The role of sucrose metabolizing proteins in hyperosmotic stress tolerance in sweet sorghum (Sorghum bicolor L. Moench)

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Abstract

Sugar accumulation is a common metabolic response under drought stress conditions. However, studies on the underlying molecular mechanisms of sugar accumulation under stress remain restricted. This study explores the role of sucrose metabolizing proteins in conferring tolerance to drought-induced hyperosmotic stress, and ultimately osmotic adjustment in sorghum. The effect of stress on sugar content, enzyme activity and gene expression was investigated. Sweet sorghum varieties (ICSV213 and ICSB338) differing in levels of drought tolerance were subjected to a 10-day water deficit period. Brix plant sap analysis indicated elevated total soluble sugar levels under water stress conditions in both varieties with ICSV213 demonstrating a higher brix content than ICSB338. HPLC analysis gave a decrease in sucrose levels and an increase in glucose and fructose concentrations in both varieties with ICSV213 demonstrating higher hexose levels. Enzyme activity levels of invertase, sucrose phosphate synthase and sucrose synthase were found to increase under stress in both varieties with ICSV213 invertase displaying the highest activity when compared to other sucrose metabolizing enzymes. Transcriptional expression of invertase and sucrose phosphate synthase (SPS) genes was significantly up regulated in ICSV213 under stressed conditions, whereas sucrose synthase (Susy) levels remained low in both varieties. Data obtained for sorghum variety ICSV213 points towards a relationship between hyperosmotic stress tolerance and the accumulation of solutes and sucrose metabolism proteins. Consequently variety ICSV213 may therefore be an excellent target for future development of hyperosmotic stress tolerant sweet sorghum.

Keywords: abiotic stress; bio-energy production; drought tolerance; osmotic adjustment; sucrose metabolism enzymes; sugar metabolism.

Abbreviations: DTT_dithiothreitol; DW_dry weight; EDTA_ethylenediaminetetraacetic acid; Fru_6-P_fructose-6-phosphate; FW_fresh weight; LWRC_leaf relative water content; NADP_nicotinamide adenine dinucleotide phosphate; NI_neutral invertase; PVPP_polyvinylpolypyrrolidone; SAI_soluble acid invertase; SPS_sucrose phosphate synthase; SS_sucrose synthase; TW_turgid weight; UDP_ uridine-5’-diphosphate.

Introduction

Sweet sorghum has been proposed as the key model system for gene discovery relating to biomass yield and quality in the bio-energy grasses under drought stressed conditions (Calvino and Messing, 2011). This is attributed to high energy return obtained from high stem sugar content, and abiotic stress tolerance due to high water and nutrient use efficiencies (Shakoor et al., 2004; Mutava et al., 2011). The characterization of a diverse sorghum germplasm collection with considerable variation for energy yield and abiotic stress tolerance will result in sorghum hybrids that will sustain next-generation bio-fuel production in arid and semi-arid regions (Rooney et al., 2007; Slewinski, 2012). This can be achieved by gaining understanding of the underlying processes that lead to differential accumulation in soluble sugar content in sorghum varieties under drought conditions. Sucrose accumulation and its subsequent metabolism for cascades of cellular activities that usually ensure when plants are subjected to various abiotic stresses such as drought, cold, salinity is shown to be a general widespread response (Mahajan and Tuteja, 2005). It is considered a major osmoprotectant involved in regulating osmotic potential in plants, thereby offering resistance against hyperosmotic stress (Massacci et al., 1996; Djilianov et al., 2005). Three key enzymes regulate sucrose metabolism and have been implicated in carbon partitioning thereby influencing plant growth and development (Qazi et al., 2012). Sucrose phosphate synthase (EC 2.4.1.14) catalyses the synthesis of sucrose in photosynthetic cells using fructose-6-phosphate (Fru-6-P) and glucose, and is also active in the futile cycle of simultaneous breakdown and synthesis of sucrose in various tissues (Park et al., 2008). Invertase (EC 3.2.1.26) catalyses the conversion of sucrose to glucose and fructose and is involved in phloem unloading and the control of cell differentiation and development (Huang, 2003). Sucrose synthase (EC 2.4.1.13) reversibly converts sucrose into UDP-glucose and fructose in the presence of uridine 5’-diphospho-
ate (UDP) and its activity is suggested to be associated with sink strength (Winter and Huber, 2000; Koch (2004); previously reported on the modulation of sucrose metabolism genes under stressed conditions which resulted in sucrose flux suggesting the possible roles of the genes in stress response. The current study explores the potential role of sucrose metabolizing proteins in conferring tolerance to drought-induced hyperosmotic stress, and ultimately osmotic adjustment in sweet sorghum.

Results

**Differential response to water stress in sorghum plants**

The ten day water stress period influenced the water content in both ICSV213 and ICSB338 sorghum varieties. The leaf relative water content of ICSB338 was reduced by 46% as compared to 27% loss in ICSV213 (Fig 1).

**Effect of water stress on soluble sugars**

Stress induction elevated brix levels in the basal stems of the two varieties with ICSV213 displaying a significant 35% increase in stressed plants (Fig 2a). Analysis of the amount of stem soluble sugars (sucrose, glucose and fructose) revealed a decrease in sucrose levels under water stress in both varieties (Fig 2b). Fructose and glucose were increased under stress in both varieties with, ICSV213 accumulating significant levels compared to ICSB338 (Fig 2c and d).

**Changes in sucrose metabolizing enzymes activities during hyperosmotic stress**

Invertase activity levels were higher in ICSV213 compared to ICSB338 under both growth conditions. Soluble acid and neutral invertase activities were triggered by stress induction in both ICSV213 and ICSB338 (Table 2). SAI increased significantly under stress by 44% and 42% in ICSV213 and ICSB338, respectively. NI levels under stressed conditions increased by 50% in both ICSV213 and ICSB338. Analysis of enzyme activity revealed an increase in SPS activity under stress in both varieties with SPS levels displaying a 64% increase in ICSV213 (Table 3). Sucrose synthase induction was more favourable towards sucrose synthesis under stressed conditions with ICSV213 displaying an increase of 11% and ICSV213 an increase of 50% in activity (Table 4).

**Differential expressions of sucrose metabolizing genes in sweet sorghum stem tissues during hyperosmotic stress**

Figure 3 shows changes in gene expression levels as detected for sucrose metabolism genes under water stress. A 55-fold increase in ICSV213 invertase expression levels was observed in stressed plants compared to the controls, whereas ICSB338 displayed insignificant changes in expression levels under stress (Fig 3a). Sucrose phosphate synthase expression in stressed plants relative to controls was increased by 15 folds, with stress further repressing the low expression levels observed for ICSB338 SPS (Fig 3b). Sucrose synthase expression levels were low compared to invertase and sucrose phosphate synthases. The expression levels were further reduced by stress in both varieties ICSB338 and ICSV213 (Fig 3c).

**Discussion**

Sugar accumulation has been demonstrated as one of the defence mechanisms plants acquire in response to stressed conditions in order to maintain osmotic balance for plant metabolic activities to operate effectively (Gill et al., 2001). Thus, understanding the molecular mechanisms that regulate sugar biosynthesis or degradation, transport and allocation is vital for creating platforms to optimize whole-plant carbohydrate partitioning for increased productivity under stress. This present study focused on the evaluation of sucrose metabolism at molecular levels during drought-induced hyperosmotic stress in two sorghum varieties differing in levels of drought tolerance. The effectiveness of the induced drought stress was demonstrated by the decrease in leaf relative water content, which demonstrated the sensitivity of the sorghum varieties to water stress (Fig 1). Relative water content is considered an important indicator of water stress in leaves, which is directly related to soil water content, with its reduction resulting in changes in plant metabolic pathways (de Oliveria Neto et al., 2009; Anjum et al., 2011; Hamad and Ali, 2014). The different levels at which LRWC declined between ICSV213 and ICSB338 validated the differences in relation to water stress responses. This was further demonstrated by the difference in sugar levels under stress in the two varieties (Fig 2). The increased total soluble sugar levels (brix) under stress were attributed to hexose (fructose and glucose) accumulation as reduced sucrose concentrations were observed in both varieties. Results demonstrated stem reserve mobilization of sucrose at a higher level in ICSV213 compared to ICSB338 under stress as evident from the difference in brix and hexose levels between these varieties. These observations were previously made in soybean, pigeon pea and sugarcane mature culms under water stress (Lawlor and Cornic, 2002; Liu et al., 2004; Rodrigues et al., 2009 and Iskandar et al., 2011). The higher levels of fructose produced in stressed plants in both varieties was an indicator that fructans were possibly the main storage carbohydrates remobilized to fructose under stress with suggestive roles in osmoregulation (Pilon-Smits et al., 1999; Livingston III et al., 2009; Gaudet et al., 2011; Van den Ende and El-Esawi, 2014).

The underlying mechanisms attributing to the observed accumulation of hexoses and depletion of sucrose with stress induction were examined by analysing the sucrose metabolism enzyme activities and gene expression in response to stress induction. The effect of stress on sucrose metabolism was characterized by an increase in SPS enzyme activity in both varieties with ICSV213 displaying an increase in activity under stress as compared to ICSB338. Sucrose phosphate synthase activity levels were the same in both varieties under stress. These observations were comparable to previous reports for a C₄ resurrection plant *Sporobolus stapfianus* (Whittaker et al., 2007) and grapevines (*Vitis vinifera* cv. *Moscatel*; Maroco et al., 2002) during water deficit. The increase in SPS enzyme activity under stress was however not associated with sucrose content. Although sucrose synthase was favourable towards sucrose synthesis under stress, activity levels compared to SPS indicated that sucrose synthesis was solely dependent on the activity of SPS as previously reported in wheat stems at anthesis by Yang et al., (2004), and that this enzyme does not play a decisive role in regulating sucrose levels in sweet sorghum stems. Of the key sucrose metabolizing proteins investigated, invertases were significantly up-regulated by stress in both varieties, thus attributing to the observed
Table 1. Sorghum sucrose biosynthesis gene primer pairs used for gene expression studies.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Accession number</th>
<th>Primer sequence</th>
<th>Product size</th>
<th>Annealing temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPS</td>
<td>Sb05g007310</td>
<td>FP5’GCAAACCTTACGCTGATACTG’3 RP5’CTTGTGGTGCTTAGGTAAGG’3</td>
<td>141</td>
<td>55°C</td>
</tr>
<tr>
<td>Susy</td>
<td>Sb01g033060</td>
<td>FP5’ATGGTAGATTCCTCCGCAAGTGG’3 RP5’CCTGGCATTTTGGAGAATGT’3</td>
<td>346</td>
<td>52°C</td>
</tr>
<tr>
<td>Inv</td>
<td>Sb04g000620</td>
<td>FP5’CATCGTTGGTTATCCGATGGTGATGCG’3 RP5’GTAAGTCGATGGATCCG’3</td>
<td>134</td>
<td>56°C</td>
</tr>
</tbody>
</table>

Fig 1. Percent of leaf relative water content (LRWC) of sweet sorghum varieties ICSV213 and ICSV38 after 10 days of drought stress induction. The error bars represent the mean standard error obtained from the SAS (Version 9.3) analysis Well-Watered (WW); Drought Stressed (DS).

Table 2. Soluble acid invertase (SAI) and neutral invertase activity in mature sorghum stems. Activity is expressed in pmol mg⁻¹ protein min⁻¹. Mean ± SE, n = 3.

<table>
<thead>
<tr>
<th>Variety</th>
<th>SAI</th>
<th>NI</th>
</tr>
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<tbody>
<tr>
<td>ICSV213</td>
<td>71.5 ± 0.2*</td>
<td>47.3 ± 0.4*</td>
</tr>
<tr>
<td>ICSV213-ds</td>
<td>128.0 ± 1.2*</td>
<td>94.3 ± 0.7*</td>
</tr>
<tr>
<td>ICSV38</td>
<td>33.4 ± 1.2*</td>
<td>18.7 ± 0.4*</td>
</tr>
<tr>
<td>ICSV38-ds</td>
<td>50.2 ± 1.2*</td>
<td>32.1 ± 1.3*</td>
</tr>
</tbody>
</table>

*statistically significant LS means; DS: Drought Stressed.

Fig 2. Shows (A) brix; (B) sucrose; (C) fructose; (D) glucose accumulation in the stem of ICSV213 and ICSV38 plants subjected to 10 days of water deficit. The error bars represent the mean standard error obtained from the SAS (Version 9.3) analysis. Well-Watered (WW); Drought Stressed (DS). g l⁻¹ grams of sugar present in a liter of stem sap.
Table 3. Sucrose phosphate synthase activity in mature sorghum stems. Activity expressed in pmol mg⁻¹ protein min⁻¹. Mean ± SE, n=3.

<table>
<thead>
<tr>
<th>Variety</th>
<th>WW</th>
<th>DS</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICSV213</td>
<td>17.9 ± 0.1*</td>
<td>49.2 ± 0.2*</td>
</tr>
<tr>
<td>ICSB338</td>
<td>14.3 ± 0.2*</td>
<td>39.0 ± 0.5*</td>
</tr>
</tbody>
</table>

*Statistically significant LS means; WW - Well Watered; DS - drought stressed

Fig 3. Quantitative PCR analysis of (A) invertase, (B) sucrose phosphate synthase, (C) sucrose synthase during drought treatments of sorghum varieties. All values were normalized to the relative expression levels of 18S RNA, β-actin and ubiquitin genes. The error bars represent the mean standard error. Well-Watered (WW); Drought Stressed (DS); AU (Arbitrary Units) represents fold.

Hexose accumulation under stress. Invertase activity of ICSV213 was higher under stress compared to ICSB338. These observations are supported by findings made by Wardlaw and Willenbrink (2000) and Trouverie et al., (2003) in wheat and maize plants respectively, where an increase in hexose accumulation in response to water stress was accompanied by a rise in invertase activity. The observed higher invertase activity compared to SPS in both varieties under stress was an indication that although SPS activity was increased by stress, its activity was below a threshold level necessary for sucrose to accumulate significantly. Transcriptional regulation which is considered to be one of the major regulatory forms in controlling metabolic alteration in plants (Xue et al., 2008) was also determined. Gene expression analysis showed an increase in invertase levels under stress for variety ICSV213. Increased invertase expression levels have previously been reported in maize, arabidopsis and sugarcane tissues (Kim et al. 2000; Qi et al. 2007) resulting in increased osmotic pressure that led to drought resistance.

In relation to the high invertase activity obtained, lower gene expression levels were observed under stress in variety ICSB338. This could be attributed to factors such as substrate concentration and specificity contributing to the observed high enzyme activity levels in this variety. Sucrose phosphate synthase transcripts were also increased by stress induction in variety ICSV213, an observation previously reported in wheat tissues subjected to water stress (Xue et al., 2008) whereas, ICSB338 displayed low transcript levels. The higher enzyme levels of SPS compared to the mRNA levels in ICSV213 could be attributed to substrate concentration and specificity, as previously suggested. The low sucrose synthase mRNA levels in both varieties indicated that this enzyme may not play a decisive role in regulating sucrose levels in sweet sorghum stems. A comparison between SPS and invertase gene expression levels indicated that invertase was more up-regulated compared to SPS, a similar observation to the enzyme activity data. The results therefore suggest that the expression of sucrose metabolism proteins in ICSV213 was independent of the osmotic effect as no relationship was established between sugar content and protein expression. The results further suggest that the differential stress-responsive mechanisms of proteins in this variety under stress could represent part of a general response to the allocation of carbohydrates during acclimation period rather than changes in sucrose concentration. The higher
levels of enzyme activity, gene expression, soluble sugar accumulation and lesser water reductions in ICSV213 compared to ICSB338 suggests that the sucrose metabolizing proteins tested have a cause and effect relationship with the differences between the two sweet sorghum varieties. The relationship established between hexose accumulation, sucrose depletion, and the up-regulated enzyme and gene levels in ICSV213 indicated that this variety was able to correctly proportion assimilates to the essential parts of the plants for longer survival under stress. It further highlighted the possible role of sucrose metabolizing proteins in osmotic adjustment and ultimately protection against drought induced hyperosmotic stress.

Materials and Methods

Cultivation of plant material and treatment

Cultivation of sorghum (Sorghum bicolor (L.) Moench) at optimum conditions

Seeds of Sorghum bicolor (L.) Moench ICSV213 (more drought tolerant) and ICSB338 (less drought tolerant) were provided by the International Crop Institute for the Semi-arid Tropics (ICRISAT), India. The seeds were sown at the Agricultural Research Council glasshouse in Stellenbosch (South Africa). The glasshouse conditions included 41/21 °C day/night temperature cycle and natural daylight. Pots with a diameter of 25 cm and height of 25 cm containing a sterilized equal proportion mix (1:1) of loam soil and inorganic nutrient solution (Ca(NO3)2 (164.1 g l⁻¹); KNO3 (101.1 g l⁻¹); MgSO4 (120.4 g l⁻¹); KH2PO4 (136.1 g l⁻¹) and FeEDTA (16.6 g l⁻¹) were used for sowing the sorghum seeds.

Drought treatment

The experiment was laid out in a randomized complete block design (RCBD) with ten biological replications for each sorghum variety. Sorghum plants were grown until the early stages of anthesis before drought was imposed by withholding water on half of the biological replicates of each variety for a 10-day period. Leaves from control and stressed plants were harvested and used immediately to measure leaf relative water content (LWRC) according to the method of Barrs and Weatherley (1962). Mature sweet sorghum internodes from the treatment and control experiments were quickly submerged in liquid nitrogen and used for sugar analysis. Ultimately, the internodes were stored at -80°C and used for protein and total RNA extraction.

Plant Extraction and determination of total soluble sugars

Brix analysis

Sorghum stems (500 mg) were grouped in triplicates for control and stressed plants for sugar analysis. The stems were finely crushed using pre-cooled pestle and mortar. The homogenized tissue was transferred into a 10 ml syringe and the sap collected by pressing the tissue with a plunger into a pre-cooled 1.5 ml microcentrifuge tube. The centrifuged extract was analyzed for Brix content using a general laboratory refractometer (OTAGO™, Germany).

HPLC analysis

Stem powder was homogenized in 80% acetone (v/v), followed by incubation at 80 °C for 1 hour. The supernatant was removed, concentrated using the Savant speedvac (Thermo Scientific, USA) and re-suspended in 1 ml distilled water. Soluble sugars were analyzed by high performance liquid chromatography (HPLC, Dionex™ Ultimate 3000) using a Rezex RHM monosaccharide column, temperature: 25 °C; speed: 0.6ml min⁻¹, detector: Shodex RI-101, mobile phase. Sucrose, glucose and fructose were identified and quantified by comparison with retention times and peak areas of external standards (Sigma-Aldrich, Germany). Analyses were performed in triplicate using three separate extractions for each treatment. The sugar content was expressed as g l⁻¹.

Protein extraction and quantification

Frozen sorghum internode groups from the treatment and control plants were pooled to minimize inter plant variability and ground to fine powder in liquid nitrogen prior to protein extractions. Protein was extracted using extraction buffer consisting of 100 mM Hepes-KOH (pH 7.5), 2 mM EDTA, 5 mM DTT, 4 mM MgCl2, 10% glycerol and 2% PVPP. Extracts were centrifuged at 12000 rpm for 4 min at 4 °C. The supernatants were desalted with Sephadex G-50 spin columns pre-equilibrated in extraction buffer before centrifugation at 4000 rpm for 2 min at 4 °C. The soluble protein fraction of the supernatants were recovered and used for activity assays. Total protein concentration in the supernatants was determined according to Bradford (1976) using bovine serum albumin as a standard.

Table 4. Sucrose synthase activity in mature sorghum stems in the breakdown and synthesis direction. Activity expressed in pmol mg⁻¹ protein min⁻¹. Mean ± SE, n = 3.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Sucrose breakdown</th>
<th>Sucrose synthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICSV213</td>
<td>3.1 ± 0.6</td>
<td>11.9. ± 0.2 *</td>
</tr>
<tr>
<td>ICSV213-ds</td>
<td>3.7 ± 0.7</td>
<td>23.6. ± 0.2*</td>
</tr>
<tr>
<td>ICSB338</td>
<td>6.5 ± 0.3</td>
<td>12.5. ± 0.2*</td>
</tr>
<tr>
<td>ICSB338-ds</td>
<td>7.4 ± 0.7</td>
<td>14.1. ± 0.2 *</td>
</tr>
</tbody>
</table>

*Statistically significant LS means; DS: Drought Stressed
Sucrose metabolizing enzyme activity assays

Invertase assay
Soluble acid and neutral invertase activities were determined according to Rossouw et al. (2010). Soluble acid activity at 35°C was started by adding desalted extract to assay buffer of 30 mM citrate phosphate (pH 5.5) and 125 mM sucrose. Reactions were incubated at 35 °C for 30 min and stopped by boiling the reaction solution for 2 min. Neutral invertase activity was started by adding desalted extract to assay buffer of 50 mM Hepes (pH 7.5) and 125 mM sucrose. The reaction was stopped by the addition of 2M Tris-HCl (pH 8.0) and 22 mM ZnSO4 solution. The amount of reducing sugars produced in the samples was measured using an NAD+ coupled reaction according to Huber and Akazawa (1986). NADP production was monitored at 340 nm.

Sucrose phosphate synthase assay
Sucrose phosphate synthase activity was assayed according to Baxter et al. (2003). Activity at 35 °C was started by adding 50 µl of desalted extract to assay buffer containing 50 mM HEPES (pH 7.5), 20 mM KCl and 4 mM MgCl2, 12 mM UDP-Glc, 10 mM Fruc 6-P and 40 mM Glc-6-P. The reaction was incubated at 35 °C for 30 min and stopped by boiling for 5 min. Sucrose produced was determined using the anthrone method (Van Handel, 1972).

Sucrose synthase assay (synthesis direction)
Sucrose synthase activity was assayed using a coupled reaction according to Schafer et al. (2004). Activity in the synthesis direction was started by the addition of 10 mM fructose to desalted extract incubated in assay buffer consisting of 100 mM Tris-HCl (pH 8.0), 10 mM MgCl2, 20 mM UDP-glucose, 0.2 mM NADP, 1 mM phosphoenolpyruvate and 0.45U ml-1 pyruvate kinase/lactate dehydrogenase (EC 2.7.1.40 and EC 1.1.1.27). The decrease in the absorbance of NADP was monitored at 340 nm. Activity was calculated in terms of pmols NADP oxidized per mg protein per min.

Sucrose synthase assay (cleavage direction)
The catalytic activity of sucrose synthase was assayed using a NAD-coupled reaction according to Schafer et al. (2004). Activity in the cleavage direction was started by the addition of 1.5M UDP to desalted extract incubated in assay buffer containing 100 mM Tris-HCl (pH 8.0), 2 mM MgCl2, 400 mM sucrose, 2 mM NAD+, 1.5 mM Na4P2O7, 4U ml-1 phosphoglucomutase (EC 5.4.2.2) and 4U ml-1 glucose-6-phosphate dehydrogenase (EC1.1.1.49).

NADP production was monitored at 340 nm. Sucrose synthase activity was calculated in terms of pmols NADP produced per mg protein per min.

Expressional analyzes of sucrose metabolizing genes

Total RNA isolation, cDNA synthesis and PCR amplification
Frozen sorghum internodes from the treatment and control plant groups were pooled to minimize inter plant variability and were ground to fine powder in liquid nitrogen prior to RNA extractions. RNA was extracted from ground tissue using the SV 96 Total RNA isolation system (#Z3105, Promega, USA) following the manufacturer’s instructions. Extracted RNA was quantified using a NanoDrop ND-1000 Spectrophotometer (Thermo Scientific, USA) at 260 nm and electrophoresed on a 3 % agarose gel to verify its integrity. Approximately 0.5 µg of RNA from each sample was reverse-transcribed into cDNA using the High Capacity cDNA synthesis Kit (#4368814, Life Technologies, USA) following the manufacturer’s instructions.

Relative quantification of sucrose metabolizing genes by RT-qPCR
The RT-qPCR reactions were carried out using an ABI 7900HT Real Time PCR system following manufacturer’s instructions. Gene-specific primers were designed so they would bind to all isoforms of the target genes. The primers were designed across the exon/exon boundary using PerlPrimer (http://perlprimer.sourceforge.net/) using primers listed on Table 1. The thermal cycling conditions used were as follows: initial PCR activation (95 °C for 15 sec); denaturation (95 °C for 15 sec; 40 cycles); extension (60 °C for 15 sec; 40 cycles); final extension (72 °C for 20 s). PCR products were detected by monitoring the increase in fluorescence of the SYBR Green dye during the extension phase of each cycle when the SYBR Green dye binds to double-stranded DNA. The results obtained for the different cDNAs were normalized against the expression levels of three stable housekeeping genes: sorghum β-actin (X79378); ubiquitin (Sb04g031060) and 18S RNA (Sb03g017560).

Statistical analysis
The experiments for determining leaf relative water content, sugar levels, activities for sucrose metabolizing enzymes and gene expression consisted of three biological replicates. All experiments were performed three times, independently. Statistical tests were performed with the statistical software SAS version 0.3 (SAS, 2012) to determine the significant changes obtained with drought stress induction using a generalized linear model procedure. Means were compared according to the Student-t and LSD (least significant differences) at 5 % level of significance.

Conclusion
The results observed highlight the use of sucrose metabolizing proteins as potential targets for metabolic engineering in order to improve stem soluble sugar concentrations (essential for biofuel production) and grain quality in sorghum varieties exposed to extreme water stress conditions in the field. Further studies are however required to examine the role of cellular localization or developmental stages on the expression levels of these proteins. These investigations would further aid in elucidating gene regulatory networks for controlling sucrose metabolism in sorghum under stress, thus contributing to the development of a robust variety that is highly hyperosmotic stress tolerant, or possibly resistant to drought stress.

Acknowledgments
Special thanks to the Agricultural Research Council and the Department of Science and Technology (South Africa) for financial assistance.
References


