CSIRO PUBLISHING

Functional Plant Biology, 2014, **41**, 954–962
http://dx.doi.org/10.1071/FP13299

Alterations in stem sugar content and metabolism in sorghum genotypes subjected to drought stress

Hilal A. Qazi[A], *Pinnamaneni Srinivasa Rao*[B], *Akanksha Kashikar*[C], *Penna Suprasanna*[D] *and Sujata Bhargava*[A,E]

[A]Department of Botany, University of Pune, Pune 411007, India.
[B]Research program on Dryland Cereals, International Crops Research Institute for Semiarid Tropics, Patancheru 502324, India.
[C]Department of Statistics, University of Pune, Pune 411007, India.
[D]Functional Plant Biology Section, Bhabha Atomic Research Centre, Mumbai 400085, India.
[E]Corresponding author. Email: sujata@unipune.ac.in

Abstract. Changes in stem sugar concentrations due to drought stress at the early reproductive stage were studied in seven sorghum (*Sorghum bicolor* (L.) Moench) genotypes that differ in their stem sugar storage ability. Total sap sugar concentration increased in most genotypes. ANOVA showed a significant contribution of genotype and treatment to the variation in sugar levels. Two genotypes showed little variation in total sugar levels at the fifth internode from the peduncle and five genotypes showed significant increases in total sugar levels under drought; these groups were used to compare sugar metabolism. Drought led to a decrease in catabolic sucrose synthase activity in both groups. Invertase activities increased significantly in two genotypes and correlated with the increase in reducing sugar concentrations under drought. Stem sugar hydrolysis probably had a role in osmotic adjustment under drought and correlated with retention of sap volume. However, the activities of sugar-metabolising enzymes did not correlate with their gene expression levels. After resuming irrigation, grain yields, stalk yields and juice volume at physiological maturity were lower in plants recovering from drought stress compared with the controls. In some genotypes, there were similar losses in grain yields and stem sugars due to drought, indicating photoassimilate source limitation; in other genotypes, grain yield losses were less than stem sugar losses in drought-exposed plants compared with the controls, suggesting mobilisation of sugars from the storage internodes to the developing panicle. Accumulation of stem sugars appears to be an adaptive strategy against drought stress in some sorghum genotypes.

Additional keywords: panicle emergence, photoassimilates, physiological maturity, plant response, *Sorghum bicolor* (L). Moench, sugar transport.

Received 14 October 2013, accepted 6 April 2014, published online 29 April 2014

Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is the fifth most important grain crop used for food and fodder. Some genotypes of sorghum are known to store stem sugar as, do several other members of the Andropogoneae tribe of grasses, namely maize (*Zea mays* L.) and sugarcane (*Saccharum officinarum* L.) (Dillon *et al*. 2007; Glassop *et al*. 2010). In comparison to sugarcane and maize, sorghum shows adaptation to drought and is hence being explored as a source of biofuel in rainfed regions.

In the vegetative stage of sorghum plants, most of the photoassimilates are transported to newly developing leaves and roots. During the panicle emergence stage, sugar from source leaves is transported to two additional sink tissues, namely the developing panicle and the storage internodes of the stem, though sugar accumulation in the latter is not correlated with the onset of the reproductive phase (Hoffmann-Thoma *et al*. 1996; Gutjahr *et al*. 2013*b*). Although sucrose is the main form in which long-distance transport of sugar through the phloem takes place, its transport across the cell membranes of the source or sink tissues of sugarcane was shown to involve interconversion between sucrose and its hydrolysis products (glucose and fructose) in order to ensure directional flow against the concentration gradient (Rohwer and Botha 2001). Evidence for sucrose transport between the apoplast and symplast, without its hydrolysis, has also been reported and its concentration in these two compartments is thought to play an important role in turgor maintenance (Walsh *et al*. 2005). Sucrose transport to sink tissues in sorghum is apoplastic and though it is mostly stored in vacuoles of mature stem storage parenchyma cells (Tarpley *et al*. 1996; Tarpley and Vietor 2007), sucrose content in the nonvacuolar and extracellular compartments has also been reported (Slewinski 2012). Sucrose accumulation occurs from the bottom to the top of the stem and a reverse gradient has been observed for its hydrolysis products, glucose

and fructose. Little variation in stem sucrose concentration has been reported in the period from flowering to physiological maturity in sweet sorghum genotypes growing under irrigated conditions (Gutjahr *et al*. 2013*b*). However, some reports indicate that sucrose accumulation in sorghum continues past the grain maturation stage, during which physiological tradeoffs between grain yield and stem sugar yield are minimum (Ganesh Kumar *et al*. 2010).

Activities of sucrose-metabolising enzymes are involved in maintaining the flow of sucrose from phloem to the storage parenchyma cells in sugarcane (Sturm and Tang 1999; Wang *et al*. 2013). In sorghum, the activities of sucrose phosphate synthase (SPS; EC 2.4.1.14), sucrose synthase (SUSY; EC 2.4.1.13) and invertase (EC 3.2.1.26) were shown to vary during the internode elongation and storage phases, and a decline in their activities was correlated with stem sugar accumulation in sweet sorghum genotypes (Qazi *et al*. 2012; Gutjahr *et al*. 2013*a*). Invertases were also known to play a key role in stem sugar accumulation in sugarcane, and lower transcript levels as well as enzyme activity were reported in maturing culms (Wang *et al*. 2013). However, there have been reports on a lack of correlation between the activities of sucrose-metabolising enzymes and sucrose accumulation (Tarpley *et al*. 1994).

When subjected to drought stress, stomatal closure and growth arrest are the two immediate responses shown by most plants (Bhargava and Sawant 2013). These responses help in conserving water within the plant and they also restrict the use of metabolic energy for biomass building, diverting it instead for protective processes like osmolyte synthesis and antioxidant metabolism. The water requirement of sorghum is less than that of maize or sugarcane (Gnansounou *et al*. 2005), and this crop species shows the ability to tolerate and survive under conditions of continuous or intermittent drought stress (Ibrahim *et al*. 2013). Storage of sugar in sorghum stems may have an important role to play in the plant's adaptive responses under stress conditions. It could serve as a reserve stock that could be used in sustaining respiration and providing energy for metabolic processes under stress conditions (Rosa *et al*. 2009). It may be mobilised from storage internodes to the developing panicle to augment grain yields when photoassimilates are in short supply due to stress. It could also serve as an osmolyte and prevent water loss from the storage parenchyma cells. There is some evidence of the mobilisation of stem sugar to the developing inflorescence in sugarcane (Silva and Caputo 2012), though irrigated plants showed stem sugar accumulation even after completing panicle development. A decrease in stem sugar accumulation has been reported in sugarcane plants subjected to drought stress, which was attributed to source limitations arising from the inhibition of photosynthesis (Zingaretti *et al*. 2012). In maize plants subjected to water stress, hexoses accumulated in most organs and correlated with higher vacuolar invertase activity (Kim *et al*. 2000). However, the role played by sorghum stem sugar reserves in stress tolerance has not been investigated.

In the present work, attempts have been made to study the variation in sugar accumulation and its metabolism in seven field-grown sorghum genotypes subjected to drought stress at the early reproductive stage. The genotypes chosen were sweet and grain sorghum cultivars. The objective of this work was to understand the ecophysiological function of stem sugar accumulation in sorghum.

Materials and methods

Plant material and drought stress application

Seven genotypes of S*orghum bicolor* (L.) Moench, namely ICSV 25275, ICSV 25280, RSSV 9, ICSSH 58, PVK 801, M 35–1 and SPV 1411, were used in the present study. These were procured from the International Crops Research Institute for Semiarid Tropics (ICRISAT), Patancheru, India (Table S1, available as Supplementary Material to this paper). All genotypes were grown in two adjacent plots in the alfisols at ICRISAT. Irrigation was applied once every 10 days. When the plants were at the early reproductive stage (see Table S2, available as Supplementary Material to this paper), one irrigation application was withheld in one of the plots to impose drought stress. Hence drought-stressed plants received no irrigation for a total period of 20 days, in contrast to the irrigated controls, which received irrigation every 10 days. Relative water content measurements were carried out using flag leaf tissue from stressed and nonstressed plants at the end of stress exposure (Table S2). De-rinded peduncles (subtending the emerging panicle) and 5th internodes (from the peduncle) were used for analysis. Irrigation at 10 day intervals was resumed in both plots and the plants grown to physiological maturity. Agronomic parameters like plant height, number of internodes, stalk yield, juice yield, sugar concentration in juice and the grain yield were measured from three replicates of 10 plants each at the physiological maturity stage. The drought-exposed plants were harvested about one to two weeks before the normally irrigated plants; since they reached the physiological maturity stage earlier (see Table S2, available as Supplementary Material to this paper).

Sap extraction

For sap extraction, 1 g of hulled internode tissue was homogenised directly in a precooled (rinsed with liquid nitrogen) mortar and pestle. The homogenate was inserted into a 10-mL syringe and the sap was collected by pressing the tissue with the plunger into a precooled microfuge tube until no further sap was expressed. The collected sap was centrifuged at 13 000*g*) and 4°C for 10 min (5804R centrifuge Eppendorf, Hamburg, Germany). The volume of the sap was measured and expressed in terms of mL g^{-1} DW of internode tissue. The sap was used immediately after extraction or stored at −20°C for a maximum period of 5 days before use.

Estimation of reducing and total sugars

Reducing sugars were estimated in terms of glucose equivalents using the dinitrosalicylic acid (DNSA) reagent method (Miller 1959). The assay mixture (1 mL) contained 0.5 mL DNSA reagent, 10 µL plant sap and distilled water. The tubes were placed in a boiling water bath for 10 min and then cooled to room temperature. Reducing sugars were estimated by measuring the absorbance at 540 nm (U2800 spectrophotometer, Hitachi, Tokyo, Japan). In control samples, sap was substituted with distilled water. Estimation of the total sugars was done by first

incubating the sap with yeast invertase (0.75 U mL^{-1}, Hi-Media, Mubai, India) for 10 min at 25°C for converting the nonreducing sugars to reducing sugars, which were then estimated using the procedure for reducing sugars.

Measurement of osmolarity

The osmolarity (mmol kg^{-1}) of the sap was measured on the basis of vapour pressure depression using an osmometer (Wescor 5500XR, Vapor pressure osmometer, Logan, UT, USA). The osmolarity of 0.01 mL of undiluted sap was measured in terms of mmol kg^{-1} as mentioned in Qazi *et al.* (2012).

Preparation of enzyme extract and assay of invertase activity

Homogenisation of the hulled fresh internode tissue (125 mg) was carried out in liquid nitrogen and the powdered tissue was added to microfuge tubes containing 1 mL of an extraction buffer consisting of 50 mM KPO_4 (pH 7.5), 5 mM $MgCl_2$ and 1 mM EDTA (Merck, Darmstadt, Germany) (Lingle and Dunlap 1987). The tubes were centrifuged in a refrigerated centrifuge (5804R, Eppendorf) at 15 000*g* and 4°C for 10 min. The collected supernatant was dialysed overnight at 4°C against 1 L of 10 mM KPO_4 (pH 7.5) using dialysis tubing (10 kD cutoff; Sigma, St Louis, MO, USA). The dialysate was used immediately for invertase assays.

The assay for invertase activity was carried out as per Lingle and Dunlap (1987) and Tarpley *et al.* (1994) with some modifications. The reaction mixture (1 mL) contained 50 mM KPO_4 (pH 7.0), 80 mM sucrose and crude enzyme extract for assaying neutral invertase activity, whereas for assaying acidic invertase activity, 50 mM sodium acetate (pH 5.0) was used instead of KPO_4 (pH. 7.0). The reaction was carried out by incubating the samples at 30°C for 30 min, after which the reaction was stopped by adding 0.5 mL DNSA. The tubes were placed in a boiling water bath for 10 min. The final volume was made up to 3 mL with distilled water after cooling the tubes to room temperature. The absorbance was measured at 540 nm using a U2800 spectrophotometer (Hitachi). For the controls, the enzyme extract was replaced with water. Invertase activity was determined in terms of mmol glucose formed min^{-1} mg^{-1} protein. Proteins of the dialysed extract were measured using Bradford's method (Bradford 1976).

Preparation of enzyme extract for assaying SUSY and SPS activity

The fresh internode tissues (125 mg) were homogenised in liquid nitrogen to a fine powder and added to 1 mL of an extraction buffer containing 50 mM HEPES-NaOH (pH 7.5), 7.5 mM $MgCl_2$, 2 mM EGTA, 2% (w/v) polyethylene glycol 8000 (PEG 8000; Sigma), 2% (w/v) polyvinylpyrrolidone and 5 mM DTT (Hoffmann-Thoma *et al.* 1996). The tubes were centrifuged at 15 000*g* (5804R centrifuge, Eppendorf) and 4°C for 10 min, and the supernatant was dialysed against 1 L of 10 mM HEPES-NaOH buffer (pH 7.5) (Hi-Media) overnight at 4°C. The dialysed extract was used immediately for SUSY and SPS assays.

Assay for SUSY enzymes (synthesis direction)

An assay using a coupled reaction was used for determining SUSY activity in the synthesis direction. The method used was based on that reported by Morell and Copeland (1985), with some modifications. The final volume of the reaction mixture (1 mL) contained 20 mM HEPES-NaOH buffer (pH 7.5), 5 mM $MgCl_2$, 20 mM KCl, 12 mM fructose, 0.4 mM phosphoenolpyruvate, 2 mM UDP-glucose, 20 U pyruvate kinase, 20 U lactate dehydrogenase, 0.15 mM NADH and the dialysed enzyme extract. The rate of reaction was measured at 340 nm (U2800 spectrophotometer, Hitachi) in terms of the decrease in absorbance of NADH. Enzyme activity was calculated in terms of µmol NADH oxidised min^{-1} mg^{-1} protein, which was stoichiometrically related to UDP released due to SUSY activity in forming sucrose from fructose and UDPglucose.

Assay for SUSY enzyme (cleavage direction)

The enzyme activity of SUSY in the cleavage direction was estimated by calculating the reduction of NAD^+ in presence of UDP-glucose dehydrogenase. The reaction mixture (1 mL) contained 20 mM HEPES-NaOH buffer (pH 7.5), 100 mM sucrose, 2 mM UDP (Sigma), 2 mM $MgCl_2$, 0.005 U UDP-glucose dehydrogenase (Sigma), 1.5 mM NAD^+ (Sigma) and the enzyme extract (Morell and Copeland 1985). The increase in absorbance of NADH at 340 nm (U2800 spectrophotometer, Hitachi) was used to determine the activity of SUSY, which was expressed as µmol NAD^+ reduced min^{-1} mg^{-1} protein during conversion of UDP-glucose to UDP-glucuronate and NADH by UDP-glucose dehydrogenase.

Assay for SPS enzymes

SPS activity was also measured in terms of NADH oxidised using a method similar to that used for estimating SUSY (synthesis direction) activity. The only difference was that 15 mM fructose-6-phosphate (Sigma) was used in the place of fructose in this reaction mixture (Harbron *et al.* 1980). The activity was calculated in terms of µmol NADH oxidised min^{-1} mg^{-1} protein.

RNA isolation, cDNA preparation and PCR amplification

Hulled internode tissues were used for the isolation of total RNA using Tri-reagent (Ambion, Carlsbad, CA, USA). A complementary DNA template was prepared from the RNA samples, using 1 µg of RNA and 0.5 µg of oligodT primer (Promega, Madison, WI, USA) in a reaction mixture containing ImProm-II reverse transcriptase (Promega) as per the manufacturer's instructions. The cDNA prepared was either used immediately for semiquantitative reverse transcription–PCR amplification or frozen at −20°C after adding 0.5 µL of RNase inhibitor (RNasin, Promega). The mRNA sequences for genes coding for SUSY, SPS and invertases in sorghum were obtained from the Gramene database (http://www.gramene.org). Using Primer3 software (http://fokker.wi.mit.edu/primer3/), specific primers for these genes were designed (Table S3). Primers were also designed for amplifying the constitutively expressed *Elongation factor 1α* (*EF1α*) sequence of sorghum, which was used as the loading

control. The PCR reaction mixture of 20 µL contained 1× reaction buffer, 1.5 mM $MgCl_2$, 1 U Taq DNA polymerase (Fermentas, Burlington, ON, Canada), 0.1 mM deoxyribose nucletotide triphosphate mixture (dNTPs), and 40 pmol of each primer. The cycling conditions used were: 94°C for 5 min, followed by 35 cycles of 94°C for 1 min, varying the annealing temperatures for different primer pairs, (as given in Table S3) for 0.45 min and 72°C for 1 min. A final extension was carried out at 72°C for 7 min. After amplification, separation of the PCR amplified product was done on 1.5% agarose gel prepared in 1× Tris-acetate EDTA (TAE) buffer, stained with ethidium bromide (0.2 nM). The gel images were captured using a gel documentation system (Gel Doc XR+, Bio-Rad, Richmond, CA, USA).

Yield parameters

When plants reached physiological maturity (35 days after 50% flowering), three groups of 10 plants each were used for estimating plant height, internode number, grain yield and stalk weight. The stems were then crushed to extract juice and the Brix value of the juice (% w/w) was determined using a hand-held refractometer (ATAGO, Tokyo, Japan). The weight of the juice from 10 plants was also determined. An estimate of sucrose concentration in the juice was made by multiplying the Brix value by the juice weight, assuming that sucrose was the main constituent of the juice.

Statistical analysis

Five independent plants were used for determining sap volume, osmolarity and sugar levels. The activities of sugar metabolising enzymes were determined from three independent plants. ANOVA was carried out using total sugars or reducing sugars as responses and genotypes, as well as treatments within genotypes as factors, at the early reproductive stage using MINITAB statistical software, (http://www.minitab.com, accessed 11 April 2014). The same analysis was carried out at the physiological maturity stage for different yield parameters with three replicates. Values for the responses were log-transformed before using them for ANOVA in order to make the variances comparable. Student's *t*-test (Microsoft Excel; Microsoft Corporation, Redmond, WA, USA) was used to identify significant variations between the irrigated and drought-stressed genotypes for the various parameters studied. Multivariate ANOVA analysis was carried out to determine the homogeneity (of the parameters measured) within two groups that were created on the basis of total sugar concentration in the fifth internodes.

Results

Drought effects at the early reproductive stage

Sorghum genotypes exposed to drought stress by withholding irrigation for 20 days (as against 10 days in the controls) at the early reproductive stage showed a decrease in the relative water content (%) of flag leaf and in sap volume (mL g^{-1} DW) of the peduncle and fifth internode tissue (Table S2). This decrease correlated with an increase in the total sugar concentration and osmolarity of the sap (Table 1). ANOVA showed a significant contribution of genotypes to the variation in total and reducing sugar levels observed (Table S4) as well as a significant contribution of the treatments within each genotype.

On comparison of total sugars in plants exposed to drought-stressed or irrigated conditions for each genotype (Fig. 1), it was seen that sorghum genotypes showed an increase in total sugar levels in the sap extracted from the peduncles of drought-stressed plants. However, sugar levels in the sap extracted from the fifth internodes of sorghum genotypes subjected to drought stress showed two distinct trends. Group I genotypes (ICSSH 58 and SPV 1411) did not show a significant difference in sugar levels under the respective treatments, whereas Group II genotypes (ICSV 25275, ICSV 25280, PVK 801, RSSV 9 and M 35–1) showed a significant difference. Sap volume, osmolarity, total sugar and reducing sugar levels, and enzyme activities were compared between these two groups by taking mean values of all the genotypes falling within each group. Group I did not show a significant change in sap volume, total sugars and osmolarity of sap in response to drought treatment, but showed a significant increase in the levels of reducing sugars (Table 2). On the other hand, Group II genotypes showed a reduction in sap volume in response to drought, which was accompanied by a significant increase in total sugars and osmolarity. Though the levels of reducing sugars in Group II genotypes increased in response to drought stress, the increase was not as significant as that observed in

Table 1. Correlation coefficients (*r*) for the sap volume, reducing sugar levels, total sugar levels and osmolarity of sap extracted from the peduncle and fifth internode (from the top) of seven sorghum genotypes subjected to continuous irrigation or drought stress at the early reproductive stage

The data represents five replicates of irrigated and drought-stressed plants for each genotype (70 observations) and were used for determining the *r* values. Significant correlations ($P < 0.05$) are marked with asterisks

	Internode position	Sap volume mL g^{-1} DW	Reducing sugar levels mg glucose mL^{-1} sap	Total sugars
Reducing sugar levels	Peduncle	–0.192	–	–
	Fifth internode	0.163	–	–
Total sugars	Peduncle	–0.592*	0.411*	–
	Fifth internode	–0.525*	0.232	–
Osmolarity	Peduncle	–0.590*	0.427*	0.902*
	Fifth internode	–0.631*	0.080	0.808*

Group I genotypes (Table 2). Multivariate analysis showed that osmolarity, sap volume, total sugars and reducing sugars did not differ significantly within each group (Table S5).

Of the various sugar-metabolising enzymes assayed in sap extracted from tissues of the fifth internode, SUSY activity in the synthesis direction did not show any change between the

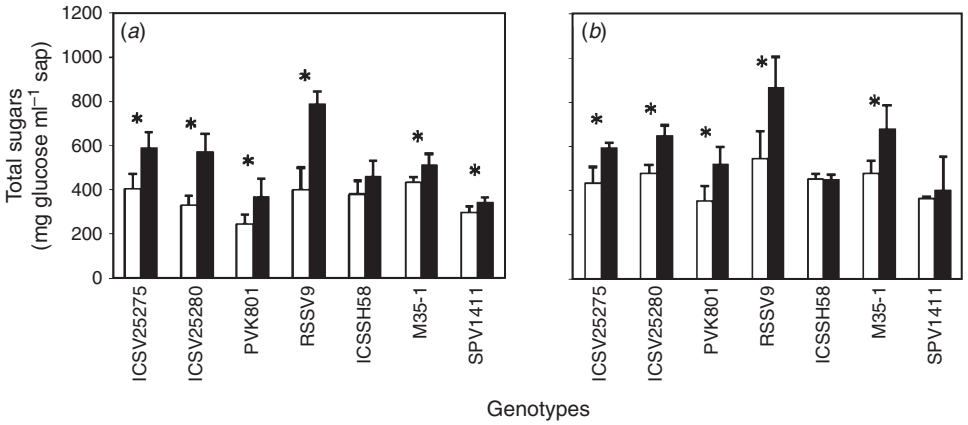

Fig. 1. Variation in the total sugar content of (*a*) peduncles and (*b*) fifth internodes of seven sorghum genotypes, either irrigated (clear bars) or subjected to drought stress for 20 days (black bars) at the early reproductive stage. Significant differences ($P < 0.05$) between the two treatments for each genotype are indicated by asterisks.

Table 2. Sap volume, osmolarity and levels of sugars in sap extracted from the fifth internode of two groups of sorghum genotypes at the early reproductive stage

C, controls (irrigated plants); D, plants subjected to drought stress for 20 days. Data represent the mean values of the two genotypes in Group 1 ($n = 10$) and five genotypes in Group II ($n = 25$). Significant differences ($P < 0.05$) in treatment means within a group are marked with asterisks

Group	Treatment	Sap volume (mL g^{-1} DW)	Osmolarity of sap (mmol kg^{-1})	Total sugars (mg glucose mL^{-1} sap)	Reducing sugars (mg glucose mL^{-1} sap)
Group I: ICSSH 58 and SPV 1411	C	4.42	662	405	138
	D	4.92	605	423	274*
Group II: ICSV 25275, ICSV 25280, PVK 801, RSSV 9 and M 35–1	C	4.35	693	456	147
	D	3.65*	833*	660*	181*

Table 3. Activities of sugar metabolising enzymes, sucrose synthase, sucrose phosphate synthase (SPS), neutral invertase (INVN) and acid invertase (INVA) in sap extracted from the fifth internode of two groups of sorghum genotypes

SUSYS, sucrose synthase in the synthesis direction; SUSYC, sucrose synthase in the cleavage direction; C, controls (irrigated plants; D, plants subjected to drought stress for 20 days at the early reproductive stage. Data represent the mean values of the two genotypes in Group 1 ($n = 6$) and five genotypes in Group II ($n = 15$). Significant differences ($P < 0.05$) in treatment means within a group are marked with asterisks

Group	Treatment	SUSYS	SUSYC	SPS	INVN	INVA
		(μmol NADH min^{-1} mg^{-1} protein)			(mmol glucose min^{-1} mg^{-1} protein)	
Group I: ICSSH 58 and SPV 1411	C	2.27	1.58	2.11	0.62	0.56
	D	2.05	0.46*	2.40	1.80*	2.06*
Group II: ICSV 25275, ICSV 25280, PVK 801, RSSV 9 and M 35–1	C	2.10	1.45	2.78	0.76	0.78
	D	1.85	0.40*	2.99	0.89	0.95

two groups under irrigated or drought treatments, whereas SUSY activity in the cleavage direction showed a significant decrease in the drought-stressed plants of both groups (Table 3). SPS activity was higher in the drought-exposed plants compared with the irrigated ones, but the difference was not significant. Striking differences in invertase activities was observed between the two groups. Drought treatment led to a more than threefold increase in the activities of both neutral (cytosolic) and acid (vacuolar) invertases in Group I but not in Group II genotypes, which correlated with the higher levels of reducing sugars observed (Table 3). Multivariate analysis showed that the enzyme activities did not differ significantly within each group (Table S6).

Expression levels of the genes coding for different isoforms of sugar-metabolising enzymes were studied using RNA extracted from the fifth internodes of two genotypes belonging to Group I (ICSSH 58 and SPV 1411) and Group II (ICSV 25280 and M 35–1) respectively. All the five isoforms of sucrose phosphate synthase (*SPS1*, *-2*, *-3*, *-4* and *-5*) and three isoforms of invertase (*INV2*, *-3* and *-4*) showed increased transcriptional expression in ICSSH 58; however, in the case of SPV 1411, in addition to invertase (*INV2*, *-3* and *-4*), it showed an increased expression of *SPS2* and *SUS2* under drought (Fig. 2; Table 4). Among the Group II genotypes, M 35–1 showed an increased expression of three invertase isoforms (*INV2*, *-3* and *-4*), like the Group I genotype ICSSH 58. However, unlike ICSSH 58, M 35–1 showed an increased expression of two sucrose synthase isoforms (*SUS1* and *SUS2*) but not of sucrose phosphate synthase isoforms under drought stress (Fig. 2; Table 4).

Drought effects at the physiological maturity stage

Irrigation was resumed after 20 days of drought stress and the plants were grown to physiological maturity, when the grain showed a distinct black hilum (Rao *et al*. 2013). Plants subjected to drought treatment reached the physiological maturity stage about 1–2 weeks earlier than irrigated plants (Table S2). Sufficient plants of one of the genotypes (SPV 1411) were not available and this genotype was omitted from the analysis carried out at this stage. A decrease in plant height, stalk yields, juice content in stalks and grain yields was observed in all genotypes on exposure to drought stress during the early reproductive stage, irrespective of the differences in these parameters observed under irrigated conditions in the different genotypes (Table 5). ANOVA showed a significant contribution of genotype and treatment within genotypes to the variation in the stalk yield, grain yield, juice yield and the Brix values at physiological maturity stage (Table S7). Stem sugar concentrations in the total juice extracted from 10 plants were calculated from the Brix values and were seen to decrease by 20–46% when the genotypes were subjected to drought stress.

Discussion

Drought-induced changes in sugar metabolism at the early reproductive stage

Withholding irrigation for a period of 20 days at the early reproductive phase brought about changes in water relations

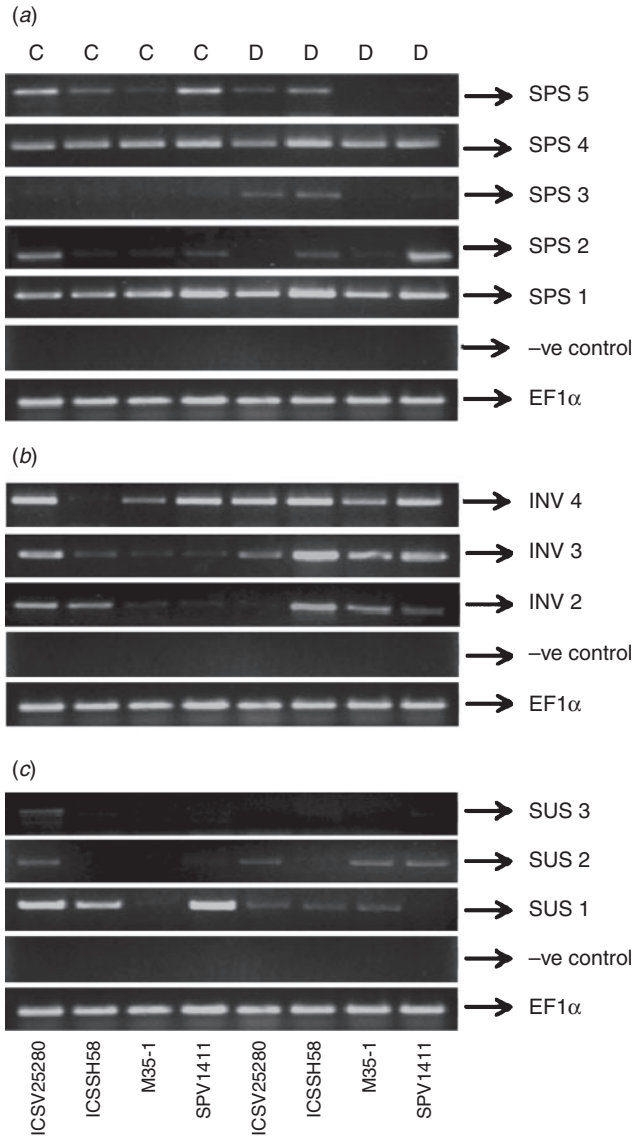

Fig. 2. Semiquantitative reverse transcription–PCR amplification of (*a*) five sucrose phosphate synthase (SPS) genes, (*b*) three invertase (INV) genes and (*c*) three sucrose synthase (SUSY) genes in two sorghum cultivars belonging to Group I (ICSSH 58 and SPV 1411) and two sorghum cultivars belonging to Group II (M 35–1 and ICSV 25280) grown under irrigated conditions (C) or subjected to drought stress for 20 days (D) at the early reproductive stage. Pooled RNA of three plants per genotype was used for reverse transcription–PCR amplification. Constitutively expressed *EF1α* was used as the loading control.

and sugar metabolism in the sorghum genotypes studied. Water loss was not so significant in the sugar-storing fifth internode of most genotypes compared with water loss in the peduncle. Total sugar concentrations in the sap increased in most genotypes, mainly as a consequence of water loss due to imposition of drought stress. However, two genotypes, ICSSH 58 and SPV 1411, did not show an increase in total sugar concentrations at the fifth internode after stress exposure (Fig. 1), possibly because they were able to maintain water content in the sap. These genotypes showed increased levels

Table 4. Fold expression of genes coding for sucrose phosphate synthase (*SPS1*, *SPS2*, *SPS3*, *SPS4* and *SPS5*), sucrose synthase (*SUSY1* and *SUSY2*) and invertase (*INV2*, *INV3* and *INV4*) amplified using semiquantitative reverse transcription–PCR in two sorghum cultivars belonging to Group I (ICSSH 58 and SPV 1411) and two sorghum cultivars belonging to Group II (M 35–1 and ICSV 25280), grown under irrigated conditions or subjected to drought stress for 20 days at the early reproductive stage (see Fig. 2)

The ratios for these fold expressions were determined from normalised band intensities under irrigated and drought conditions. nd, not detected in irrigated or drought-stressed plants; □, not detected in irrigated but detected in drought-stressed plants; ●, detected in irrigated but not detected in drought-stressed plants

	Fold expression (drought-stressed plants ÷ irrigated plants)									
	SPS1	SPS2	SPS3	SPS4	SPS5	SUSY1	SUSY2	INV2	INV3	INV4
Group I										
ICSSH 58	2.1	2.5	□	1.2	1.1	0.4	nd	3.2	10.8	23.0
SPV 1411	1.0	6.5	nd	1.0	0.1	●	3.3	3.6	14.4	1.3
Group II										
ICSV 25280	1.1	●	□	0.8	0.3	0.2	0.8	0.1	0.5	0.8
M 35–1	0.7	0.5	nd	0.6	●	1.4	□	4.3	6.9	1.1

Table 5. Plant height, internode number, and stalk, grain and juice yields in six sorghum genotypes subjected to continuous irrigation (controls (C)) or to a brief drought stress (D) during the early reproductive phase

All plants were harvested at their respective physiological maturity stages (Table S2). Values represent the means of three replicates of 10 plants each. Significant differences between mean values under drought-stressed and irrigated conditions for each genotype ($P < 0.05$) are indicated by asterisks

Variety	Treatment	Plant height (cm)	Internode number	Stalk yield (kg per 10 plants)	Grain yield (kg per 10 plants)	Juice yield (kg per 10 plants)	Degrees Brix (% w/w of juice)	Sugar content[A] in total juice from 10 plants (kg)
ICSV 25275	C	310	13	4.63	0. 31	1.23	17.33	0.213
	D	290*	13	4.21*	0. 25*	0.94*	18.17	0.171
ICSV 25280	C	340	12	4.45	0. 27	1.32	17.37	0.229
	D	305*	12	4.06*	0. 23	0.92*	17.00	0.156
PVK 801	C	250	14	4.50	0. 38	1.06	12.00	0.127
	D	240*	14	3.05*	0. 25*	0.66*	9.00*	0.059
RSSV 9	C	280	12	4.59	0. 26	0.94	14.17	0.133
	D	265*	12	3.15*	0. 21	0.73*	12.40*	0.091
ICSSH 58	C	360	14	4.70	0. 31	1.85	15.00	0.278
	D	320*	14	4.21*	0. 23	1.19*	17.00*	0.202
M 35–1	C	260	14	4.44	0. 38	0.94	12.67	0.119
	D	250*	14	3.78*	0. 31	0.84*	11.33	0.095

[A]Calculated from the degrees Brix values.

of reducing sugar, which may have a role to play in preserving the osmolarity of sap. Hexose accumulation and no alteration in moisture levels were also reported in sugarcane internodes when plants were subjected to water deficit (Iskandar *et al*. 2011). Maintenance of sap volume in ICSSH 58 and SPV 1411 under drought stress could arise due to stomatal closure, efficient water absorption by roots or accumulation of osmoprotective compounds, all of which constitute stress acclimation mechanisms (Lei *et al*. 2006; Xue *et al*. 2008). Isohydric responses arising from stomatal closure have also been reported in other sorghum cultivars (Tsuji *et al*. 2003). The higher levels of reducing sugars observed in these genotypes would have arisen from sucrose hydrolysis, since sucrose is the main storage sugar in sorghum (Slewinski 2012). Starch hydrolysis could also lead to higher levels of reducing sugars, but it seems unlikely, as starch content accounts for less than 0.2% of the nonstructural carbohydrates in sorghum stems (Arai-Sanoh *et al*. 2011). Lower levels of sucrose in the fifth internode in the drought exposed plants could be attributed to source limitations arising from a reduction in photosynthesis due to stomatal closure (Lemoine *et al*. 2013).

On the other hand, genotypes like ICSV 25275, ICSV 25280, PVK 801, RSSV 9 and M 35–1 were unable to conserve water in the fifth internode, as seen from a reduction in sap volume and an increase in sugar concentration. However, the levels of sugar in sap were higher (45%) than what could be accounted for by water loss alone (16%) (Table 2). This indicated photoassimilate influx into the fifth internode of these genotypes under drought stress conditions. Anisohydric plants are able to maintain high stomatal conductance under drought, thereby permitting photosynthetic assimilation for a longer period, at the cost of water loss (Blum and Arkin 1984). The Group II sorghum genotypes appeared to display anisohydric stomatal behaviour, as has also been reported in other sweet sorghum cultivars (Mastrorilli *et al*. 1999), where photosynthesis takes place at the cost of water loss. The Group II sorghum genotypes showed an increase in the levels of reducing sugars and invertase activity, but it was not as significant as the increase observed in Group I genotypes.

At the transcriptional level, two genotypes, belonging to Group I (ICSSH 58) and Group II (M 35–1) respectively, showed an induction of vacuolar invertase (*INV3*) genes as

well as an induction of cell wall invertases (*INV2* and *INV4*) in the fifth internode on exposure to drought stress. Transcriptional activation of vacuolar invertase was also reported in sink tissues of maize plants growing under drought conditions. This correlated with higher invertase activity and was thought to play an important role in osmotic adjustment by bringing about an accumulation of hexoses (Kim *et al*. 2000). M 35–1 also showed an induction of genes coding for SUSY isoforms, though the activity of the former was reduced in response to drought. This lack of correlation between gene expression and enzyme activity may arise from post-translational modifications like phosphorylation or oligomerisation, which have been reported to regulate SUSY activity (Hardin *et al*. 2003; Duncan and Huber 2007). Our experiments also showed a lack of correlation in the transcriptional expression of genes coding for sugar-metabolising enzymes and the stem sugar-accumulating ability of the respective genotypes, as has been recently reported in a comparative study of vegetative tissues of various sweet, grain and forage sorghum genotypes (Shakoor *et al*. 2014).

Sugar metabolism at physiological maturity in plants recovering from drought stress

Sorghum genotypes exposed to drought stress reached the physiological maturity stage about 7–13 days earlier than the plants that received continuous irrigation. A reduction in the period of attaining physiological maturity was also observed in some photoperiod-sensitive sorghum genotypes when they were grown under an unfavourable photoperiod (Gutjahr *et al*. 2013*a*). However, these genotypes showed relatively stable stem sugar concentrations in spite of phenotypic plasticity, as was also seen in the sorghum genotypes used in this study. Stem sugar accumulation therefore appeared to depend on source–sink balance rather than on phenology. Drought stress exposure at the early reproductive stage led to a reduction of stalk weights and grain yields at physiological maturity. In sugarcane plants subjected to drought stress, photoassimilates were shown to be diverted to stems for storage, rather than being used for supporting growth (Inman-Bamber and Smith 2005). However, in the sorghum genotypes exposed to drought stress, both growth and sugar accumulation decreased in the stems. In some genotypes like ICSV 25275, ICSSH 58, PVK 801 and M 35–1, there were similar losses in grain yields and stem sugars (20%, 25%, 45% and 20% respectively; Table 5) due to the brief drought exposure, indicating a source limitation of photoassimilates to the panicle and stem internodes, both of which served as independent sinks. However, in other genotypes like ICSV 25280 and RSSV 9, grain yield losses were ~1.5- to 2-fold lower than stem sugar losses in drought-exposed plants compared with the controls (Table 5). This could arise either due to panicles being preferred sinks over stem internodes for photoassimilate partitioning or due to mobilisation of sugars from the storage internodes to the developing panicle to ensure viable progeny (Slewinski 2012). Hence accumulation of stem sugars and their role as buffer stocks to be used in grain filling appeared to be an adaptive strategy against drought stress in two of the six sorghum genotypes studied, whereas the panicle and stem appeared to serve as independent sinks in the other four genotypes.

Acknowledgements

SB and HQ thank the Department of Atomic Energy, Board of Research in Nuclear Sciences for providing financial support and ICRISAT for providing field facilities to grow the sorghum plants.

References

Arai-Sanoh Y, Ida M, Zhao R, Yoshinaga S, Takai T, Ishimaru T, Maeda H, Nishitani K, Terashima Y, Gau M, Kato N, Matsuoka M, Kondo M (2011) Genotypic variation in non-structural carbohydrate and cell wall components of the stem in rice, sorghum and sugarcane. *Bioscience, Biotechnology, and Biochemistry* **75**, 1104–1112. doi:10.1271/bbb.110009

Bhargava S, Sawant K (2013) Drought stress adaptation: metabolic adjustment and regulation of gene expression. *Plant Breeding* **132**, 21–32. doi:10.1111/pbr.12004

Blum A, Arkin GF (1984) Sorghum root growth and water-use as affected by water supply and growth duration. *Field Crops Research* **9**, 131–142. doi:10.1016/0378-4290(84)90019-4

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. *Analytical Biochemistry* **72**, 248–254. doi:10.1016/0003-2697(76)90527-3

Dillon SL, Shapter FM, Henry RJ, Cordeiro G, Izquierdo L, Lee LS (2007) Domestication to crop improvement: genetic resources for *Sorghum* and *Saccharum* (Andropogoneae). *Annals of Botany* **100**, 975–989. doi:10.1093/aob/mcm192

Duncan KA, Huber SC (2007) Sucrose synthase oligomerization and F-actin association are regulated by sucrose concentration and phosphorylation. *Plant & Cell Physiology* **48**, 1612–1623. doi:10.1093/pcp/pcm133

Ganesh Kumar C, Fatima A, Srinivasa Rao P, Reddy BVS, Rathore A, Rao RN, Khalid S, Kumar AA, Kamal A (2010) Characterization of improved sweet sorghum genotypes for biochemical parameters, sugar yield and its attributes at different phenological stages. *Sugar Tech* **12**, 322–328. doi:10.1007/s12355-010-0045-1

Glassop D, Ryan LP, Bonnett GD, Rae AL (2010) The complement of soluble sugars in the *Saccharum* complex. *Tropical Plant Biology* **3**, 110–122. doi:10.1007/s12042-010-9049-y

Gnansounou E, Dauriat A, Wyman CE (2005) Refining sweet sorghum to ethanol and sugar: economic trade-offs in the context of North China. *Bioresource Technology* **96**, 985–1002. doi:10.1016/j.biortech.2004.09.015

Gutjahr S, Vaksmann M, Dingkuhn M, Thera K, Trouche G, Braconnier S, Luquet D (2013*a*) Grain, sugar and biomass accumulation in tropical sorghums. I. Trade-offs and effects of phenological plasticity. *Functional Plant Biology* **40**, 342–354. doi:10.1071/FP12269

Gutjahr S, Clement-Vidal A, Soutiras A, Sonderegger N, Braconnier S, Dingkuhn M, Luquet D (2013*b*) Grain, sugar and biomass accumulation in photoperiod-sensitive sorghums. II. Biochemical processes at internode level and interaction with phenology. *Functional Plant Biology* **40**, 355–368. doi:10.1071/FP12177

Harbron S, Woodrow IE, Kelly GJ, Robinson SP, Latzko E, Walker DA (1980) A continuous spectrophotometric assay for sucrose phosphate synthetase. *Analytical Biochemistry* **107**, 56–59. doi:10.1016/0003-2697(80)90491-1

Hardin SC, Tang GQ, Scholz A, Holtgraewe D, Winter H, Huber SC (2003) Phosphorylation of sucrose synthase at serine 170: occurrence and possible role as a signal for proteolysis. *The Plant Journal* **35**, 588–603. doi:10.1046/j.1365-313X.2003.01831.x

Hoffmann-Thoma G, Hinkel K, Nicolay P, Willenbrink J (1996) Sucrose accumulation in sweet sorghum stem internodes in relation to growth.

Physiologia Plantarum **97**, 277–284. doi:10.1034/j.1399-3054.1996.970210.x

Ibrahim AH, El-Shahaby OA, Abo-Hamed SA, Younis ME (2013) Parental drought and defoliation effect on yield, grains biochemical aspects and drought performance of sorghum progeny. *Journal of Stress Physiology & Biochemistry* **9**, 258–272.

Inman-Bamber NG, Smith DM (2005) Water relations in sugarcane and response to water deficits. *Field Crops Research* **92**, 185–202. doi:10.1016/j.fcr.2005.01.023

Iskandar HM, Casu RE, Fletcher AT, Schmidt S, Xu J, Maclean DJ, Manners JM, Bonnett GD (2011) Identification of drought-response genes and a study of their expression during sucrose accumulation and water deficit in sugarcane culms. *BMC Plant Biology* **11**, 12. doi:10.1186/1471-2229-11-12

Kim JY, Mahe A, Brangeon J, Prioul JL (2000) A maize vacuolar invertase, *IVR2*, is induced by water stress. Organ/tissue specificity and diurnal modulation of expression. *Plant Physiology* **124**, 71–84. doi:10.1104/pp.124.1.71

Lei Y, Yin C, Lia C (2006) Differences in some morphological, physiological and biochemical responses to drought stress in two contrasting populations of *Populus przewalskii. Physiologia Plantarum* **127**, 182–191. doi:10.1111/j.1399-3054.2006.00638.x

Lemoine R, La Camera S, Atanassova R, Dedaldechamp F, Allario T, Pourtau N, Bonnemain JL, Laloi M, Coutos-Thevenot P, Maurousset L, Faucher M, Girousse C, Lemonnier L, Parrilla J, Durand M (2013) Source-to sink transport of sugar and regulation by environmental factors. *Frontiers in Plant Science* **4**, 272. doi:10.3389/fpls.2013.00272

Lingle SE, Dunlap JR (1987) Sucrose metabolism in netted muskmelon fruit during development. *Plant Physiology* **84**, 386–389. doi:10.1104/pp.84.2.386

Mastrorilli M, Katerji N, Rana G (1999) Productivity and water use efficiency of sweet sorghum as affected by soil water deficit occurring at different vegetative growth stages. *European Journal of Agronomy* **11**, 207–215. doi:10.1016/S1161-0301(99)00032-5

Miller GL (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry* **31**, 426–428. doi:10.1021/ac60147a030

Morell M, Copeland L (1985) Sucrose synthase of soybean nodules. *Plant Physiology* **78**, 149–154. doi:10.1104/pp.78.1.149

Qazi HA, Paranjpe S, Bhargava S (2012) Stem sugar accumulation in sweet sorghum – activity and expression of sucrose metabolizing enzymes and sucrose transporters. *Journal of Plant Physiology* **169**, 605–613. doi:10.1016/j.jplph.2012.01.005

Rao SS, Patil JV, Prasad PVV, Reddy DCS, Mishra JS, Umakanth AV, Reddy BVS, Kumar AA (2013) Sweet sorghum planting effects on stalk yield and sugar quality in semi-arid tropical environment. *Crop Ecology & Physiology* **105**, 1458–1465.

Rohwer JM, Botha FC (2001) Analysis of sucrose accumulation in the sugar cane culm on the basis of *in vitro* kinetic data. *Biochemical Journal* **358**, 437–445. doi:10.1042/0264-6021:3580437

Rosa M, Prado C, Podazza G, Interdonato R, Gonzalez JA, Hilal M, Prado FE (2009) Soluble sugars – metabolism, sensing and abiotic stress. *Plant Signaling & Behavior* **4**, 388–393. doi:10.4161/psb.4.5.8294

Shakoor N, Nair R, Crasta O, Morris G, Feltus A, Kresovich S (2014) A *Sorghum bicolor* expression atlas reveals dynamic genotype-specific expressin profiles for vegetative tissues of grain, sweet and bioenergy sorghums. *BMC Plant Biology* **14**, 35–48. doi:10.1186/1471-2229-14-35

Silva MA, Caputo MM (2012) Ripening and the use of ripeners for better sugarcane management, crop management – cases and tools for higher yield and sustainability. In 'Crop management – cases and tools for higher yield and sustainability'. (Ed FR Marin) pp. 1–23. (InTech: Rijeka, Croatia)

Slewinski TL (2012) Non-structural carbohydrate partitioning in grass stems: a target to increase yield stability, stress tolerance and biofuel production. *Journal of Experimental Botany* **63**, 4647–4670. doi:10.1093/jxb/ers124

Sturm A, Tang GQ (1999) The sucrose-cleaving enzymes of plants are crucial for development, growth and carbon partitioning. *Trends in Plant Science* **4**, 401–407. doi:10.1016/S1360-1385(99)01470-3

Tarpley L, Vietor D (2007) Compartmentation of sucrose during radial transfer in mature sorghum culm. *BMC Plant Biology* **7**, 33. doi:10.1186/1471-2229-7-33

Tarpley L, Lingle SE, Vietor DM, Andrews DL, Miller FR (1994) Enzymatic control of non structural carbohydrate concentration in stems and panicles of sorghum. *Crop Science* **34**, 446–452. doi:10.2135/cropsci1994.0011183X003400020026x

Tarpley L, Vietor D, Miller F (1996) Metabolism of sucrose during storage in intact sorghum stalk. *International Journal of Plant Sciences* **157**, 159–163. doi:10.1086/297334

Tsuji W, Ali MEK, Inanaga S, Sugimoto Y (2003) Growth and gas exchange of three sorghum cultivars under drought stress. *Biologia Plantarum* **46**, 583–587. doi:10.1023/A:1024875814296

Walsh KB, Sky RC, Brown SM (2005) The anatomy of the pathway of sucrose unloading within the sugarcane stalk. *Functional Plant Biology* **32**, 367–374. doi:10.1071/FP04102

Wang J, Nayak S, Koch K, Ming R (2013) Carbon partitioning in sugarcane (*Saccharum* species). *Frontiers in Plant Science* **4**, 201. doi:10.3389/fpls.2013.00201

Xue GP, McIntyre CL, Glassop D, Shorter R (2008) Use of expression analysis to dissect alterations in carbohydrate metabolism in wheat leaves during drought stress. *Plant Molecular Biology* **67**, 197–214. doi:10.1007/s11103-008-9311-y

Zingaretti SM, Rodrigues FA, Graca JPD, Pereira LDM, Lourenco MV (2012) Sugarcane responses at water deficit conditions. In 'Water stress'. (Eds IMM Rahman, H Hasegawa) pp. 255–276. (InTech: Rijeka, Croatia)