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ORIGINAL ARTICLE

Differential responses of sorghum genotypes to drought stress revealed by physio-chemical and transcriptional analysis

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Abstract

Sorghum is an essential food crop for millions of people in the semi-arid regions of the world, where its production is severely limited by drought stress. Drought in the early stages of crop growth and development irreversibly interferes, which leads to poor yield. The effect of drought stress in sorghum was studied at physiological, biochemical, and molecular levels in a set of two genotypes differing in their tolerance to drought. Drought stress was imposed by restraining water for 10 days on 25 days old seedlings. A significant influence of water stress was observed on the considered morpho-physiological and biochemical traits. The genotype DRT1019 exhibited physiological and biochemical indicators of drought avoidance through delayed leaf rolling, osmotic adjustment, ideal gas-exchange system, solute accumulation, an increased level of enzyme synthesis and root trait expression as compared to the ICSV95022 genotype. Furthermore, differences in the metabolite changes viz. total carbohydrate, total amides, and lipids were found between the two genotypes under drought stress. In addition, transcript profiling of potential candidate drought genes such as *SbTIP3-1*, *SbDHN1*, *SbTPS*, and *SbDREB1A* revealed up-regulation in DRT1019, which corresponded with other important physiological and biochemical parameters exhibited in the genotype. In conclusion, this study provides an improved understanding of whole plant response to drought stress in sorghum. Additionally, our results provide promising candidate genes for drought tolerance in sorghum that can be used as potential markers for drought tolerance breeding programs.

Keywords Physiology · Biochemistry · Sorghum · Candidate genes · Drought tolerance

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Introduction

Drought is an edaphic stress that affects plant growth and limits agricultural productivity worldwide [1]. It has become common in semi-arid regions due to insufficient, unevenly distributed, and unpredictable rainfall [2]. There is an increasing demand to produce crops for intense environmental situations, viz. drought, to maximize crop productivity. Sorghum [*Sorghum bicolor* (L.) Moench] is the major food crop for many parts of the tropics, and is also an important feedstock with a high industrial value [3]. Also, it is considered an essential staple cereal crop for more than 500 million people in more than 30 countries worldwide [4]. However, sorghum production is widely affected by frequent drought stress [5, 6]. Drought is fast becoming a significant stress affecting sorghum cultivation in semi-arid regions [7], where sorghum is an important crop. Generally, sorghum drought tolerance is categorized at pre-flowering and post-flowering stages. Pre-flowering drought stress is a critical phase to consider, as it occurs during the panicle development stage, which affects the survival, crop establishment, flowering, and fodder yield [6, 8]. On the other hand, post-flowering stress happens during the grain filling phase resulting in premature senescence causing reduced seed size and grain yield [9]. Sorghum drought tolerance is an intricate characteristic governed by genotype and environmental interaction that varies from one genotype to another due to morpho-physiological alterations [10]. In most of the plants, drought tolerance has been associated with reduced stomatal conductance and delayed leaf rolling controlled by root signals and leaf water potential, respectively [11]. In sorghum, drought stress response could be through morphological, physiological, or anatomical mechanisms for assisting in maintaining an effective water balance or allowing drought tolerance at reduced leaf water potential [6, 12, 13]. Drought stress widely affects water relations and photosynthesis functions in many plants, including sorghum [5, 14, 15]. Plants can withstand stress conditions through osmotic adjustment by solute accumulation, which has been an indication of stress or a defensive mechanism for reducing the effect of stress [16, 17]. Sorghum drought tolerance mechanisms include leaf rolling, leaf waxiness, stay-green, stomatal closure, osmotic adjustment, root morphological adjustments, and solute accumulation [6].

At the molecular level, plants respond to drought stress through metabolic alteration, signal transduction, and differential gene expression [18]. The stress responsive genes induced by abiotic stress to protect cellular damage in plants are important in tolerance mechanism at molecular level [19]. Hence, understanding the expression of these genes under stress conditions is important in stress adaptation [20]. In sorghum, previous studies have reported the expression of many drought-responsive candidate genes determining the drought adaptation mechanisms including aquaporins (AQP), dehydrin proteins (DHN), drought-responsive element binding (DREB) proteins, trehalose phosphate synthase (TPS), and ABA responsive proteins [21–24]. These drought responsive genes are involved in several mechanisms including osmotic adjustment, solute accumulation, root water uptake, cell membrane stability, protection of protein structures, activation of drought responsive genes and defense systems [22, 25–28]. During drought stress, understanding the functional trait association of the whole plant by investigating the physiological and biochemical changes with molecular marker mechanisms continues to be a challenge in most plants [29, 30], including sorghum. As stated earlier, in sorghum, drought stress responsive mechanisms at the vegetative stage is more important as this stage is critical for survival and crop establishment.

Therefore, in this study, the emphasis is given to sorghum whole plant vegetative drought stress responses at physiochemical and molecular levels with respect to plant metabolism and differential gene expression patterns.

Materials and methods

Plant materials and experimental conditions

Initially a total of 100 sorghum genotypes were evaluated for drought stress tolerance at the field level. The genotypes were collected from the Department of Plant Genetic Resources, Tamil Nadu Agricultural University (TNAU), Coimbatore, India. The experiment was conducted in pot culture under a completely randomized block design (CRBD) at the Department of Crop Physiology, TNAU, Coimbatore, India [31]. The selected genotypes were screened for drought tolerance as tolerant and susceptible. Out of this screening, a set of genotypes were selected i.e., DRT1019 (drought tolerant) and ICSV95022 (drought susceptible) and were sown in standard-size (15 cm × 20 cm) plastic pots filled with soil, sand, and manure in the proportion of 2:1:1. One plant per pot and three replications per genotype were maintained, with each replication had eight plants. All plants were watered with 1 L of water every day until 25 days after sowing (DAS). At 25 DAS, plants were subjected to moisture stress for 10 days by withholding water and allowing the soil moisture to reach 30%. Control plants were maintained at > 75% soil moisture content by applying 1 L of water every 24 h. The soil moisture content was monitored daily with a portable moisture meter (Delta Systems, UK).

Morpho-physiological traits

Observations on the morpho-physiological characteristics were made on three plants for each genotype. Traits viz., leaf rolling (LR), SPAD chlorophyll content (CHP), relative water content (RWC), stomatal conductance (gs), transpiration rate (E), net photosynthetic rate (P_N), epicuticular wax (ECW), and root length (RL) were recorded. Leaf rolling was measured as described by [32] using a scale of rolling from 1 to 5 (1 being only the evidence of rolling, while 5 was severest with the leaf being a closed cylinder). The SPAD leaf chlorophyll was measured in both drought-stressed and control plants with Minolta SPAD-502 chlorophyll meter (Minolta Camera Co., Ltd). RWC was estimated using the method as suggested by [33] and expressed as a percentage. Leaf gas-exchange variables (P_N, E, and gs) were measured from each genotype, as described by [34]. The epicuticular wax was estimated by the method indicated by [35]. The osmotic adjustment was measured using a VAPRO 5520 vapour pressure osmometer (Wescor, Logan, UT) as suggested by [36]. For estimating root length, the plastic pots were shrunk, and the roots were exposed to measure root length by a ruler scale and expressed in centimeters.

Quantification of biochemical responses

The biochemical assays such as nitrate reductase activity (NR), proline content (PRL), and metabolite changes were conducted using the leaf samples of control and drought-stressed sorghum genotypes. The fully expanded leaves were collected from each genotype for control and drought stress treatment at 12:00 to 12:30 noon and immediately flash frozen with liquid nitrogen and stored at – 80 °C for further biochemical analyses. The NR activity was estimated based on the method suggested by [37]. Proline was estimated using Bates et al. [38] methodology.

Changes in metabolite content

Fully expanded leaf tissues of sorghum genotypes were excised and flash-frozen in liquid nitrogen. The FT-IR (Fourier Transformation-Infrared Analysis) analysis was performed according to [39] using Jasco FT/IR 6800 model to measure the changes in metabolites (lipids, amides, and carbohydrates) and the data were recorded in the absorbance range of 600–4000 cm^{-1}. For instance, the spectral regions of macro-molecules for lipids were recorded at ~ 3050–2800 cm^{-1}, proteins at ~ 1750–1250 cm^{-1}, and carbohydrates at ~ 1250–900 cm^{-1}as previously described by [40–42].

Molecular analysis

Quantitative RT-PCR (qRT-PCR) analyses

Total RNA was isolated from drought-stressed and control plants using Trizol (Sigma Aldrich) according to [43]. The Transcriptor High Fidelity cDNA Synthesis Kit (Roche, Germany) was used to synthesize complementary DNA (cDNA) from 1 µg of total RNA following the manufacturer's protocol. Reverse transcription was performed at 45 °C for 30 min, followed by 85 °C for 5 min. Gene-specific primers (Table 1) were developed using Primer3 [44] and 18S rRNA was used as an internal control [45]. qRT-PCR was carried out on a Real-Time PCR system (ABI Biosystem, Step One Plus) using the SYBR Green Master Mix (Roche, Diagnostics). The 15 µL reaction volume contained 8µL of SYBR Green ready mix, 1 µL (10 pmoL) each of the forward (F) and reverse (R) gene-specific primers, 2 µL of template cDNA (50 ng), and 3 µL of double-distilled water. The thermal cycling conditions were as follows: initial denaturation at 95 °C for 10 min; 40 cycles of denaturation at 95 °C for 15 s; annealing at 55 °C for 1 min; and extension at 60 °C

Table 1 List of genes and primer sequence used for RT-PCR analysis

No	Gene ID	Annotated function	Primer sequence	Amplicon size (bp)
1	Sb01g018430.1	Similar to Aquaporin TIP3-1 (Tonoplast intrinsic protein) (*SbTIP3-1*)	F: GCCGCCATCTCCGAGTTCAT R: TGCTCGCCGTGTCGTGGTAGTA	100
2	Sb09g018420.1	Similar to (Dehydrin) DHN1 (*SbDHN1*)	F: GCGGAAGGAGGAAGAAGGGA R: GTGTGTTCTTGCTGCCCGTAG	121
3	Sb08g014070.1	Similar to ABA-responsive protein;putative; expressed (*SbABA*)	F: GCACGCTCTACCTGACCAACA R: GGAGTCCACGGTGACGATGT	194
4	Sb07g020270.1	Similar to Putative (trehalose6-phosphate synthase) (*SbTPS*)	F: TCCTGATGTGCGTTGGCAATGA R: ACCTCTGGTGCTGTGGGTGATA	100
5	Sb02g030310.1	(Drought response element Binding 1A) (*SbDREB1A*)	F: GTGAAGGCAGGTGAAACAGCG R: AGGCTGGCGTAGTACACATC	104
6	Sb18sRNA	Endogenous control	F: TGATAACTTGACGGATCGC R: CTTGGATGTGGTAGCCGTTT	200

for 30 s. The melt curve analysis was performed to confirm the specificity of amplification.

Statistical analysis

The significance of morpho-physiological and biochemical data was tested by Duncan's Multiple Range Test at $p < 0.05$ using XLSTAT software Addinsoft, Paris, France (2018).

Results and discussion

Effect of drought stress on morpho-physiological characters

It was observed that the two genotypes i.e. (susceptible ICSV93022; tolerant DRT1019) selected for drought susceptibility and tolerance, respectively showed differential responses under control conditions indicating the existing genetic ability or inherent genetic potential for drought susceptibility or drought tolerance [46, 47]. Under drought stress these two genotypes exhibited significant differences for all the morpho-physiological and biochemical traits analysed (Fig. 1). In this study, delayed leaf rolling (score 1) was observed in DRT1019 compared to ICSV95022 (score 4) under drought stress conditions (Fig. 1a). The adaptive ability of these genotypes to drought tolerance could be due to the genotypic variations in leaf rolling. Delayed leaf rolling and lower stomatal conductance in sorghum have been widely recognized as the most reliable drought avoidance mechanisms [48, 49].

The SPAD chlorophyll content in DRT1019 was reduced by 19.02%, while in ICSV95022 it was 7.61% percent, indicating the effect of drought stress on the genotypes [50]. The chlorophyll content of drought stressed plants was reduced relative to the control plants. However, under control conditions the chlorophyll content in DRT1019 were (38.10) and in ICSV95022 (19.70). This variation is due to the intrinsic

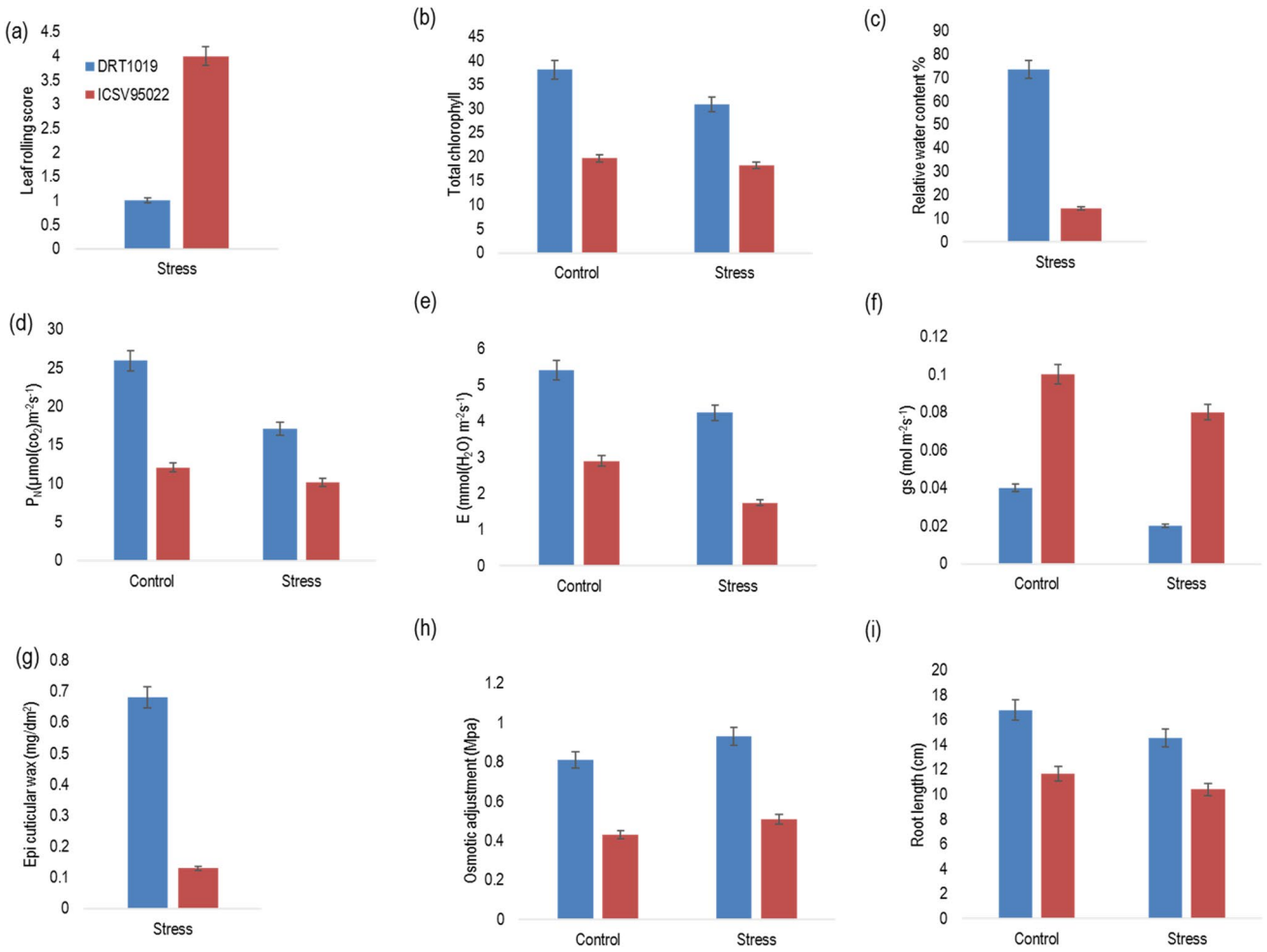

Fig. 1 Effect of drought stress on morpho-physiological traits **a** leaf rolling, **b** total chlorophyll, **c** relative water content, **d** net photosynthetic rate, **e** transpiration rate, **f** stomatal conductance, **g** epicuticular wax, **h** osmotic adjustment and root length (**i**). The values represent mean ± SE

genetic potential of individual genotypes. Further, the SPAD chlorophyll content of the two genotypes were significantly affected by drought stress. In stress conditions, the DRT1019 genotype (30.85) had a significantly higher chlorophyll level compared with ICSV95022 (18.20) genotype (Fig. 1b). Generally, drought stress results in pigment photo-oxidation and degradation of chlorophyll, eventually affecting a plant's chlorophyll content [51]. A higher SPAD chlorophyll content under drought stress has been widely reported in tolerant sorghum genotypes [52, 53].

Relative water content (RWC) is an important indicator of plant water status [54], which is essential for normal growth and physiological functions of plants. Maintenance of higher RWC during water stress indicates a genotype's improved tolerance to drought [55, 56]. In this study, a significant difference was observed in the RWC between the genotypes. Under drought stress conditions, a low level of RWC was observed in ICSV95022 (14.16%) compared to DRT1019 (73.52%) (Fig. 1c). Likewise, [57] reported that the tolerance of drought in sorghum is associated with higher RWC. This difference in RWC between genotypes may indicate the genotype's ability to obtain more soil water or the ability to prevent water loss [58].

Quantifying the changes in gas exchange parameters is considered a key method for assessing the ability of a genotype under limited water conditions. In this study, the net photosynthetic rate (P_N) and transpiration rate (E) was reduced in the DRT1019 genotype by 33.94% and 21.67%, respectively, while inICSV95022 the reduction of P_N was (15.98%) and E (40.48%). The reduction percentages indicate the effect of drought stress on these genotypes. However, under control conditions, the gas exchange parameters viz. P_N, E, and stomatal conductance (gs) in DRT1019 were higher than the susceptible genotype ICSV950222 (Fig. 1). In stress conditions, the results indicated that a significant reduction of P_N was observed in ICSV95022 (10.15 µmol (CO_2) m^{-2} s^{-1}) compared to DRT1019 (17.13 µmol (CO_2) m^{-2} s^{-1}) (Fig. 1d). Maintaining higher P_N even under drought stress conditions is a sign of tolerance to drought stress in sorghum [59]. Similarly, the transpiration rate (E) was significantly different between genotypes under drought stress conditions (Fig. 1e). The genotype DRT1019 (4.23 µmol (H_2O) m^{-2} s^{-1}) had a relatively higher E as compared with ICSV95022 (1.72 µmol (H_2O) m^{-2} s^{-1}) under drought stress conditions. This result indicated that a higher transpiration rate might make the genotype's internal plant system cooler by relieving the plant's heat. In addition, higher yields of sorghum could be associated with a higher transpiration rate under moisture stress. Stomatal conductance (gs) is an important parameter for dehydration avoidance by most plants [60]. In our study, drought stress significantly affected the stomatal conductance pattern between genotypes (Fig. 1f). The genotype DRT1019 (0.02 µmol m^{-2} s^{-1}) had lower stomatal conductance than ICSV95022 (0.08 µmol m^{-2} s^{-1}). This result indicated that under drought stress conditions in DRT1019 the lower stomatal conductance was accompanied by higher transpiration efficiency as compared to ICSV95022. Similarly, [59, 61] reported that, in sorghum, rice and wheat lower stomatal conductance is associated with higher transpiration efficiency i.e. ratio of net photosynthetic rate to transpiration rate.

Epicuticular wax is an effective component of drought avoidance mechanism in sorghum, as genotypes with higher epicuticular wax are reported to be drought tolerant [62]. In our study, a significant difference was found for the accumulation of epicuticular wax between the genotypes (Fig. 1g); DRT1019 (0.68 mg/dm^2) had higher epicuticular wax than ICSV95022 (0.13 mg/dm^2). This result indicated that a high concentration of leaf epicuticular wax in DRT1019 might contribute to its enhanced drought tolerance through a reduction in water vapor loss from leaves compared to ICSV95022.

Osmotic adjustment is a key physiological process in drought response and involves the accumulation of cellular compatible solutes [63]. Under control conditions, DRT1019 (0.81 MPa) had higher osmotic adjustment than ICSV95022 (0.43 MPa) genotype (Fig. 1h). The difference for osmotic adjustment under control condition is due to genotypic inherent potential. Under stress conditions, the osmotic adjustment was significantly affected (Fig. 1h). The genotype DRT1019 (0.93 MPa) had higher osmotic adjustment than ICSV95022 (0.51 MPa) under drought stress conditions. Under drought stress, the osmotic adjustment in DRT1019 was increased relative to control by 12.90%, while it was increased by 15.69% in ICSV95022. However, the results suggested that the increased osmotic adjustment under drought stress in tolerant genotype was able to maintain greater cell membrane stability, which in turn leads to higher cell water potential under drought stress conditions for normal physiological functioning [64].

Root traits are essential parameters to assess drought tolerance in most plants [65]. For example, root length is an important component for deep water utilization [66], which is attributed to a greater ability to maintain high and relatively stable xylem water potential during drought stress conditions [1]. In this study, under control conditions the root length was higher in DRT1019 (16.80 cm) than in ICSV950222 (11.69 cm) (Fig. 1i). Stress conditions significantly reduced the root length in both genotypes. (Fig. 1i). Under drought stress, the genotype DRT1019 (14.55 cm) had a higher root length than ICSV95022 (10.39 cm). In terms of % reduction, the root length was reduced in both genotypes i.e., in DRT1019 it was slightly higher (13.39%) than ICSV95022 (11.12%). Although, there is a reduction in DRT1019, the mean root length was higher than ICSV95022

under drought stress, which allows the roots to access water from deeper soil. The difference in the root length is due to the individual genotype's ability to respond drought stress [66]. In addition, the variation in root length between genotypes may be attributed to the difference in their ability to uptake water from deeper soil and was found to be higher in the drought tolerant genotype compared to the susceptible [67].

Effect of drought stress on biochemical traits

Nitrate reductase (NR) enzyme is involved in the metabolic regulation of nitrogen assimilation in plants. In this study, due to drought stress the reduction of NR enzyme activity was more in ICSV95022 (16.87%) than DRT1019 (9.31%) indicating the genotype's response to drought stress. Otherwise, in control conditions, the NR level was higher in DRT1019 (2.90 µmol NO_2 g^{-1} h^{-1}) than in ICSV95022 (1.60 µmol NO_2 g^{-1} h^{-1}). Under drought stress, a significant difference was found between the genotypes for NR levels. DRT1019 (2.63 µmol NO_2 g^{-1} h^{-1}) had higher NR activity than ICSV95022 (1.33 µmol NO_2 g^{-1} h^{-1}) under drought stress conditions (Fig. 2a). The decline of NR activity during drought stress has accompanied by reduced NR transcript level and leads to reduction of rate protein synthesis due to an inhibition of transcription. Our results are in line with previous findings that higher NR activity is involved in stable protein synthesis thereby avoiding the inactivation of essential enzymes under drought stress conditions [68]. Therefore, NR activity under drought stress conditions can be an index for assessing plants' drought tolerance capacity [69].

Proline accumulation was found to be significantly increased with drought stress in both genotypes (Fig. 2b). The proline content were increased by 17% in DRT1019 and 28.57% in ICSV95022. The increase in proline content within genotypes indicates the drought stress effect on these genotypes. Further, the results revealed that in control conditions, the proline level was lower in ICSV95022 (0.75 µmol/g^{-1}) than in DRT1019 (2.10 µmol/$g^{-}1$). In stress conditions, the proline level was significantly higher in DRT1019 (2.53 µmol/g^{-1}) than in ICSV95022 (1.05 µmol/g^{-1}) as shown in the Fig. 2b. The higher level of proline in DRT1019 might serve as a primary combat response to maintain osmotic pressure in the cells, which is associated with drought tolerance through higher osmotic adjustment that stabilizes membranes, enzymes, and proteins necessary for normal cellular functions [14]. In sorghum, proline accumulation in leaves under moisture stress is also associated with the genotypic potential of stress recovery [70].

Metabolomic approaches have revealed that drought-stressed plants accumulate a variety of metabolites such as such as amino acids, organic acids, polyamines, and lipids to protect plant cells against oxidative stresses [71]. In our study, relative changes of metabolites such as total carbohydrate, amides, and lipids were investigated in two sorghum genotypes (Fig. 3). The results revealed that there was a change in these metabolites in the genotypes under drought stress i.e. DRT1019 had a higher absorbance value than the ICSV95022 genotype. Such alterations in the levels of metabolites in response to drought stress possibly play key roles in adjusting cellular metabolism of water stressed plants [72]. It is also revealed that the tolerant genotype would alter the protein composition, metabolic pathways, and utilization of metabolites when acclimating to drought stress [73].

Sorghum transcriptional response to drought stress

Five putative drought genes were selected for analyzing the drought responsiveness of the selected genotypes to drought stress. The qRT-PCR analysis in leaf tissue revealed that four genes viz. *SbTIP3-1, SbDHN1, SbTPS,* and *SbDREB1A* were up-regulated, and *SbABA* was down-regulated in DRT1019 under drought stress (Fig. 4). In contrast, all five

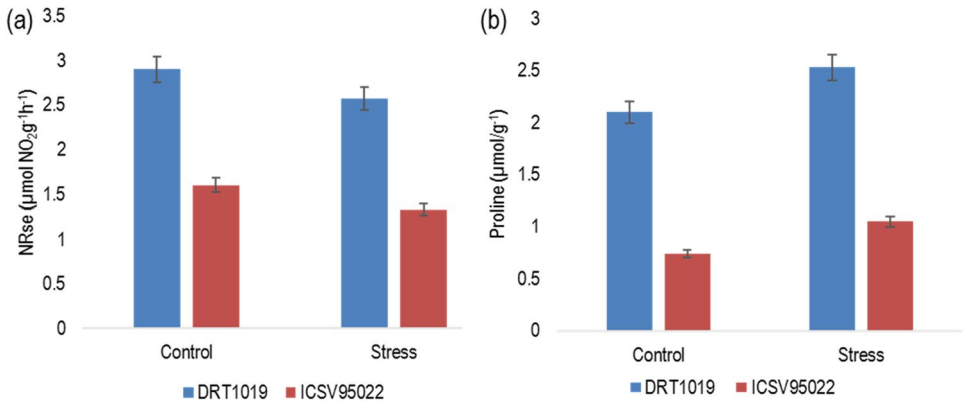

Fig. 2 Effect of drought stress on biochemical traits **a** nitrate reductase activity (NRse) and **b** proline content. The values represent mean ± SE (n = 3)

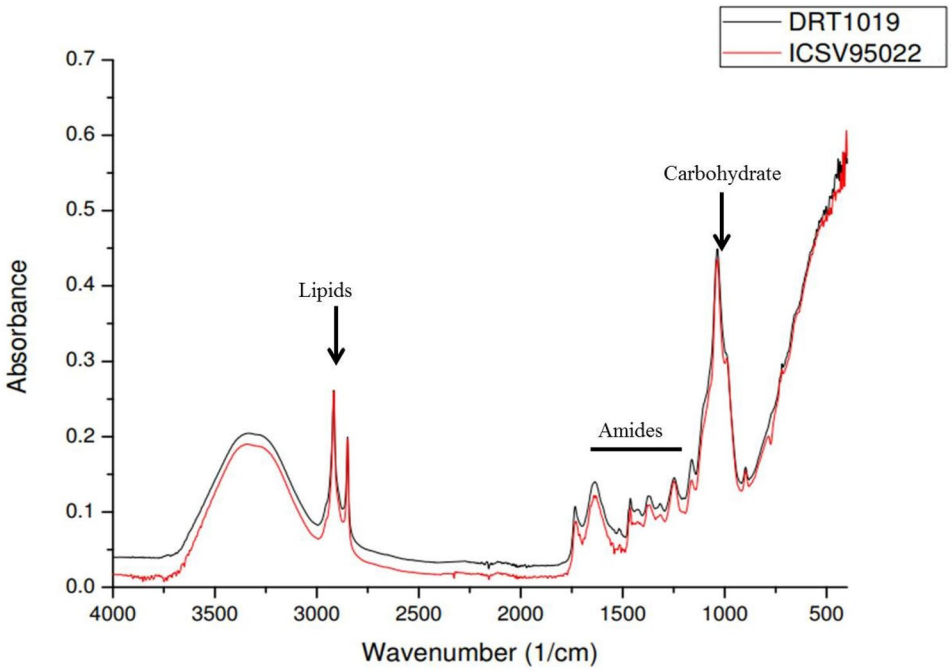

Fig. 3 FT-IR Spectra from tolerant (DRT1019) and susceptible (ICSV95022) genotypes under drought stress conditions. FT-IR raw data were plotted in Origin Pro 8 (OriginLab, Northampton, MA)

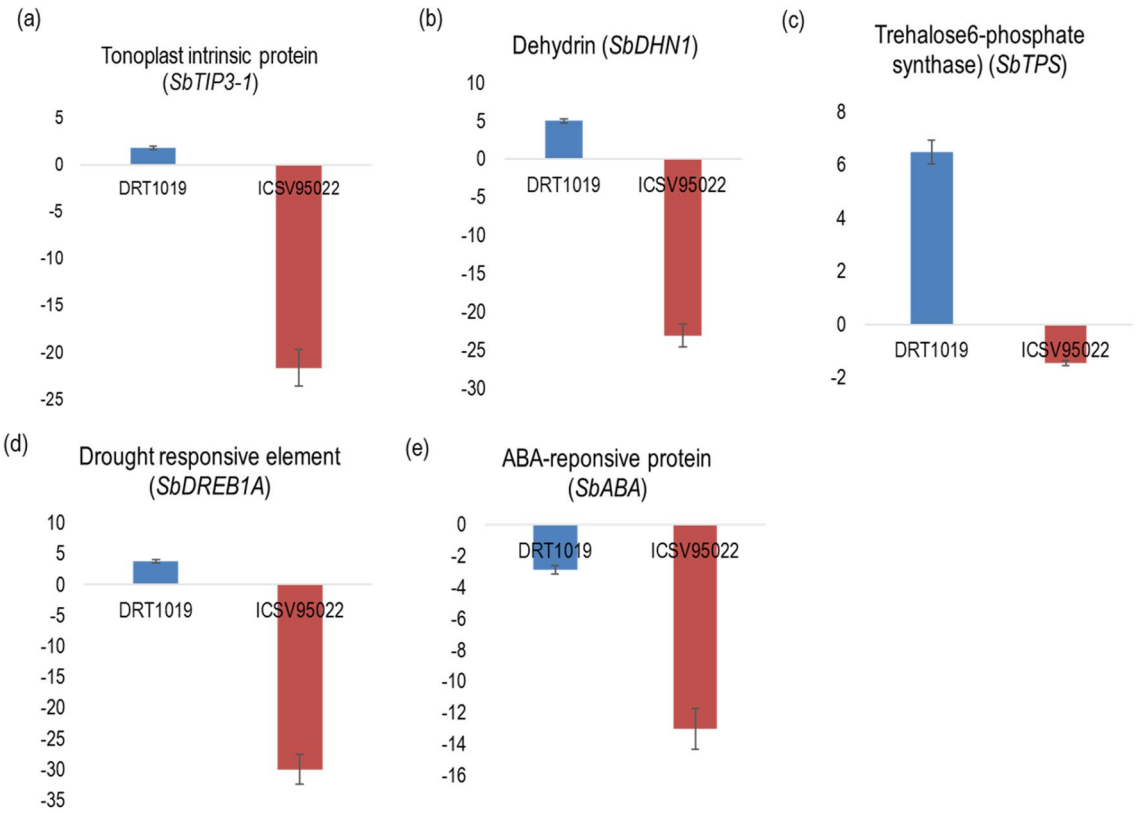

Fig. 4 Relative fold changes of SbTIP3-1 (**a**), SbDHN1 (**b**), SbTPS (**c**), SbDREB1A (**d**) and SbABA (**e**) under drought stress condition in two sorghum genotypes. The values represent mean ± SE. 18 s rRNA was used as endogenous control

genes (*TIP3-1, DHN1, TPS, ABA, and SbDREB1A*) were down-regulated in ICSV95022 (Fig. 4a–e).

In the DRT1019 genotype, *SbTIP3-1*was found to exhibit a 1.8 fold up-regulation as compared to in ICSV95022 during drought stress (Fig. 4a). Increased expression of *SbTIP3-1* encoding aquaporin could be involved in water uptake, regulating the passive exchange of water across membranes [25]. Dehydrin protein belongs to a set of late embryogenesis abundant protein (LEA) accumulating under drought stress and protecting the plants by acting as molecular chaperones and maintaining the ion balance and cell membrane stability [22]. In our study, tolerant genotype DRT1019 showed significant up-regulation of *SbDHN1* by 5.0 fold (Fig. 4b), indicating that water stress leads to the increased role of *SbDHN1* in this genotype. Furthermore, the higher expression of *SbDHN1* confers drought tolerance in sorghum by protecting the protein structures and enhancing the water holding capacity of the genotype [26].

Trehalose functions in sugar metabolism and acts as osmoprotectant in response to salt and drought stress [27]. In our study, gene expression of *SbTPS* (Trehalose 6 phosphate synthase) was investigated in two sorghum genotypes under control and drought stress conditions. The DRT1019 genotype exhibited up-regulation of *SbTPS* by 6.50-fold, while the same gene was down-regulated in ICSV95022 (Fig. 4c). Similarly, genes involved in trehalose synthesis have been significantly up-regulated by drought stress in maize [74], and rice [75]. Hence, overexpression of this gene might have contributed to trehalose accumulation, which may increase the carbohydrate content in the genotype for higher energy supply and acts as osmoprotectant rendering the genotype DRT1019 more stable under drought stress conditions. However, future analysis of trehalose levels in the drought-stressed DRT1019 and ICSV95022 sorghum genotypes is required.

Transcription factors (TFs) like drought responsive element binding (DREB) proteins are involved in drought tolerance [76, 77]. In this study, *SbDREB1A* showed over-expression (3.75-fold) in the tolerant genotype under drought stress (Fig. 4d). Overexpression of *SbDREB1A* in DRT1019 genotype could help in activating ABA-independent pathways to drought stress [78]. In addition, overexpression of DREB1/*CBF* exhibited activation of many stress-responsive genes for enhanced drought tolerance in sugarcane [28]. Therefore, these four promising candidate genes (*SbTIP3-1*, *SbDHN1*, *SbTPS*, and *SbDREB1A*) could be involved in drought tolerance and adaptability of the DRT1019 genotype under drought stress.

In this study, ABA-responsive protein under drought stress was down-regulated by 2.85- and 13.0-fold in tolerant and susceptible genotypes, respectively (Fig. 4e). Similarly, in *A. thaliana*, the ABA-responsive genes involving plant developmental process, tolerance to environmental stresses, and defensive mechanisms were down-regulated, [79]. The downregulation of the ABA-responsive gene could indicate ABA-dependent gene expression in plant growth and developmental processes of both genotypes under drought stress conditions.

Conclusion

In this study the genotype DRT1019 showed increased physiological and biochemical functions compared to ICSV9001, which may be indicative of genotype stability under drought stress. The DRT1019 genotype exhibited drought avoidance through delayed leaf rolling, higher osmotic adjustment, optimal gas exchange system, increased epicuticular wax, solute accumulation and enhanced metabolic alterations. In addition, drought stress greatly affected the expression of the genes and induced changes in the sorghum transcript profiles. The genes exclusively up-regulated in DRT1019 possibly indicate the most promising candidates for drought tolerance in sorghum. Based on the known functions of these genes from other crop plants, the tolerant genotype DRT1019 is possibly capable of higher root water uptake (*SbTIP3-1*), higher cell membrane stability (*SbDHN1*), higher osmotic adjustment by sugar accumulation (*SbTPS*), and stimulation of various stress-responsive genes (*SbDREB1A*) involved in normal physiological functions during drought stress. Overall, this study offers a glimpse of sorghum whole plant morpho-physiological, metabolic, and molecular mechanisms associated with drought stress. In future, the genotype DRT1019 could be useful in the identification of drought tolerant QTLs in breeding programs through development of recombinant inbred lines and segregating populations.

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Author contributions KR, KG, MR and PJ conceived the idea. KR, MR, and PS wrote the main manuscript text. KR, PTP, AY and CS prepared the manuscript. KR, KG, MR and PTP revised the manuscript at different stages of the writing process and read and approved the revised manuscript.

Declarations

Conflict of interest All the authors declared that no conflicts of interest are associated with this publication.

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