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Comparative Physiological, Biochemical and Transcript Response to Drought in Sorghum Genotypes

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Authors' contributions

This work was carried out in collaboration between both authors. Author JNA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors JNA and DAB managed the analyses of the study. Author JNA managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Sorghum [*Sorghum bicolor* (L.) Moench] is considered as an important staple crop in the tropical regions. However, the productivity of this useful crop is hindered by drought which contributes to significant yield reduction. The present study aimed to decipher the effects of drought stress on physiological, biochemical and gene expression changes in sorghum genotypes and to ascertain the differences in their response to drought stress. To achieve these objectives, six sorghum genotypes were grown in pot culture in a greenhouse, in a randomized complete block design and exposed to water stress treatment for 10 days. From the study, drought stress caused a significant ($P < .05$) reduction in plant height, leaf water and chlorophyll contents while the proline, malondialdehyde (MDA), soluble sugar, electrolyte leakage (EL), hydrogen peroxide (H_2O_2) and antioxidant enzymes activity increased significantly ($P < .05$) and differentially in all sorghum genotypes. Among the genotypes investigated, PI 585456 showed enhanced performance and was considered as the most tolerant to drought in relation to plant growth and water relation, membrane status, photosynthetic activity, ROS and osmolytes accumulation and antioxidant enzymes activity. Furthermore, the

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transcript expression analyses of different categories of drought-responsive genes, viz; antioxidant-related, osmolytes biosynthesis-related, dehydrin-related, photosystem-related and transcription-related were differentially upregulated in sorghum genotypes investigated. The results revealed the differences in metabolic response to drought among the genotypes, which accentuated the physiological, biochemical and molecular mechanism related to a specific response that may play a vital role in drought tolerance in sorghum.

Keywords: *Sorghum*; *genotype*; *drought stress*; *metabolic response*; *transcript analysis*.

1. INTRODUCTION

Sorghum [*Sorghum bicolor* (L) Moench] is an important staple crop mostly found in the tropical areas of Africa. Sorghum normally requires 450–700 mm of water during its 120-130 days life cycle [1]. However, the onset of drought during the growth and development of sorghum hinders its productivity [2]. Furthermore, the widely cultivated variety of sorghum in the northern part of Ghana is the landrace type, which is low yielding and susceptible to pests, disease and drought [3]. Attributed the lack of improved varieties to be a major problem associated with low yield in sorghum production. Therefore, engineering and breeding of drought-tolerant sorghum variety are crucial to increase the production and distribution of sorghum. To meet this overarching objective, comprehensive knowledge of the metabolic response under drought in sorghum plants is highly essential. Drought may induce various physiological, biochemical changes and gene expression regulations which inhibit a plant's growth [4, 5]. As a valuable crop, sorghum undergoes a series of metabolic changes to remedy the various forms of damage associated with water deficit.

The focus of previous studies on sorghum has been on the impact of biotic factors [6-11], with little emphasis on drought and abiotic stresses. Therefore, investigations of the metabolic response of sorghum under water deficit conditions will help to safeguard against yield loss. Our mock experiment led to the discovery of the sorghum variety PI 585456, as the best performing and most tolerant to 10 d drought treatment in relation to plant growth, leaf water content and enzyme antioxidant activities among dozens of sorghum varieties (data not provided).

This study aimed to (i) evaluate the growth performance and physiological responses of sorghum genotypes exposed to 10 d water stress treatment with reference to leaf water content, chlorophyll content, cell membrane status, reactive oxygen species (ROS) and enzyme

antioxidant activities and the pattern of drought-responsive genes; (ii) compare the tolerance and difference in metabolic response among six sorghum genotypes to water stress. We hypothesise that the sorghum genotypes may respond differently to drought. The study will help us to unravel the physiological, biochemical and gene expression response of sorghum to drought and provide us with the theoretical base for improving and developing drought adaptable sorghum genotypes.

2. MATERIALS AND METHODS

2.1 Plant Materials, Growth, Experimental Design and Water Stress Treatment

Sorghum [*Sorghum bicolor* (L) Moench] genotypes; PI 585451, PI 585452, PI 585453, PI 585454, PI 585455 and PI 585456 were investigated in the study. The seeds were obtained from the National Plant Germplasm System (NPGS), United States Department of Agriculture (USDA, USA).

Sorghum seeds were initially surface cleaned in 75% ethanol for 5 min, 5% sodium hypochlorite for 5 min and finally washed in distilled water for 5 min, for three consecutive times. The seeds were allowed to germinate on a moist filter paper in the dark and then transferred into plastic pots filled with soil (Sunshine mix #1).

Subsequently, 20 plants of each genotype were maintained under a controlled greenhouse in randomized complete block design. The growth conditions were similar for all plants till they were at fully expanded 2nd – 3rd leaf stage (Z12 stage of Zadoks's scale [12]). The plants were watered daily and nutrient was supplied in the form of ½ strength Hoagland media [13]. After the plants reached the fully expanded 2nd – 3rd leaf stage (Z12 stage of Zadoks's scale), the pots of each genotype were divided into two sets. One set represented the well-watered (control) plants and the second the drought-treated (treatment) plants. Each set comprised eight to ten pots per

genotype. To induce drought stress, water was withheld for treatment plants for 10 d while control plants were watered daily. Uniform growth stage plants were sampled and stored at -80°C until use after the treatment time elapsed. The average day/night temperature in the greenhouse was 26°C with 60% relative humidity, 16/8 h (day/night) and a $300\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$ light intensity.

2.2 Measurement of Plant Growth and Leaf Water Content

The height of the plants was measured from the ground to the top axial part of the shoot using a measuring tape. Leaf RWC was determined by assaying the fresh (FW), turgid (TW) and dry weight (DW) of the leaves heating them for 30 min at 100°C and drying in an oven at 70°C for 48 h. RWC of the leaves was then calculated as [14]:

$$\text{RWC (\%)} = \frac{\text{FW} - \text{DW}}{\text{FW}} \times 100 \quad (1)$$

2.3 Quantification of H_2O_2 Accumulation

Hydrogen peroxide (H_2O_2) was assayed by a modified protocol of [15]. Briefly, 100 mg of leaf samples were homogenised in 1.0 mL of TCA (0.1%, w/v) and centrifuged at $12\ 000 \times g$ for 15 min. 0.5 mL of the supernatant was carefully collected and 0.5 ml of a phosphate buffer (pH 7.0) along with 1.0 mL of potassium iodide (1 M) was added. The absorbance of the mixture was read at 410 nm. H_2O_2 concentration was estimated expressed as $\mu\text{mol g}^{-1}$ FW.

2.4 Measurement of Electrolyte Leakage (EL)

EL was quantified by following the method by [16]. Sorghum leaves were cut into strips and incubated in distilled water for 12 h. The initial (C_1) and final (C_2) conductivity were measured with a meter (Hana Instruments, USA) and the EL was estimated using the equation (2).

$$\text{EL (\%)} = 1 - (C_1/C_2) \times 100 \quad (2)$$

2.5 Lipid Peroxidation Analysis

The modified method for quantifying malondialdehyde (MDA) content, previously reported by [17] was used to determine the lipid peroxidation. A 0.1 g of leaf sample was extracted in 1 ml of 0.1% trichloroacetic acid

(TCA) and centrifuged at $12\ 000 \times g$ for 5 min. A 1 ml supernatant was mixed with 0.5 mL 0.5% thiobarbituric acid (TBA) and the mixture was boiled for 30 min, the reaction was stopped by placing the reaction tubes in an ice bath for 20 min. The mixture was placed on ice for 25 min to stop the reaction and the centrifuge at $12\ 000 \times g$ for 10 min. The specific and non-specific absorbance was recorded at 532 and 600 nm, respectively, using a spectrophotometer (UV-2550, Shimadzu, Japan).

2.6 Osmolytes Accumulation Analysis

Proline was assayed using the ninhydrin method described by [18]. A 100 mg leaf sample was homogenized in 1 ml of 3% sulfosalicylic acid. The reaction mixture was centrifuged at $12\ 000 \times g$ for 10 min, glacial acetic acid and 0.5 ml ninhydrin and the mixture was heated for 1 h at 100°C and cooled on ice for 20 min. A 1 ml toluene was added for the extraction of the organic phase and absorbance recorded at 520 nm. The calibration curve expressed as $\mu\text{mol proline g}^{-1}$ FW was used to quantified the concentration of proline.

For soluble sugar, a 0.1 g leaf sample was ground and mixed with 80% ethanol at 80°C for 20 min. The mixture was centrifuged at $12\ 000 \times g$ for 10 min. The residue was extracted twice with 80% ethanol and 80% ethanol was mixed with the three supernatants to constitute 5 mL. were combined and 80% ethanol was added to a total volume of 5 mL and absorbance was read at 620 nm [19].

2.7 Chlorophyll Estimation

The total chlorophyll (chlorophyll a and b) content were quantified according to a modified procedure of [20]. A 0.1g leaf samples were homogenized in 0.1 ml 80% acetone and centrifuged at $12\ 000 \times g$ for 5 min and chlorophyll 'a', 'b' and total chlorophyll content were determined by equations (3), (4) and (5), respectively.

$$\text{Chlorophyll 'a' } (\mu\text{mol g}^{-1}\ \text{FW}) = [(12.7 \times A663 - 2.69 \times A645) V/\text{FW}] \quad (3)$$

$$\text{Chlorophyll 'b' } (\mu\text{mol g}^{-1}\ \text{FW}) = [(22.9 \times A645 - 4.68 \times A663) V/\text{FW}] \quad (4)$$

$$\text{Total chlorophyll 'b' } (\mu\text{mol g}^{-1}\ \text{FW}) = [(20.2 \times A645 - 8.02 \times A663) V/\text{FW}] \quad (5)$$

Where, A = The absorbance at specific wavelengths, V = The final volume of chlorophyll extract and FW = leaf fresh weight.

2.8 Quantification of Antioxidant Enzymes Activity

The enzyme antioxidants (SOD, CAT and POD) activity was quantified spectrophotometrically according to a modified procedure by [21,22]. The protein content was estimated using the Bradford assay, using BSA as a standard [23]. The SOD activity was quantified by monitoring the inhibition of photochemical reduction of nitro blue tetrazolium (NBT). The CAT activity was assayed spectrophotometrically by monitoring a reduction in H₂O₂ absorbance at 240 nm. The POD activity was assayed according to the guaiacol test, as reported by [24].

2.8.1 RNA isolation and cDNA synthesis

The Plant Total RNA kit (Invitrogen, USA) was used for the extraction of total RNA from leaf samples, according to the manufacturer's instructions. To ensure the quality and quantity of RNA, a nanophotometer (Implen Inc., Westlake Village, CA, USA) was used to achieve this purpose. The ethidium-bromide stain analyses using agarose gel electrophoresis was used to check the purity of total RNA and the Power cDNA synthesis kit (Intron Biotechnology Inc., USA) was used to reverse transcribe DNA free total RNA into cDNA.

2.8.2 Quantitative real-time polymerase chain reaction (qPCR) analysis

To decipher the expression pattern of drought-responsive genes used in the study (Table 1), the CFX 96 Real-Time system (Bio-Rad, Richmond, CA, USA) with SYBR-green fluorescence was used and analyses of the results were done by using the $\Delta\Delta CT$ method. The conditions for the thermal cycle was 95°C for 5 min and 40 cycles of 95°C for 15 s, 55°C for 15 s and 72°C for 30 s. Experiments were triplicated, using the *Actin* as internal control for standardizing the relative transcript levels.

2.9 Statistical Analysis

Ten (10) plants of each genotype and treatments were employed in the experiments. The experiment was laid in Randomized complete block design and samples were collected from each pot and measurements performed in 3–5 biological replicates per genotypes and treatments. Factorial analysis of variance (ANOVA) was conducted using SIGMAPLOT software package (version 14.0), for evaluating the effect of genotypes (G), drought treatment (T) and G × T. Besides, Tukey's post hoc test was also used for the determination of the statistically significant difference between the mean values at the P = .05 and .01 probability level, using the R (v.3.5.1).

Table 1. List of primers used for quantitative real polymerase chain reaction (qPCR)

Transcript identity	Primer sequence
<i>SOD1</i>	5'- CGTGTGGATTCTGGTGATG - 3' 3'- AGCCACATATGCGAGCTTCT - 5'
<i>CAT1</i>	5'- CGTGTGGATTCTGGTGATG - 3' 3'- AGCCACATATGCGAGCTTCT - 5'
<i>POD1</i>	5'- CGTGTGGATTCTGGTGATG -3' 3'- AGCCACATATGCGAGCTTCT -5'
<i>P5CS</i>	5'- GGGAAAGGTGGAAGATTGGC-3' 3'- AGCCTTCCCATCAAGTTCCA - 5'
<i>ERD1</i>	5'- ATCATCGGCCGCGAGAGATTA -3' 3'- GGTGGAGTTTGTGCGAGGAGA- 5'
<i>NAC1</i>	5'- GCAACAACGATGGTGAAATG- 3' 3'- TAACACGGTAGCACGGATCA- 5'
<i>MYB2</i>	5'- GGTGAATTTTCCACCTCCA - 3' 3'- CAATAATGTGCCCGCTACCT - 5'
<i>PsbA</i>	5'- ATCACGTAATTGGCCTCCAC - 3' 3'- TCGACCGTCTAGCAGATTCC - 5'
<i>WRKY1</i>	5'- AGGATGGTGGTGGTGATGAT - 3' 3'- TCCTGGGGAAGTTGTTGTTTC - 5'
<i>Actin</i>	5'- CGTGTGGATTCTGGTGATG - 3' 3'- AGCCACATATGCGAGCTTCT - 5'

3. RESULTS

3.1 Plant Growth, Water Content, Membrane Status and ROS Accumulation under Drought

From Fig. 1A-C, drought stress significantly reduced the plant height, leaf dry weight (LDW) and RWC. Comparatively, the maximum reduction in plant height (21.18 cm), LDW (80.41%) and RWC (28.79%) were observed in drought-stressed plants of PI 585454, PI 585456 and PI 585451, respectively, while PI 585455, PI 585451 and PI 585451 showed the lowest decline in plant height, LDW and RWC, respectively (Fig. 1A-C). All measured parameters were assessed with a two-way ANOVA (Table 2). The genotype (G), treatment (T) and their interaction affected the LDW and RWC significantly ($P = .05$), except G and G \times T of plant height (Table 1).

Furthermore, the accumulation of H₂O₂ content, EL and MDA increased significantly in genotypes (Fig. 1D-F). H₂O₂ accumulation ranged from 47.76% (PI 585453) to 29.34% (PI 585456), EL

increased by 85.20% (PI 585453) and 52.03% (PI 585454), while the MDA levels increased by 68.42% (PI 585452) and 47.02% (PI 585452) compared to the control plants. G, T and G \times T significantly ($P < 0.05$) affected the MDA level and H₂O₂ accumulation, except G and G \times T of EL in the two-way ANOVA (Table 2).

3.2 Proline, Soluble Sugar and Enzyme Antioxidant Activities

Free proline and soluble sugar increased remarkably in all sorghum genotypes compared with the controls (Fig. 2 A and B). The highest proline content of 91.85% and soluble sugar of 24.11% was detected in PI 585454 and PI 585456, respectively, while the lowest proline content (38.95%) and the soluble sugar (15.19%) content was both recorded in the drought-stressed plant of PI 585451 compared to the control. The chlorophyll content significantly decreases at a greater magnitude (23.59%) in PI 585453 (Fig. 2C). Besides, proline, soluble sugar and chlorophyll were greatly affected by G, T and G \times T in the two-way ANOVA (Table 2).

Table 2. Analysis of variance of shoot trait of PI 585451, PI 585452, PI 585453, PI 585454, PI 585455 and PI 585456 under progressive drought stress

Trait (/plant)	Sources of variations		
	Genotype (G) (df = 5)	Treatment (T) (df = 1)	Genotype \times Treatment (G \times T) (df = 5)
Plant height (cm)	.833	***	0.431
Leaf DW (g)	***	***	**
RWC (%)	***	***	***
Proline ($\mu\text{mol g}^{-1}$ FW)	***	***	***
MDA ($\mu\text{mol g}^{-1}$ FW)	***	***	***
H ₂ O ₂ ($\mu\text{mol g}^{-1}$ FW)	***	***	**
SOD ($\mu\text{mol min}^{-1} \text{g}^{-1}$ protein)	**	***	***
CAT ($\mu\text{mol min}^{-1} \text{g}^{-1}$ protein)	***	***	***
POD ($\mu\text{mol min}^{-1} \text{g}^{-1}$ protein)	.391	***	***
Electrolyte leakage (%)	.258	***	.416
Sucrose ($\mu\text{mol g}^{-1}$ FW)	***	***	.138
Chlorophyll ($\mu\text{mol g}^{-1}$ FW)	***	***	.843
SOD1	***	***	***
CAT1	***	***	***
POD1	***	***	***
P5CS	***	***	***
ERD1	***	***	***
PsbA	***	***	***
WRKY1	***	***	***
NAC1	***	***	***

** , *** Significant at the .05 and .01 probability levels

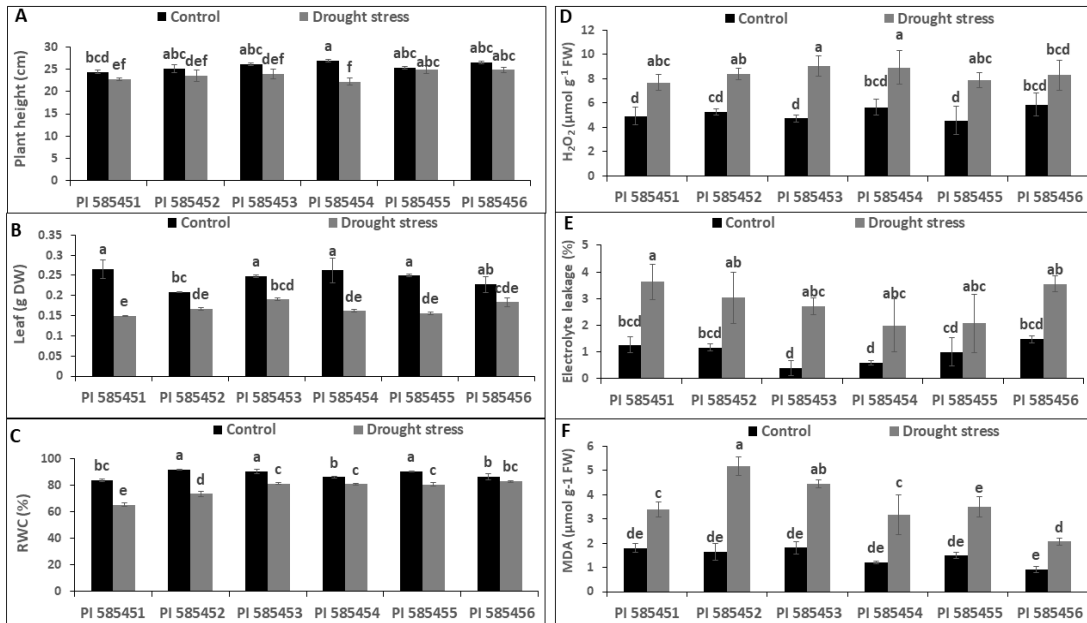


Fig. 1. Effect of drought stress on plant height (A), leaf dry weight (B), relative water content (C), hydrogen peroxide accumulation (D), electrolyte leakages (E) and malondialdehyde content (F) in PI 585451, PI 585452, PI 585453, PI 585454, PI 585455 and PI 585456 sorghum genotypes under progressive drought stress. Data show the means \pm standard deviation of three independent samples. Different letters on vertical bars indicate significant differences between means at the $P = .05$ level

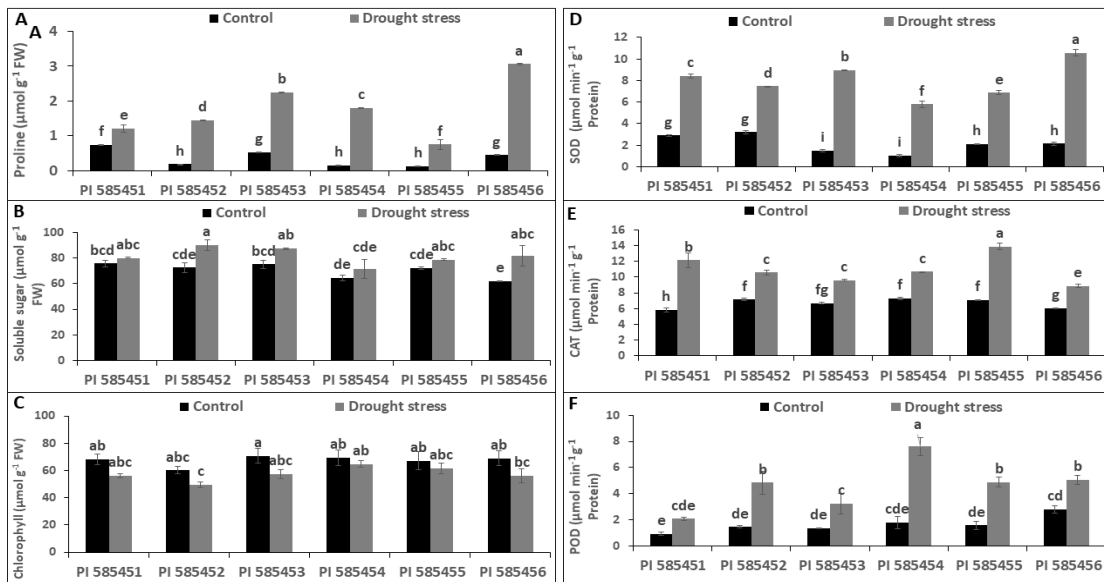


Fig. 2. Effect of drought stress on proline (A), soluble sugar (B), chlorophyll content (C), superoxide dismutase activity (SOD) (D), catalase activity (CAT) (E) and peroxidase activity (POD) (F) in PI 585451, PI 585452, PI 585453, PI 585454, PI 585455 and PI 585456 sorghum genotypes under progressive drought stress. Data show the means \pm standard deviation of three independent samples. Different letters on vertical bars indicate significant differences between means at the $P = .05$ level

Also, to understand the effect of drought on ROS production and oxidative damages, antioxidant enzymes activity was analysed. The SOD, CAT and POD were higher in drought-stressed plants than control (Fig. 2 D–F). For instance, SOD activity was maximum at 83.27% (PI 585453) and minimum at 57.14% (PI 585452) (Fig. 2D). CAT activity ranged from 52.14% (PI 585451) to 30.20% (PI 585453) (Fig. 2E), while the highest POD activity was recorded in PI 585454 (76.51%) and the lowest in PI 585456 (45.17%) (Fig. 2F). Besides, the ANOVA for enzyme antioxidant activities displayed strong interactions among T, G and G × T (Table 2).

3.3 The Expression Pattern of Antioxidant-related, Osmolytes Biosynthesis-related, Dehydrin-related and Photosystem-Related Genes

The expression pattern of antioxidant-related (*SOD1*, *CAT1* and *POD1*), osmolytes biosynthesis (*P5CS*), dehydrin-related (*ERD1*)

and photosystem-related (*psbA*) gene was investigated under drought stress using qPCR analysis. Compared to the control, the expression levels of *SOD1*, *CAT1* and *POD1* was relatively higher in sorghum genotypes (Fig. 3A-C). For example, the expression level of *SOD1*, *CAT1* and *POD1* increased by 5-fold (PI 585456), 9.2-fold (PI 585454) and 6.6-fold (PI 585456), respectively. The ANOVA showed that genotype (G), treatment (T) and their interaction also affected the expression patterns of *SOD1*, *CAT1*, and *POD1* (Table 2).

Similarly, the expression level of *P5CS*, *ERD1* and *psbA* was higher in all genotypes (Fig. 3D-F). The expression level of *P5CS*, *ERD1* and *psbA* increased by 8.9-fold (PI 585452), 10.64-fold (PI 585455) and 10.48-fold (PI 585454), respectively. Two-way ANOVA showed that genotype (G), treatment (T) and their interaction also significantly affected the expression levels of *P5CS*, *ERD1* and *psbA* (Table 2).

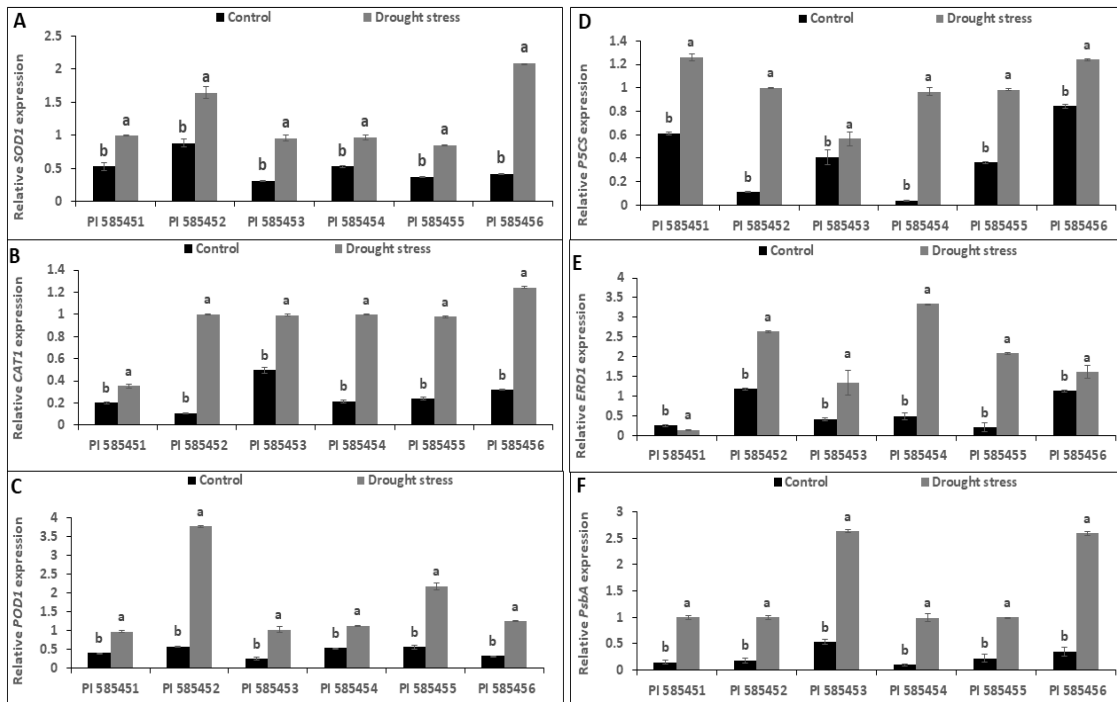


Fig. 3. Effect of drought stress on the expression pattern of superoxide dismutase (*SOD1*) (A), catalase (*CAT1*) (B), peroxidase (*POD1*) (C), *P5CS* (D), *ERD1* (E) and *psbA* (F) transcripts in PI 585451, PI 585452, PI 585453, PI 585454, PI 585455 and PI 585456 sorghum genotypes under progressive drought stress. Data show the means ± standard deviation of three independent samples. Different letters on vertical bars indicate significant differences between means at the $P = .05$ level

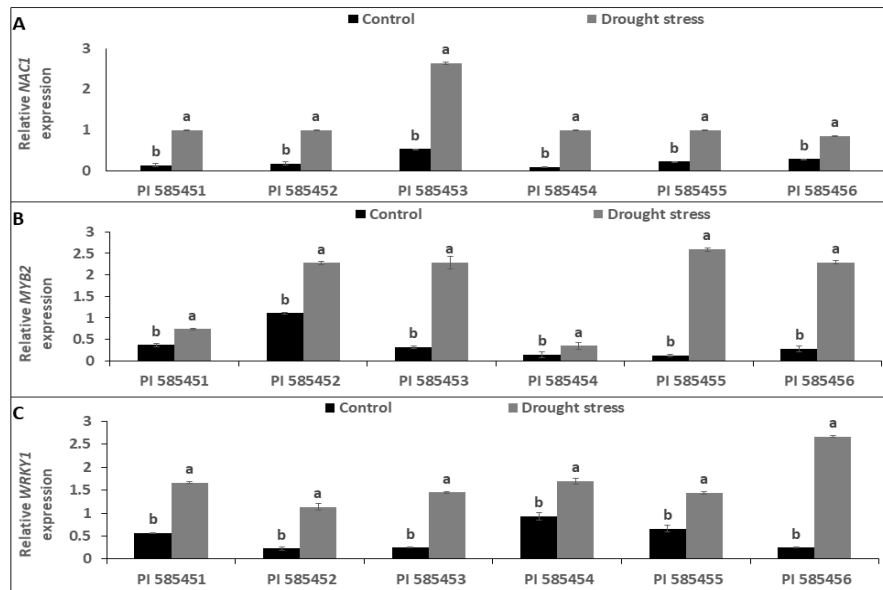


Fig. 4. Effect of drought stress on the transcript expression level of *NAC1* (A), catalase *MYB2* (B) and *WRKY1* (C) in PI 585451, PI 585452, PI 585453, PI 585454, PI 585455 and PI 585456 sorghum genotypes under progressive drought stress. Data show the means \pm standard deviation of three independent samples. Different letters on vertical bars indicate significant differences between means at the $P = .05$ level

3.4 The Expression Pattern of *NAC1*, *MYB2* and *WRKY1* Transcription-related Genes

NAC1, *MYB2* and *WRKY1* were upregulated in sorghum genotypes (Fig. 4A-C). For instance, *NAC1* increased by 10.48-fold (PI 585454) and 2.96-fold (PI 585456) (Fig. 4A), *MYB2* gene was maximum at 19.81-fold (PI 585455) and minimum at 2.0-fold (PI 585451) (Fig. 4B), while *WRKY1* gene increased by 10.47-fold (PI 585456) and 1.8-fold (PI 585454) (Fig. 4C). Two-way analysis of the variance of transcriptional-related genes showed strong interactions among treatments T, G and G \times T (Table 2).

4. DISCUSSION

4.1 Physiological Aspect of Drought among Sorghum Genotypes

In the study, the physiological response in sorghum under progressive drought was investigated. It was observed that drought stress significantly ($P = 0.05$) resulted in differential growth, development and related characteristics of the sorghum genotypes investigated. With plant growth and water content, an increase in drought stress resulted in decreased plant height

and RWC. However, the magnitude of the decline in such traits was more prominent in PI 585453 (Plant height), PI 585451 (RWC) and PI 585456 (LDW) (Fig. 1A-C). The relationship between plant height and leaf water contents under drought stress for sorghum genotypes has been previously shown [25-27]. The reduction in transpiration water loss illustrates an effective approach used by plants to cope with drought, by conserving adequate water. Even so, measurement of plant height and leaf water content reflects a plant's growth, water status and physiological functioning under water deficit conditions [28]. Therefore, it can be argued that the lower reduction in plant height and RWC in drought-stressed plants of PI 585453 and PI 585456, respectively, indicated that these genotypes showed enhanced performance to growth and higher water status, better control of stomatal water loss, enhanced osmotic adjustment towards the maintenance of tissue turgor and physiological activity.

4.2 The Biochemical Aspect of Drought Response among Sorghum Genotypes

Furthermore, drought induces the accumulation of ROS and damages to the cellular membrane

which impedes the various metabolic process of plants [29]. From the study, drought led to a noticeably higher accumulation of ROS and membrane damage in all genotypes (Fig. 1D-F). Nonetheless, the effect of drought stress was more pronounced in PI 585453 (H_2O_2 accumulation), PI 585456 (EL) and PI 585452 (MDA) (Fig. 1D-F). Previous reports have showed that drought stress induced higher H_2O_2 accumulation, lipid peroxidation and EL in drought-sensitive maize, barley and tobacco genotypes than that in drought-tolerant genotypes [17,30,31]. Similarly, the results from this study indicate that the sorghum genotypes PI 585453, PI 585456 and PI 585452 suffered greater cell membrane damage than other genotypes and demonstrate a possible correlation to drought sensitivity. Also, drought stress resulted in differentially higher enzyme antioxidant (SOD, CAT and POD) activities in sorghum genotypes (Fig. 2D-F). Comparatively, the antioxidant enzymes activity was relatively higher in PI 585453 (SOD), PI 585451 (CAT) and PI 585454 (POD) than other genotypes. Consistent with our findings, higher enzyme antioxidant activities have been reported in drought-tolerant wheat genotypes than that in the sensitive type [32, 33], indicating that the sorghum genotypes (PI 585453, PI 585452 and PI 585456) exhibited enhanced ROS scavenging system than other genotypes.

Water stress stimulates the production of proline and soluble sugars and these osmoprotectants are highly linked to drought tolerance in wheat [34,35]. However, there is limited information about the role of proline and soluble sugars under progressive drought conditions in sorghum. From our study, we showed that drought stress increased proline and soluble sugars in all genotype (Fig. 2A-B). Specifically, proline and soluble sugar were higher in PI 585454 and PI 585456, respectively than in other genotypes. These results suggest the osmoregulatory role of the osmoprotectants under water deficiency conditions, enabling sorghum to maintain low water potential, as well as protecting protein from oxidative damages and enhanced enzyme antioxidant activities. Also, drought stress fast-tracks the decomposition of chlorophyll and the ability of a plant to maintain a higher chlorophyll content under water deficiency conditions contributes to drought adaptability [17]. In the study, the reduction in the chlorophyll content was observed in drought-stressed sorghum genotypes. However, the magnitude of the

decline in chlorophyll content among the genotypes did not refer to the observed physiological parameter and differences in their tolerance to stress. Thus, similar chlorophyll content was found in PI 585451, PI 585452, PI 585453 and PI 585456 (Fig. 2A), suggesting that although chlorophyll content is an indicator of photosynthetic activity under drought in all genotypes, it is not the main factor responsible for the differences among the genotypes.

4.3 Transcriptional Response to Drought among Sorghum Genotypes

It is well-known that water stress regulates the expression of numerous genes in plants [36]. From the study, the expression pattern of nine drought-expressed genes was elucidated under water deficit condition. The genes which included antioxidant, osmolytes biosynthesis, dehydrin, photosystem-related genes and transcriptional-related genes were differentially upregulated in sorghum genotypes (Figs. 3 and 4).

Several pieces of research have reported the response of key enzyme antioxidants (SOD, CAT and POD) to the effect of drought in different plant species [37, 38]. Yet, information on the transcript expression pattern of these enzymes in response to drought remains unelucidated. From the study, a noticeably increased expression of *SOD1*, *CAT1* and *POD1* were observed for sorghum genotypes (Fig. 3A-C). Similar observations were made for *SOD1* [39, 40], *CAT1* [41] and *POD1* [42] transcripts in transgenic rice and Kentucky bluegrass, *Arabidopsis thaliana* and Sesame genotype, respectively. The higher levels of enzyme antioxidant-related genes (*SOD1*, *CAT1* and *POD1*) in drought-stressed plants might protect the plant cells from the damages associated with drought stress.

Furthermore, *P5CS* (Δ^1 -pyrroline-5-carboxylate synthetase) plays a crucial role in the accumulation of proline under drought conditions [43]. As an important osmoprotectant, proline has been used to assess the metabolic function of a plant under drought [44]. The higher expression of *P5CS*, particularly in drought-stressed plants of PI 585451 and PI 585456 indicated a greater accumulation of proline, which prevents them from membrane injury and maintain better growth especially in PI 585456 compared to other genotypes (Fig. 3D). The early response to drought (ERD) genes plays an important function in preventing dehydration of plant tissues and

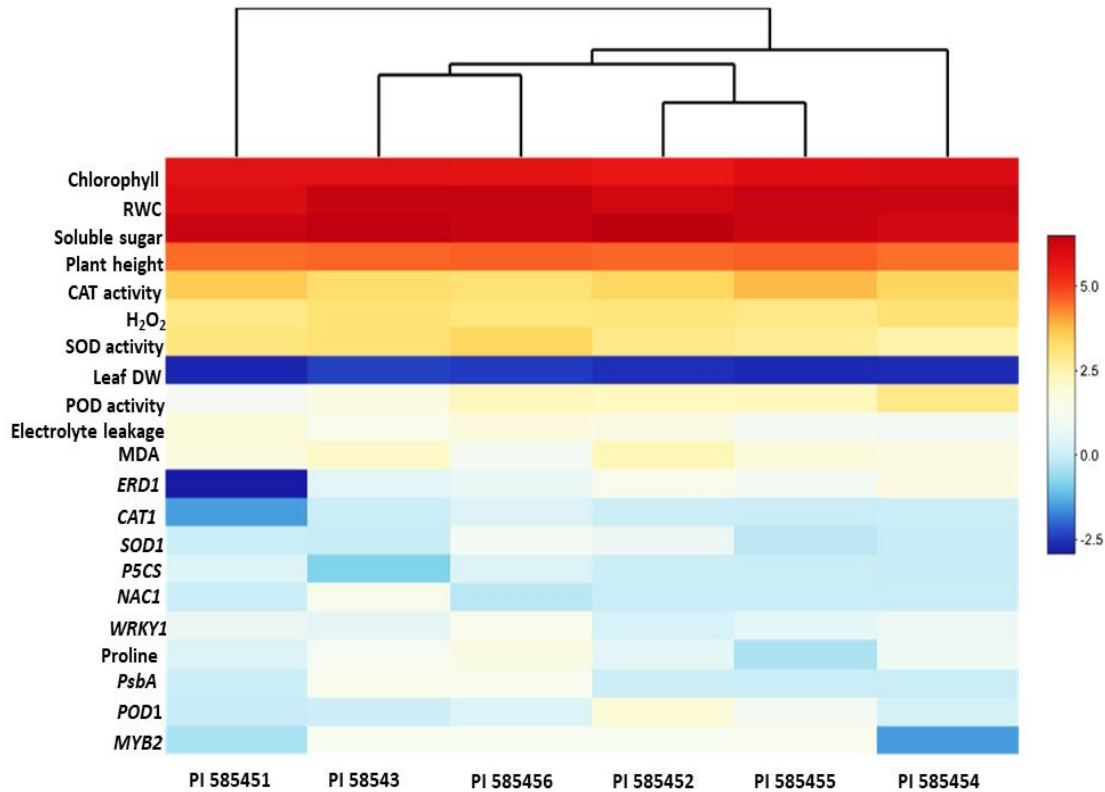


Fig. 5. Total assessments of physiological responses in six (6) sorghum genotypes during drought stress. The relative values of physiological parameters for the heat map analysis. Different colour represents different concentration of metabolites

cells during drought conditions [45]. From the study, although *ERD1* gene was highly and differentially expressed in genotypes, the marked increase in the expression pattern of *ERD1* was noticed in PI 585456 compared to other genotypes (Fig. 3E), suggesting that PI 585456 played an active role the maintenance of cell membrane integrity and function in defence against water deficit conditions. Besides, the higher transcript level of the photosystem II-related gene (*psbA*) was observed in drought-stressed plants of PI 585453 and PI 585456 (Fig. 3F), indicating that the chloroplast-encoded gene significantly increased in PI 585453 and PI 585456 than other genotypes, under water stress condition. The result from this study presents a new insight into the role of photosystem-related genes and also indicated that PI 585453 and PI 585456 exhibited powerful photosynthetic performance under drought stress.

Also, *NAC1*, *MYB2* and *WRKY1* plays a vital role in the transcriptional regulation and signal transduction in response to drought stress [46-

48]. Significantly and differentially increase in the expression level of these genes was observed under 14 d drought treatment (Fig. 4A-C). Specifically, *NAC1* was highly induced in PI 585453 (Fig. 4A), *MYB2* was significantly expressed at a greater magnitude in drought-stressed plants of all genotypes except PI 585451 and PI 585454 (Fig. 4B) while PI 585456 showed a relatively increased expression pattern of *WRKY1* than that in other genotypes (Fig. 4C), suggesting the potential regulatory role of *NAC1*, *MYB2* and *WRKY1*, in sorghum adaptation to drought stress.

4.4 Analysis of the Metabolic Traits and Tolerant Abilities under Drought in Sorghum Genotypes

From Fig. 5, the heat map provides a summary of the overall evaluation of metabolic functions under drought in sorghum genotypes. The heat map showed significant variation among the genotypes investigated. Thus, under water stress, the relative values of chlorophyll, RWC

and plant height, leaf WD, significantly decline in all genotypes, while the soluble sugar content, MDA level, H₂O₂ content, antioxidant enzymes (SOD, CAT and POD) activity and EL significantly increased in all genotypes. Also, the antioxidant-related, osmolytes biosynthesis, dehydrin-related, photosystem-related and transcription-related genes were significantly and differentially upregulated in the genotypes investigated

5. CONCLUSION

Comprehensive knowledge of the metabolic response under drought in sorghum plants is highly essential because it would increase the production and distribution of sorghum. Findings from the study showed that drought stress resulted in significant reduction in plant height, LDW, LRWC, altered membrane integrity, inhibition of photosynthetic activity, an increased ROS and osmolytes (soluble sugar and proline) accumulation, antioxidant enzymes activity and increased expression of the nine (9) drought genes in all tested genotypes. Thus, the genotypes showed a difference in physiological, biochemical and gene expression responses to the effect of drought. Nonetheless, PI 585456 showed a superior drought-tolerant performance in relation to its ability to cope with cellular dehydration, enhanced growth and the maintenance of a higher RWC, higher ROS and proline accumulation, as well as a lesser cellular membrane damage. Also, considering the totality of all metabolic parameters investigated, PI 585453 and PI 585454 showed a higher susceptibility to drought. Thus, PI 585453 and PI 585454 had a greater accumulation of soluble sugars, inhibited photosynthetic activity, higher MDA content, lower proline content and a greater accumulation of soluble sugars. Similar, but a varied magnitude of biochemical and physiological response was observed in PI 585451 and PI 585455 under drought. The results suggested that PI 585451, PI 585455 and PI 585456 genotypes showed active physiological, biochemical and transcriptional expression pattern, conferring a greater capacity to withstand water drought and are adaptable to drought stress than PI 585453 and PI 585454. In summary, the results from the study suggest that the although sorghum genotypes investigated had a narrow genetic variation, they employed different mechanisms to withstand drought. The findings from the study will provide valuable information for initiating breeding program for improving sorghum.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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