leaves was negatively impacted by a restricted water supply in all the cotton genotypes (36). Similar results were observed in the present study such as when exposed to drought stress, maximum ELWL was recorded in TVH007 at both squaring (88.04 %) and flowering (90.11 %) stage (Fig. 3). Whereas drought cultures/varieties KC3 recorded lower water loss of 78.18 % and 80.2 % at squaring and flowering respectively.

Leaf area was reduced under drought stress (Fig. 4). Higher reduction in leaf area was recorded in TVH007 (21.33 %) and TVH003 (19.31 %) at stage. Similar trend was observed at flowering stage. Whereas KC3 and TVH002 recorded less reduction in leaf area under drought conditions. These results coincide with the previous reports of reduction in leaf size occurred because of water-deficit stress. This decrease in leaf area was due to impaired mitosis, cell elongation and development.

Physiological and biochemical traits in cotton cultures/ varieties under drought stress

Proline accumulation was increased under water deficit condition in cotton plants. This accumulation of proline during water stress condition is directly associated with stress adaptation in plants (37). The primary osmoregulatory substance in plants during periods of drought stress is proline. It enhances water movement within the plant by lowering the cell's osmotic potential, thus ensuring the maintenance of turgor pressure and facilitating cell growth (38). At squaring stage KC3 recorded 62.06 % increase in proline content followed by TVH002 (57.28 %) which was higher than other TNAU cotton cultures. TVH007 recorded minimum increase in proline content of 46.55 % under drought stress condition. Similar trend was recorded at flowering stage signifying comparatively higher tolerance of TVH002 and KC3 under water deficit condition compared to TVH003 and TVH007. Above result was supported by previous works (39) who recorded similar trend of proline accumulation in cotton leaves under drought stress condition (Fig. 4).

CSI is an important parameter to assess drought stress tolerance (40). CSI was higher in drought-tolerant genotypes when compared to susceptible genotypes (41). KC3 and TVH002 recorded higher CSI under water deficit condition at both squaring and flowering stage indicating its tolerance to stress condition compared to other cultures (Data not shown). TVH007 exhibited maximum CSI reduction percent of 6.07 % during squaring and 7.10 % during flowering among all the cultures/varieties. Higher CSI due to stable chloroplast results in increase photosynthetic rate, dry matter production (42). The above-mentioned results were in accordance with early results (43).

Nitrate reductase (NRase) is regarded as the pivotal enzyme in the overall process of nitrogen assimilation and is essential for the existence of plants (44). Plants exposed to stress experience a reduction in their protein synthesis, leading to a decrease in nitrate reduction activity due to less nitrate influx (45). Decrease in NRase activity was recorded under drought conditions. Flowering stage in cotton recorded higher decrease in NRase activity compared to squaring stage. KC3 showed the least reduction of 5.18 % at squaring and 9.83 % at flowering. However, among the cultures TVH007 recorded highest reduction percentage of 10.86 % and 16.22 % at squaring and flowering respectively. The results are in line with the finding of some previous works (46).

Increase in antioxidant enzyme is directly correlated to drought stress tolerance in plants (47). In accordance with some findings (47), the tolerant genotype KC3 and TVH002 recorded high % increase in catalase activity under drought conditions. KC3 recorded highest increase of 66.27 % during squaring stage and 48.71 % at flowering stage over control. Among the cultures TVH002 (squaring: 51.85 %, flowering: 46.77 %) recorded on par with TVH003 (squaring: 50.60 %, flowering: 46.56 %). However, TVH007 (squaring: 45.96 %, flowering: 44.54 %) recorded comparatively lower increase in catalase activity under drought condition. This result coincides with previous findings (48) who reported FH -114 and FH-326 variety of cotton had much higher catalase activity under drought condition than VH-327, which had significantly lower catalase activity. Plants exposed to drought stress conditions displayed a significant increase in CAT activity (49, 50).

Conclusion

The findings of this study highlight the significant impact of drought stress on cotton growth and yield, demonstrating that its effects are more detrimental compared to heat stress. This is evident from the greater reduction in yield observed under drought conditions. The study confirms that the flowering stage in cotton is more vulnerable to both drought and heat stress compared to the squaring stage, as indicated by the higher percentage reduction in yield at the flowering stage. This suggests that any stress management interventions should be particularly focused on the flowering period to mitigate yield losses effectively. Among the tested pre-release TNAU cotton cultures, TVH002 exhibited strong tolerance to both drought and heat stress, performing on par with the check variety KC3. This makes TVH002 a promising candidate for future cotton cultivation programs in regions prone to climatic stress. Further research and field trials across diverse agro-climatic conditions would be beneficial to validate its resilience and ensure its suitability for large-scale adoption.

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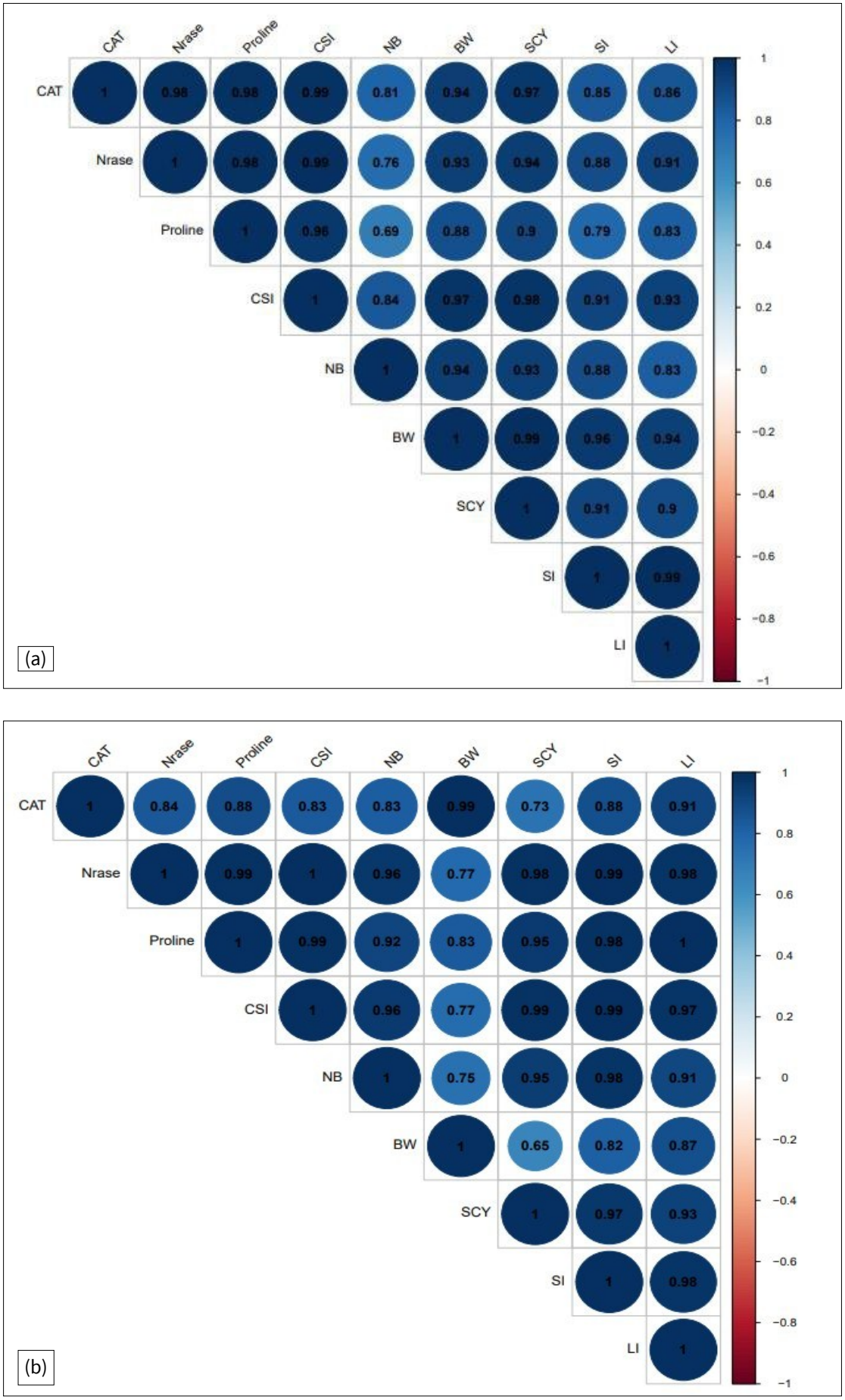

Fig. 4. Correlating biochemical analysis of TNAU cotton cultures with the yield under stress condition. (a) Squaring stage (b) Flowering stage.

CAT: Catalase, NRase: Nitrate Reductase, CSI: Chlorophyll Stability Index, NB: Number of Bolls, BW: Boll Weight, SCY: Seed Cotton Yield, SI: Seed Index, LI: Lint Index

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Authors' contributions

Conceptualization was done by RM and SR. RM, SR and BN carried out the methodology. Formal analysis was carried out by KM and VN. The investigation was done by DR. Resources were gathered by VN. Original draft was prepared by RM, SR and BN. Review and editing was done by RM, SR, BN and KM. All authors read and approved the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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