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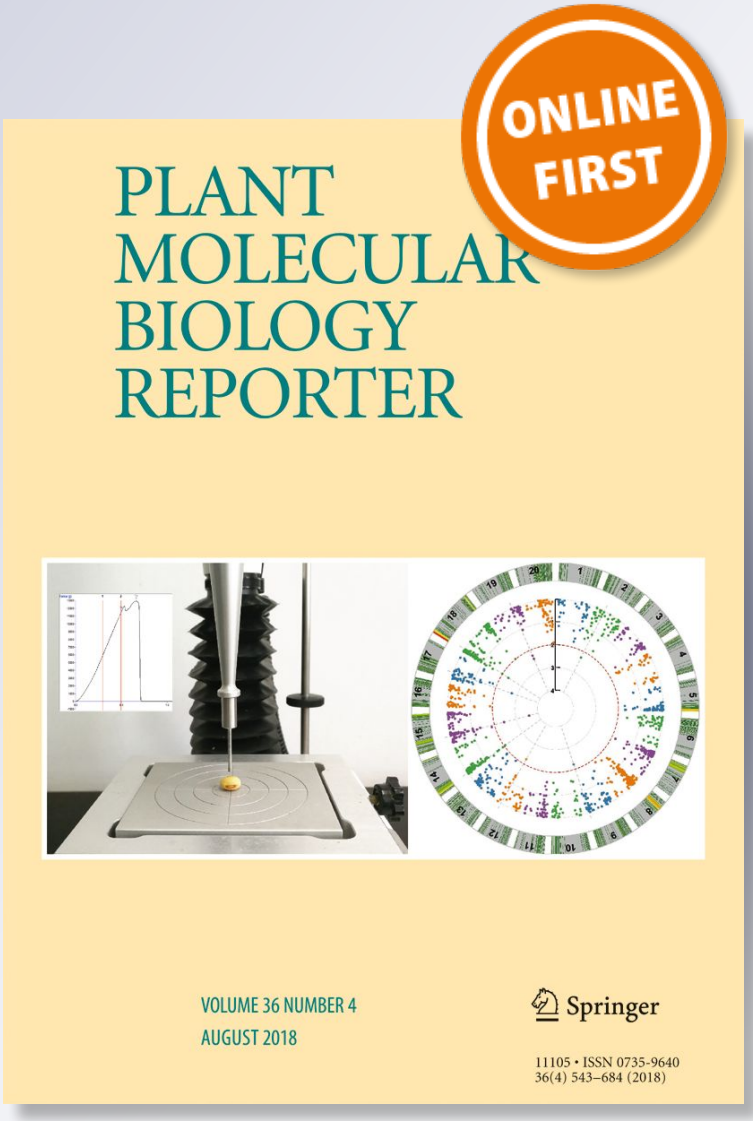

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ORIGINAL PAPER

Role of ABA Signaling in Regulation of Stem Sugar Metabolism and Transport under Post- Flowering Drought Stress in Sweet Sorghum

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Abstract

Sugar remobilization from vegetative to reproductive tissues is an important process that determines grain yield in crops. Sweet sorghum stems store sugar and introgression of Stay green1 (Stg 1) locus from the grain sorghum genotype B35 into the sweet sorghum genotype S35 was previously shown to bring about a 2-fold higher stem sugar accumulation in the near-isogenic line (NIL) S35SG06040. We hypothesized that remobilization of stem sugar augments grain yield on exposure to drought stress and that the phytohormone abscisic acid (ABA) has a role to play in this process. ABA levels were three times higher in the NIL as compared to those in S35 on drought stress exposure. Remobilization of stem sugars in the NIL was evident by the observed decrease in reducing sugar content in the stem but not in the peduncle in response to prolonged drought stress exposure. Drought-induced expression of some ABA response factors (ABFs) as well as invertase and sucrose transporter genes was seen to be higher in the NIL as compared to S35. An over-representation of ABA-responsive elements (ABREs) and sugar signaling motifs in the differentially expressed genes indicated the involvement of ABA and sugar signaling in regulation of their expression. Two *ABF* genes located on the Stg1 locus showed single nucleotide polymorphism, which possibly accounted for their differential regulation in S35 and the NIL. The results suggest that ABA signaling plays an important role in post-flowering drought-induced remobilization of sugars to the reproductive sinks.

Keywords Abscisic acid · Sugar accumulation · Drought stress · Hormonal regulation · Signaling

Introduction

Sweet sorghum (*Sorghum bicolor* Moench.) is known to accumulate sucrose in the stem, which occurs mostly after completion of vegetative growth (Slewinski 2012). Sucrose is transported from source leaves to the stem via phloem and is stored in vacuoles of stem parenchyma cells. Stem sugars in grasses are thought to represent stores of excess photoassimilates, hence reducing feedback inhibition of photosynthesis (Pollock et al. 2003). Sucrose synthase and invertases play an important role in the regulation of sugar accumulation in sink tissues (Braun et al. 2014). In addition, sucrose transporters (SUTs and SWEETs) also play an important role in the regulation of sugar fluxes in source and sink tissues (Mizuno et al. 2016).

Stem carbohydrates have been shown to remobilize to the panicle during grain filling (Ruuska et al. 2006) and are also thought to act as a buffer when photoassimilation decreases

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under stress conditions like water limitation, thus ensuring viable seed production (Blum et al. 1997; Slewinski 2012). A trade-off between grain yield and non-structural carbohydrate accumulation in the stem has been observed in sweet sorghum, where the panicle weight negatively correlated to stem sugar content (Shukla et al. 2017). Similarly, in post-rainy season-grown sweet sorghum, where water is often limiting, stem sugar yield reduced significantly, while the grain yield increased (Vadez et al. 2011). Remobilization of carbohydrates from stem to grains was shown to increase in wheat to compensate for the insufficiency of photosynthetic products formed under water stress (Yang et al. 2004). Remobilization of reserves therefore is an important drought tolerance strategy in crops (Blum 2005).

Abscisic acid plays an important role in regulating plant responses to drought. On exposure to drought stress, ABA accumulation occurs in plants either through activation of ABA biosynthesis genes like 9-cis-epoxycarotenoid dioxygenase (NCED) or by hydrolysis of its glycosyl ester (Yang and Tan 2014). ABA accumulation leads to the activation of ABA response factors (ABFs), which belong to the bZIP family of transcription factors (Jakoby et al. 2002). The sorghum ABFs belong to class 3 of the bZIP family of transcription factors (Wang et al. 2011) and are orthologous to the group 1 bZIP family from *Arabidopsis* (Jakoby et al. 2002). The ABFs bind to ABA-responsive elements (ABREs), which are *cis*-elements having the sequence ACGTG(G/t)C, with AC, CC, or TG preceding it (Choi et al. 2000). ABREs are present in the promoters of many drought response genes, including the ABA biosynthesis genes and ABF genes (Fujita et al. 2005).

ABA signaling is also known to bring about transcriptional or post-transcriptional regulation of genes coding for enzymes involved in sucrose metabolism like invertases and sucrose synthases (Koch 2004). For example, ABA led to an induction of vacuolar invertase activity and higher expression of the vacuolar invertase (*INV2*) gene in maize leaf (Trouverie et al. 2003). ABA accumulation under water stress was also shown to reduce the transport of sucrose into maize kernels and regulate the activities of enzymes of sugar to starch conversion in rice spikelets (Trouverie et al. 2003; Wang et al. 2015). Further, an *ABF* was shown to bind to promoters of sugar transporter and amylase genes, and activate their expression, leading to accumulation of sugar in apple fruits (Ma et al. 2017). Sugar-based signals in turn are known to play a role in regulation of ABA biosynthesis and signaling, as evidenced by the fact that sugar response mutants were often ABA-related (Rook et al. 2006).

Besides hormonal regulation, alteration in gene expression is known to occur due to the presence of single nucleotide polymorphism (SNP) within *cis*-elements present in the promoter regions of genes. For example, a SNP in the promoter of *TaGW2–6*, which codes for a ubiquitin E3 ligase in wheat, was shown to alter the CGCG motif that is involved in Ca^{2+}/calmodulin-mediated regulation of this gene, leading to a change in its expression (Jaiswal et al. 2015). Regulation of gene expression is also known to occur at the post-transcriptional level through microRNA (miRNA)-mediated cleavage/translation inhibition of target mRNAs. Presence of SNPs in either miRNAs or their target sites was shown to alter the expression of several development-related genes in cucumber genotypes (Ling et al. 2017).

In the present paper, attempts have been made to understand remobilization of sugars in sweet sorghum, which stores photoassimilates in two independent sinks, namely the stem and panicle. We hypothesized that sweet sorghum can augment grain yield when subjected to drought stress through efficient remobilization of stem sugars, hence ensuring food security. For testing this hypothesis, a sweet sorghum genotype S35 and its NIL S35SG06040, which has introgression of a stay green locus (Stg1) from the grain sorghum genotype B35, have been used. Stay green phenotypes in sorghum have been reported to confer post-flowering drought tolerance due to delayed onset of leaf senescence (Harris et al. 2006). Marker-assisted foreground (Stg1 locus) selection using simple sequence repeat (SSR) alleles of B35 adjacent to the locus and background selection using SSR alleles of S35 located elsewhere in the genome was carried out on the third generation backcross of the F1 plants with recurrent parent S35 (BC_3F_1) (Vadez et al. 2013). The Stg 1 locus, which comprises of 342 genes, not only confers post-flowering drought tolerance to the NIL (Vadez et al. 2011) but also increases the stem sugar content as compared to the parent S35 (Ghate et al. 2017). The sweet sorghum line S35 and its NIL S35SG06040 were compared for sugar and ABA levels on exposure to post-flowering drought stress. Use of an introgression line enabled comparison over a fairly uniform genetic background and reduced genotype-dependent differences. Gene expression levels and promoter motif analysis of invertase (*INV*) genes, sucrose transport genes (*SUTs* and *SWEETs*), and the *ABF*s indicated a cross-talk between the ABA and sugar signaling pathways, which may play a role in regulating sugar remobilization from stem to panicle. Presence of SNP variants was observed in the *ABF2* and *ABF5* genes, which could possibly lead to differential expression of these genes.

Materials and Methods

Plant Material and Growth Conditions

Sweet sorghum (*Sorghum bicolor* (L.) Moench, Poaceae) varieties S35 and NIL S35SG06040 (with Stg1 QTL) were used for the experiments. Seeds were sown in field in the alfisols at ICRISAT (International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India) in January

2017. Two plots having 6 rows each (2 m × 0.5 m per row, separated by 0.75 m). Both the plots were irrigated till 80 days. Plants of both S35 and the NIL had reached the flowering stage at this time-point. Drought stress was applied by drying down, from 80 days post sowing (dps) onwards. The third leaf from panicle, the peduncle, and the fifth internode from panicle, from five individual plants were collected at two time points: first at 90 dps (post-anthesis stage) and second at 100 dps (milky dough stage) from irrigated controls and drought stressed plants. Harvesting was done between 8 and 9 a.m., and the tissues from both harvests were stored at − 80 °C. The soil moisture content at the end of drought stress application was seen to be 4% *w/w*. Relative water content (RWC) of tissues was determined using the formula: RWC (%) = 100*[(FW-DW)/(TW-DW)], where FW = fresh weight, DW = dry weight, and TW = weight of the turgid tissue (Ghate et al. 2017).

Reducing Sugars and Total Sugars Estimation

Reducing sugars and total sugars were estimated by using the dinitrosalicylic acid (DNSA) reagent method (Miller 1959). To 100 mg tissue (leaves, peduncle and internodes), 500 µL ethanol was added, vortexed for 5 min, and then centrifuged at 10,000 rpm (Eppendorf 5804 R) for 10 min. For estimation of total sugars, yeast invertase (0.75 U/mL) was added to the supernatant and samples were incubated for 10 min at 25 °C for conversion of non-reducing sugars to reducing sugars. To 100 µL of each sample, 0.5 mL DNSA reagent and 0.4 mL distilled water were added and the tubes placed in a water bath for 10 min at 100 °C. Absorbance was measured at 540 nm (Hitachi-U2800, Japan) after cooling the tubes to room temperature and making the volume to 3 mL in each tube. Reducing sugars and total sugar content was calculated in terms of glucose equivalents by comparing the absorbance against a standard curve of glucose.

Plant Extract for Invertase Assay

Leaf (central portion, excluding midrib), de-rinded internode and peduncle samples (100 mg) were homogenized in liquid nitrogen and the homogenate suspended in 1 mL extraction buffer consisting of 50 mM KPO_4, pH 7.5, 5 mM $MgCl_2$, and 1 mM EDTA (Lingle and Dunlap 1987). The tubes were centrifuged at 8000 rpm (4 °C) for 10 min (Eppendorf 5804R). The supernatant was dialyzed overnight using dialysis tubing (10 kD cut off; Sigma, USA), against 1 L of 10 mM KPO_4 buffer (pH 7.5) at 4 °C. Dialysate was used for invertase assay. One milliliter reaction mixture containing 50 mM sodium acetate (pH 5) 80 mM sucrose and crude enzyme extract was used for determining acidic invertase (INVA) activity. Tubes were incubated for 30 min in a water bath at 30 °C. The reaction was stopped by adding 0.5 mL DNSA, and the reducing sugars formed by invertase activity were estimated in terms of glucose formed min^{-1} mg^{-1} protein.

Abscisic Acid Extraction

ABA was estimated from leaf and de-rinded internode tissues using the method of Nakurte et al. (2012). Samples were ground in liquid nitrogen and extracted with 80% methanol. The extract was cleared by centrifugation at 4000 rpm (Eppendorf 5804 R) for 10 min at room temperature. The resulting supernatant was transferred to a new tube and concentrated in a lyophilizer (Martin Christ GmbH, Alpha 2–4 LD plus, Germany) to 1/10th the original volume. One volume of PO_4 buffer (pH 8) was added to the concentrated extract and the extract partitioned against petroleum ether (60–80 °C bp). The aqueous and organic phases were separated by centrifugation at 8000 rpm for 5 min (Eppendorf 5804 R), and the lower aqueous phase was transferred to a new tube. The pH of the solution was lowered to 2.8 with 1 N HCl. The acidic sample was partitioned against ethyl acetate and centrifuged at 8000 rpm (Eppendorf 5804 R) for 10 min. The upper organic phase was recovered, completely dried, and then dissolved in 300 µL of the mobile phase. HPLC separation was carried out by using a reverse-phase Zorbax Eclipse XDB-C8 reverse-phase 4.6 × 150 mm, 5 µm column (Agilent Technologies, Germany). The mobile phase consisted of a mixture of methanol and 1% acetic acid (60:40 $v\ v^{-1}$). The HPLC run was carried out in an isocratic mode at a flow rate of 1 mL min^{-1} using a UV detector, at 270 nm. The ABA peak was confirmed by spiking the plant extract sample with standard ABA (Fig. S1a). A standard curve using pure ABA (A-1049, Sigma Chemical Company, USA) was used for quantitating the ABA levels from plant samples (Fig. S1b).

Real-Time Polymerase Chain Reaction

Leaf and internode (de-rinded) tissues from both control and drought-treated samples were ground in liquid nitrogen. Total RNA was extracted from 100 mg tissue using Trizol reagent (Sigma, USA). One microgram RNA was used for synthesis of cDNA using ImPromII reverse transcriptase (Promega) and oligodT primers. q-RT PCR was performed for 5 *ABF*, 5 *SUT*, 2 *SWEET*, and 3 *INV* genes. Details of the genes and primers used for q-RT PCR are given in supplementary data (Table S1). A 10 µL reaction mixture was prepared containing 0.3 µM of each primer, 1 µL of cDNA, and 5 µL of 2× Roche Mastermix (Roche, USA). PCR was carried out at 95 °C for 3 min followed by 40 cycles at 95 °C for 10 s, 58 °C for 20 s, and 60 °C for 20 s (Eppendorf Realplex^2, Germany). PCR specificity was checked by performing melting curve analysis. *EF1α* was used as an internal control for normalizing the expression levels of the genes. The mean of five biological

replicates, each with three technical replicates, was used to determine the fold change in gene expression under drought as compared to irrigated controls by the using $2^{-\Delta\Delta ct}$ method (Livak and Schmittgen 2001).

Bioinformatics Analysis

Two thousand bases upstream regions of *INV*, *SUT*s, *SWEET*s, and *ABF* genes were downloaded from the Gramene database (http://www.gramene.org). A PERL program developed by Lindlof et al. (Lindlöf et al. 2009) was used to identify motifs over-represented in these promoters (based on motifs described in the PLACE database (http://www.dna.affrc.go.jp/htdocs/PLACE/) and other collected motifs (Lindlof et al. Lindlöf et al. 2009). ABA and sugar-related motifs present in promoters of at least half of the 43 sugar and ABA-related genes selected (including 3 *INV*, 5 *SUT*, 2 *SWEET*, and 5 *ABF* genes) as against their occurrence in all the genes (32403) present in sorghum genome were identified using the PERL program. The two ratios were compared using Fischer's one-sided exact test, and the motifs showing significance values $p \leq 0.05$ were considered as over-represented.

Data on single nucleotide polymorphism (SNP) variants in the Stg1 QTL region in S35 and the NIL using the genotyping by sequencing method was provided by the Department of Genomics, ICRISAT, Patancheru, India (Table S3; personal communication). The 2-kb upstream region of *ABF2* (Sb03g040970; *GBX4*) gene was analyzed to identify all the transcription factor binding sites using PlantPAN database (http://plantpan2.itps.ncku.edu.). The presence of an upstream SNP identified in this gene, within a TF-binding site, was detected by manual comparison. The psRNA target server (http://plantgrn.noble.org/psRNATarget/) was used for prediction of miRNA target sites in the *ABF5* (Sb03g040510; *ABI5*) gene from S35 and the NIL. Two SNPs were present in the 5′UTR region of the *ABI5* transcript.

Statistical Analysis

For physiological parameters, ANOVA was carried out using the studied parameters as responses and treatments within genotypes as factors (SYSTAT statistical software, https://systatsoftware.com/). Readings from five biological replicates representing five individual plants ($n = 5$) were used for this analysis. Tukey's pairwise comparison test was performed to evaluate statistical significance between two treatment means for each response. Significant differences in the gene expression levels were determined using Student's *t* test. Ratios of ABA and sugar-related motifs present in promoters of at least half of the sugar and ABA-related genes, as against their occurrence in the total number of genes (32403) present in sorghum genome, were compared using Fisher's one-sided exact test and the motifs showing significance values ($p \leq 0.05$) were considered as over-represented.

Results

Drought Stress Leads to Alteration in Sugar and Abscisic Acid Levels in Source (Leaf) and Sink (Internode) Tissues

Progressive drought stress exposure of the plant lines by withholding irrigation at post-flowering stage led to about 50 to 60% reduction in the water content in leaves, peduncles, and the sugar storing fifth internode, at the end of stress application. The NIL S35SG06040 showed better water retention as compared to the parent line S35 (Tables 1, 2, and 3). The sugar content of source leaves decreased in both lines in response to drought exposure. Under irrigated conditions, the NIL showed 2-fold higher sugar accumulation in the sugar storing fifth internode, while the sugar content of peduncle, which transports photoassimilates to the developing panicle, was similar in the two lines. In response to drought stress, the fifth internode accumulated 6-fold and the peduncle accumulated about 3-fold more sugars, as compared to the parent line S35, while starch content decreased in all tissues (Tables 1, 2, and 3). The percentage of reducing sugars in the total sugars increased in the leaves of both lines on exposure to drought stress, but in peduncles, S35 showed a decrease in proportion of reducing sugars, while the NIL showed an increase. The proportion of reducing sugars in the fifth internodes of S35 decreased and then recovered at the second time point of drought stress sampling, while in the NIL, the proportion of reducing sugars increased and then reduced. The reducing sugar level fluctuations under drought correlated to changes in acid invertase (INVA) activity (Tables 1, 2, and 3). Leaves of S35 showed about 2-fold and the internodes about 60% higher INVA when the plants were subjected to drought stress. However in the NIL, INVA activity increased about 2-fold in all tissues but reduced at the second time point of drought stress sampling.

Levels of ABA increased in response to drought stress in both lines, with the NIL showing over 8-fold and 7-fold increases in leaves and internodes respectively as compared to 3-fold and 5-fold increases in the respective tissues of S35 (Tables 1 and 3).

Drought Stress Induces Differential Expression of Abscisic Acid- and Sugar-Related Genes

Since ABA levels increased significantly in the NIL S35SG06040, attempts were made to study the expression of ABA response factors (*ABFs*), which regulate the expression of ABA-responsive genes. Of the 5 *ABFs* studied, leaves of the NIL showed 5–15-fold increase in the expression of 4 of

Table 1 Changes in relative water content (RWC), total and reducing sugars content, starch content, ABA levels, and activities of neutral invertase (INVN) and acid invertase (INVA) in leaves of sweet sorghum line S35 and its NIL S35SG06040 in response to progressive drought exposure of 10 days (D1) and 20 days (D2) at post-flowering stage. Values represent means of five biological replicates and their SDs. Different letters within a column indicate significant ($p < 0.05$) differences as determined by ANOVA and Tukey's pairwise comparison

Plant genotype (treatment)	RWC (%)	Total sugars mg g^{-1} DW	Reducing sugars mg g^{-1} DW	Starch mg g^{-1} DW	ABA nmol g^{-1} DW	INVN mmol glu min^{-1} mg^{-1} protein	INVA mmol glu min^{-1} mg^{-1} protein
S35 (C)	88 ± 2a	97 ± 5b	30 ± 2d	46 ± 3a	0.9 ± 0.02e	0.3 ± 0.04c	0.5 ± 0.03c
S35 (D1)	47 ± 3c	73 ± 4c	35 ± 2d	29 ± 2c	2.2 ± 0.06d	0.4 ± 0.05b	0.4 ± 0.01c
S35 (D2)	38 ± 3d	66 ± 4c	49 ± 3c	26 ± 2c	3.1 ± 0.12c	0.3 ± 0.03c	0.9 ± 0.04b
S35SG06040 (C)	86 ± 4a	121 ± 5a	69 ± 4b	36 ± 3b	1.1 ± 0.19e	0.4 ± 0.03b	0.4 ± 0.03d
S35SG06040 (D1)	53 ± 2b	112 ± 6a	94 ± 7a	24 ± 2c	7.2 ± 0.65b	0.6 ± 0.04a	1.4 ± 0.07a
S35SG06040 (D2)	44 ± 3c	90 ± 4b	60 ± 4b	21 ± 3c	8.9 ± 0.76a	0.3 ± 0.04c	0.3 ± 0.01d

the 5 *ABFs*, while the fifth internodes showed 2–3-fold higher expression of *ABFs 2*, *3*, and *5* (Fig. 1). The *ABF* expression levels did not vary significantly in S35.

Given the drought-induced changes observed in invertase activity, expression levels of the three acid invertase genes reported in sorghum was studied. In leaves of S35, expression of *INV2* and *IVN3* increased 15-fold and 8-fold respectively, while in the internodes, S35 showed about 3- to 4-fold higher expression of these two *INV* genes (Fig. 2). In leaves of the NIL, expression of all *INV* genes was reduced in response to drought, but in the 5th internode, there was about 10-fold increase in *INV3* expression, which reduced to 2-fold higher levels at the second time point of drought sampling.

Expression of sucrose transporter genes (5 *SUTs* and 2 *SWEETs*) was also seen to alter in response to drought stress application. *SUT2*, *SUT3*, and *SUT5* genes showed about 5- to 8-fold higher expression in leaves of the NIL as compared to those of S35 (Fig. 3). The two *SWEET* genes, namely *SWEET4* and *SWEET8*, which have been reported to play a role in stem sugar accumulation and in phloem loading respectively, showed about 10-fold higher expression in the NIL as compared to S35 in response to drought stress. In internodes, *SUT2* and *SUT5* expression increased 20-fold in S35, and 40- to 50-fold in the NIL, while expression of the 2 *SWEET* genes increased 10-fold in the NIL, but not in S35.

Interaction Between Abscisic Acid and Sugar Signaling Pathways Regulates Expression of Abscisic Acid- and Sugar-Related Genes

Over-representation of ABREs and sugar-related motifs was scored in 2000 bp upstream region from the transcription start site of the genes used for expression studies, namely, the *ABFs*, *INV*, *SUT*, and *SWEET* genes. The ABRE motif TACGTG (Zolotarov and Stromvik 2015) was significantly over-represented in *ABF2* and *ABF5*, *INV3*, *SUT2*, and *SUT5* and in both the *SWEETs* (Table 4). These genes showed an increased expression that correlated to drought-induced increase in ABA accumulation (see Figs. 1, 2, and 3). Some of the genes also showed over-representation of another ABRE motif CACGTG (Menkens et al. 1995).

Of the several sugar-related motifs studied (Table S2), three motifs were over-represented in the genes studied. The motif CGACG (sugar starvation in amylase genes, Hwang et al. 1998) was over-represented not only in the

Table 2 Changes in relative water content (RWC), total and reducing sugars content, starch content, ABA levels, and activities of neutral invertase (INVN) and acid invertase (INVA) in peduncles of sweet sorghum line S35 and its NIL S35SG06040 in response to progressive drought exposure of 10 days (D1) and 20 days (D2) at post-flowering stage. Values represent means of five biological replicates and their SDs. Different letters within a column indicate significant ($p < 0.05$) differences as determined by ANOVA and Tukey's pairwise comparison

Plant genotype (Treatment)	RWC (%)	Total sugars mg g^{-1} DW	Reducing sugars mg g^{-1} DW	Starch mg g^{-1} DW	INVN mmol glu min^{-1} mg^{-1} protein	INVA mmol glu min^{-1} mg^{-1} protein
S35 (C)	87 ± 3a	67 ± 3d	38 ± 4c	55 ± 4b	0.6 ± 0.05a	0.5 ± 0.03d
S35 (D1)	62 ± 3b	52 ± 5e	22 ± 3d	52 ± 5b	0.4 ± 0.02a	0.6 ± 0.04c
S35 (D2)	34 ± 4d	63 ± 4d	22 ± 3d	43 ± 4c	0.4 ± 0.02b	0.5 ± 0.03d
S35SG06040 (C)	85 ± 2a	131 ± 10c	83 ± 9b	62 ± 5a	0.3 ± 0.01c	0.6 ± 0.02c
S35SG06040 (D1)	57 ± 2b	221 ± 15a	149 ± 13a	45 ± 3c	0.5 ± 0.04a	1.7 ± 0.3a
S35SG06040 (D2)	47 ± 4c	177 ± 13b	138 ± 13a	40 ± 3c	0.5 ± 0.03a	0.9 ± 0.1b

Table 3 Changes in relative water content (RWC), total and reducing sugars content, starch content, ABA levels, and activities of neutral invertase (INVN) and acid invertase (INVA) in fifth internodes of sweet sorghum line S35 and its NIL S35SG06040 in response to progressive drought exposure of 10 days (D1) and 20 days (D2) at post-flowering stage. Values represent means of five biological replicates and their SDs. Different letters within a column indicate significant ($p < 0.05$) differences as determined by ANOVA and Tukey's pairwise comparison

Plant genotype (Treatment)	RWC (%)	Total sugars mg g^{-1} DW	Reducing sugars mg g^{-1} DW	Starch mg g^{-1} DW	ABA nmol g^{-1} DW	INVN mmol glu min^{-1} mg^{-1} protein	INVA mmol glu min^{-1} mg^{-1} protein
S35 (C)	86 ± 4a	83 ± 6e	50 ± 4c	70 ± 4b	0.4 ± 0.06d	0.4 ± 0.03c	0.5 ± 0.03d
S35 (D1)	52 ± 3b	98 ± 9d	34 ± 3d	42 ± 3e	1.0 ± 0.03c	0.5 ± 0.05b	0.6 ± 0.02c
S35 (D2)	30 ± 3d	43 ± 4f	28 ± 3d	37 ± 6e	2.1 ± 0.02b	0.3 ± 0.01d	0.7 ± 0.04b
S35SG06040 (C)	88 ± 4a	201 ± 21c	91 ± 9b	99 ± 7a	0.6 ± 0.04d	0.5 ± 0.03b	0.8 ± 0.04b
S35SG06040 (D1)	46 ± 1b	312 ± 26a	202 ± 10a	69 ± 3b	2.2 ± 0.07b	0.6 ± 0.04a	2.0 ± 0.05a
S35SG06040 (D2)	42 ± 2c	272 ± 10b	88 ± 7b	54 ± 4c	4.5 ± 1.02a	0.3 ± 0.02d	0.5 ± 0.02c

sugar-related genes, but also in all the *ABFs* (Table 4). The motif TTATCC, which represents a sugar-repressive element (Tatematsu et al. 2005), was over-represented in all *SUTs*, and also in three *ABF*s, namely *ABF1*, *2*, and *3*, while the third over-represented motif TGGACGG (carbohydrate metabolite-responsive signal element, Morikami et al. 2005) was over-represented in the two drought-responsive *SUT*s and in two *ABF*s, but not in any of the *INV* genes. Hence, sugar-related motifs were over-represented in *ABF*s and ABREs were over-represented in the sugar-related genes, suggesting cross-talk between these two signaling pathways.

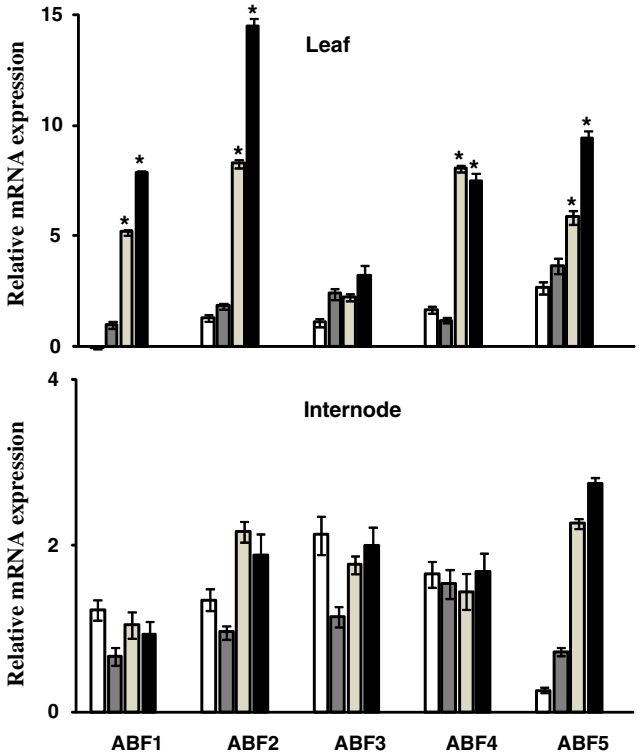

Fig. 1 Expression of ABA binding factor (ABFs 1–5) coding genes in leaves and internodes of sweet sorghum lines S35 and S35SG06040. White bars = S35, at 10 days drought stress; dark gray bars = S35, at 20 days drought stress; light gray bars = S35SG06040, at 10 days drought stress; black bars = S35SG06040, at 20 days drought stress. Values plotted represent the fold change in gene expression under drought as compared to the respective irrigated controls. The mean of five biological replicates was used to determine the fold change in gene expression. Significant differences in the gene expression levels on exposure to drought stress were determined using Student's *t* test and are indicated by asterisks

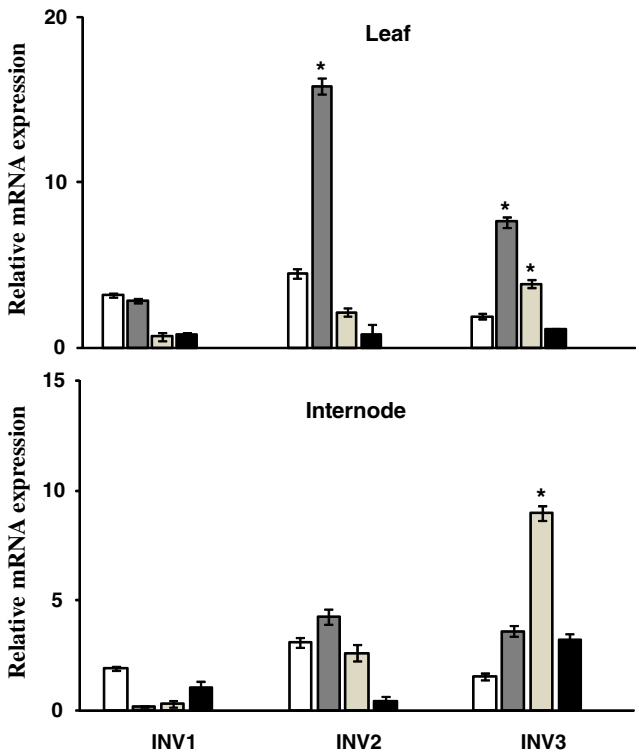

Fig. 2 Expression of cell wall invertase (*INV1*, *INV2*) and vacuolar acid invertase (*INV3*) coding genes in leaves and internodes of sweet sorghum lines S35 and S35SG06040. White bars = S35, at 10 days drought stress; dark gray bars = S35, at 20 days drought stress; light gray bars = S35SG06040, at 10 days drought stress; black bars = S35SG06040, at 20 days drought stress. Values plotted represent the fold change in gene expression under drought as compared to the respective irrigated controls. The mean of five biological replicates was used to determine the fold change in gene expression. Significant differences in the gene expression levels on exposure to drought stress were determined using Student's *t* test and are indicated by asterisks

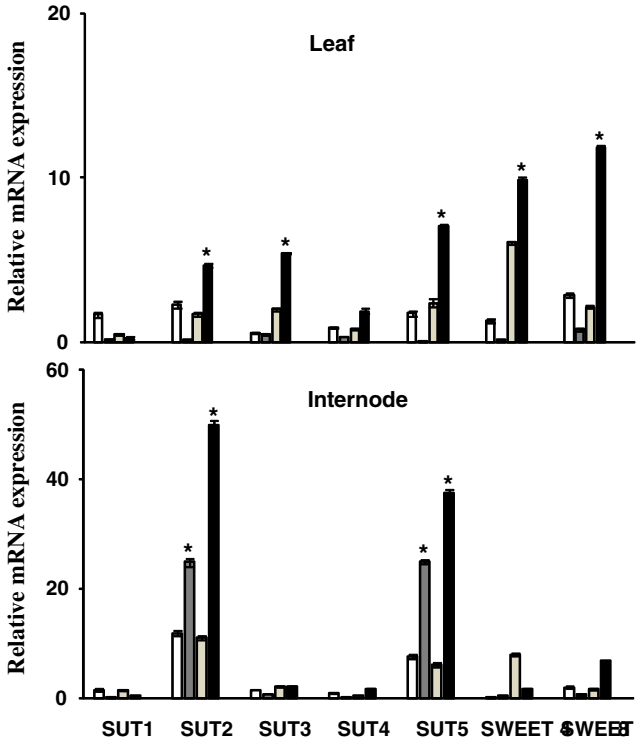

Fig. 3 Expression of sucrose transporter (SUTs1–5) and SWEET transporter (SWEET 4 and SWEET 8) coding genes in leaves and internodes of sweet sorghum lines S35 and S35SG06040. White bars = S35, at 10 days drought stress; dark gray bars = S35, at 20 days drought stress; Light gray bars = S35SG06040, at 10 days drought stress; black bars = S35SG06040, at 20 days drought stress. Values plotted represent the fold change in gene expression under drought as compared to the respective irrigated controls. The mean of five biological replicates was used to determine the fold change in gene expression. Significant differences in the gene expression levels on exposure to drought stress were determined using Student's *t* test and are indicated by asterisks

Abscisic Acid Response Factors Located in Stg1 QTL Show Single Nucleotide Polymorphism Variants

The Stg1 locus that was introgressed in the NIL carried two *ABF*s, namely, *ABF2* and *ABF5* (Ghate et al. 2017). These two genes showed the SNP variants seen in stay green parent B35 (Table S3). The SNPs were either missense or synonymous SNPs and were located either in the promoter or 3′UTR regulatory regions, or in the coding region of the *ABF*s. One SNP variant that was present in the upstream regulatory region of *ABF2* was in an ABRE motif, which altered the *ABF* binding site in the NIL (Table 5) and could possibly account for the differential expression of *ABF2* in the two sorghum lines (see Fig. 1). Two variants present in the coding sequence region were within a miRNA5386 target site. The two SNPs led to alteration of the miRNA binding site in the NIL, but not in S35 (Table 6), suggesting that *ABF5* was probably regulated post-transcriptionally, which led to miRNA-induced downregulation of ABF5 in S35. The low transcript levels of *ABF5* observed in S35 supported the possibility of miRNA 5386-induced downregulation of this *ABF* in S35 but not in the NIL (see Fig. 1). The miRNA5386 was located in a region spanning a part of the coding region and an adjacent intron of a gene coding for an Aux/IAA protein (Sb07g000990), which belongs to the family of repressors of auxin response genes (Fig. 4a). Transcript levels of this *Aux / IAA* gene were similar in S35 and the NIL, in the absence of drought stress. Exposure to drought stress led to a 2- and 4-fold increase in transcript abundance of miRNA5386 precursor in the NIL and S35 respectively (Fig. 4b). Expression of the *Aux/IAA* gene also differed in the two sorghum lines in response to drought. In the NIL, a 4-fold higher expression of *Aux/IAA* (Sb07g000990) was observed as compared to its expression in S35 (Fig. 4b).

Discussion

The NIL S35SG06040, but not the parent line S35, was seen to accumulate sugars in the stems when subjected to drought stress at post-flowering stage. Accumulation of soluble carbohydrates is known to occur in plants exposed to drought stress, which is attributed to a decrease in carbon demand due to growth arrest occurring before decrease in carbon supplied via photosynthesis (Muller et al. 2011). Photosynthetic activity was shown to decrease to a similar extent in S35 and the NIL, when subjected to drought stress during early reproductive stage (Ghate et al. 2017). Besides photosynthesis, starch hydrolysis represents an important source of soluble sugars, especially in response to stress (Ma et al. 2017). However, starch levels decreased in both S35 and the NIL and therefore could not account for the observed difference in sugar levels between them. An interesting observation was the observed increase in soluble sugar levels in peduncles of the NIL in response to drought exposure. Since the peduncle serves as a conduit for transport of photoassimilates to the growing panicle, our results suggest a greater flux of sugars to the panicle of the NIL as compared to that of S35. Remobilization of sugars from vegetative parts to the panicle has been reported in other cereal crops during senescence caused by soil drying (Yang and Zhang 2006). Since sugar content in the leaves decreased in response to drought stress in both the sorghum lines studied, contribution of source leaves to the increase in sugar levels in peduncle due to prolonged photosynthetic activity (in the NIL) seems unlikely. Remobilization of sugars from the senescing leaves could account for an increase in the sugar levels in peduncles of S35, but not in the leaves of NIL, which remained green during the period of drought application (results not shown). The observation that reducing sugar levels first increased (at the first time point of drought sampling) and then decreased (at the second time point of drought sampling) in the fifth internode of the NIL was suggestive of

Table 4 Over-represented ABRE and sugar-responsive motifs in promoters of *ABF*s, *SUT*s, *SWEET*s, and *Inv* genes. The ratio of occurrence of the specific motif among the selected genes as against occurrence of the specific motif among all other genes in sorghum genome were calculated using the Fisher's one-sided exact test. Only 5 of the 28 motifs searched (see Supplementary Table S2) showed significant over-representation in the genes studied ($p < 0.05$). Number of times a significantly over-represented motif occurred in the 2000 nucleotide upstream region from transcription start site of each gene is indicated in the columns

	ABRE motifs		Sugar-responsive motifs		
Sequence	TACGTG	CACGTG	CGACG	TTATCC	TGGACGG
P value	0.049	0.009	0.022	0.026	0.040
ABFs					
ABF1 Sb03g037740	0	0	3	2	0
ABF2 Sb03g040970	2	2	3	1	1
ABF3 Sb02g026570	0	2	1	2	0
ABF4 Sb04g034190	0	0	7	0	2
ABF5 Sb03g040510	1	2	3	0	0
SUTs					
SUT1 Sb01g045720	1	0	2	1	0
SUT2 Sb04g038030	3	0	3	2	2
SUT3 Sb01g022430	0	2	6	1	0
SUT4 Sb08g023310	0	0	1	1	0
SUT5 Sb04g023860	3	0	0	8	1
SWEETs					
SWEET4 Sb04g015420	1	0	0	1	1
SWEET8 Sb08g013620	2	0	1	0	0
Invertases					
Inv1 Sb01g008910	1	0	2	2	0
Inv2 Sb03g047060	0	0	2	0	0
Inv3 Sb04g000620	1	2	11	1	0

remobilization of sugars from the internode to the peduncle in the NIL, but not in the parent line S35. Remobilization of sugars was also supported by the observation that the 5th internode of the NIL showed higher expression of *SUT2*, *SUT5* and the two *SWEET*s as compared to S35.

Besides sugar accumulation, drought stress exposure led to accumulation of ABA, which was about three times higher in the NIL as compared to S35. ABA is known to bring about the activation of ABFs that regulate the stress response phenotype (Fujita et al. 2005). Some of the ABFs in turn regulate the expression of genes involved in ABA biosynthesis by binding to the ABA-responsive *cis*-elements (ABREs) present in the promoters of ABA biosynthesis genes and bring about higher ABA accumulation. For example, the activity of 9-cis-epoxycarotenoid dioxygenase (NCED), which is a rate-limiting step in ABA biosynthesis, was reported to have two ABREs in its promoter and was subjected to regulation by *ABF*s, besides other transcription factors (Liu et al. 2016). In the two sorghum lines studied, stress-induced ABA accumulation correlated to higher expression of the *ABF*s, in an ABA concentration-dependent manner. Two *ABF*s, *ABF2*, and *ABF*5 that were induced by ABA had over-representation of an ABRE (TACGTG) in their promoters, suggesting that they were responsive to ABA signaling. Besides *ABF*s, the invertase gene *INV3* representing a vacuolar invertase; sucrose transporter genes *SUT2* and *SUT5*; and the two *SWEET* genes, *SWEET4* and *SWEET8*, also showed an over-representation of this ABRE, suggesting that these genes were ABA responsive as well. ABA has also been shown to regulate the expression of vacuolar invertases (Rook et al. 2006) and sugar transporter genes (Gibson 2004) in other plants. Besides ABREs, the *ABF*s, *SUT*s, and *INV* genes also showed an over-representation of a sugar-related motif CGACG, indicating that the *ABF*s and sugar-related genes were probably transcriptionally regulated both by ABA and sugars. ABA-responsive genes were also shown to be sugar responsive

Table 5 Location of an SNP variant within an ABRE element present at − 111 bp position in the promoter region of *ABF2* (Sb03g040970)

SNP	Position	Similar Score	Sequence in B35	Sequence in S35	Motif name
C / T	− 111	0.8	+strand CGGTGC -strand GCCACG	+ strand TGGTGC -strand ACCACG	ABRE3 HVA22 (PlantPAN TF matrix ID_034)

Table 6 Location of two SNP variants (bold and underlined) in the coding region of *ABF5* (Sobic.003G363400) at +515(T/G) and + 522(T/C) positions, which are a part of the target site for microRNA5386 (sbi-miR5386)

miRNA and Target Accession	Alignment	Inhibition
sbi-miR5386	miRNA 20 GUCGCGCGCGCUGUCGCUGC 1	
Sobic.003G363400	:::: :::.:.:::.::	
	Target 504 GAGCGGCGGCG**G**CGGCGG**C**G 523	
	S35 GAGCGGCGGCG**G**CGGCGG**C**G	Cleavage
	B35 GAGCGGCGGCG**T**CGGCGG**T**G	No binding

and were regulated by both ABA and sugar in other plants (Ruan 2014). For example, an *ABF* (*AREB1*) was shown to respond to ABA and sugar signal in carrot (Guan et al. 2009), while a *SUT2* from apple was thought to function as a sugar sensor, which positively regulated the expression of an *ABF*, *AREB2* (Ma et al. 2017).

One difference observed in the NIL S35SG06040 and the parent line S35 was that the *ABF2* and *ABF5* genes, both of which were located in the Stg1 locus, showed SNP variants. SNP variant in the promoter region of delta1-pyrroline-5-carboxylate synthase 2 (*P5CS2*) gene, which codes for an important step in proline biosynthesis, was located in the

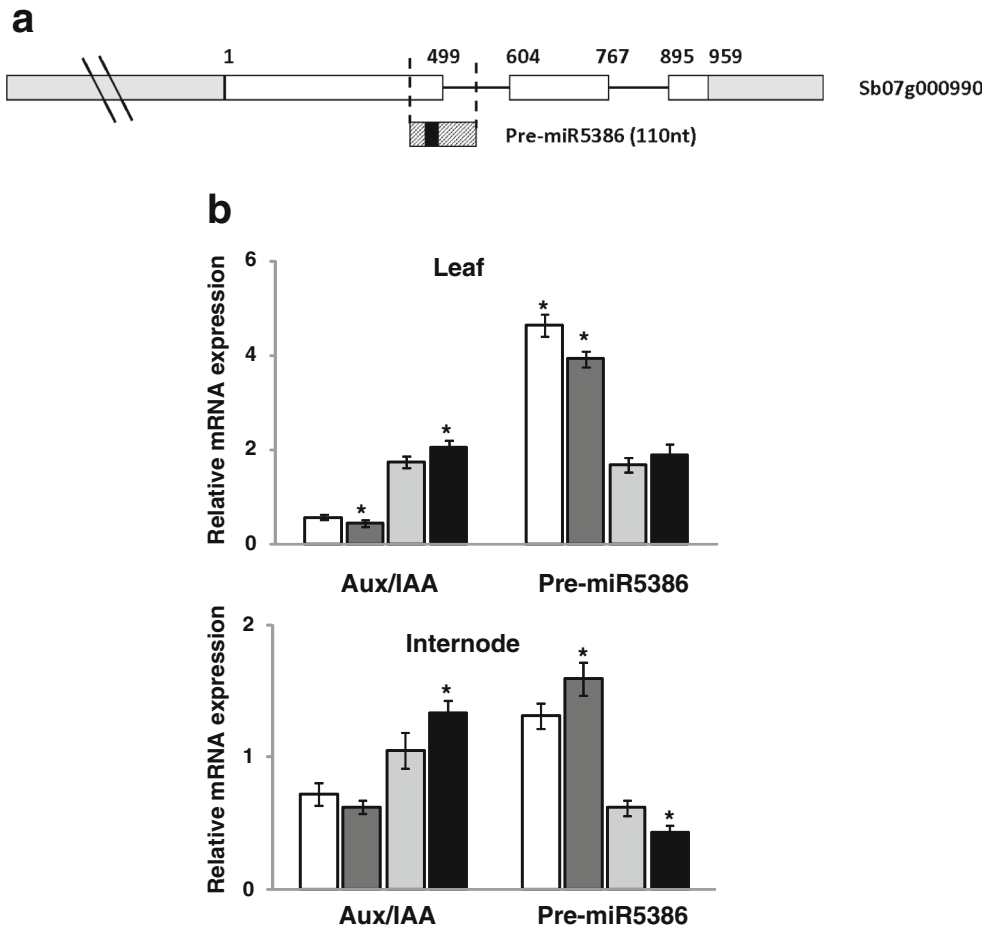

Fig. 4 **a** Location of miRNA 5386 in the Aux/IAA gene Sb07g000990. Gray shaded area depicts the UTRs and introns outside the coding sequence of this gene. White portions represent the coding sequence and black lines represent the introns within the coding region. Position of the pre-miRNA5386 (110 nt) is shown by dotted lines, and it covers a part of the exon and adjacent intron. The mature miRNA5386 is shaded black within the pre miRNA. **b** Expression of miRNA5386 precursor and the *Aux/IAA* gene in which it is located in leaves and internodes of sweet sorghum lines S35 and S35SG06040. White bars = S35, at 10 days drought stress; dark gray bars = S35, at 20 days drought stress; light gray bars = S35SG06040, at 10 days drought stress; black bars = S35SG06040, at 20 days drought stress. Values plotted represent the fold change in gene expression under drought as compared to the respective irrigated controls. The mean of five biological replicates was used to determine the fold change in gene expression. Significant differences in the gene expression levels on exposure to drought stress were determined using Student's *t* test and are indicated by asterisks

Stg1 locus and led to differential expression of this gene in the stay green line B35 and the senescent sorghum line R16 (Johnson et al. 2015). Using bioinformatics tools, attempts were made to correlate the SNPs to observed differential expression of these *ABF*s in the NIL and S35. SNP variant in the upstream region of *ABF2* gene led to disruption of the ABRE motif in S35, but not in the NIL, which may account for its differential expression in the two lines on drought stress exposure. In barley, the presence of an ABRE3 (GCCACGTA CA) and a coupling element CE1 (TGCCACCGG) was seen to be essential for high-level ABA induction, and replacement of either of these sequences abolished ABA responsiveness (Shen and Ho 1995).

Similarly, two SNPs in the *ABF5* coding sequence appeared to abolish a miRNA 5386 binding site in the NIL, suggestive of a post-transcriptional regulation of *ABF5* expression, which could account for the differential accumulation of *ABF5* transcripts in the two lines. Allelic variation in miRNA 5386 was also observed between the grain sorghum line BTx623 and the sweet sorghum line Rio (Calviño et al. 2011). While these authors indicated that the target genes for this miRNA did not include genes related to flowering or carbohydrate metabolism, our observations suggest that *ABF5* could be a predicted target of this miRNA in S35, which possibly leads to reduced transcript accumulation of this gene in response to drought stress. In the NIL, the SNP variants appeared to abolish the miRNA target site and correlated to higher accumulation of *ABF5* transcripts. The miRNA 5386 precursor was located within a region spanning a part of an exon and an adjacent intron of an Aux/IAA gene, which codes for a transcription factor that represses auxin responses. Excision of the miRNA5386 could lead to a truncated Aux/IAA transcript. Several truncated Aux/IAA proteins have been reported in plants (Luo et al. 2018). Alternative splice variants due to excision or inclusion of an intronic miRNA have also been reported in Arabidopsis (Yan et al. 2012). Our experiments provide indirect evidence on the origin of miRNA5386 and its role in inhibition of the expression of its target *ABF5*. Further studies would be required to prove this experimentally.

Conclusions

- Introgression of Stg1 locus into the sweet sorghum senescent line S35 led to higher sugar accumulation in the stem. Stem sugar augmented the sugar flux to developing panicle when post-flowering drought stress led to source limitation on photoassimilation.
- Levels of ABA, which were much higher in the NIL as compared to S35, possibly had a role to play in stem sugar remobilization, since many of the genes involved in sugar metabolism and transport showed over-representation of ABA-related motifs in their promoters. The Stg1 locus, which carried two *ABF* genes from the B35 parent, showed differential regulation in S35 and the NIL, most likely due to the presence of SNPs that altered their expression levels and hence ABA signaling.
- Introgression of the Stg1 locus therefore led to better photoassimilation and drought tolerance in the NIL as compared to the sweet sorghum parent, S35. However, since the Stg1 locus carries over 300 genes, field experiments to measure phenology and agronomic parameters would be required before the NIL can be cultivated as a drought-tolerant line, capable of augmenting grain yield under drought through efficient remobilization of stem sugars.

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References

Blum A (2005) Drought resistance, water-use efficiency, and yield potential—are they compatible, dissonant, or mutually exclusive? Aust J Agri Res 56:1159–1168

Blum A, Golan G, Mayer J, Sinmena B (1997) The effect of dwarfing genes on sorghum grain. filling from remobilized stem reserves, under stress. Field Crop Res 52:43–54

Braun DM, Wang L, Ruan YL (2014) Understanding and manipulating sucrose phloem loading, unloading, metabolism, and signaling to enhance crop yield and food security. J Exp Bot 65:1713–1735

Calviño M, Bruggmann R, Messing J (2011) Characterization of the small RNA component of the transcriptome from grain and sweet sorghum stems. BMC Genomics 12:356–367

Choi H, Hong J, Ha J, Kang J, Kim SY (2000) ABFs, a family of ABA-responsive element bindingfactors. J Biol Chem 275:1723–1730

Fujita Y, Fujita M, Satoh R, Maruyama K, Parvez MM, Seki M, Hiratsu K, Ohme-Takagi M, Shinozaki K, Yamaguchi-Shinozaki K (2005) AREB1 is a transcription activator of novel ABRE-dependent ABA signaling that enhances drought stress tolerance in *Arabidopsis*. Plant Cell 17:3470–3488

Ghate T, Deshpande S, Bhargava S (2017) Accumulation of stem sugar and its remobilisation in response to drought stress in a sweet sorghum genotype and its near-isogenic lines carrying different stay-green loci. Plant Biol 19:396–405

Gibson SI (2004) Sugar and phytohormone response pathways: navigating a signaling network. J Exp Bot 55:253–264

Guan Y, Ren H, Xie H, Ma Z, Chen F (2009) Identification and characterization of bZIP-type. transcription factors involved in carrot (*Daucus carota* L.) somatic embryogenesis. Plant J 60:207–217

Harris K, Subudhi PK, Borrell A, Jordan D, Rosenow D, Nguyen H, Klein P, Klein R, Mullet J (2006) Sorghum stay-green QTL individually reduce post-flowering drought-induced leaf senescence. J Exp Bot 58:327–338

Hwang YS, Karrer EE, Thomas BR, Chen L, Rodriguez RL (1998) Three cis-elements required for. rice α-amylase Amy3D expression during sugar starvation. Plant Mol Biol 36:331–341

Jaiswal V, Gahlaut V, Mathur S, Agarwal P, Khandelwal MK, Khurana JP et al (2015) Identification of novel SNP in promoter sequence of TaGW2-6A associated with grain weight and other agronomic traits in wheat (*Triticum aestivum* L.). PLoS One 10(6):e0129400

Jakoby M, Weisshaar B, Droge-Laser W, Vicente-Carbajosa J, Tiedemann J, Kroj T, Parcy F (2002) bZIP transcription factors in Arabidopsis. Trends Plant Sci 7:106–111

Johnson SM, Cummins I, Lim FL, Slabas AR, Knight MR (2015) Transcriptomic analysis. comparing stay-green and senescent *Sorghum bicolor* lines identifies a role for proline biosynthesis in the stay-green trait. J Exp Bot 66:7061–7073

Koch K (2004) Sucrose metabolism: regulatory mechanisms and pivotal roles in sugar sensing and plant development. Curr Opin Plant Biol 7:235–246

Lindlöf A, Bräutigam M, Chawade A, Olsson O, Olsson B (2009) In silico analysis of promoter. regions from cold-induced genes in rice (*Oryza sativa* L.) and *Arabidopsis thaliana* reveals the importance of combinatorial control. Bioinformatics 25:1345–1348

Ling J, Luo Z, Liu F, Mao Z, Yang Y, Xie B (2017) Genome-wide analysis of microRNA targeting impacted by SNPs in cucumber genome. BMC Genomics 18:275–287

Lingle SE, Dunlap JR (1987) Sucrose metabolism in netted muskmelon fruit during development. Plant Physiol 84:386–389

Liu S, Li M, Su L, Ge K, Li L, Li X, Liu X, Li L (2016) Negative feedback regulation of ABA biosynthesis in peanut (*Arachis hypogea*): a transcription factor complex inhibits *AhNCED1* expression during water stress. Sci Rep 6:37943–37954

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time. quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25:402–408

Luo J, Zhou JJ, Zhang JZ (2018) Aux/IAA gene family in plants: molecular structure, regulation and function. Int J Mol Sci 19:259–276

Ma QJ, Sun MH, Lu J, Liu YJ, Hu DG, Hao YJ (2017) Transcription factor AREB2 is involved in. soluble sugar accumulation by activating sugar transporter and amylase genes. Plant Physiol 174: 2348–2362

Menkens AE, Schindler U, Cashmore AR (1995) The G-box: a ubiquitous regulatory DNA element in plants bound by the GBF family of bZIP proteins. Trends Biochem Sci 20:506–510

Miller GL (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 31:426–428

Mizuno H, Kasuga S, Kawahigashi H (2016) The sorghum *SWEET* gene family: stem sucrose. accumulation as revealed through transcriptome profiling. Biotechnol Biofuels 9:127–138

Morikami A, Matsunaga R, Tanaka Y, Suzuki S, Mano S, Nakamura K (2005) Two cis-acting. regulatory elements are involved in the sucrose-inducible expression of the sporamin gene promoter from sweet potato in transgenic tobacco. Mol Gen Genomics 272:690–699

Muller B, Pantin F, Genard M, Turc O, Freixes S, Piques M, Gibon Y (2011) Water deficits. uncouple growth from photosynthesis, increase C content, and modify the relationships between C and growth in sink organs. J Exp Bot 62:1715–1729

Nakurte I, Keisa A, Rostoks N (2012) Development and validation of a reversed-phase liquid. chromatography method for the simultaneous determination of indole-3-acetic acid, indole-3-pyruvic acid, and abscisic acid in barley (*Hordeum vulgare* L.). J Anal Methods Chem 2012:103575–103580

Pollock C, Farrar J, Tomos D, Gallagher J, Lu C, Koroleva O (2003) Balancing supply and. demand: the spatial regulation of carbon metabolism in grass and cereal leaves. J Exp Bot 54:489–494

Rook F, Hadingham SA, Li Y, Bevan MW (2006) Sugar and ABA response pathways and the control of gene expression. Plant Cell Environ 29:426–434

Ruan YL (2014) Sucrose metabolism: gateway to diverse carbon use and sugar signaling. Annu Rev Plant Biol 65:33–67

Ruuska SA, Rebetzke GJ, van Herwaarden AF, Richards RA, Fettell NA, Tabe L, Jenkins CL (2006) Genotypic variation in water-soluble carbohydrate accumulation in wheat. Funct Plant Biol 33:799–809

Shen Q, Ho TH (1995) Functional dissection of an abscisic acid (ABA)-inducible gene reveals two independent ABA-responsive complexes each containing a G-Box and a novel cis-acting element. Plant Cell 7:295–307

Shukla S, Felderhoff TJ, Saballos A, Vermerrisa W (2017) The relationship between plant height. and sugar accumulation in the stems of sweet sorghum (*Sorghum bicolor* (L.) Moench). Field Crop Res 203:181–191

Slewinski TL (2012) Non-structural carbohydrate partitioning in grass stems: a target to increase yield stability, stress tolerance, and biofuel production. J Exp Bot 63:4647–4670

Tatematsu K, Ward S, Leyser O, Kamiya Y, Nambara E (2005) Identification of cis-elements that regulate gene expression during initiation of axillary bud outgrowth in *Arabidopsis*. Plant Physiol 138:757–766

Trouverie J, Thevenot C, Rocher JP, Sotta B, Prioul JL (2003) The role of abscisic acid in the response of a specific vacuolar invertase to water stress in the adult maize leaf. J Exp Bot 5:2177–2186

Vadez V, Deshpande SP, Kholova J, Hammer GL, Borrell AK, Talwar HS, Hash CT (2011) Stay green quantitative trait loci's effects on water extraction, transpiration efficiency and seed yield depend on recipient parent background. Funct Plant Biol 38:553–566

Vadez V, Deshpande S, Kholova J, Ramu P, Hash CT, Varshney R Tuberosa R. (2013) Molecular breeding for stay-green: progress and challenges in sorghum. In: Translational Genomics for Crop Breeding: Abiotic Stress, Yield and Quality, Wiley, New York, pp 125–141

Wang J, Zhou J, Zhang B, Vanitha J, Ramachandran S, Jiang SY (2011) Genome-wide expansion and expression divergence of the basic leucine zipper transcription factors in higher plants with an emphasis on sorghum. J Integr Plant Biol 53:212–231

Wang Z, Xu Y, Chen T, Zhang H, Yang J, Zhang J (2015) Abscisic acid and the key enzymes and genes in sucrose-to-starch conversion in rice spikelets in response to soil drying during grain filling. Planta 241:1091–1107

Yan K, Liu P, Wu CA, Yang GD, Xu R, Guo QH, Huang JG, Zheng CC (2012) Stress-induced alternative splicing provides a mechanism for the regulation of microRNA processing in *Arabidopsis thaliana*. Mol Cell 48:521–531

Yang YZ, Tan BC (2014) A distal ABA responsive element in *AtNCED3* promoter is required for positive feedback regulation of ABA biosynthesis in *Arabidopsis*. PLoS One 9:e87283

Yang J, Zhang J (2006) Grain filling of cereals under soil drying. New Phytol 169:223–236

Yang JC, Zhang JH, Wang ZQ, Zhu QS, Liu LJ (2004) Activities of fructan- and sucrose-metabolizing enzymes in wheat stems subjected to water stress during grain filling. Planta 220:331–343

Zolotarov Y, Stromvik M (2015) De novo regulatory motif discovery identifies significant motifs in promoters of five classes of plant dehydrin genes. PLoS One 10:e0129016