

**RESEARCH ARTICLE** 



# Morphological, physiological and biochemical trait analysis of maize inbreds under drought conditions

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# Abstract

Maize (Zea mays L.) is a crucial cereal crop that is highly sensitive to drought, which disrupts its morphological, physiological and biochemical traits, impairing photoassimilate allocation. A 2-year field experiment was conducted to assess drought tolerance mechanisms in 6 maize inbred lines-CBM-DL 38, CBM-DL 111, CBM-DL 238, CBM-DL 448, CBM-DL 360 and UMI 1200-by evaluating morphological, physiological, biochemical changes as well as carbohydrate assimilation during sensitive growth stages. Drought stress was applied at different growth stages and observations were recorded 10 days after stress initiation. Drought stress significantly affected growth stages, canopy traits, carbon assimilation and yields. The chlorophyll index decreased by 17-23%, the vegetative index by 33-36% and chlorophyll fluorescence by 47-48%. Meanwhile, the leaf angle increased to 25-30° and the flagging point ratio was reduced to 0.8, resulting in 56-59% yield reduction. However, CBM-DL 38, CBM-DL 111, CBM-DL 448 and CBM-DL 360 demonstrated enhanced drought tolerance, with reduced malondialdehyde (1.5-1.8 times), increased proline (75-93%), improved antioxidant activities [catalase (52-76%), peroxidase (45-57%)] and higher leaf tissue water content (43-59%). Improved leaf architecture enhanced light captures and resource allocation, reducing oxidative damage and maintaining yields. In contrast, CBM-DL 238 and UMI 1200 showed greater reductions in cob weight and 100-grain weight (47-49%). Drought stress during 35-75 days after sowing (DAS) severely impaired photosynthesis, leading to reduced yields. Enhanced canopy traits and biochemical resilience made CBM-DL 38, CBM-DL 111, CBM-DL 448 and CBM-DL 360 more drought-tolerant.

# **Keywords**

drought; maize; photo assimilate mobilization; physio-biochemical; yield traits

# Introduction

Agriculture is highly susceptible to extreme weather and climatic events because it relies heavily on precipitation, temperature and other natural resources. The effects of climate change are evident in agriculture as it slows the growth and development of crops, leading to a yield reduction of 23% in wheat, maize, millet, sorghum and rice by disrupting physiological and biochemical processes (1). Changing precipitation regimes result in drought and may trigger oxidative stress in plants. Drought tolerance refers to plant's ability to sustain physiological and biochemical functions under water-deficit conditions and is often achieved through mitigating oxidative stress and photosynthetic adjustments and stomatal regulation (2).

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Therefore, improving physiological and biochemical traits is critical for maintaining yields under drought conditions. Maize (Zea mays L.) is a C4 cereal crop that is widely cultivated and consumed in tropical, subtropical and temperate regions. In India, maize is grown in all seasons, including kharif (monsoon), post-monsoon, rabi (winter) and spring. A report published by the Ministry of Agriculture and Farmers Welfare 2023-24 revealed that the kharif maize production in India is 224.82 lakh MT, which is 5% greater than the average production (3). Moreover, maize production in Tamil Nadu in 2020 increased from 0.95 million tonnes in 2017 to 2.35 million tonnes in 2020, with an average annual rate of 54.98% (4). Thus, proper water management is essential for maize growth and reproduction to attain maximum yield (5). Water-deficit conditions can lead to yield reductions of 30 to 90% in maize, depending on the severity and timing of exposure. During the reproductive stage, drought may cause yield losses of 21 - 50% in maize crops.

Drought stress negatively affects morphological, physiological and biochemical characteristics, leading to significant yield reductions in maize. Changes such as leaf angle, leaf orientation value (LOV), the F<sub>v</sub>/F<sub>m</sub> ratio, reduced leaf area, leaf area index (LAI) and chlorophyll content may lead to cellular damage in leaves, which leads to reduced carbon assimilation (6). Drought triggers the formation of reactive oxygen species (ROS) (7). The over production of ROS is scavenged by enzymatic antioxidants such as catalase (CAT) and peroxidase (POX). Reduced leaf water content (RWC) may cause cellular and lipid damage (8). Drought at any growth stage can impact plant yield. In maize, specific growth stages (vegetative, flowering and grain filling) that adversely hinder growth, development and pave the way for maximum yield reductions of 25%, 50% and 21% respectively (9).

Based on these observations, it is hypothesized that maize-inbreds possess novel characteristics that enhance morphologically, physiologically and biochemically efficiency and sensitive to drought during various developmental stages. The present study was projected to investigate the morphophysiological and biochemical changes of maize genotypes under drought stress. This study also aimed to identify critical growth stages where drought stress causes significant yield reduction and to screen maize inbreds for their tolerance to drought at these stages.

# **Materials and Methods**

#### Plant materials and screening protocol

Different maize inbred lines were evaluated for drought tolerance during the kharif season (August to November) of 2023. Sensitive growth stages were identified based on morphophysiological traits and confirmed using biochemical studies in the summer (mid-March to June) of 2024. Field trials were conducted at the Eastern Block Farm at Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu, at 11.0168°N latitude, 76.9558°E longitude and 426.72 m above mean sea level. A factorial completely randomized block design (plot size: 30 m × 1.2 m) with three replications was used. Two-year weather parameters were recorded, as shown in Fig. 1a, b.

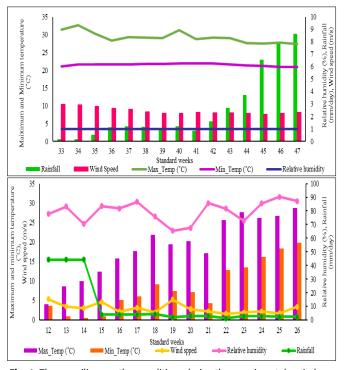


Fig. 1. The prevailing weather conditions during the experimental period are shown:

a) Standard weeks from 37-44 indicate the period of drought treatment during Kharif 2023.

b) Standard weeks from 16-22 indicate the period of drought treatment during the summer of 2024 from the Agro Climate Research Centre (ACRC), TNAU, Coimbatore, Tamil Nadu.

# **Experimental design and treatments**

The first factor was subjected to stress treatments: C: absolute control; drought (D) from 25-45 DAS; D from 35-55 DAS; D from 55 -75 DAS and D from 60-80 DAS. The second factor included 6 maize inbreds (CBM-DL 38, CBM-DL 111, CBM-DL 238, CBM-DL 448, CBM-DL 360 and UMI 1200) sown in mid-August, so the stress imposition stage did not coincide with the northeast monsoon. Four genotypes (CBM-DL 38, CBM-DL 111, CBM-DL 448 and CBM-DL 360) were selected based on their superior morphophysiological characteristics and yield potential, particularly their better physiological performance and efficient translocation of photoassimilates. The growth stages (D from 35-55 DAS and D from 55-75 DAS) with the greatest sensitivity to drought stress were then identified.

#### Soil and irrigation

A drip irrigation system was adopted as the most efficient method for water management, particularly under water-deficit conditions. The experimental soil was texturally categorized as a sandy clay loam with a field capacity of 26.52%, a permanent wilting point of 13.53% and a bulk density of 1.33 g cc<sup>-1</sup>. The pH of the soil was 7.53, with an EC of 0.76 dS m<sup>-1</sup> and a 0.32% organic carbon content. The soil had a low nitrogen content, medium phosphorus content and high potassium content for both seasons of the cropping period during kharif and the summer of 2023 and 2024.

# **Treatment imposition**

Irrigation was arrested by closing the tape for 20 days ( $T_2$ : 25-45 DAS,  $T_3$ : 35-55 DAS,  $T_4$ : 55-75 DAS and  $T_5$ : 60-80 DAS) to impose drought stress *via* the drip system. The soil moisture levels were monitored every 5 days interval by using Delta-T Soil Moisture Kit

(Model: SM150, Delta-T Devices, Cambridge) and the soil moisture data are collected and presented in Table 1a, b. Samples were collected 10 days after the onset of stress to capture dynamic changes in physiological, biochemical and morphological traits during drought stress progression. Based on analyses of morphological traits (leaf area, leaf area index, leaf angle and orientation value, anthesis silking interval-ASI), physiological traits [chlorophyll index, normalized difference vegetation index-(NDVI), photosystem II (PSII) efficiency (F<sub>v</sub>/F<sub>m</sub> ratio), yield and carbon translocation traits of the droughttolerant inbred lines and sensitive growth stages were screened. These findings were further confirmed by analyzing physiological traits (relative water content (RWC), proline (PRO) and biochemical such as malondialdehyde (MDA), catalase (CAT) and peroxidase (POX)) traits in the summer of 2024. The above morpho-physiological and biochemical observations were recorded in tagged plants 10 days after drought was imposed.

#### **Traits recorded**

a. Kharif

The total leaf area was calculated *via* a leaf area meter [( $cm^2$  plant<sup>-1</sup>), LICOR, Model LI 3000]. Leaf area index (LAI) was determined as the ratio of the total leaf area of a single plant to the ground area it occupied (60 cm × 30 cm) following the method outlined previously (10). The anthesis and silking interval (ASI) were calculated as the difference between the tasseling and silking dates.

Canopy traits, i.e., the leaf angle ( $\theta$ ), were measured using a clinometer. The total leaf length (TLL) was calculated by summing the length of the leaf from the beginning of the ligula to the flagging point (LfA) and the length from the flagging point to the leaf tip (LfB), which was expressed as

#### TLL=LfA+LfB.

These measurements were taken from all the leaves of the tagged plants and were used to determine the leaf orientation value (LOV) using the following formula (11):

$$LOV = 1/n \Sigma (90 - \theta) \times (LfA/LL) ni = 1$$

Table 1. Growth stage, time of drought imposition and soil moisture depletion (%)

Where, LL represents the leaf length and n represents the number of leaves measured.

The pre and post anthesis partitioning efficiencies were calculated at the vegetative and harvest stages because they represent critical phases in the plant's growth and resource allocation. At the end of the growing season, 5 plants (above ground parts) in each treatment plot were harvested by hand and separated into stems, leaves and roots, which were air-dried before the grain yield was recorded. All the samples were oven-dried at 105 °C for 30 min and weighed after drying at 70°C to a constant weight. The translocation efficiency (TE) was calculated (12):

$$TE(\%) = [(C_{max} - C_{har})/C_{max}] \times 100$$

Where,  $C_{max}$  is the maximum amount of carbon (C) accumulated in a particular tissue during stress and  $C_{har}$  is the amount of C measured at harvest.

The chlorophyll index was recorded with a Soil Plant Analytical Development (SPAD) meter (Model: Minolta SPAD-502) (13). A chlorophyll fluorescence meter (Model: Opti-Sciences OS1p) was used to measure the PS II efficiency via the dark adaptation method. The third leaf from the top of the plant was fixed with leaf clips for 30 min. After that, the clip slit was opened and the fluorescence instrument automatically calculated in the leaf important parameters of initial fluorescence (F<sub>o</sub>), maximum fluorescence (F<sub>m</sub>), variable fluorescence ( $F_v$ ) and the  $F_v/F_m$  ratio (PS II efficiency). The normalized difference vegetation index (NDVI) was assessed via a green seeker (Trimble) by measuring the amount of reflected near-infrared radiant energy from the plant on the leaf tissues, which reflects the greenness of the plant (14). The instrument was placed above the canopy and readings were recorded 10 days after drought induction in all the treatments.

The accumulated proline content in the leaf (mg  $g^1$ ) was calculated (15) by macerating the sample with 10 mL of 3% sulfosalicylic acid and centrifuging at 3000 rpm for 10 min. The mixture (2 mL of supernatant, acid ninhydrin, glacial acetic acid and orthophosphoric acid) was prepared and incubated in a hot

Treatments	BBCH Scale	Drought period	Soil moisture percentage (%)	
Absolute control	-	-	92.5	
Drought 25 to 45 DAS	15 to 39: Leaf development, stem elongation and booting	14.09.2023 to 04.10.2023	39.5	
Drought 35 to 55 DAS	20 to 51: Advanced stem elongation, tassel begins to emerge	24.09.2023 to 14.10.2023	37.8	
Drought 55 to 75 DAS	51 to 75: Beginning of silk emergence, kernel dough and early dent stage	14.10.2023 to 04.11.2023	37.2	
Drought 60 to 80 DAS	60 to 80+: Flowering to early ripening begins	19.10.2023 to 09.11.2023	35.0	
b. Summer				
	Date of sowing: 18.03.2024			
Treatments	BBCH Scale	Drought period	Soil moisture percentage (%)	
Absolute control	-	-	92.5	
Drought 35 to 55 DAS	20 to 51: Advanced stem elongation, tassel begins to emerge	22.4.2024 to 12.05.2024	71.1	
Drought 55 to 75 DAS	51 to 75: Beginning of silk emergence, kernel dough and early dent stage	12.05.2024 to 02.06.2024	72.1	

Date of sowing: 11.08.2023

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water bath for 1 hr. The contents were subsequently cooled and transferred to a separating funnel, 4 mL of toluene was added to the separating funnel and the mixture was shaken for 30 sec until a pale pink color developed; the color was read at 520 nm *via* a UV spectrophotometer.

The relative water content (RWC) of the leaves was assessed (16) in the leaf discs by fresh weight (Fw), turgid weight [(Tw), immersed in water for 1 hr] and dry weight [(Dw), dried at 70 °C in a hot air oven].

# RWC (%) = $(Fw-Dw) / (Tw-Dw) \times 100$

The MDA content was assessed through the thiobarbituric acid (TBA) reaction (17). Leaf tissue (0.5 g) was homogenized with 5 mL of 0.1% trichloroacetic acid (TCA) and centrifuged at 10000 rpm for 5 min. Approximately 4 mL of 20% trichloroacetic acid (TCA) containing 0.5% thiobarbituric acid (TBA) was added to a 1 mL aliquot and heated at 95 °C for 30 min. Afterwards, the tubes were cooled immediately and centrifuged at 10000 rpm for 10 min at 25 °C, the supernatant was collected and the absorbance (A) was measured at 532 and 600 nm and expressed as nmol g<sup>1</sup> fresh weight.

MDA equivalents (nmol/ml) = {(A532-A600)/155000} × 106

Where, 532 nm represented the maximum absorbance of the TBA-MDA complex, 600 nm the correction for nonspecific turbidity and 155000 the molar extinction coefficient for MDA.

For the preparation of the enzyme extracts used to assess antioxidant enzyme activity, the leaf samples were powdered in liquid nitrogen and mixed with 10 mL of extraction buffer (0.1 M phosphate buffer). After filtration through cheese cloth, the homogenate was centrifuged (15000 rpm) for 20 min at 4°C. The resulting supernatant served as the enzyme extract.

Catalase activity was analyzed (18). Approximately 0.5 mL of 75 mM H<sub>2</sub>O<sub>2</sub>, 1.5 mL of 0.1 M phosphate buffer (pH 7.0) and 50  $\mu$ L of enzyme extract were added to a test tube and the volume of the reaction mixture was increased to 3 mL by adding distilled water. The reaction was started by the addition of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). The decrease in absorbance at 240 nm was recorded every 15 sec for 1 min and catalase activity was computed by calculating the amount of H<sub>2</sub>O<sub>2</sub> decomposed. For catalase activity, the extinction coefficient is 39.4 mM<sup>-1</sup> cm<sup>-1</sup> and is expressed in terms of  $\mu$ g of H<sub>2</sub>O<sub>2</sub> reduced in mg protein<sup>-1</sup>min<sup>-1</sup>.

Peroxidase activity was analysed in the leaves using pyrogallol as a substrate (19). Fresh leaves were collected and immediately placed in an ice bath to prevent enzyme degradation. The preserved leaves were homogenized with cold 0.1 M phosphate buffer (pH 6.8) at a ratio of 500 mg fresh tissue to 10 mL buffer via a prechilled mortar and pestle. The contents were subsequently centrifuged at 5000 rpm for 15 min. The reaction mixture was prepared by combining 1 mL of the leaf extract, 3 mL of pyrogallol solution (0.3%) and 0.5 mL of hydrogen peroxide solution (usually 0.1 M) in a cuvette.

The components were mixed gently and incubated at room temperature for 10-30 min to allow the reaction to proceed. After incubation, the absorbance was measured at 430 nm *via* a spectrophotometer, as this wavelength corresponds to the product formed during the oxidation of pyrogallol by peroxidase. Peroxidase activity was calculated based on the change in absorbance over time. A higher absorbance increase indicates greater enzyme activity. The activity was expressed as the change in the OD at 430 nm min<sup>-1</sup> g<sup>-1</sup>. The yield attributes of cob weight (g) and 100-grain weight (g) were recorded via an electronic balance after harvest.

# **Statistical Analysis**

For statistical analysis, IBM SPSS Statistics version 23.0 (http:// www.spss.com) software was used. The mean values of each characteristic were determined using Duncan's multiple range test, different letters adjacent to data in the same column indicate significance at  $P \le 0.05$  and two-way analysis of variance (ANOVA) was used to determine their significance (5%). Fairly close correlation between parameters were revealed by correlation analysis with the same package. Furthermore, figures were generated *via* GraphPad Prism 8 software.

# Results

The drought-stressed inbred lines CBM-DL 448, CBM-DL 111, CBM-DL 360 and CBM-DL 38 exhibited larger leaf laminae with areas and corresponding LAIs of 8512.4 cm<sup>2</sup> plant<sup>1</sup>/4.7, 7656 cm<sup>2</sup> plant<sup>1</sup>/4.3, 6161.6 cm<sup>2</sup> plant<sup>1</sup>/3.4 and 4882.4 cm<sup>2</sup> plant<sup>-1</sup>/2.7 respectively (Fig. 1a, b). In contrast, CBM-DL 238, with 4189.2 cm<sup>2</sup> plant<sup>-1</sup>/2.3 and UMI 1200, with 3479.5 cm<sup>2</sup> plant<sup>-1</sup>/1.9, exhibited

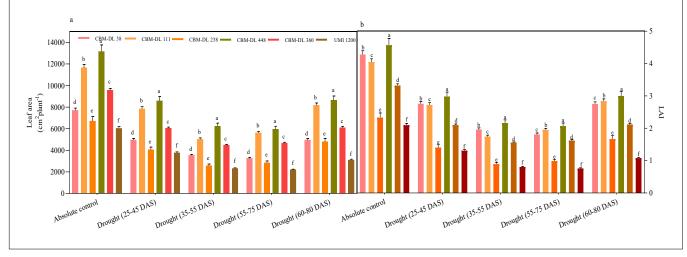


Fig. 2. Effects of drought on the leaf area [a, (cm<sup>2</sup> plant<sup>-1</sup>)] and [b, leaf area index [b, (LAI)]. There is no statistical difference between means shown with the same letters in the same column (P≤ 0.05).

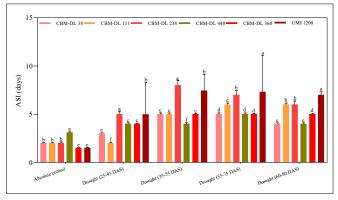
the lowest leaf area and LAI (Fig. 2a, b). Regardless of the inbreds, drought during 35 to 55 DAS and 55 to 75 DAS, resulted in significant percentage reduction (45 and 65% respectively), leading to substantial yield losses.

Canopy traits, including leaf angle, were greater in the control plants compared to drought-susceptible plants, with leaf angles decreasing under drought conditions (Fig. 3a, b). Among the 6 maize inbreds, CBM-DL 238 and UMI 1200 recorded wider leaf angle than CBM-DL 38, CBM-DL 111, CBM-DL 448 and CBM-DL 360. In terms of LOV, CBM-DL 38, CBM-DL 111 and CBM-DL 448 recorded optimum values, but CBM-DL 360 had a higher LOV.

The anthesis-silking interval (ASI) is a crucial trait in maize as it directly influences pollination synchronization and kernel development. Maize inbred lines showed a significant extent in the ASI ranging from 2-7 days under drought (Fig. 4). CBM-DL 38, CBM-DL 111, CBM-DL 448 and CBM-DL 360 recorded 4 days, while CBM-DL 238 and UMI 1200 recorded 6 days for ASI. Among the growth stages, drought during 35-55 DAS and 55-75 DAS (>5 days) led to significant changes compared to the control.

Physiological traits such as chlorophyll index, normalized difference vegetation index (NDVI) and chlorophyll fluorescence, were significantly altered ( $P \le 0.05$ ) during different growth stages

of maize inbred lines (Fig. 5). Drought at 35-55 DAS and 55-75 DAS resulted in greater reduction in the chlorophyll index (23% and 26%), NDVI (33% and 36%) and  $F_v/F_m$  ratio (47% and 48%), respectively (Fig. 5a-c). Among the inbred CBM-DL 448 (46.3), CBM-DL 360 (44.9), CBM-DL 111 (44.0) and CBM-DL 38 (43.6) lines presented the highest chlorophyll indices, whereas the UMI 1200 (37.5) and CBM-DL 238 (34.8) inbred lines presented reduced chlorophyll content. In terms of the NDVI, a greater percentage reduction was observed in CBM-DL 238 (35%) and UMI 1200



**Fig. 4.** Effects of drought on the anthesis silking interval (days, ASI). In Duncan's multiple range test, different letters next to data within the same column indicate a significant difference at  $P \le 0.05$ .

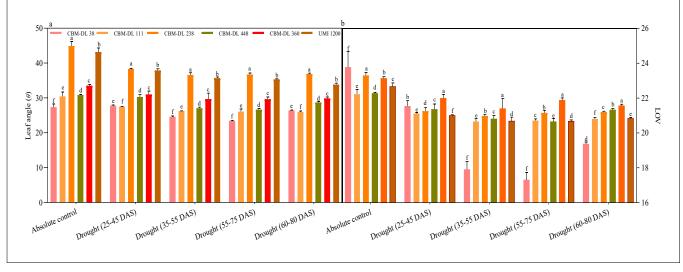


Fig. 3. Effects of drought on the leaf angle  $[a, (\theta)]$  and leaf orientation value [b, (LOV)]. In Duncan's multiple range test, different letters next to data within the same column indicate a significant difference at P  $\leq$  0.05.

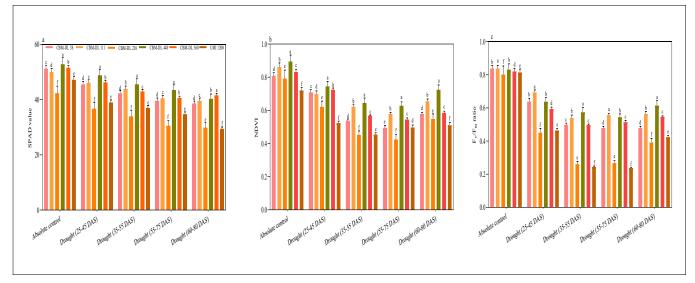


Fig. 5. Effects of drought on the chlorophyll index [a, (SPAD value)], normalized difference vegetative index [b, (NDVI)] and chlorophyll fluorescence [c,  $(F_v/F_m ratio)]$ . In Duncan's multiple range test, the presence of different letters next to data in the same column a significantly different at P $\leq$  0.05.

#### (31%) under drought.

Drought significantly altered chlorophyll fluorescence in control plants, initially showing an increasing trend followed by a decrease after drought exposure. Among the six maize inbred lines, a greater  $F_v/F_m$  ratio was recorded for CBM-DL 448 and CBM -DL 111, with a value of 0.64, which was statistically significant and a lower  $F_v/F_m$  ratio was recorded for the UMI 1200 (0.43) and CBM-DL 238 (0.43) inbred lines.

Biomass and carbon assimilation in different maize inbred lines was recorded during the drought periods and at harvest stage, with the values presented in Table 2. The biomass of different parts of maize inbred plants (leaf, stem and root) showed significant differences during drought conditions, with significantly greater reductions detected at 35-55 DAS and 55-75 DAS in all the stressed inbred lines. The percentage of carbon translocation varied significantly between the leaves and stems (Fig. 6). Leaf's contribution to biomass development decreased by (24%) under drought at 25-45 DAS, followed by drought at 60-80 DAS (26%). Stem contribution was reduced by (15%) under drought at 55-75 DAS. Under drought conditions, the root contribution was greater (>30%) than that under all the treatments except drought at 35-55 DAS (28%).

Regardless of drought stress, the percentage of translocation to economic parts was as follows: CBM-DL 38 (26%), CBM-DL 111 (31%), CBM-DL 238 (26%), CBM-DL 360 (19%), CBM-DL 448 (34%) and UMI 1200 (31%). Like carbon assimilation,

In the confirmation trial (experiment 2), physiological such as relative water content (RWC), proline (PRO) and biochemical like malondialdehyde (MDA), catalase (CAT) and peroxidase (POX) traits were evaluated. Compared with the control, drought at different growth stages caused significant changes in maize leaves. A rapid reduction was recorded during the 35-55 DAS (52.4%) and drought 55-75 DAS (37.6%) drought stages. However, CBM-DL 38 (60.2%), CBM-DL 111 (58.6%), CBM-DL 448 (63%) and CBM-DL 360 (60.5%) inbred lines maintained high RWC under drought.

Analysis of variance (ANOVA) for proline revealed significant differences (P≤0.05) between the stress level and inbred line. The results of the mean comparison revealed that drought stress increased the osmolyte content (Table 3a). The increased concentration of PRO was recorded from 35-55 DAS (61 mg g<sup>-1</sup>) and 55-75 DAS (67.4 mg g<sup>-1</sup>). Unlike CBM-DL 38 (51.2 mg g<sup>-1</sup>), CBM-DL 111 (57.1 mg g<sup>-1</sup>), CBM-DL 448 (54 mg g<sup>-1</sup>) and CBM-DL 360 (55.4 mg g<sup>-1</sup>) inbreds.

Drought significantly increased the MDA content in maize inbred lines (P $\leq$ 0.05) (Table 3b). The lowest MDA content was recorded in CBM-DL 38 [0.34 µmol g<sup>1</sup> fresh weight (FW)], CBM-DL 360 (0.36 µmol g<sup>1</sup> FW), CBM-DL 111 (0.38 µmol g<sup>1</sup> FW) and CBM-DL 448 (0.46 µmol g<sup>1</sup> FW) during experiment II.

Table 2. Effects of drought on carbon accumulation in different plant parts (stem, leaves and roots)

						Ster	n (g plant <sup>-1</sup> )							
Treatmen	ts	Absolute control		Drought (25-45 DAS)				i) Drougł	Drought (55-75 DAS)		Drought (60-80 DAS)		Mean	
	S	tress Har	vest S	tress	Harvest	Stress	Harvest	Stre	ss Harve	st Stress	Harvest	Stress	Harvest	
CBM-DL 38	22.6ª	17	.8ª 1	.6.0 <sup>c</sup>	12.5°	13.2 <sup>d</sup>	11.1 <sup>d</sup>	12.2 <sup>e</sup>	11.7 <sup>e</sup>	16.3 <sup>b</sup>	13.2 <sup>b</sup>	16.1	13.3	
CBM-DL 111	20.6ª	15	.9ª	.8.8 <sup>c</sup>	11.7°	15.4 <sup>d</sup>	10.7 <sup>e</sup>	13.8 <sup>e</sup>	11.2 <sup>d</sup>	19.1 <sup>b</sup>	12.3 <sup>b</sup>	17.5	12.4	
CBM-DL 238	13.5ª	11		.2.2 <sup>b</sup>	8.0 <sup>c</sup>	9.8 <sup>d</sup>	7.2 <sup>e</sup>	8.5 <sup>e</sup>	7.4 <sup>d</sup>	12.2 <sup>b</sup>	8.4 <sup>b</sup>	11.2	8.5	
CBM-DL 448	21.7ª	18	.1ª 1	9.9°	13.5°	16.5 <sup>d</sup>	12.5°	14.6 <sup>e</sup>	13.1 <sup>d</sup>	20.1 <sup>b</sup>	14.0 <sup>b</sup>	18.6	14.2	
CBM-DL 360	18.5ª	13	.9ª 1	.7.1 <sup>b</sup>	10.4 <sup>c</sup>	13.9 <sup>d</sup>	9.1 <sup>e</sup>	12.4 <sup>e</sup>	10.1 <sup>d</sup>	17.1 <sup>b</sup>	10.9 <sup>b</sup>	15.8	10.9	
UMI 1200	14.7ª	10	.1ª 1	.3.3 <sup>b</sup>	7.5°	10.6 <sup>d</sup>	6.7 <sup>e</sup>	9.4 <sup>e</sup>	7.2 <sup>d</sup>	13.3 <sup>b</sup>	8.0 <sup>b</sup>	12.3	7.9	
Mean	18.6	14		16.2	10.6	13.2	9.5	11.8	10.1	16.3	11.1			
	Stress Harvest													
CD (P≤0.05)		G		Т		G×T			G		Т	G	i×Т	
. ,		0.7		0.	6		1.5		0.2		0.1	(	).4	
	Leaves (g plant <sup>-1</sup> )													
	Absolut	te control	Droug	ht (25-	45 DAS)	Drought	(35-55 DAS)	Drought	(55-75 DAS)	Drought (e	0-80 DAS)	Me	ean	
Treatments -	Stress		t Stres		rvest	Stress	Harvest	Stress	Harvest		Harvest	Stress	Harvest	
CBM-DL 38	28.7ª	22.3ª	25.	1 <sup>c</sup>	18.5°	22.8 <sup>d</sup>	15.3 <sup>d</sup>	21.4 <sup>e</sup>	15.0 <sup>e</sup>	26.3 <sup>b</sup>	18.8 <sup>b</sup>	24.9	18.0	
CBM-DL 111	33.4ª	27.5ª	29.	5 <sup>c</sup>	22.9°	26.2 <sup>d</sup>	18.6 <sup>d</sup>	25.1°	18.1 <sup>e</sup>	30.8 <sup>b</sup>	23.4 <sup>b</sup>	29.0	22.1	
CBM-DL 238	22.4ª	17.8ª	18.		13.8°	17.1 <sup>d</sup>	11.6 <sup>d</sup>	15.8 <sup>e</sup>	11.1 <sup>e</sup>	20.3 <sup>b</sup>	14.3 <sup>b</sup>	18.9	13.7	
CBM-DL 448	27.3ª	23.8ª	24.	2 <sup>c</sup>	19.6°	22.0 <sup>d</sup>	15.9°	20.6 <sup>e</sup>	16.0 <sup>d</sup>	25.1 <sup>b</sup>	20.3 <sup>b</sup>	23.8	19.1	
CBM-DL 360	26.9ª	20.7ª	23.	5 <sup>c</sup>	17.1 <sup>c</sup>	21.4 <sup>d</sup>	14.0 <sup>d</sup>	20.1 <sup>e</sup>	13.4 <sup>e</sup>	24.6 <sup>b</sup>	17.3 <sup>b</sup>	23.3	16.5	
UMI 1200	24.9ª	20.4ª	21.	3°	16.2 <sup>c</sup>	19.1 <sup>d</sup>	14.0 <sup>d</sup>	18.0 <sup>e</sup>	12.6 <sup>e</sup>	22.5 <sup>b</sup>	16.6 <sup>b</sup>	21.2	15.9	
Mean	27.3	22.1	23.		18.1	21.4	14.9	20.2	14.4	24.9	18.4			
				Stress	5					Harv	est			
CD (P≤0.05)		G		Т		G×T		G		٦	-	G×T		
. ,	0.3			0.3			0.7		0.2	0.	2	0	.5	
							Root (g	plant⁻¹)						
Treatments	reatments Absolute control					Drought (	35-55 DAS)	Drought	(55-75 DAS)	Drought	60-80 DAS	) М	ean	
	Stress	Harvest	Stress	Har	vest	Stress		Stress	Harvest	Stress	Harvest	Stress	Harvest	
CBM-DL 38	18.3ª	12.8ª	15.6 <sup>c</sup>	10	.3°	14.0 <sup>d</sup>	9.5 <sup>d</sup>	14.0 <sup>d</sup>	9.1 <sup>e</sup>	17.1 <sup>b</sup>	10.8 <sup>b</sup>	15.8	10.5	
CBM-DL 111	22.7ª	13.7ª	19.2 <sup>c</sup>	11	.4 <sup>c</sup>	17.4 <sup>e</sup>	10.9 <sup>d</sup>	17.6 <sup>e</sup>	10.2 <sup>e</sup>	21.1 <sup>b</sup>	12.3 <sup>b</sup>	19.6	11.7	
CBM-DL 238	11.7ª	8.7ª	9.6°	7.	0 <sup>c</sup>	8.6 <sup>d</sup>	6.4 <sup>d</sup>	8.6 <sup>d</sup>	6.2 <sup>e</sup>	10.4 <sup>b</sup>	7.3 <sup>b</sup>	9.8	7.1	
CBM-DL 448	19.8ª	17.4ª	16.9°	14	.4 <sup>c</sup>	15.0 <sup>d</sup>	13.9 <sup>d</sup>	15.0 <sup>d</sup>	13.1 <sup>e</sup>	18.8 <sup>b</sup>	15.6 <sup>b</sup>	17.1	14.9	
CBM-DL 360	19.4ª	11.5ª	16.6 <sup>c</sup>	9.	6 <sup>c</sup>	14.8 <sup>d</sup>	9.0 <sup>e</sup>	14.8 <sup>d</sup>	9.3 <sup>d</sup>	18.2 <sup>b</sup>	10.2 <sup>b</sup>	16.8	9.9	
UMI 1200	13.9ª	9.4ª	11.6°	7.	7 <sup>c</sup>	10.3 <sup>d</sup>	<b>7.4</b> <sup>d</sup>	10.3 <sup>d</sup>	7.1 <sup>e</sup>	13.0 <sup>b</sup>	8.3 <sup>b</sup>	11.8	8.0	
Mean	17.6	12.2	14.9	10	).1	13.3	9.5	13.4	9.2	16.4	10.8			
				Stres	s					Harvest				
CD (P≤0.05)		G T		G	Б×Т		G		Т		G×T			
	0.2			0.2		0.4		0.2		0.2		0.4		

Duncan's multiple range test was used for statistical analysis. Different letters adjacent to values in the same column indicate significant differences at p ≤ 0.05.

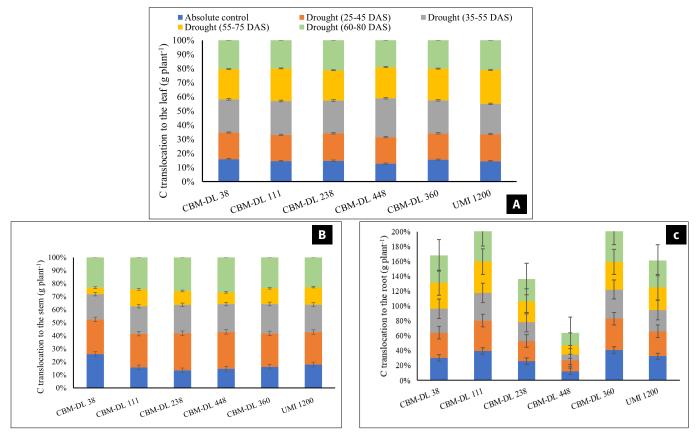


Fig. 6. Effects of drought on the carbon translocation rates (%) in the leaves (a), stems (b) and roots (c) of maize inbred lines. DAS: days after sowing.

**Table 3a.** Effects of drought on the physiological traits of maize inbred plants. The mean data of three replications and the interactions between factor I [control, drought (35-55 DAS), drought (55-75 DAS)] and factor II (CBM-DL 38, CBM-DL 111, CBM-DL 448, CBM-DL 360) are presented. For Duncan's multiple range test, different letters adjacent to the data in the same column indicate significance at  $P \le 0.05$ . DAS: days after sowing, NS: Nonsignificant

	I	Relative water	content (%)	Proline (µg g⁻¹ of fresh weight)					
Treatments		Drought (DAS)				Droug			
	Control	35-55	55-75	- Mean	Control	35-55	55-75	– Mean	
CBM-DL 38	93.4ª	48.5 <sup>d</sup>	38.6 <sup>b</sup>	60.2	32.8 <sup>d</sup>	58.0 <sup>d</sup>	62.7 <sup>d</sup>	51.2	
CBM-DL 111	92.2 <sup>b</sup>	50.5°	33.2 <sup>d</sup>	58.6	36.7ª	64.0ª	70.5ª	57.1	
CBM-DL 448	90.8°	56.3ª	41.8ª	63.0	35.1 <sup>b</sup>	59.9°	67.1 <sup>c</sup>	54.0	
CBM-DL 360	90.4 <sup>d</sup>	54.1 <sup>b</sup>	36.9 <sup>c</sup>	60.5	35.0 <sup>c</sup>	62.1 <sup>b</sup>	69.2 <sup>b</sup>	55.4	
Mean	91.7	52.4	37.6		34.9	61.0	67.4		
	G	Т	G×T		G	Т	G×T		
CD (P≤0.05)	2.2	1.9	3.9		3.2	2.8	NS		

**Table 3b.** Effects of drought on the biochemical traits of maize inbred plants. The mean data of three replications and the interactions between factor I [control, drought (35-55 DAS)] and factor II (CBM-DL 38, CBM-DL 111, CBM-DL 448, CBM-DL 360) are presented. By Duncan's multiple range test, different letters adjacent to data in the same column indicate significance at  $P \le 0.05$ . DAS: days after sowing, NS: Nonsignificant

Treatments <sup>-</sup>	Malondialdehyde content (µmol g <sup>.1</sup> fresh weight)				с	atalase activ	Peroxidase activity (ΔA430 nm min <sup>-1</sup> g <sup>-1</sup> )					
	Control	Drought (35-55 DAS)	Drought (55-75 DAS)	Mean	Control	Drought (35-55 DAS)	Drought (55-75 DAS)	Mean	Control	Drought (35-55 DAS)	Drought (55-75 DAS)	Mean
CBM-DL 38	0.12 <sup>d</sup>	0.40 <sup>c</sup>	0.50 <sup>c</sup>	0.34	18.2 <sup>c</sup>	29.0 <sup>b</sup>	34.8 <sup>b</sup>	27.3	14.9 <sup>c</sup>	19.4 <sup>d</sup>	21.0 <sup>d</sup>	18.4
CBM-DL 111	0.22 <sup>b</sup>	0.40 <sup>c</sup>	0.51 <sup>b</sup>	0.38	15.7 <sup>d</sup>	28.6 <sup>c</sup>	31.9 <sup>d</sup>	25.4	15.6 <sup>b</sup>	23.1 <sup>b</sup>	24.7 <sup>b</sup>	21.1
CBM-DL 448	0.23ª	0.55ª	0.60ª	0.46	27.0ª	30.9ª	35.5°	31.1	14.3 <sup>d</sup>	24.8ª	24.6°	21.2
CBM-DL 360	0.19 <sup>c</sup>	0.45 <sup>b</sup>	0.44 <sup>c</sup>	0.36	18.9 <sup>b</sup>	28.5 <sup>d</sup>	33.8 <sup>c</sup>	27.1	17.0ª	21.9 <sup>c</sup>	26.6ª	21.8
Mean	0.19	0.45	0.51		20.0	29.3	34.0		15.5	22.3	24.2	
	G	Т	G×T		G	Т	G×T		G	Т	G×T	
CD (P≤0.05)	0.02	0.02	0.04		1.6	1.4	2.8		1.2	1.1	2.1	

CAT activity generally increased under drought, but inbred lines presented different responses in their CAT activity. The greatest values under drought conditions were detected in CBM-DL 448, followed by CBM-DL38, CBM-DL 360 and CBM-DL 111 (Table 3b). On the other hand, drought at 55-75 DAS resulted in increased CAT activity. The greatest POX activities were observed in CBM-DL 360, CBM-DL 448, CBM-DL 111 and CBM-DL 38 (21.8, 21.2, 21.1 and 18.4 changes in  $\Delta$ A430 nm min<sup>-1</sup>g<sup>-1</sup>) respectively.

Yield attributes including 100-grain weight and cob weight, were significantly lower in the drought treatment compared to the control (Fig. 7). During the drought 35-55 DAS and 55-75 DAS caused significant percentage reductions in cob weight (56-59 % respectively) and 100-grain weight (56-59% respectively). Among the stressed plants, the cob weight of the CBM-DL 38 (38%), CBM-DL 111 (37%), CBM-DL 448 (39%) and CBM-DL 360 (39%) lines presented relatively lower percentage reduction. A similar trend was observed for 100-grain weight. UMI 1200 and CBM-DL 238

resulted in greater percentage reductions in cob weight (49% and 47%) and 100-grain weight (49% and 47%).

The correlation analysis results (Fig. 8) revealed a significant ( $P \le 0.05$ ) relationship among the morphophysiological and yield traits. The strength of the correlation is illustrated by 2 different color shades. Dark green color indicates the strong positive correlation (+1), dark peach color indicates the strong negative correlation (-1) and the lighter shades indicate the weaker strength among the traits. From the analysis, morphological traits (leaf area and LAI) were strongly positively correlated with the physiological traits such as chlorophyll index, NDVI, chlorophyll fluorescence (r = 0.7, 0.9 and 0.8 respectively) and yield traits like cob weight and 100-grain weight (r = 0.8 and 0.4) under drought. The ASI was negatively correlated with the yield attributes (r= -0.5, -0.7). The leaf angle was negatively correlated with 100-grain weight and weakly correlated with the cob weight of maize inbred plants under drought.

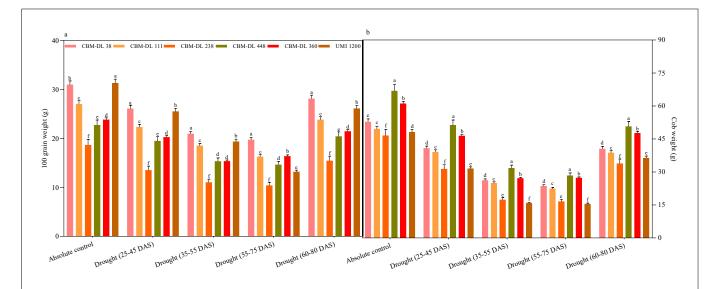
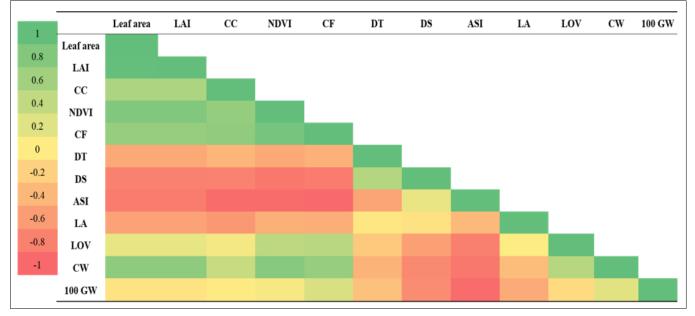


Fig. 7. Effects of drought on 100-grain weight [a, (g)] and cob weight [b, (g)]. For Duncan's multiple range test, different letters adjacent to the data in the same column indicate significance at P≤0.05.



**Fig. 8.** The correlogram shows the strength and direction of the linear relationship between pairs of variables. LAI: leaf area index, CC: chlorophyll index (SPAD value), NDVI: normalized difference vegetative index, CF: chlorophyll fluorescence (F<sub>v</sub>/F<sub>m</sub> ratio), ASI: anthesis silking interval, LA: leaf angle, LOV: leaf orientation value, CW: Cob weight, 100 GW: 100 grain weight.

# Discussion

Drought stress affects the cellular processes such as cell division and expansion that are responsible for leaf growth. Under waterlimited conditions, these processes are inhibited, leading to a reduction in leaf area by 30% to 60% compared with wellwatered condition, particularly in environments with a high vapor pressure deficit (VPD) (20). In the present study, it was revealed that drought-stressed CBM-DL 448, CBM-DL 111, CBM-DL 360 and CBM-DL 38 inbred lines exhibited larger leaf area and higher LAI (Fig. 1a, b). These traits enabled efficient utilization of available sunlight and space for with maximum photosynthesis, contributing to increased carbohydrate production under drought stress (21). However, CBM-DL 238 and UMI 1200 inbred lines demonstrated the lowest leaf area and LAI (Fig. 2a, b). This reduction could be attributed to the adverse stress conditions experienced by plants, which likely accelerated chlorophyllase (22), reduced CO<sub>2</sub> assimilation and impaired the photosynthetic rate, ultimately hindering leaf development. Hence, the two inbred lines (CBM-DL 238 and UMI 1200) used in the present experiment did not have the capacity to perform under drought. This decrease in leaf area directly affected the plant's ability to photosynthesize, leading to reduced growth rates and lower biomass production (23). Among the growth stages, the periods from 35 to 55 DAS and 55 to 75 DAS were particularly sensitive to drought leading to significant loss in grain yield. These results show that, drought during the reproductive phase (silking and milking) caused the greatest decrease in leaf area (over 25%) in maize plants compared to the seedling and joining stages, aligning with findings from other study (24). Based on their larger leaf area, higher LAI and potential for increased carbohydrate production under drought, CBM-DL 448, CBM-DL 111, CBM-DL 360 and CBM-DL 38 maize inbred lines can be considered drought-tolerant (25). The earlier findings corroborated well with the present study.

Canopy traits, including the leaf angle and LOV, are critical for understanding plant responses to drought. These traits influence how plants capture sunlight, manage water use and ultimately affect their growth and yield under stress. The efficiency of light capture and photosynthesis in maize ideotypes is significantly influenced by leaf orientation, structure and size. The leaf characteristics of the leaf angle and LOV are important factors that profoundly impact on plants light capture capacity. The results of the present study revealed that among the six maize inbreds, lines with compact and well-oriented leaf angles, like those seen in CBM-DL 38, CBM-DL 111 and CBM-DL 448, maintain photosynthetic efficiency better under drought stress. This aligns with modern breeding strategies focused on compact leaf architecture to enhance tolerance to high planting densities and drought conditions, which also improves yield stability under stress (26). Moreover, lines like CBM-DL 360, which exhibit a higher LOV, demonstrate that their leaves are optimally positioned to intercept light, making them efficient in photosynthesis during drought. These traits collectively contribute to better drought tolerance, as plants are better adapted to use available resources, particularly under waterlimited environments (27).

Optimized leaf orientation enhances photosynthetic efficiency, ensuring that plants can utilize available light more effectively for growth and carbohydrate production.

Additionally, plants adjust their leaf orientation to minimize water loss through transpiration while maximizing light capture. A previous study supports this notion, revealing that changes in leaf angle and orientation value are important adaptive mechanisms for optimizing light interception and water use efficiency (WUE) in plants under drought conditions (28). The observed changes in leaf angle and orientation value under drought stress align with the findings of the role of the above traits in optimizing light interception and WUE (28). Thus, based on these architectural advantages, CBM-DL 38, CBM-DL 111, CBM -DL 448 and CBM-DL 360 can be considered drought-tolerant lines.

Drought can significantly impact the flowering and pollination process in maize affecting both the number of days to flowering and ASI (29). This might be due to the greater sensitivity of maize to drought stress at the flowering stage, which can lead to a marked delay in silk extrusion and significant extension of the ASI. A considerable extent of ASI was recorded during drought at 35-55 DAS and 55-75 DAS in all inbred lines. Drought during the flowering stage can delay or inhibit plant growth and tassel development, ultimately leading to a prolonged ASI, which ultimately reduces the grain yield of maize (29). Early flowering is an important trait observed in the drought-tolerant inbred lines CBM-DL 38, CBM-DL 111, CBM-DL 448 and CBM-DL 360. The findings of this study are consistent with the results reported in other study (30). Physiological traits, such as chlorophyll index, NDVI and F<sub>v</sub>/F<sub>m</sub> ratio, in maize under drought are essential for assessing plant health, understanding photosynthetic efficiency, guiding breeding efforts, enabling early stress detection and ensuring food security in the face of climate change. Drought during different growth stages of maize led to a reduction in physiological parameters namely chlorophyll index, NDVI and F<sub>v</sub>/ F<sub>m</sub> ratio. A decrease in the chlorophyll index is one of the earliest responses caused by drought. It was reported that the NDVI was related to changes in the chlorophyll index (31). The NDVI measures leaf expansion and determines the ability of plants to intercept light and convert it into biomass, which is directly correlated with the amount of chlorophyll present, which can decrease in response to drought stress. In the present study, drought at tasseling and silking stages resulted in greater reduction in the chlorophyll index, NDVI and F<sub>v</sub>/F<sub>m</sub> ratio.

Among the inbreds CBM-DL 448, CBM-DL 360, CBM-DL 111 and CBM-DL 38 lines presented the highest chlorophyll index, whereas the UMI 1200 and CBM-DL 238 inbred lines recorded a reduced value. The decline in the chlorophyll (SPAD) under severe drought stress might be due to the inhibition of carbon and nitrogen metabolic activities, which causes carbon and nitrogen deficiency and affects the development of chloroplast in yellow plant leaves (32). In terms of the NDVI, a greater percentage reduction was observed in CBM-DL 238 (35%) and UMI 1200 (31%) inbred lines under drought. Similar findings were reported in pea leaves under drought conditions, in which chlorophyll levels decreased substantially, possibly due to impairment of the photosynthetic apparatus or activation of chlorophyll-degrading enzymes such as phosphoenolpyruvate carboxylase and ribulose-1,5-bisphosphate carboxylase (33). This might be the reason behind the lowest chlorophyll index and NDVI in maize inbred lines under drought stress. The present investigation supports earlier findings.

Chlorophyll fluorescence can reflect the efficiency of the reaction center of the photosystem II (PSII) by analyzing plant responses to light absorption and carbon assimilation. The Fv/Fm ratio can be affected by drought, indicating reduced photosynthetic efficiency. The present study revealed a significant difference in the control plants, with an increasing trend observed and a decreasing trend recorded in drought. This reduction might be due to the water-limited condition, where photoelectron plants can deactivate transport and photophosphorylation, leading to a decrease in the captured energy leads to a reduction in photosynthetic efficiency (31). Based on the findings of the present study, the maize inbred lines CBM-DL 448, CBM-DL 360, CBM-DL 111 and CBM-DL 38, which recorded lesser reductions in chlorophyll index, Fv/Fm ratio and NDVI under drought stress, can indeed be considered droughttolerant. On the other hand, CBM-DL 238 and UMI 1200, which exhibited significant reductions in these traits, can be classified as drought-sensitive. The results of the present study in wheat for drought tolerance (34).

The partitioning and transportation of assimilates play a crucial role in the reproductive growth and development of maize, especially during drought, where insufficient assimilates supply to the ear can result in substantial grain yield reduction (6). A reduced aboveground biomass contribution under drought stress was an adaptive strategy employed by the plant to maintain overall plant productivity. Drought stress leads to a decrease in biomass, particularly stem and leaf, as a result it adversely affects plant growth, flowering and yield in maize (35). Additionally, in the present study increased root biomass and reduced leaf biomass were observed in the drought stressed maize inbred lines, which is consistent with the findings (35).

During periods of drought stress, crops tend to allocate biomass preferentially to their root systems to increase their water absorption ability while simultaneously decreasing the allocation of dry matter to stems and leaves above ground, thereby reducing transpiration and water loss. This strategy, which involves decreasing aboveground biomass distribution while increasing root biomass, is a mechanism employed by plants to adapt to drought conditions. The present study results revealed that, during the harvest stage, the biomass of all the plant parts decreased. The findings revealed that the produced photoassimilates were efficiently translocated to sink tissues ensuring that yield was maintained in drought-stressed inbred plants (36).

Under water stress conditions, a decrease in photoassimilates results in reduced plant biomass. The exposure of maize to drought stress during the mature stage can directly reduce yield to a considerable extent (5). In addition, exposure of maize to drought at the tasseling stage also results in significant yield loss. Compared with the other inbred lines, the four inbred lines (CBM- DL 38, CBM-DL 111, CBM-DL 448 and CBM-DL 360) used in the present study presented greater aboveground biomasses, which suggests their ability to allocate resources to sinks (cobs) under drought stress. However, the CBM-DL 238 and UMI 1200 inbred lines showed poor economic translocation, which may have caused a yield reduction. In addition, a reduction in root growth capacity is also observed under drought conditions, which might be due to disruption of the plant water relationship (35).

From the six inbred lines, four maize inbred lines (CBM-

DL 38, CBM-DL 111, CBM-DL 448 and CBM-DL 360) were selected and considered drought tolerant lines. These lines were confirmed (summer-2024) with their physiological traits (Table 3a), i.e., relative water content (RWC) and proline (osmolyte) content and biochemical traits (Table 3b), i.e., malondialdehyde (MDA) content and antioxidant activity, catalase (CAT) and peroxidase (POX) activity. Under drought, a decrease in leaf RWC leads to physiological changes, including stomatal closure, to minimize water loss. This response can limit photosynthesis and overall plant growth. In the present study, maize lines (CBM- DL 38, CBM-DL 111, CBM-DL 448 and CBM-DL 360) that maintain higher RWC during drought stress are better able to sustain photosynthetic activity and growth, ultimately leading to improved drought resilience (37). The RWC of drought-tolerant inbreds can be restored after drought stress without significant cellular damage, resulting in resilience with effective osmotic adjustment and turgor maintenance mechanisms (38).

Proline (PRO) is a crucial amino acid and an important physiological compound that plays a significant role in plant responses to drought stress. The accumulation of PRO is associated with the physiological adaptation in plants under drought conditions. PRO helps to regulate stomatal conductance, allowing plants to manage water loss while facilitating the gas exchange necessary for photosynthesis. High PRO levels are correlated with improved physiological traits, such as increased chlorophyll content and enhanced photosynthetic efficiency, which are critical for plant survival during drought (39). The findings of the current study indicate that the inbred lines CBM-DL 38, CBM-DL 111, CBM-DL 448 and CBM-DL 360 performed similarly. Malondialdehyde (MDA) is a lipid peroxidation product and an indicator of oxidative damage induced by drought stress. Under drought stress, MDA content can increase approximately 1.2-fold, 1.8-fold and 2.9-fold compared with that of control plants subjected to 75%, 50% and 30% field capacity, respectively (8). In general, drought-tolerant genotypes accumulate lower MDA levels than sensitive genotypes thus, the four genotypes (CBM-DL 38, CBM-DL 360, CBM-DL 111 and CBM-DL 448) exhibited the lowest degree of cell damage (40). Studies revealed that oxidative damage was lower in cultivars tolerant to drought stress, thus less lipid peroxidation occurred than in sensitive cultivars due to increased antioxidant activity in wheat (41). Drought imposed from the tasseling stage led to higher MDA levels than drought stress was imposed later in the growth cycle (42). Based on the results of the present study and supporting literature, the CBM-DL 38, CBM-DL 360, CBM-DL 111 and CBM-DL 448 lines are classified as drought-tolerant.

The reactive oxygen species (ROS) produced during oxidative stress are balanced by those scavenged by antioxidant enzymes, *viz.*, CAT and POX, in maize leaves. The CAT enzyme is responsible for the eliminating reactive oxygen species (ROS) produced under oxidative stress by converting them into water and molecular oxygen (43). The CAT activity of maize inbred lines generally increased with drought; however, different inbred lines exhibited varying responses in CAT activity. In general, droughttolerant genotypes presented increased CAT enzyme activities under drought stress, which helps mitigate oxidative damage and maintain cellular integrity. This enhanced antioxidant defense is closely linked to improved physiological traits, such as better water use efficiency and ultimately higher yield under drought conditions. In the present study, CAT activity played a

protective role in drought tolerance in the maize inbred lines (CBM-DL 38, CBM-DL 360, CBM-DL 111 and CBM-DL 448). Malondialdehyde (MDA) is a key byproduct of lipid peroxidation that occurs when plant cells experience oxidative stress, particularly under adverse conditions (drought). In addition, drought decreases the amount of MDA in the above four inbred lines with increasing tolerance to the inbred lines, indicating decreased oxidative damage to the membranes. CAT may inhibit the production of free radicals in all inbreds and thus may result in less lipid peroxidation (44). The results for POX enzyme activities were similar to those for CAT enzyme activities. POX plays a crucial role in catalysing the oxidation of substrates via the use of hydrogen peroxide  $(H_2O_2)$  as a co-substrate, thereby detoxifying the ROS (45). There was an increase in POX activity in all inbred lines in response to drought stress. The most significant increases compared with those in the control plants were detected in the drought-tolerant inbred lines. Four maize inbreds (CBM-DL 360, CBM-DL 448, CBM-DL 111 and CBM-DL 37) were able to protect the membranes of the POX enzyme under water-limited conditions. Additionally, the MDA levels of these inbreds were lower. POX activity reduces the level of H<sub>2</sub>O<sub>2</sub>, a potent ROS that can cause oxidative damage to cellular components such as lipids, proteins and nucleic acids. It has been reported that in drought-resistant varieties, POX activity increases, which enhances the ability of plants to cope with oxidative stress in barley (23).

Water-limited stress leads to a reduction in cob weight due to its impact on biomass remobilization (46). In the present study, a similar trend was observed. When drought stress occurs during the reproductive period, it stimulates the remobilization of prestored assimilate reserves, leading to the movement of large amounts of carbohydrates from the stem to the grains to compensate for the lack of current photosynthesis (6). Additionally, drought stress affects the ASI and grain filling of maize, leading to a reduction in the 100-grain weight and cob weight, which ultimately reduces grain yield (47). Therefore, the reduction in cob weight and 100-grain weight in maize during drought can be attributed to the disruption of physiological processes, grain filling and overall plant growth. A marginal decrease in leaf area may lead to minor effects on yield, whereas a substantial reduction in leaf area can adversely impact crop yield (20). Additionally, the chlorophyll index and chlorophyll fluorescence are positively correlated with each other, which indicates that the photosynthetic potential of dark-adapted leaves may be a proxy for the leaf chlorophyll index (LCI), particularly under drought (21). Furthermore, LAI and yield strongly positively correlated, which might be due to the increase in the LAI can be able to contribute to the increase in leaf chlorophyll, which affects the photosynthetic capacity and yield of the plant (6, 48). A negative correlation was found between the characteristics of the ASI and grain yield, which might be due to an increased ASI leading to improper pollination and kernel setting (49). An appropriate leaf shape with a smaller leaf angle is an important morphological factor contributing to greater biomass accumulation and rice yield (50).

# Conclusion

The evaluation of maize inbred lines under various growth stages of drought stress through morphological, physiological and

biochemical traits as well as photoassimilate translocation is a crucial area of research, especially in the context of improving drought tolerance. Several studies have focused on the resilience of maize to drought, as well as the efficiency of CO<sub>2</sub> assimilation in maize plants. Based on the results of this study, drought at 35-55 DAS and 55-75 DAS caused a greater percentage reduction in yield. Among the six maize inbred lines, CBM-DL 38, CBM-DL 111, CBM-DL 448 and CBM-DL 360 performed well under drought conditions in various aspects, such as morphophysiological (enhanced photosynthetic traits, maintaining relative water content and proline content), biochemical (improved antioxidant activities) and yield-related parameters. These findings suggest that the efficiency of CO<sub>2</sub> assimilation and the morphological characteristics (leaf angle and leaf orientation value) of maize plants play significant roles in their resilience to drought stress. These findings are valuable for the development of drought-tolerant maize varieties through breeding efforts by selecting maize inbred lines that exhibit enhanced photosynthetic traits, improved water retention, higher proline content and robust antioxidant activities

# Acknowledgements

under drought stress.

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# **Authors' Contributions**

All authors contributed to the study's conception and design. The study, material preparation, data collection, analysis and manuscript preparation were carried out by LP. Review and technical guidance were provided by MKK, AS and NS. LP, MKK and MU were involved in the study and manuscript preparation. RK, MU, SP and GV reviewed and edited the manuscript. All authors read and approved the final 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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